The development and evaluation of a hydrogel drug delivery system for dithranol.

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THE DEVELOPMENT AND EVALUATION OF A HYDROGEL DRUG DELIVERY SYSTEM FOR DITHRANOL

AROONSRI PRIPREM

A thesis submitted in partial fulfilment of the requirement of the Council for National Academic Awards for the Degree of Doctor of Philosophy

School of Pharmacy
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To my Father

who

provides

and

loves

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ABSTRACT

The Development and Evaluation of a Hydrogel Drug Delivery System for Dithranol

Dithranol is widely used in the treatment of psoriasis but it has the drawbacks of instability, staining of skin and clothes and irritation, especially of non-involved skin. A polyurethaneurea hydrogel in sheet form has been used to investigate the possibility of developing a drug delivery system which would confine dithranol to the psoriatic plaque whilst also providing appropriate stability and drug delivery rates.

Analytical methods were developed and evaluated to ensure that dithranol stability could be monitored and release rates measured. An hplc method enabled the assay of dithranol, danthrone and dimer at concentrations appropriate for stability studies and longer-time release experiments. A more sensitive fluorimetric method enabled assay of the dithranol in the low concentrations occurring in the early stages of release experiments.

Hydrogels were loaded using a dithranol solution. The blend of solvents for the loading solution were selected to optimize dithranol solubility and hydrogel swelling and minimise oxidation of dithranol. Storage conditions for the loading films were also optimized until a zero order half-life of 30 days for the loaded film was obtained.

In vitro release studies indicated that the process was slower and less extensive into aqueous media than into non-aqueous media. Stratum corneum, however, acts as a rate-limiting membrane so that the release rate became independent of receptor medium hydrophobicity. A rate constant of dithranol release from hydrogel through stratum corneum of 0.2 $\mu g/cm^2/hr$ was obtained.

The hydrogel was evaluated on 5 normal and 8 psoriatic patients. Clinical response was measured by scoring scaliness, thickness, erythema and staining. Detailed time course evaluation on two psoriatic patients indicated that there was a lag time of 7 hours after application before the first clinical response was obtained. Comparative studies on 5 normal volunteers indicated that there was an approximate bioequivalence between the hydrogel and 0.1% Dithrocream. This was confirmed with 6 psoriatic patients which also showed that both are more effective than occlusion alone or blank hydrogel..

From the data obtained some suggestions about the possible effective concentration and the site and mechanism of action of dithranol in psoriasis are discussed.

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CHAPTER 1

INTRODUCTION

1.1 THE HUMAN SKIN

The entire surface of the human body is covered by a layer of skin which presents a tough but flexible barrier to the exterior [Wood & Bladon, 1985]. It is the largest [McClintic, 1975] and heaviest [Barry, 1983] organ of the body. Humans owe their ability to survive in an uncompromising environment to the skin. It may be regarded as having two functions [Barry, 1983]: communication between the inside and the outside environments, and control of the former. For example, it separates and protects the internal body structures from the external environmental factors such as temperature, humidity, radiation, and pollution and it restricts the passage of substances into and out of the body. It also plays a vital role in the regulation of body temperature and blood pressure and is the mediator of sensations of touch, pain, heat and pressure. Synthetic and metabolic processes also occur in the skin such as the production of vitamin D in the presence of light.

The average adult skin has a surface area 1.75 m^2 (about 3000 in^2) [McClintic, 1975, Barry, 1983] and comprises about 7-12 % of the body weight [Wood & Bladon, 1985]. It receives about one third of the blood supply [McClintic, 1975].

The human skin comprises two distinct, but mutually dependent, tissues: the cellular epidermis and an underlying dermis of connective tissue. The epidermis arises from the embryonic ectoderm whereas the dermis the embryonic mesoderm. Beneath the dermis lies a layer of fatty adipose tissue called the subcutaneous layer or hypodermis [Barry, 1983]. Because psoriasis is a skin disease which results from an epidermal response and dermal participation [van Scott & Flaxman, 1973], these parts of the skin will be briefly reviewed along with the keratins.

The epidermis

The thickness of the epidermis varies, depending on cell size and the number of cell layers, ranging from about 0.8 mm on the palms and the soles down to 0.06 mm on the eyelids but is between 0.06-0.1 mm in most other regions. The epidermis, which is attached to the dermis at a basement membrane, consists of epithelial cells that pass through 5 subsequent layers namely (from the deepest to the most superficial layer of the skin): the stratum basale or germinativum, stratum spinosum (sometimes called stratum germinativum), stratum granulosum, stratum lucidum, and stratum corneum.

The mitotic proliferation of the basal cells in the stratum basale continuously renews the epidermis[Barry, 1983]. Attempts to estimate the epidermal turnover times have found that a cell from the basal layer takes at least 14 days in normal skin to reach the stratum corneum and the normal turnover time in the stratum corneum was some 13 or 14 days, thus the average total turnover time is 28 days [Barry, 1983, Wood & Bladon, 1985].

As the cells produced by basal cell mitosis move outward towards the surface, they undergo a series of alterations, including keratinization. In the stratum spinosum, the nuclei in the cells shrink and the cells flatten, becoming polygonal in shape, and producing fine

prickles or spines, called prickle or spinous cells or keratinocytes. Each prickle cell encloses an extension of the cytoplasm, and the opposing tips of the prickles of adjacent cells adhere to form intercellular bridges, the desmosomes. The desmosomes correspond to points of attachment of bundles of filaments, called tonofilaments, to the plasma membrane of the cell, and thus maintain the integrity of the epidermis. Tonofilaments are made of the insoluble fibrous protein, keratin.

As the keratinocytes move into the stratum granulosum, they produce basic staining particles, the keratohyalin granules. This is an active, not a degenerative, process which creates a transitional region between living cells and dead keratin [Barry, 1983]. Polypeptide building blocks present in this layer aggregate to form insoluble fibrous keratin molecules. This transitional zone between the living cells and dead keratins is a region of intense biochemical activity and morphological changes [Barry, 1983].

After leaving the stratum granulosum, lysosomal enzymes are activated, resulting eventually in the breakdown of the nucleus and all other cell organelles [Wood & Bladon, 1985]. In the palms and soles, the stratum lucidum a single layer of anucleate translucent cells, could be seen lying just above the granular layer. Finally, 10-15 layers of keratinized dead cells form an inert and relatively impervious layer called stratum corneum (horny layer). The flattened cells overlap and interdigitate their lateral edges. A lamellar material, possibly keratin, is thought to form several layers of broad sheets attached to the outer surfaces of the cells, with adjacent cells so as to form cohesive lamellae.

The division of the basal cells balances the constant shedding of fully keratinized dead squamous cells (an average daily loss of 0.5-1 g from the whole body skin surface), as a result of which, the epidermis remains

constant in thickness. When dry, stratum corneum may be only 10 μm thick with a density of about 1.5 g cm^{-3}, but it can swell in water to several times this thickness. Besides containing keratin, the cells are also the final repository of the end products of epidermal metabolism.

Other cells of the epidermis are the dendritic Langerhan's cells, which organize the function of the squamous epidermis and are involved in immune responses in the skin: Merkel's corpuscles, which are associated with the sensation of touch: and the dendritic melanocytes which are responsible for skin pigmentation.

Lipids make up as much as 10% of the dry weight of epidermis [Barry, 1983]. 90% of the skin surface lipids is produced by the sebaceous glands which are abundant on the scalp, forehead, and upperback [Wood & Bladon, 1985]. The remainder, which is mainly sterols, is produced in the lower layers of the epidermis. Overall, 60% of the lipid is fatty acids (predominately C16 and C18), sterol esters or wax esters. The quantity of fat ranging between 5-150 $\mu g/cm^2$ corresponds to 0.05-1.5 μm hypothetical thickness [Schaefer et al, 1982]. The fat film is highly viscous but fluid at normal skin temperature and is mechanically displaceable and dispersible.

The dermis(or corium)

The dermis (3-5 mm thick), underlying the epidermis, forms the bulk of the skin. The main structural feature of the dermis is a network of mechanically strong fibrous proteins (woven from approximately 75% collagen, 4% elastin, 0.4% reticulin) embedded in a matrix of amorphous ground substance of mucopolysaccharide which provides about 20% of the mass. In contrast to the epidermis, the dermis contains few cells. Those present are fibroblasts (the majority of which are responsible for secreting the dermal constituents), mast cells, macrophages, lymphocytes, and melanocytes. Blood vessels and the lymphatic system form the vascular plexuses to the matrix

of the dermis, as well as the nerves and skin appendages [Barry, 1983]. They do not enter the epidermis and so the dermis has to support and supply the need of the epidermis with respect to nourishment, warmth, moisture, defensive systems, etc.

The keratins and keratohyalin granules

Keratins consist of chains of amino acids which are synthesised by the usual DNA-RNA mechanisms [Mier & Cotton, 1976]. Analysis of the keratins extracted from human epidermis reveals a series of larger "or mature" polypeptides and smaller ones called "prekeratin" [Wood & Bladon, 1985]. Prekeratins are thought to undergo several processes such as disulphide cross-linking to form keratins. These processes begin in the basal cells and terminate in the horny layer. Bundles of the keratin polypeptide chains usually terminate in membrane-bound desmosomes.

Barry (1983) believed that keratohyalin granules are an early form of keratin or cell organelles which are partially destroyed by hydrolytic enzymes. It is thought [Wood & Baldon, 1985] that keratohyalin granules contain the precursor of a protein called filaggrin (filament-aggregation protein) and that the filaggrin is involved in some as yet unknown way with the transportation of large amounts of keratin filaments into an intracellular matrix.

1.2 PSORIASIS

Prevalence and clinical features of psoriasis

Psoriasis is one of the most common of all the skin diseases. It occurs world-wide, affects persons of all ages, and almost all ethnic groups [Farber & Nall, 1984]. Estimates of the prevalence of psoriasis in the general population of the world range from 0.1 to 2.8% [Farber & Nall, 1984]. It occurs in males and females with nearly

equal frequency. The average age at onset is in the 20s, ranging from birth to 80 or 90 years. It is a chronic, lifelong disorder causing physical disfigurement and disability and producing psychological and economic stress. Lesions are commonly observed on the scalp, elbows, trunk, and the lower extremities, but can occur on all body parts including fingernails and toe-nails. A temporary or permanent remission may occur in the course of the disease. The reason for this is not known.

Psoriatic skin is characterised by the appearance of sharply defined erythematous patches covered with a distinctive crumbling silvery grey scale [Barry, 1983]. When the scales are removed, small punctate bleeding points become visible. Its clinical appearance can vary widely and may be classified as:

Psoriasis vulgaris
Guttate psoriasis
Pustular psoriasis
Psoriatic erythroderma
Psoriasis and arthritis

Two categories of psoriatic skin are defined: (1) visible psoriasis, (2) non-visible psoriasis. The latter includes residual psoriasis (clinically non-apparent, but histologically recognizable), pre-psoriasis (epidermal thickening, increased DNA synthesis, histochemical parakeratosis, infiltration of macrophages, lymphocytes in the dermis, capillary dilatation), and latent psoriasis (absence of any measurable change but susceptibility to visible psoriasis after injury - the so called "Koebner reaction".

Cellular features and cause of psoriatic skin

Psoriatic skin exhibits a complex irregularity [Steigleder, 1981] in the mechanisms which control the epidermal cell division. The characteristic scales which are different to normal stratum corneum and overlie the epidermal are the result of altered keratinization [Marks et al, 1979]. It is established that in individuals with

a genetic predisposition to the disease, physical and chemical trauma, infections, inflammation, ecdocrine changes such as those occurring at puberty, some drugs, and emotional stress can provoke the course of the disease or exacerbate it if it is already present [Wood & Bladon, 1985].

Table 1.1 Histopathological changes in untreated psoriatic plaques [from Wilborn & Montes, 1974 and Steigleder, 1981])

Targets	normal	psoriasis
parakeratosis	non	appear
cell size	normal	larger
skin thickness	normal	thicker
tonofilaments	numerous	reduced
intercellular space	narrow	large
mitochondria	few, small	increased, enlarged
keratohyalin	numerous	decreased

Histological sections through psoriatic lesions (Table 1.1) show that the granular layer is reduced or absent and there is parakeratosis with an increase in mitotic activity in the basal layer cells [vanScott & Ekel, 1963, vanScott & Flaxman, 1973, Wilborn & Montes, 1974, Wood & Bladon, 1985]. The size of psoriatic epidermal cells are larger than normal ones. Mahrle (1981) showed that profound alterations of cell membranes and increases in the cell surface area of psoriatic keratinocytes accelerate cell mitosis. The increase in the skin thickness or epidermal hyperplasia is shown to be the result of an increase in cell size [Flaxman & Chopra, 1972]. The type and number of tonofilaments of psoriatic skin are different from the normal ones and the intercellular spaces in the psoriatic lesions are dilated [supported by Steigleder, 1981]. Table 1.1 also shows that the number and size of mitochondria in psoriatic plaques are different from normal skin.

Evidence shows an increase in mitotic rate of the basal cell but the cause is not clear [Baker, 1975a]. It has been proposed that the level of chalone, a mitotic

inhibitory substance involved in the negative feedback mechanism [Marks et al, 1979], is decreased, resulting in increased mitotic rates. Thus, the presence of the parakeratotic keratin in psoriasis is a positive feedback in the mitotic control and leads to psoriasis becoming a self-perpetuating disease.

The factors involved in the cell divisions associated with psoriatic epidermal hyperplasia are in dispute [Baker, 1975a]. For example, Weinstein & Frost (1968) estimated that the whole cell cycle time is decreased from 457 hours (normal) to 37 hr (involved psoriatic), but Gelfant (1976) disagreed with these figures. He showed that not all the cells in the proliferative pool are actively involved in cell division and that a recruitment of about half of the cells, stimulated by some unknown factors, causes the epidermal hyperplasia. However, there is a reasonable agreement on the duration of the "synthesis" (S) phase of the mitotic cycle in psoriasis of 8.5 hours [Weinstein & Frost, 1968] and 10 hours [Goodwin et al, 1974].

Steigleder (1981) and Bauer et al (1981) indicate not only that there are higher proliferation rates in psoriatic keratinocytes than normal cells, but also altered keratinisation. Hodgson (1962) and Rothberg (1960) demonstrated the presence of abnormal protein fractions in psoriatic epidermal keratins. Abnormal keratins in psoriasis could, in turn, provoke the compensatory increase in cell division [Marks et al, 1979, Mier & Cotton, 1976]. Whether abnormal keratins are formed prior to increased cell division or vice versa is not known. However, the abnormality of the keratins causes a decrease in the water holding capacity of the epidermis, thus total epidermal water loss in psoriatic skin is higher than that in normal skin [Serup & Blichmann, 1987].

Examples of other biochemical and immunological deviations from normal skin have been implicited. Hammar (1970) reported a significant rise in the activity of glucose-6-phosphate dehydrogenase in the uninvolved skin and within the psoriatic lesion. An alteration in arachidonic acid metabolism in psoriasis has been reported [Muller, 1988, Kragballe & Voorhees, 1987]. Defects in sterol metabolism, fatty acid metabolism and the esterification process have been demonstrated [Yardley, 1969]. Esterified cholesterol is a vital component in the ordered structure of the keratin fibrils. An incomplete catabolism of phospholipids during keratinisation and a deficiency in a phophorylation mechanism in psoriasis were shown [Yardley & Godfrey, 1964]. The levels of cAMP [Prunieras, 1979, Plummer, 1979, Zachariae, 1979] and folic acid derivatives [Mier & Cotton, 1976] which deviated from normal could indicate the non-clinical presence of psoriasis. The immunological factors IgA and complement are found deposited in the lesion after hyperproliferation [Steigleder, 1981], whilst T-lymphocytes are lower than normal [Zachariae, 1979]. Polymorphonuclear leukocytes show an enhanced chemotactic response to various chemo-attractants which dithranol reduced this activity and becomes normal [Michaelsson, 1980 and Schroder et al, 1985].

Defects in the dermis

Histological sections show that there is papillary oedema, dilatation and tortuosity of the papillary capillaries [Ross et al, 1964]. This explains why removal of the scales damages not only the epidermis but also the underlying capillaries, resulting in a characteristic punctate bleeding point [Wood & Bladon, 1985, Barry, 1983]. Inherent vasodilatation and a characteristic psoriatic capillary network have been observed [Ryan, 1979] whilst Kemeny (1989) has shown that the capillary resistance of psoriatic patients is significantly lower than that of healthy individuals.

Treatment

There is no cure for psoriasis. Several treatments are available, which varies from topical to systemic modalities administered singly or in combination, are effective in controlling of the disease. Remission may occur in the course of the disease. Treatment regimens may be complex and tend to vary from one dermatologist to another. The advantages and disadvantages of the treatments available are summarized as follows:

Treatment	Advantage	Disadvantage
dithranol	safety efficacy	staining irritancy
tars	safe	efficacy variation inconvenient inconsistent unacceptable preparation
corticosteroid	patient acceptance	short term use only rebound flare-ups systemic side effects
photochemotherapy (Psoralen + UltraViolet A or PUVA)	rapid effective	high recurrent rate possibly cancer inducer chronic toxicity risks acute side effects
antimitotic drugs	highly effective	very potent/toxic more severe in recurrent
cyclosporin A	effective	rebound effect toxicity
retinoids	effective	teratogenicity hypervitaminosis A

DITHRANOL will be discussed later in detailed in this chapter.

TARS have been used in the treatment of psoriasis for a long time. Crude coal tar containing thousands of compounds leads to lack of knowledge of mode of action and standardised preparations [Baker, 1975b]. Various tar products are available commercially as solutions, for baths, paints, ointments, sticks and shampoos.

The use of CORTICOSTERIODS, such as clobetasol propionate and fluocinolone acetonide, in the treatment of psoriasis is common. It is acceptable among the patients despite many complications of steriods and rebound effects because of their cosmetically elegant properties, i.e. odourless, non-staining. The combined use of steriods and dithranol therapy has been studied in an attempt to reduce dithranol side effects and increase steriod therapeutic efficacy [Grattan et al, 1988]. But earlier clinical response as well as relapse are observed.

Natural sun LIGHT was found to be beneficial in 80% of 5600 psoriatic patients [Farber & Nall, 1984]. This gave rise to extensive studies of phototherapy in psoriasis. Oral psoralen and long-wave ultraviolet irradiation (PUVA) is an effective form of therapy [Wolff & Fritsch 1981]. Reports on cutaneous carcinoma [Stern et al, 1979], cataract formation [Goldberg et al, 1979], epidermal dystrophy and high recurrent rate [Abel et al, 1982] in patients treated with PUVA have raised the question of its long term safety.

ANTIMITOTIC drugs, for example methotrexate and hydroxyurea, are reserved for use in severe cases or those failing to respond to other treatment [Farber & Nall, 1984]. Methotrexate is more effective, and thus better known, than hydroxyurea [Baker, 1975 b]. It is used orally or by intramuscular injection with close monitoring of liver, kidney and bone marrow functions.

An immunosuppressive drug, CYCLOSPORIN A, is given orally or intravascular injection and found to be effective in severe psoriasis [Griffiths et al, 1989].

Vitamin A has long been recognised as being vital to general growth and skin tissue, and synthetic RETINOIDS are demonstrated to be effective in psoriasis [Orfanos & Runne, 1976]. However, the major side effects, including skeletal damage and diffuse hair loss, and high and severe

remission not to mention teratogenicity have limited the use of retinoids in psoriasis.

Occlusive dressing therapy

Previous studies have suggested a potential therapeutic role for occlusion on psoriasis. In 1970, Fry and colleagues reported the clinical improvements of a significant decrease in mitotic counts, and the reformation of the granular cell layer in psoriatic lesions by covering them with plastic dressings only. These were seen after 2 weeks of occlusion but not in unoccluded controls. Baxter and Stoughton (1970) have also found a reduction in mitotic index (number of mitoses per 1000 cells) after occlusions alone in psoriatic skin. More recently, Friedman (1987) reported significant clinical improvements in psoriatic patients using an adhesive hydrocolloid occlusive dressing (DuoDerm). The proposed mechanism by which occlusion exerts its effect on psoriasis [Friedman, 1987] involves trapping moisture by acting as an artificial water and mechanical barrier for the abnormal stratum corneum. This would enable the epidermis to reduce cell division, increase cellular differentiation and facilitate desquamation. Monitoring by clinical assessments for psoriatic plaque, namely thickness, scaling, and erythema shows that the thickness and scaling, but not erythema, were significantly reduced after 2 weeks [Gottlieb et al, 1988] or up to 10 weeks in 46 patients [Telfer et al, 1988]. Telfer and colleagues observed the reformation of granular cell layer but emphasised that, after 10 weeks of treatment, none of the patients has completely recovered from psoriasis.

Thus, combined therapies using occlusion and conventional methods, such as steriods, phototherapy or dithranol have been widely studied. Short contact dithranol therapy under occlusions has been used to compare with either the dithranol or occlusions alone [Lever & Marks, 1988]. All three therapies give significant improvements after 3 weeks. It was found that

the occlusion was at least as effective as short contact therapy using 0.1% Dithrocream, and that combined treatment could be more effective than either treatment alone. Additionally, dithranol irritation is not increased with the combined treatment and the patients found it reasonably convenient to use.

Adverse effects of occlusion on skin such as potential increase in bacterial infections or discomfort after prolonged applications are likely. Sulzberger and Witten (1961) showed that application of a plastic (vinylidine) film to psoriatic skin causes sweating underneath the sheet and the consequent discomfort was the most common complaint. Bacterial counts increased and pH fell (from 7 to 4) after 4 days of occlusion on normal skin using the same plastic film [Aly et al, 1978]. Under hydrocolloid occlusion in psoriatic patients, Cherry et al (1988) demonstrated that the bacterial counts found were much less than with the plastic film, whilst an increase in maceration was seen in all patients with the plastic film but in none with the hydrocolloid film.

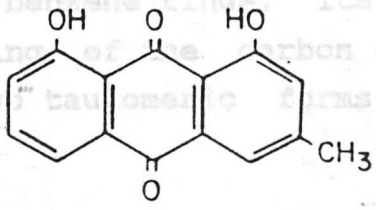
1.3 DITHRANOL

The clinical efficacy of topically applied dithranol for the treatment of psoriasis including its high degree of safety and efficacy are well established [Ingram, 1953, Farber & Harris, 1970, Baker, 1975b]. Despite being used for almost a century, it has not yet been possible to satisfactorily elucidate all aspects of its pharmacology and pharmaceutics, possibly due to its relatively low concentration in use and the lack of sensitive analytical procedures [Mustakallio, 1981b] and knowledge of the causes of psoriasis. Patient acceptance of dithranol preparations and compliance with the treatment regimens is often limited due to unpleasant staining and contact irritation of uninvolved skin. Although its mechanism of

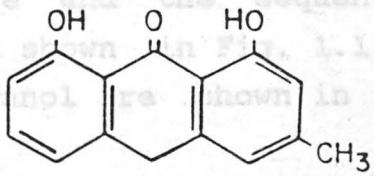
action is not known at present, it appears that these side effects and its effectiveness are correlated. Formulation development for the topical use of dithranol which can improve patient compliance and product shelf-life is one of the numerous variations on the use of dithranol evolved in different regions of the world [Farber, 1983].

History

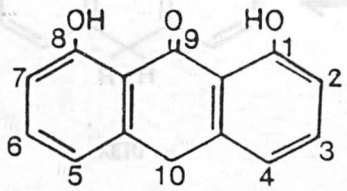
Goa powder (known as poh de Bahia in China and araroba powder in Brazil), the natural source of dithranol, was first used in the treatment of fungal infections of the skin by Balmanno Squire, a British dermatologist in 1874 [quoted from Ashton et al, 1983, Farber, 1983]. Workmen employed in cutting up Goa trees and pounding the sap were obliged to protect their faces because of its irritation.



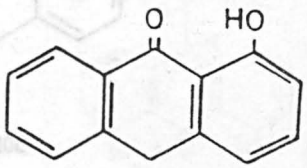
a) chrysophanic acid



b) chrysarobine



c) dithranol



d) 1-hydroxy-9-anthrone

Fig.1.1 Chemical structures of a) chrysophanic acid, b) chrysarobine, c) dithranol with numbering, and d) 1-hydroxy-9-anthrone.

Goa powder, extracted from the medulla of the stem and branches, was first analysed in London in 1875 when it was found that 85% of the powder was the benzene soluble

chrysophanic acid (Fig 1.1a) which could be used as a purgative. In 1878, Squire published a book "On the treatment of psoriasis by an ointment of chrysophanic acid" [quoted by Fisher & Maibach, 1975, Farber, 1983].

It was later shown that the active antipsoriatic component in Goa powder was not chrysophanic acid but a reduced product of it, known as chrysarobine (Fig 1.1b). The first synthetic compound (dithranol or anthralin shown in Fig.1.1c) was produced in 1916 and named Cignolin. 0.05 - 0.1 % dithranol in acetone or together with 0.5% salicylic acid and liquor carbonis detergens in an ointment base were used as effective formulations.

Chemistry

Dithranol is an aromatic compound consisting of three fused benzene rings, its structure and the sequential numbering of the carbon atoms are shown in Fig. 1.1(c). The two tautomeric forms of dithranol are shown in Fig 1.2.

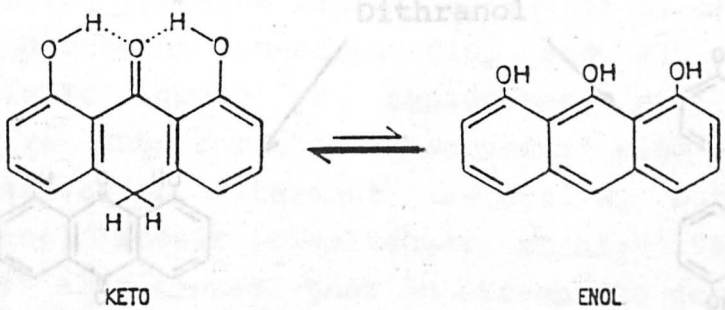


Fig. 1.2 Tautomeric forms of dithranol.

Dithranol is less stable in its enol form than the ketonic one because of the intermolecular hydrogen bond formation in the latter form [Whitefield, 1981a]. From the key structure-activity studies of dithranol [Krebs et al, 1981], 1-hydroxy-9-anthrone, Fig.1.1 (d) is suggested as the "minimum structure of the antipsoriatic

anthrones" containing anthracene rings with a carbonyl group at C9 and a methylene group at C10. Thus, chrysarobine, mentioned earlier, is effective because it contains this minimum structure of anthrone [Schaltegger et al, 1987]. The molecular structure of dithranol also enables an efficient penetration through the epidermis since it consists of hydrophilic part (the hydroxyl and carbonyl groups) and a hydrophobic (the anthracene nucleus) parts of the molecule.

Dithranol is an strong reductant which is readily oxidised by light [Raab & Gmeiner, 1975] and oxygen at C10, possibly via 2 different and independent pathways [Whitefield, 1981a], shown in Fig. 1.3, giving free radicals or the stable compound danthrone (1,8-dihydroxy anthraquinone). The decomposition of dithranol is greatly enhanced in a basic aqueous solution [Upadrashta and Wurster, 1988ab].

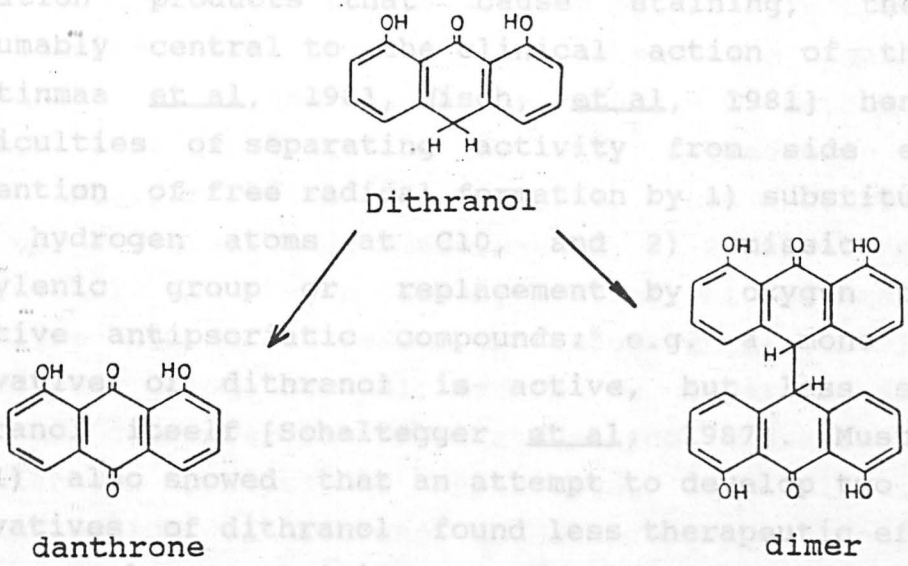


Fig. 1.3 Oxidation pathways of dithranol with two of the decomposition products: danthrone and dithranol dimer.

The release of a hydrogen atom from the methylene group at C10 initiates the oxidative decomposition of dithranol and the formation of free radicals [Krebs et al,

1981, Colin et al, 1981] (Fig. 1.3). The free radicals produced are biologically active and able to produce further active radicals in a chain reaction [Martinmaa et al, 1981, Whitefield, 1981a, Fusch et al, 1990]. For example, it is suggested that dithranol could sensitize the formation of $^1O_2$ and $O_2^{\bullet -}$ [Muller, 1988]. The formation of $^1O_2$ is a function of the deprotonation at C1 associated with the carbonyl group at C9 of the molecule: that of $O_2^{\bullet -}$ is initiated by the formation of a free radical of dithranol at C10. It was shown that the generation of the superoxygen anion ($O_2^{\bullet -}$) occurs within the plasmalemma [Schroder et al, 1985]. The highly reactive dithranol free radical reacts efficiently with oxygen to form an intermediate peroxy radical (R-O-O$^{\bullet}$) in the chain reaction towards anthraquinones. As a result danthrone and dithranol dimer (10,10'-dehydrodimer) are formed in the presence of oxygen. Although these radicals may be responsible for skin irritation and produce oxidation products that cause staining, they are presumably central to the clinical action of the drug [Martinmaa et al, 1981, Misch, et al, 1981] hence the difficulties of separating activity from side effects. Prevention of free radical formation by 1) substitution of both hydrogen atoms at C10, and 2) omission of the methylenic group or replacement by oxygen produced inactive antipsoriatic compounds: e.g. a mono 10-acyl derivative of dithranol is active, but less so than dithranol itself [Schaltegger et al, 1987]. Mustakallio (1981) also showed that an attempt to develop two 10-acyl derivatives of dithranol found less therapeutic effect as well as less staining. Schaltegger et al (1987) demonstrated that for a valid antipsoriatic effect, it is necessary that the C10-position be free and easily liberated.

Danthrone shows a stronger colour than dithranol, whereas the dithranol dimer has the same colour as dithranol itself [Fisher & Maibach, 1975]. This dimer can

undergo further oxidation [Whitefield, 1981a] producing an anthraquinone dimer linked by a double bond which has more extensive conjugation and results in a much darker colour responsible for the purple/brown staining when in contact with clothes and the skin [Colin et al, 1981]. The anthraquinone dimer undergoes polymerisation to form an insoluble "dithranol brown" the structure of which is not yet known.

Determination of dithranol

The lack of sensitive and specific analytical procedures for the investigation of dithranol has delayed the answer to such therapeutic parameters of dithranol as effective dose and site of action [Mustakallio, 1981]. Early official assay procedures for dithranol as the starting material in the preparation of ointment or in the ointment itself had been employing UV spectrophotometry [B.P., 1980 and U.S.P.,XX]. The absorption spectra [Caron and Shroot, 1981] indicate that the UV methods suffer from overlapping and interference between the absorption maxima of dithranol, dimer and danthrone. In the UV methods, it is assumed that danthrone is the only degradation product which interferes with the analysis, thus a correction by the use of absorbance measurements at 2 wavelengths and the use of the known absorptivities of dithranol and danthrone had to be made. Elsabbagh et al (1979) used a thin-layer chromatographic method to separate dithranol from its decomposition derivatives and emphasised that a simultaneous process of separation, identification and quantification of dithranol and its decomposition products is necessary for specificity of the assay. Two hplc methods have been developed and used to show that the UV method provided different assay results from hplc which could also determine the quantities of the decomposition compounds [Caron & Shroot, 1981]. The systems which Caron & Shroot (1981) developed employed either normal (C_{18}) or reversed phase (SiO_2) columns. A normal phase hplc system is currently the official method in both B.P.(1988) and

U.S.P. (XXI). Although both normal and reversed phase systems have been used [Cavey et al, 1982, Burton & Gadde, 1985, Lee, 1987, Wurster & Upadrashta, 1986, Kneczeke et al, 1989], a reversed-phase hplc system, has been reported to provide better separation between dithranol and danthrone [Burton and Gadde, 1985, Wurster and Upadrashta, 1986].

The other possibility for dithranol determination is by using spectrofluorimetry which is a very sensitive method of analysis. Dithranol is known to be fluoresce [Selim et al, 1981]. It does not fluoresce in neutral aqueous solution, however [Melo et al, 1983]. Recently, it has been shown that the anionic form of dithranol is a photosensitive species and also tends to be the most unstable species [Upadrashta and Wurster, 1988]. Another fluorescence technique reported involves the formation of a dithranol-borate complex [Mustakallio & Lamminsivu, 1981], which is not a selective determination for dithranol.

Therefore, the analysis method of choice for dithranol is hplc whilst fluorimetric analysis, which might increase sensitivity of detection, has not been thoroughly investigated.

Formulation

The use of dithranol had not been improved until Ingram regimen was introduced. Ingram (1953) showed that, when applied onto the skin, dithranol ointment (melting point between 38-60°C) will spread beyond the original site of application, thus a paste should be used to prevent the spread of the dithranol onto normal uninvolved skin and the stiffness of the paste is important. It is recommended that a paraffin wax is added to the petrolatum to increase the texture of the paste [Ingram, 1953]. Additionally, the effect of milling on even distribution of dithranol and in the stiffness of the paste and the importance of the techniques used to prepare the paste

were stressed by Seville (1966). It has been suggested that the incorporation of dithranol into an ointment and/or paste can be improved by dissolving dithranol in chloroform before levigating with the base if milling is not possible [Hulsebosch & Ponec-Waelsch, 1972]. These workers also indicate the problem of incorporating small amount of dithranol into the vehicle base. Moreover, the addition of salicylic acid to zinc oxide paste so as to maintain stability of dithranol was suggested by Ingram (1953). That salicylic acid might be entirely converted into zinc salicylate which does not stabilise dithranol nor improve its clinical efficacy has been suggested by several workers. Comaish et al (1971) demonstrated that salicylic acid was present in the paste and that zinc oxide paste without salicylic acid slowly decolourised when dithranol was added because of a zinc-oxide-dithranol complex formation. The complex is not therapeutically active. Hulsebosch & Ponec-Waelsch (1972) suggested that zinc oxide is hydrolysed and consequently formed a complex with dithranol which accelerates dithranol decomposition. Subsequently, Ponec-Waelsch & Hulsebosch(1974) showed that the decomposition of dithranol paste is initiated on the surface of zinc oxide and this could occur on other surfaces if water was present. Additionally, they demonstrated that salicylic acid in the formulation used in the Ingram regimen protected the zinc oxide-dithranol complex by formation of a layer of zinc salicylate on the surface of zinc oxide which is then deactivated.

Attempts have been made to increase the cosmetic acceptability of dithranol preparations. This is because ointment and paste bases are not well absorbed and remained on the surface, so that dithranol staining becomes a problem. Also, dithranol pastes are difficult to apply to and remove from the skin. This is an especially necessary procedure in short contact therapy [Schaefer, et al, 1980]. Dithranol creams [Seville et al, 1979] and dithranol sticks have become commercially available during

the past decade. Creams achieve patient acceptability only when they are completely rubbed in and leave no residues on the plaque. However, irritation and staining of the surrounding skin is often found [Wilson & Ive, 1980]. Sticks consisting of a hard paraffin wax with dithranol suspended in the base were claimed not cause dithranol spreading to the surrounding area [Brandt & Mustakallio, 1981].

Of all the preparations developed dithranol ointments are the mainstay in clinical use. The solubility of dithranol in petrolatum is about 0.2% [Whitefield, 1981a], thus above this strength the excess is held in suspension. The ointment is stable for only short periods of time, unless an antioxidant is added and protection from light and air is provided.

Dithranol regimens

Attempts have been made to minimise dithranol side effects by formulation and the development of clinical regimens.

CONVENTIONAL REGIME - Ingram (1953) introduced a combined treatment using tar, ultraviolet light and a dithranol paste. Patients, under the guidance of trained medical staff, were given a tar bath and exposed to varying increments of UV light to maintain near erythema dosage. The stiff paste was then applied to the psoriatic plaques, which were powdered with talcum, and the patient was wrapped with a protective stockinette dressing.

The Ingram regimen has been found to be effective and reliable. The side effects of dithranol are limited as a result of the stiffness of the paste which prevents spreading to normal uninvolved skin. However, the regimen is not suitable for outpatient care, and it relies on homogeneous mixing and the availability of dithranol from the paste [Farber & Nall, 1981].

There are several modifications of Ingram regimens involving the concentration of dithranol, the formulation, or the period of application [Comaish et al, 1971, Farber & Nall, 1984] which are the basis of the methods used at the present.

LOW DOSE-LONG TERM APPLICATION - It has been suggested that a low dose dithranol ointment (0.01 - 0.05%) does not irritate normal skin and would be effective if left in contact with the psoriatic plaque for a long time [Brody 1981]. However, Lowe and Breeding (1981) have shown that 0.05% dithranol o/w cream give equal clinical response to vehicle alone.

HIGH DOSE/SHORT TERM APPLICATION OR SHORT CONTACT THERAPY - Kammerau et al (1975) used tritiated dithranol to show that the maximal epidermal concentrations of dithranol (from 0.1% in petrolatum) occurred between 60 to 300 minutes after application. Schaefer et al (1980) found enhanced penetration of dithranol into epidermis from which the horny layer had been removed by tape-stripping, and that the difference between penetration into normal skin and tape-stripped skin was more noticeable after a short (30 minutes) than after a longer (1000 minutes) period. They concluded that removal of dithranol after 30 minutes should give a relatively higher amount of dithranol penetrating into psoriatic skin, assuming that skin with horny layer removed was a reasonable model for psoriasis. Thus, it was suggested that 1% dithranol (including 1% salicylic acid) in petrolatum be applied for 1 hour and then washed off [Schaefer, 1980]. Runne & Kunze (1982) used 1-3% dithranol ointment for 10 to 20 minutes. Short contact therapy was shown to be equally effective as conventional dithranol paste therapy and it is suggested as being suitable for outpatient therapy [Marsden et al, 1983].

ADJUNT THERAPY - Several combinations have been studied in order to optimize the beneficial effects from dithranol

and the other therapies. Examples of these are dithranol combined with ultraviolet A (UV-A) [Reshad et al, 1984] or PUVA [Morison et al, 1978]. These were claimed to be better than either of them alone, although Grattan et al (1988) disagreed. The retinoid, etretinate, was also investigated with dithranol therapy [Orfanos & Runne, 1976] and found to reduce clearance time when compared to dithranol alone.

Summarised recommendations for the use of dithranol are as follows. Concentrations should start with 0.1% and be increased gradually provided no irritation occurs. Creams should be rubbed in well and any excess should be rubbed off. Should staining on clothes occur, do not use soap but rinse well with running water. Should irritation or burning occur on the skin, stop the therapy until this settles and then resume cautiously at a lower strength. Hand washing after applying dithranol is necessary. Avoid applying dithranol near eyes.

Penetration studies

Penetration studies of dithranol, using both radiolabeling and fluorescence as the detection methods, indicate that the molecule has a rather high flux, and that a high concentration in the upper epidermis is obtained after topical application in vaseline [Shroot et al, 1981]. The penetration of dithranol through the defective skin barrier of the psoriatic lesion is faster than in normal skin [Schalla et al, 1981, Timmerman et al, 1990]. Schaefer (1980) showed that the penetration of dithranol into the epidermis was increased when the horny layer was removed: the effect being greater after 30 minutes of dithranol contact than after 1000 minutes. This provided the rational for the so called short contact therapy. A study using radioactive dithranol [Selim et al, 1981] showed that the penetration in vitro occurs shortly after application and the distribution rapidly became stabilised so that there was little difference between 1 hour and 24

hours of application. Dithranol attained its highest concentration in the upper part of the normal epidermis with a sharp decrease in the concentration in the lower epidermis and the dermis. The structure of dithranol, particularly its hydrophobicity [Whitefield, 1981a], contributes greatly to the penetration as mentioned earlier.

The penetration of dithranol through the epidermis can be influenced by the vehicles [Kammerau et al, 1975]. The more hydrophobicity was the vehicle the more was the penetration into the epidermis (petrolatum > water in oil cream > oil in water cream > single phase water miscible cream). Using tritiated dithranol in their study, a two stage kinetic process of penetration of dithranol was shown with lag time periods of 1 and 8 hours [Schalla et al, 1981].

After application, dithranol is oxidised in the skin to danthrone and dithranol brown. Danthrone is excreted in the urine and the insoluble dithranol brown polymer shed with the keratin lamella [Ippen, 1981]. Schalla (1981) suggested that danthrone and dithranol brown were not the only metabolites on the skin, but that some unidentified substance(s) were also found.

Mechanism of action

Histopathological changes in psoriatic epidermis after dithranol therapy [Wilborn & Montes, 1974].

Targets	untreated	After 1 month	After 3 months
parakeratotic cells	many	few	infrequent
cell size	larger than normal	variation	uniform
nuclear bodies	numerous	fewer	fewer
chromatin	dispersed	some clumping	much clumping
ribosome	numerous	fewer	fewer
tonofilaments	reduced	increased	increased
mitochondria	increased,enlarged	fewer, normal	fewer, normal
intercellular space	large	reduced	small
skin thickness	thick	thinner	thin

The overall mechanism of action of dithranol is not clear. The role of dithranol inhibition on DNA synthesis and its antimitotic effect has been well presented in either normal or psoriatic skin [Swanbeck & Thyresson, 1965, Swanbeck & Liden, 1966, Baxter & Stoughton, 1970, Liden & Michalesson, 1974, Fisher & Maibach, 1975]. It has not yet been clarified on which specific sites of DNA dithranol acts. Binding of dithranol with DNA and consequently altered DNA synthesis have been demonstrated *in vitro* [Swanbeck & Thyresson, 1965, Swanbeck & Liden, 1966] although recently it has been shown that dithranol is more likely to bind to tissue proteins such as albumins than to DNA [Caron *et al*, 1982, Melo *et al* 1983]. Nevertheless, the mitotic counts of the epidermal cells in normal skin after dithranol treatment were decreased [Baxter & Stoughton, 1970]. A decrease in the number of DNA-synthesising cells in the psoriatic epidermis was correlated with the disappearance of parakeratosis by Liden and Michalesson (1974). Danthrone does not inhibit DNA activitiy in normal skin [Clark & Hanawalt, 1982].

It has been postulated that the action of any drug on DNA synthesis would only possess an effect on psoriasis if it preferentially reacts with mitochondria [Zetterberg & Swanbeck, 1971]. Hammar (1975) showed that in parakeratotic epidermis dithranol reduces the elevated level of ICD-NAD (isocitrate dependent-nicotinamide adenine dinucleotide) which is found exclusively in mitochondria. Shroot *et al* (1981) indicated that nuclear DNA was not altered by dithranol. The structure of the dithranol molecule appears to favour its penetration into mitochondrial DNA rather than the nucleus [Swanbeck & Lundquist, 1972]. Dithranol interferes with mitochondria and their functions and consequently cell division is likely to be suppressed by the lack of energy supply [Morliere *et al*, 1985]. Reichert *et al* (1985) showed that inhibition of mitochondrial respiration was the primary target of dithranol action.

Dithranol has been shown to have a wide range of other activities. Jacques & Reichert (1981) showed that dithranol and dithranol dimer inhibit tritiated thymidine incorporation. Dithranol reduced the elevated polyamine levels in psoriasis to normal and this effect correlated with the reduction of proliferative activity [Henry et al, 1981]. The synthesis of prostaglandin E (PGE) which affects cAMP level was increased by dithranol [Ippen, 1981]. Dithranol inhibited the activity of enzyme glucose-6-phosphate dehydrogenase [Cavey et al, 1982], glycolysis [Diezel et al, 1975], lipid peroxidation [Diezel et al, 1975, Finnen et al, 1984, Fusch et al, 1990], superoxide dismutase and superoxide catalase [Kemeny et al, 1989, Fusch et al, 1990]. These enzymatic effects, however, are more likely to involve free radical activity [Fusch et al, 1990]. The motility and chemotactic migration of polymorphonuclear (PMN) leukocytes pretreated with dithranol were shown to be suppressed [Schroder et al, 1985].

During this past decade, there has been an increasing interest in the active involvement of free radicals in the action of dithranol [Shroot et al, 1981, Cavey et al, 1986]. In 1916, Unna [quoted from Muller et al ,1986] elucidated an interaction of chrysarobine and oleic acid and later Muller et al (1986) found that oxidative decomposition of dithranol in the presence of unsaturated fatty acid resulted in the formation of peroxides and free radicals. Arachidonic acid is one target of the free radicals proposed by Muller et al (1988). The formation of reactive oxidant radicals, especially $O_2^{\bullet -}$, H_2O_2 and $^{\bullet}OH^-$, is influenced by many processes in vivo, such as phagocytosis by PMN leukocytes, arachidonate metabolism and prostaglandin formation [Waldman & Murad, 1987]. These radicals are potent antiproliferative, immunosuppressive agents, and activators of cGMP synthesis. It has been shown that PMN leukocytes

pretreated with dithranol inhibited $O_2^{\bullet -}$ generation [Schroder et al, 1985].

A study of the effect of dithranol decomposition on the inhibition of glucose-6-phosphate-dehydrogenase revealed that dithranol, danthrone and dimer inhibited and interacted with the enzyme but the most active derivative was some unidentified decomposition product(s) [Cavey et al, 1982]. Using ESR, it was revealed that free radicals form as the major fraction of dithranol derivatives in the skin after 18 hours of application [Shroot & Brown, 1986]. The radicals could be the cause of dithranol irritation [Mustakallio, 1979, Martinmaa et al, 1981, Farber, 1983] and therapeutic action [Martinmaa et al, 1981]. It was suggested that the biological targets of the active free radical species were DNA, enzymes, and polyunsaturated fatty acids associated with arachidonic acid [Muller, 1988]. The $^1O_2$ caused DNA damage, inactivated enzymes and destroyed membranes by affecting cholesterol and polyunsaturated fatty acids [Schroder et al, 1985, Muller, 1988]. The mechanism by which $O_2^{\bullet -}$ might be deleterious to cells remains unclear because it is poorly active in aqueous solution. It has been reported that $O_2^{\bullet -}$ is responsible for skin irritation although $^{\circ}OH$ derived from $O_2^{\bullet -}$ is more likely to be the toxic species than $O_2^{\bullet -}$ itself [Muller & Kappus, 1988].

In general, therefore, it appears that the free radicals derived from dithranol are most likely to exert their effect on membranes [Mustakallio, 1981, Schroder et al, 1985] and mitochondrial DNA [Shroot et al 1981].

Overall both dithranol and free radicals generated in the chain reaction, interfere with metabolism, energy supply or electron transport systems and oxidative phosphorylation of dividing epidermal cells. Mitochondria are, therefore, of increasing interest as the target of action of dithranol. These are affected in both psoriatic and normal skin but the former is more susceptible because

of its increased cell division. This could, however, explain why dithranol exerts its therapeutic effect on psoriatic skin but only irritation on normal skin. Moreover, because of its optimal molecular size and hydrophobicity, it might penetrate into and dissolve in the lipid part of the bilayers of the membrane, whilst danthrone and dimer do not have these optimum properties. Future work on the possible intramembraneous site of dithranol action and its antirespiratory effect are required to obtain a full understanding in the way(s) in which dithranol reacts to suppress cell division and growth.

1.4 HYDROGEL

Ideally following administration, a drug should reach its sites of action at an optimum concentration which is maintained for the desired duration of the treatment. With conventional formulations this goal is usually found to be only partially achieved. In this review, their use for topical delivery only is considered. Present day topical drug delivery systems make use of inert polymers the release from which is dependent on the physical and chemical properties of the drug and the polymer. [Bodde et al, 1989].

One particular group of polymeric systems which have been evaluated for use as drug delivery systems are hydrogels [Roorda et al, 1986]. Most hydrogels are biocompatible because they have soft, tissue-like consistency which minimizes frictional irritation and their porous character enables the extraction of metabolic residues which are a potential source of inflammation. This could be the result of the capacity of the hydrogels not only to hold but also to exchange water with the normal skin when used for long term application [Bodde et al, 1989]. Hydrocolloidal dressings, which are based on hydrophilic polymer matrices, appear to hydrate

psoriatic skin and cause a clinical improvement [Friedman, 1987].

In 1960, Wichterle and colleagues suggested that polymerization of 2 monomers based on polymethacrylate could yield a transparent gel which would be compatible with biological materials. Since then, hydrogels have been used for a wide range of biomedical applications such as coating, contact lenses, replacement of vitreous humor, burn wound dressings, synthetic cartilage, haemodialysis membrane [Roorda et al, 1986]. There has also been an increasing interest in the possibility of optimizing drug delivery because, in addition to good biocompatibility, hydrogels have an ability to release entrapped solutes when in contact with water. The process could be regulated by controlling the swelling, chemical composition and the geometry of the hydrogels. An exact definition of "hydrogel" does not exist [Roorda et al, 1986]. Hydrogels can be described as network polymers which can swell and absorb a considerable amount of water without dissolving. Thus, they can be regarded as two component systems, one component being a hydrophilic, insoluble, three-dimensional polymer network, and the other being water. However, it is not possible to differentiate between these components since they form a continuous and completely interpenetrating system. The processes responsible for the water sorption include capillary, osmotic and hydration forces, and are strong enough to exert considerable influence on the structure of both components.

More recently, hydrophilic polyurethane networks have been used to form new types of hydrogels. They are prepared by reaction of poly(ethylene oxide) (PEO) or polyethylene glycol(PEG) with aliphatic or aromatic diisocyanates (such as Desmodur = dicyclohexylmethane-4,4-diisocyanate) in the presence of a small amount of a compatible triol [Gander et al, 1986].

The sorption of water by a dry hydrogel is a complicated process [Roorda et al,1986]. Dry hydrogels are glassy at room temperature. Given a favourable thermodynamic compatibility between the hydrogel matrix and the penetrating solvent, the latter will have a plasticising effect on the polymer chains lowering the glass transition temperature below the experimental temperature. This results in a change from a rigid glassy state to a soft rubbery state and permits the considerable swelling observed. However, this expansion is counteracted by elastic contractions of the stretched polymer network, leading to an equilibrium swelling when both forces are balanced [Roorda et al, 1986]. Solvents may make specific associations in hydrogels. In the anhydrous state PEG contains ordered regions with a crystalline character. Even after taking up its own weight in water, the PEG gel still shows this crystallinity. [Martin et al, 1983]. Graham et al (1988) have shown that water is strongly associated with the crystalline PEO of the polymer in the ratio of three molecules of water per ether group.

The swelling of polyurethane PEO hydrogels is thermoreversible [Graham et al, 1982, Schacht & vanBos, 1987]. The polymers have a lower critical solution temperature (LCST) and lose the water of hydration as the temperature is raised to and above the LCST.

Loading of drugs

Drug loaded hydrogels can be prepared using a one-step procedure by adding the drug to the polymerization mixture. This method is practical provided the content of residual reactants is below acceptable levels. Since this is difficult to achieve, most therapeutic hydrogels for in vivo use are prepared by a two-step procedure. In this, a hydrogel device is fabricated with the desired geometry and purified by repeated extraction. It is then loaded with drug by soaking the dry hydrogel in a drug

dissolved either in water or a volatile organic solvent. The temperature dependent swelling behaviour discussed previously can be used to achieve higher drug loadings. For example, the loading of propanolol HCl in a polyurethane hydrogel was increased by about 6 times when the loading temperature was reduced from 37° to 5°C [Schacht & vanBos, 1987]. Gander et al (1986) has found that large amounts of drugs with different solubility characteristics may be loaded into the polyurethaneurea hydrogels. Using a sparingly water soluble drug, methylcatechine (1% at 37°C), they also found that the uptake of the drug by the hydrogel varied greatly from expected levels, i.e. from 6 to 10%. This effect was not observed with a highly water soluble drug, proxyphylline (59% at 37°C). Additionally, the release of proxyphylline into water was much faster than methylcatechine. This indicates the solubility properties of solutes and solvents on the diffusion into and from the hydrogels.

Transport of solutes

Two models have been proposed to describe the transport mechanism of substances in hydrogels - referred to as the open pore membrane model and the dense membrane model [Roorda et al, 1986]. In the open pore membrane model, a hydrogel is considered to behave like a sieve by obstructing the passage of large molecules whilst permitting movement of small ones. In this model, the chemical nature of polymer would not have much influence on transport. On the other hand, in the dense membrane model, the hydrogel is considered to be homogeneous and not contain open pores. Molecules could only pass through by dissolving in the polymer matrix, followed by diffusion through it. In this case, the chemical nature of the polymer would be of great importance. In general hydrogels are considered to be structures of water-filled pores surrounded by polymer which is constantly in motion and changing in size and shape because of network

movements. From this, a third model - free volume diffusion - has been proposed. In this model it is assumed that the dry polymer consists of randomly coiled and packed macromolecules with some unoccupied space, known as the free volume. Some of this free volume is assumed to remain in the hydrated polymer, the maximum size being determined by the nature of the polymer, especially the degree of cross linking. The transport of molecules through the hydrogel is then dependent on the probability of a permeant molecule finding a suitable hole next to its location. This makes transport also dependent on the size of these molecules. The gels are thought to have two domains corresponding to the two phases. Thus, the A domain contains polymer chains, and bound and interfacial water, whereas the B domain contains bulk water. Hydrophilic solutes would diffuse almost exclusively through the B domain, hence, they permeate freely in gels with low levels of cross-linking content. Hydrophobic solutes, on the other hand, would diffuse through the A domains, giving in general, a much slower diffusion than for hydrophilic compounds, but transport through highly cross-linked gels would never be completely obstructed.

The idea of using medicated plasters for topical drugs administration might be dated several hundred years back to ancient China where medicated plasters were said to have originated [Chien, 1987]. These tended to contain multiple ingredients of herbal drugs and were indicated for localised action in the tissue directly underneath the site of application. They were also popular in Japan, where they were called cataplasms. In western medicine, medicated plasters have existed for several decades - such as belladonna plaster, mustard plaster, salicylic acid plaster. Such plasters which adhere to the skin and are attached to a dressing bring medications into close contact with the skin, whilst also affording protection, support, occlusion and a macerating action. However, these early

devices differ from hydrogels in the way in which the drug is incorporated. With plasters, it is on the surface, with hydrogels it is within the structure of the hydrogels. Both will tend to be occlusive.

1.5 PURPOSE OF STUDY

Present dosage forms of dithranol are not satisfactory for a number of reasons - dosage is not accurately controlled, irritancy is common, staining of clothes occurs and the drug spreads to adjacent "normal" skin. The aim of this project was to investigate the possibility of developing a controlled delivery system of dithranol using a hydrogel vehicle which would be capable of releasing adequate amounts of dithranol specifically into the psoriatic plaque, thereby, minimizing irritancy and eliminating staining. Additionally, the area onto which dithranol would be applied could be limited solely to that of the plaque.

A polyurethaneurea hydrogel was to be used and loaded by soaking the hydrogel in a concentrated drug solution. This method would enable preparation of a sheet of loaded hydrogel film which could be prepared to the shape of a specific plaque. The study can be subdivided into 5 main sections. 1) The formulation of dithranol solution was developed to facilitate both the loading process and dithranol stability throughout the preparation process. Factors affecting the uptake of dithranol were also investigated. 2) Developing a valid and sensitive method for the determination of dithranol in order to assay the low concentrations of dithranol released from the hydrogel. 3) Developing an assay to determine dithranol concentrations in the loaded hydrogel was necessary so that the dose of the drug administered is known. 4) Investigation of factors affecting release in both in

in vitro and in *in vivo* models. 5) A clinical evaluation of the dithranol hydrogel in comparison with a conventional dithranol dosage form.

CHAPTER 2

DEVELOPMENT OF ANALYSIS

2.1 INTRODUCTION

In order to fulfil the purpose of this study, it was essential to develop procedures to quantify and monitor dithranol and its decomposition products in a variety of media at different concentration levels. These include the determination of dithranol, danthrone, and/or dimer in, for example the loading solution, hydrogel, receptor media, and stratum corneum.

Hplc is the current method of choice [B.P. 1988]. A reversed phase hplc is established because of its higher sensitivity and better separation between dithranol and danthrone. When higher concentrations of dithranol were encountered, e.g. the loading solution or loaded film, hplc provides adequate sensitivity for the determination of dithranol, danthrone and dimer: whereas at lower levels, such as the release studies, recourse to the higher sensitivity of spectrofluorimetry was essential. Dithranol is highly fluorescent in its anionic form which tends to be the most unstable species (Upadrashta and Wurster, 1988). Hence, a spectrofluorimetric procedure had to be developed which stabilised the anionic form of dithranol and maximised the sensitivity of the assay.

The solvent of choice for dithranol solutions had to be established so that stability upon storage for subsequent analysis and hence reproducibility of

determination over long period of times is obtained. This is particularly neccessary, in release studies when such samples as that of the releasing media, the hydrogel and/or stratum corneum should be simultaneously assayed without delay so that dithranol is not further decomposed. Although stability studies of dithranol in solvents such as acetone (Schroder et al, 1985, Wurster and Upadrashta, 1986), acetonitrile (Burton and Gadde, 1985, Kneczeke et al, 1989), chloroform(Burton and Gadde, 1985, Caron and Shroot, 1981), DMSO (Fuchs et al, 1990), hexane (Caron and Shroot, 1981), methanol (Burton and Gadde, 1985) and a mixture of methanol and chloroform (Lee, 1987) have been reported, the storage conditions for dithranol in a particular solvent/solvent system which would ensure dithranol stability over a prolonged period of time has not been studied.

2.2 EXPERIMENTAL METHODS

2.2.1 MATERIALS AND EQUIPMENT

Chemicals and reagents

Dithranol was obtained from Aldrich (Milwaukee, WI, U.S.A.): Danthrone was from Sigma (St.Louis, U.S.A.): and dithranol dimer(dimer), B.P. chemical reference substance, was from the B.P.C. Laboratory (Stanmore, U.K.). Dithranol and dimer were stored in a dessicator at room temperature in the dark. Fluorene, the internal standard used in the hplc, was from Fisons (Loughborough, England). AnalaR grade ammonium ferrous sulphate, molecular weight 392.14, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, was from BDH (Poole, England).

Methanol, hplc grade, and hexane were obtained from Rathburn Chemicals (Walkerburn, U.K.). Acetone, conc. ammonia solution (SG 0.88), dimethylsulphoxide (DMSO), and polyethylene glycol400 (PEG400) were from FSA (Loughborough, England). Glacial acetic acid (GAA) was

from BDH Limited (Poole, England). Hplc water was purified by a Millipore Milli-Q system (Harrow, U.K.).

10 x 25 cm diameter dry linear polyurethaneurea hydrogel, Polymer Code 157 (Reference : PUU VI - Chris Moran), was supplied by Graham, N.B., University of Strathclyde, Glasgow. 0.1% Dithrocream was from Dermal Laboratories Ltd.(Gosmore, Herts, England).

The containers used to store solutions of dithranol and dimer were amber glass unless otherwise specified. Standard and stock solutions of danthrone were kept in transparent glassware.

All chemicals and reagents were used as received.

Chromatographic system

EQUIPMENT - A Waters Associates (Milford, MA, U.S.A.) Model 6000A solvent delivery system pump was used. Detection was by a Model LC 871 UV-VIS detector operating at 254 nm fitted with a nominal 18 µl flow cell. Injection was by a Rheodyne model 7125 valve (Cotate, C.A., U.S.A.) fitted with a 200 µl fixed volume loop. Recording of chromatograms was accomplished with a BBC chart recorder SE 120 (BBC Goerz, Austria). Columns were 100x2 mm ID slurry packed in the laboratory with C-18, 5 µm ODS Hypersil, HETP (Macclesfield, U.K.). These were used for all quantitative work. Analysis was performed at room temperature (nominally 20°C). Equilibration with the mobile phase was complete in about 30 mins. For all studies, the absorbance range used was 0.01 AUFS.

SOLVENT SYSTEMS - SYSTEM 1 - The isocratic mobile phase consisted of methanol, hplc water, and glacial acetic acid, 60: 39.5 : 0.5 %v/v (pH 2.34± 0.04, n = 4, at 20°C). The flow rate was 0.4 ml/min. The combination of mobile phase, temperature, and flow rate yielded a back pressure of about 1600 p.s.i. The mobile phase in the reservoir

was degassed using a filter prior to use. SYSTEM 2 - This was modified from system 1. The combination of the mobile phase was 60:38:2, respectively; and the flow rate was 0.6 ml/min (pH at 20°C 2.22±0.05; n=5). The back pressure was about 2500 p.s.i.

Spectrofluorimetry

A Perkin-Elmer Model LS-5B Luminescence Spectrometer was used. The excitation and emission slit widths were set at 2.5 and 10.0 nm. The calibration scale of the instrument was fixed at 1.00. The scanning speed was 30 nm/min and recorder speed 20 nm/cm. The integration time for reading the fluorescence intensity (FI) at a particular wavelength was 4.2 seconds and for the recording of spectra 16.8 secs. A 1-cm cuvette was used.

Miscellaneous

The following apparatus was used where and when required: WhirliMixer, Model WM/250/SCP/2 (Fisons, England), 50-60 Hertz, set at maximum speed (about 2,600 vibrations per min); A Model FS100 ultrasonic bath (Decon, Sussex, England); A Corning pH meter Model 10, Corning Limited (Essex, England), standardised with the pH of the buffer standards used being 4.00 and 7.00; A Sartorius Balance (Sartorius Werke AG,Gottingen, Germany) for weighing more than 50 mg weight of materials and a Mettler Model AE 240 (Mettler Instruments, Buckinghamshire, U.K.) for 5 mg or more. Centrifugation was performed using a Model MSE Micro Centaur, Fisons, (England) which was set at 13000 rpm for 2 mins.

The determination of ferrous ions (Fe^{2+}), where applicable, were performed using a Baird Atomic Absorption Spectrophotometer Model Alpha 3. Infrared spectra, where applicable, were recorded using a Perkin-Elmer Infrared Spectrophotometer Model 681 (England).

2.2.2 STOCK SOLUTIONS

Dithranol

10 ml of a solution of dithranol in glacial acetic acid of about 40 (42.5±2.9, n = 6) µg/ml, was accurately prepared (monthly) and stored at -21°C.

Danthrone

A solution of danthrone in methanol, 100 µg/ml, was used throughout the study.

Dimer

Accurate concentrations of dimer in acetone (about 100 µg/ml) were freshly prepared.

2.2.3 STANDARD SOLUTIONS, CALIBRATION AND REPRODUCIBILITY

Hplc

10 µg/ml fluorene in methanol, stored at room temperature, was used as an INTERNAL STANDARD (IS) solution.

DITHRANOL - 0.05, 0.1, 0.15, and 0.2 ml of dithranol stock solution were transferred to 10-ml flasks, 0.1 ml of the IS solution was added to each and the appropriate hplc mobile phase was used to make up to the volume. The range of concentrations covered was 0 - 1 µg/ml.

A 0.4 µg/ml solution of dithranol was used as the day to day standard.

DANTHRONE - 1 ml of danthrone stock solution was diluted to make 4 µg/ml in methanol. 0.05, 0.1, 0.15, and 0.2 ml of this solution were transferred to 10-ml flasks, 0.1 ml of the IS solution added to each and the appropriate hplc mobile phase was used to make up to the volume. The range of concentrations covered was 0 - 0.09 µg/ml.

A 0.04 µg/ml solution of danthrone was used as the day to day standard.

DIMER - 1 ml aliquot of the dimer solution was diluted to 10 ml with glacial acetic acid, the final concentration being about 10 µg/ml. 0.05, 0.10, 0.15 and 0.20 ml of this solution were transferred to 10-ml flasks, 0.1 ml of the IS solution added to each and the appropriate hplc mobile phase was used to make up to volume. The range of concentration covered was 0 to 0.2 µg/ml.

A 0.1 µg/ml solution of the dimer was used as the day to day standard.

CALIBRATION - These were performed on a monthly basis: 6 times for dithranol(both systems), 3 times for danthrone(both systems), and 4 times for dimer using system 2. Linear regression was used for analysis of the ratio of peak height (dithranol and danthrone) or peak area (dimer) on concentrations. The average correlation coefficients, slopes and intercepts are shown in Table 2.1.

Spectrofluorimetry for dithranol analysis

A freshly prepared solvent system consisting of 4.5 ml of DMSO and 0.4 ml of conc.ammonia solution was used as "am-DMSO".

FOR NON-AQUEOUS SOLUTIONS - About 100, 200, 300, 400 ng/ml of dithranol in glacial acetic acid were prepared from the stock solution. 0.1 ml of each solution was added to the "am-DMSO" to complete the final volume of 5 ml (and become "am-DMSO-GAA"). The concentration range covered was from about 0 - 8 ng/ml.

The standards used routinely daily for dithranol determinations were 2 and 8 ng/ml.

A blank solution of freshly prepared "am-DMSO-GAA" was used to set the fluorimeter to zero using the Autozero mode.

For assay purposes as a check for spectral purity, routine measurement using four wavelength pairs was employed. These pairs (WP, λ_{exc} - λ_{ems}, respectively) were as follows: WP1 390-500, WP2 390-530, WP3 440-500,and WP4 440-530 nms. The average ratio of FI of solutions at WP1 to that of WP4 (FI_{wp1}:FI_{wp4}), each of which was obtained monthly, using the FI at WP4 as the reference.

Nine calibration graphs, obtained monthly from the plot of FI against concentration, were analysed using linear regression. The average correlation coefficient, and slope and intercept are shown in Table 2.2.

FOR AQUEOUS SOLUTIONS - A series of standard solution in the presence of aqueous solutions was prepared as follows: appropriate volume (not more than 0.4 ml) of the same aqueous solution as that used in the sample was first mixed with about 3 ml DMSO, 0.4 ml conc.ammonia and 0.1 ml of about 100, 200, 300, 400 ng/ml dithranol in glacial acetic acid added. An appropriate amount of DMSO was then added to make a final volume of 5 ml. If the aqueous solution is not mixed with DMSO before either conc. ammonia or glacial acetic acid is added, failure to obtain the fluorescence spectrum of dithranol occurs.

The standards used routinely daily for dithranol determinations were 2 and 8 ng/ml.

A blank solution consisting of freshly prepared am-DMSO-GAA and the appropriate volume of the aqueous solution was used to check any interference in the analysis.

A check for spectral purity using four wavelength pairs was routinely carried out as described above.

Three calibration graphs, obtained monthly from the plot of FI against concentration, were analysed using linear regression. The average correlation coefficient, and slope and intercept are shown in Table 2.2.

2.2.4 SAMPLE PRETREATMENT AND ANALYSIS

Hplc

LOADING SOLUTION - 1 ml of loading solution, approximately 0.05% in dithranol (refer to Chapter 4), was diluted to 10 ml using glacial acetic acid. 0.1 ml of the solution was transferred to a 10-ml flask, 0.1 ml of IS solution added and the appropriate hplc mobile phase was used to make up to volume. Duplicate analysis was performed for each sample. The ratio of the peak height to IS for dithranol (and where applicable danthrone and/or dimer) for each solution was used to quantify dithranol (and where applicable danthrone and/or dimer) by comparing with appropriate standard solutions.

HYDROGEL - A hydrogel film, loaded from 1 cm diameter dry film (refer to Chapter 4), was dissolved in 2 ml of glacial acetic acid. 0.05 ml of the hydrogel solution was transferred to a 10-ml flask, and 0.1 ml of the IS solution added, and the appropriate hplc mobile phase was used to make up to volume. Duplicate analysis was performed for each sample. The ratio of the peak height to IS for dithranol (and where applicable danthrone and/or dimer) for each solution was used to quantify dithranol (and where applicable danthrone and/or dimer) by comparing with appropriate standard solutions.

CREAM - Approximately 0.0250 g of Dithrocream was accurately weighed in a tared bottle. The cream was suspended in 1 ml distilled water and 2 ml of hexane was added. The liquids were shaken using a vortex mixer for 15 mins. 1.5 ml of the hexane solution was taken and

evaporated to dryness under a stream of air. The residue was dissolved in 1 ml glacial acetic acid and the solution clarified of waxy precipitates by centrifugation. 0.5 ml of the supernatant was transferred to a 10-ml flask, 0.1 ml of IS solution added and the hplc mobile phase system 2 was used to make up to volume. For comparison purposes, a standard solution was prepared as follows: 1.5 ml of about 10 µg/ml dithranol in hexane, freshly prepared, was blown dry under a stream of air; 1 ml of glacial acetic acid used to reconstitute the residue. 0.5 ml of the solution was transferred to a 10-ml flask, 0.1 ml of IS solution added and the hplc mobile phase system 2 was used to make up to volume.

Duplicate analysis was performed for the standard and each sample. The mean ratio of the peak height to IS for dithranol and each sample solution was used to quantify the dithranol by comparing with the mean peak height ratios obtained under the same conditions for the standard solution.

Spectrofluorimetry

STRATUM CORNEUM - 0.005 g (about 1.6cm²) of dry stratum corneum was soaked in 2 ml glacial acetic acid, then placed in an ultrasonic bath for 15 minutes. The resultant supernatant was used for sample analysis. 0.1 ml of the supernatant was taken to complete the am-DMSO mixture whose FI was read off and the amount of dithranol was calculated by comparison with two-point standard solutions (2 and 8 ng/ml) which were prepared under the same conditions as the samples. Duplicate analysis was performed for each sample.

A blank was prepared in the same way using untreated stratum corneum.

AQUEOUS RECEPTOR MEDIUM (refer to Chapter 5) - An apropriate volume ,i.e from 0.1 to 0.4 ml, of acetate buffer pH 2.8 was transferred to a 5-ml flask, 0.4 ml conc. ammonia, 0.1 ml glacial acetic acid added and DMSO was used to adjust the volume. The FI of the solution was read off at WP1. The amount of dithranol in the sample was determined by comparison with two-point standard solutions (2 and 8 ng/ml) which were prepared under the same conditions as the samples. Duplicate analysis was performed for each sample.

For assay of NON-AQUEOUS MEDIUM (refer to Chapter 5), 0.1 ml of the medium was diluted to 2 ml with glacial acetic acid. 0.1 ml of the glacial acetic acid solution was added to complete the am-DMSO mixture. The FI of the solution was read off and the amount of dithranol was calculated by comparing with two-point standard solutions (2 and 8 ng/ml) which were prepared under the same conditions as the samples. Duplicate analysis was performed in each sample.

FILM REMAINING FROM THE RELEASE STUDIES - Each film was blotted dry and dissolved in 4 ml glacial acetic acid. 0.1 ml of the solution was transferred to complete the am-DMSO mixture. The FI of the solution was read off and the amount of dithranol was calculated by comparing with two-point standard solutions (2 and 8 ng/ml) which were prepared under the same conditions as the samples. Duplicate analysis was performed on each sample.

2.2.5 ASSAY VALIDATION

Hplc

HYDROGEL - A blank hydrogel solution was prepared as follows: a 1-cm diameter dry film was loaded with blank loading solution (refer to Chapter 4): it was then dissolved in 2 ml glacial acetic acid. A solution of 0.05 ml of the blank hydrogel solution and 0.1 ml of the IS

solution in 10 ml of the appropriate mobile phase was analysed: the resulting chromatogram indicated that there would be no interference from the hydrogel in the determination of dithranol, danthrone and dimer, as shown in Fig. 2.3. The peak heights and retention times of added dithranol, danthrone and dimer were the same as those of equivalent concentrations of the corresponding standard solutions without hydrogel added.

Attempts to increase the amount of the hydrogel solution injected were made. This was found to increase back pressure and cause column blockage, particularly when the solution injected contained over 2% of the hydrogel solution.

LOADING SOLUTION - A blank chromatogram of the loading solution was obtained as follows: 1 ml of blank loading solution was diluted to 10 ml using glacial acetic acid. A solution of 0.1 ml of the blank loading solution and 0.1 ml of the IS solution in 10 ml of the hplc mobile phase was analysed. The resulting chromatogram indicated that there would be no interference from the loading solution in the determination of dithranol, danthrone and dimer, as shown in Fig. 2.4. The peak heights and retention times of added dithranol, danthrone, or dimer were shown to be the same as those of equivalent concentrations of the corresponding standard solutions without loading solution.

CREAM - The complete analysis of cream was repeated six times. The average percent recovery was 85.3 (±10, n=6).

Spectrofluorimetry

STRATUM CORNEUM OR HYDROGEL - 0.05 ml of the blank stratum corneum or blank hydrogel solution was transferred to a 5-ml flask, 0.4 ml of conc. ammonia solution, 0.1 ml of glacial acetic acid added, and DMSO was used to make up to volume. Fluorescence spectra of the solutions recorded showed no interference for the determination of dithranol.

AQUEOUS RECEPTOR MEDIUM (ACETATE BUFFER, pH 2.80) - A fluorescence spectrum of a blank solution containing 0.4 ml of the buffer, 0.1 ml glacial acetic acid, 0.4 ml of ammonia solution make up to 5 ml with DMSO was recorded. This showed no interference for the determination of dithranol.

EFFECT OF ACETATE BUFFER, pH 2.80 - 0.0, 0.1, 0.3, 0.5, and 0.9 ml of the buffer were transferred to 5-ml flasks, 0.4 ml ammonia solution and 0.1 ml dithranol solution in glacial acetic acid (42 ng/ml) added, DMSO was used to make up to volume. The FI of each solution at the 4 wavelength pairs were read. A plot between percent of FI (from maximum FI) and the volume of the buffer added is shown in Fig. 2.6.

EFFECT OF AMMONIA - 0.07, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0 ml of conc. ammonia solution were transferred to 5-ml flasks, 0.4 ml ammonia solution and 0.1 ml dithranol solution in glacial acetic acid(42.2 ng/ml) added, DMSO was used to make up to volume. The FI of each solution at the 4 wavelength pairs were read. A plot of percent of FI (from maximum FI) and the volume of ammonia added is shown in Fig. 2.7.

EFFECT OF DANTHRONE AND DIMER - An aliquot of danthrone stock solution was diluted to make 0.03 µg/ml in glacial acetic acid. 0.1 ml of the solution was used to complete the "am-DMSO-GAA" which consisted of 0.6 ng/ml of danthrone. The spectra of the solutions were recorded as for dithranol.

Accordingly, a solution of 70 ng/ml dimer and 7.3 ng/ml dithranol in the solvent system was prepared and the spectra of the solution were also recorded.

2.3 RESULTS AND DISCUSSION

2.3.1 HPLC

Establishment of hplc analysis

Preliminary study of the composition of the mobile phase (MeOH:H_2O:GAA) showed that 60% methanol was the maximum limit which gave separation and resolution of dithranol, danthrone and the internal standard. Chromatograms resulting from increasing the composition of methanol exhibited incomplete separation of danthrone and dithranol. This finding is in good agreement with Albert(1985) and Burton & Gadde (1985).

Fluorene, which is not a degradation product of dithranol, was selected as the internal standard because of its good peak shape and resolution from dithranol, danthrone and dimer. A representative chromatogram using hplc system 1 for dithranol, danthrone, dimer and internal standard, fluorene, is shown in Fig. 2.1.

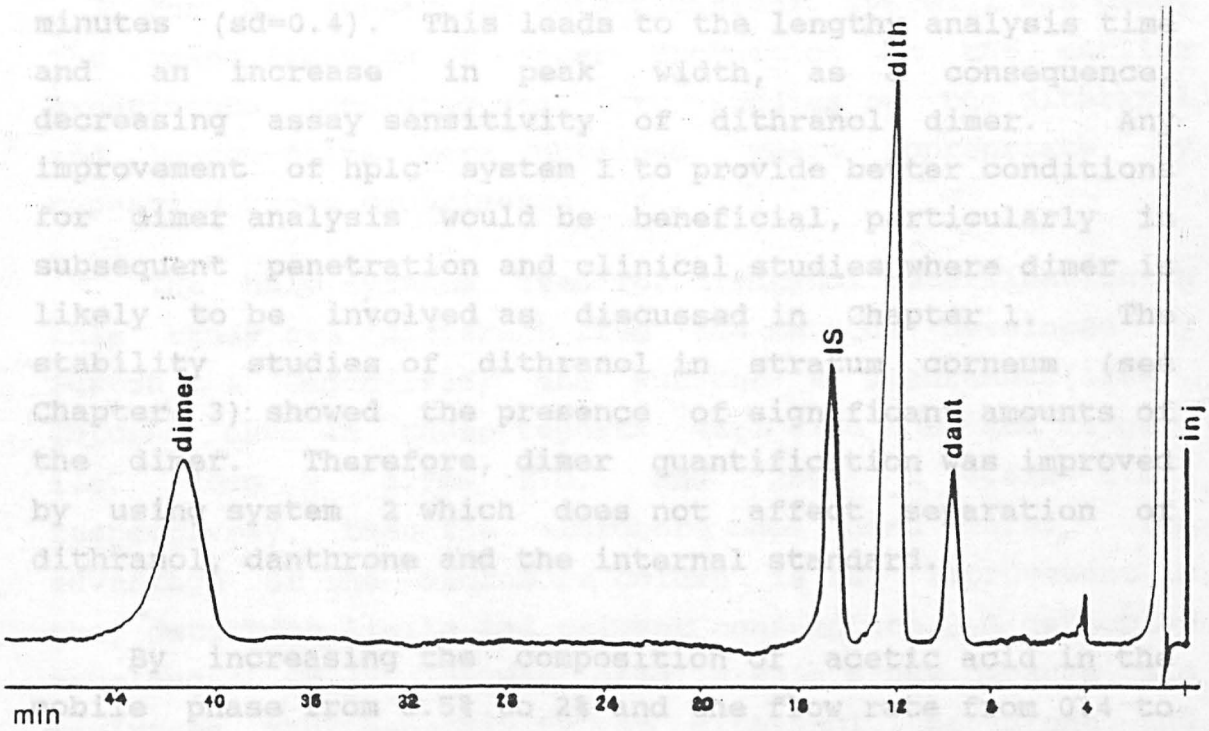


Fig.2.1 Representative chromatogram for dithranol, danthrone, dimer and fluorene(internal standard) analysed using system 1.

Initially, hplc system 1 was used for stability studies of dithranol in the loading solution and loaded film (see Chapter 3). These samples consisted of high concentrations of dithranol. Upon storage, danthrone could be detected because of its chemical stability. Dimer, on the other hand, had not been detected because it is likely to be precipitated in the solvent systems used. Additionally, the limited volume of hydrogel samples restricts the injectable amount of dimer to be less than 2 µg per 1 loaded circular film . Thus, any dimer formed in these samples appears to be further decomposed without detection, as a consequence, brown colour rather was seen (see Chapter 3). However, because dithranol is the only active ingredient required in the ultimate therapeutic treatment, the results obtained by using hplc system 1 proved to be satisfactory in terms of monitoring the stability of dithranol in the loading solution and film. Using system 1, the retention time of dimer, 44 minutes (sd= 4.5, n=10), was very long relative to that of dithranol, 11.7 minutes (sd=0.4), or danthrone, 9.4 minutes (sd=0.4). This leads to the lengthy analysis time and an increase in peak width, as a consequence, decreasing assay sensitivity of dithranol dimer. Any improvement of hplc system 1 to provide better conditions for dimer analysis would be beneficial, particularly in subsequent penetration and clinical studies where dimer is likely to be involved as discussed in Chapter 1. The stability studies of dithranol in stratum corneum (see Chapter 3) showed the presence of significant amounts of the dimer. Therefore, dimer quantification was improved by using system 2 which does not affect separation of dithranol, danthrone and the internal standard.

By increasing the composition of acetic acid in the mobile phase from 0.5% to 2% and the flow rate from 0.4 to 0.6 ml/min, all the retention times were halved, resolution maintained and sensitivity increased as shown in Fig. 2.2.

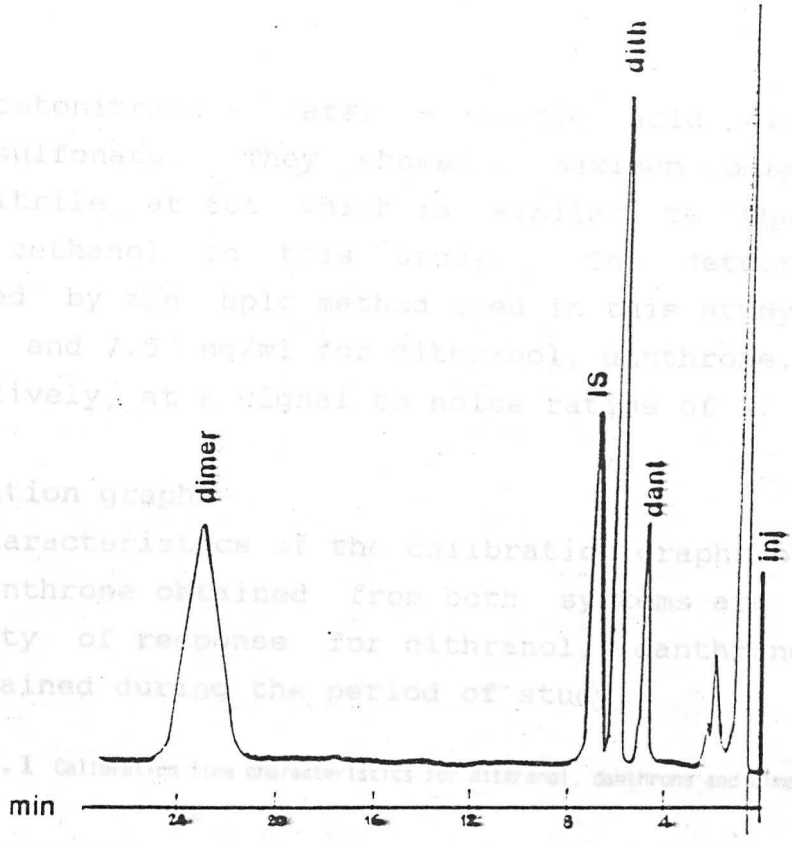


Fig.2.2 Representative chromatograms of dithranol, danthrone, dimer and fluorene(internal standard) analysed using system 2.

For all subsequent determinations, system 2 was used. The quantification of dimer production in the earlier experiments involving stability studies of the dithranol and loaded films were obtained, where appropriate, by repeating those experiments.

The hplc systems used for dithranol determination in this study are different from the methods developed by Burton & Gadde(1985) and Wurster & Upadrashta(1986). Columns used in these reports were wide bore and larger, i.e. 30cm x 3.9mm I.D. and 25cm x 4.6mm I.D., respectively, than the microbore ones used here. The advantage of the microbore column is the improvement in the detection limits and solvent consumption. A detection wavelength of 254 nm was used in this study because this maximises the sensitivity of dithranol, danthrone and dimer in mobile phases which contain either methanol or acetonitrile [Wurster & Upadrashta, 1986]. Burton & Gadde used an ion-pair chromatography system. The mobile phase

was acetonitrile - water - acetic acid eluent sodium hexanesulfonate. They showed a maximum composition of acetonitrile at 60% which is similar to that obtained using methanol in this study. The detection limits obtained by the hplc method used in this study are 15-20, 1.5-2, and 7.5 ng/ml for dithranol, danthrone, and dimer, respectively, at a signal to noise ratios of 3.

Calibration graphs

The characteristics of the calibration graphs of dithranol and danthrone obtained from both systems are identical. Linearity of response for dithranol, danthrone and dimer was obtained during the period of study.

Table 2.1 Calibration line characteristics for dithranol, danthrone and dimer, obtained using hplc.

	Calibration		
	slope Mean(sd)	correlation coefficient Mean(sd)	intercept Mean (sd)
SYSTEM 1			
dithranol n=6	4.1(0.1)	0.995(0.005)	0.03 (0.004)
danthrone n=3	4.6(0.4)	0.995(0.005)	0.01 (0.009)
SYSTEM 2			
dithranol n=6	4.1(0.1)	0.998(0.001)	0.02 (0.016)
danthrone n=3	4.0(0.0)	0.999(0.000)	-0.01(0.001)
dimer n=4	2.3(0.2)	0.996(0.001)	-0.02(0.004)

Table 2.1 shows that correlation coefficients ranging from 0.994 to 0.999 and y-intercepts equivalent to less than 0.9% of the normal response, corresponding to less than 0.006, 0.0002, 0.009 µg/ml of dithranol, danthrone and dimer, respectively, were obtained.

Assay of hydrogel, loading solution and Dithrocream

All of the samples injected were diluted with the mobile phase (1 in 10 to 1000 dilutions). This means that the polarity of the stationary phase will not be greatly affected by the presence of the other solvents used in the preparation of standard and sample solutions.

Analysis of blank hydrogel solution, as prepared under Section 2.2.5, showed no extraneous peaks which would cause interference in the chromatogram of dithranol, danthrone and dimer, as shown in Fig.2.3. There was also no significant effect on the expected responses for related standards.

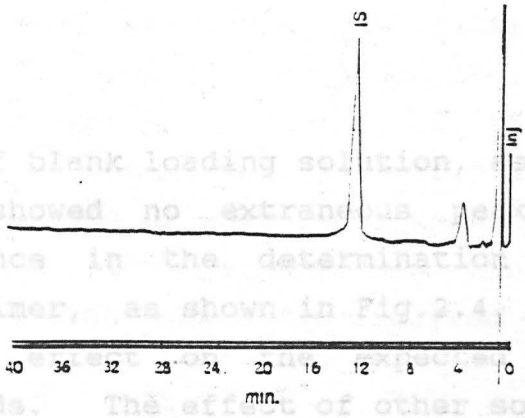


Fig.2.3 A chromatogram of the analysis of blank hydrogel solution with the internal standard (IS) added using system 2.

For stability reasons, the solutions of hydrogel were made in glacial acetic acid. Varying the amounts of glacial acetic acid present will affect the pH of the solution injected into the hplc column and this could affect the chromatogram. No changes in the chromatography were observed at concentration lower than 15%. Successive injections of solutions of glacial acetic acid of concentration higher than 15% resulted in significant broadening of the peaks. This could be the result of the interference of strong acid with the adsorption of the solutes onto the column. This phenomenon was reversible

by washing the column with water. This effect limits the amount of sample with low concentrations of dithranol which can be injected, e.g. in the hydrogel remaining from the release studies.

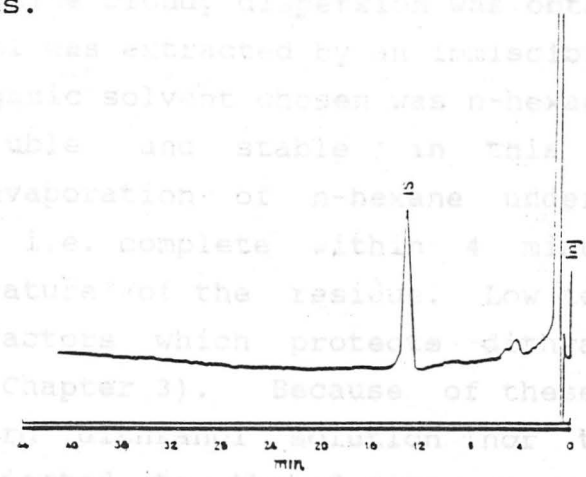


Fig.2.4 A chromatogram of the analysis of blank loading solution with the internal standard (IS) added using system 2.

Analysis of blank loading solution, as prepared under Section 2.2.5, showed no extraneous peaks which would cause interference in the determination of dithranol, danthrone and dimer, as shown in Fig.2.4. There was also no significant effect on the expected responses for related standards. The effect of other solvents from the loading solution was found to be negligible because no change in retention times occured. The method used is valid for the determination of dithranol in the loading solution.

For the purpose of this study, the actual amount of dithranol applied as the cream on the stratum corneum had to be determined. Examples of solvents used to dissolve Dithrocream were chloroform, glacial acetic acid, n-hexane, petroleum, acetone, methanol, and acetonitrile. Using these, it was found not to be possible to obtain complete solution of the cream. This could result in poor recoveries for dithranol. Another possibility was to disperse the cream in water and separate the cream base by centrifugation. Using this method, a percent recovery of

dithranol was less than 30% because dithranol prefers the cream base to water. As the result of the above, a partitioning system was devised. The cream was dispersed in water until a white cloudy dispersion was obtained from which the dithranol was extracted by an immiscible organic solvent. The organic solvent chosen was n-hexane because dithranol is soluble and stable in this solvent. Furthermore, the evaporation of n-hexane under an air stream was rapid, i.e. complete within 4 minutes, and reduced the temperature of the residue. Low temperature was one of the factors which protects dithranol from decomposition(see Chapter 3). Because of these factors, neither the standard dithranol solution nor the fresh cream extracts subjected to the drying process showed evidence of decomposition to danthrone or the dimer. The recovery of dithranol extracted from Dithrocream was 85% (sd = 10%, n = 6). The high standard deviation may well be attributable to the small sample of the cream being analysed. However, the method is sufficiently sensitive to monitor the changes of dithranol when the cream was applied onto the stratum corneum and the results are comparable because each sample was analysed using the same method.

2.3.2 SPECTROFLUORIMETRY

Establishment of spectrofluorimetric analysis

For the aqueous receptor media, direct fluorescence analysis is found impossible because of the limited solubility of dithranol in water (Melo et al, 1983). This leads to light scattering effects and poorly defined spectra. Therefore, an analytical method based on the use of non-aqueous solvents was required. The use of glacial acetic acid in the extraction of dithranol from the hydrogel means that the dithranol would be present in the non-fluorescent unionised form. Dithranol is relatively stable in its unionised form but it is the monoanionic

species which is the fluorescent form. It is also very unstable particularly at pH values above its pK_a value of 9.4 [Melo et al, 1983]. The stability of dithranol was also shown by these authors to be dependent on the solvent chosen. Basic solvents such as dimethylformamide (DMF) enhance dithranol ionisation, as a consequence, decrease its stability. This is known because the extent of ionisation proportional to the increase in the absorption at 360 nm, the λ max for the monoanionic species of dithranol.

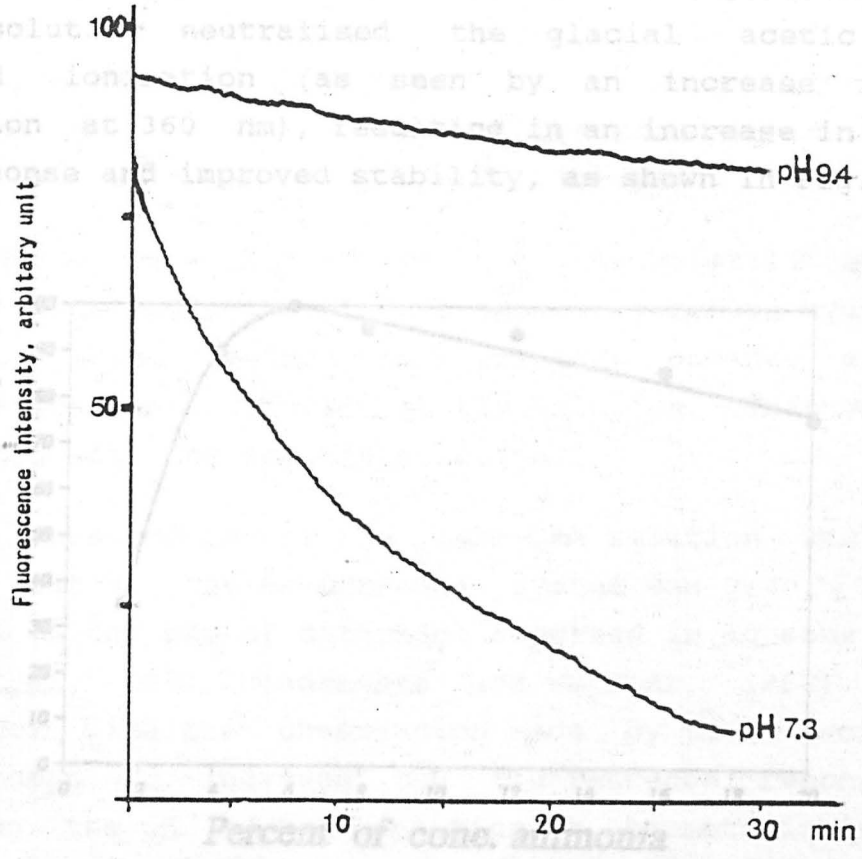


Fig.2.5 Fluorescence intensity of dithranol (8 ng/ml) in DMSO-GAA (pH 7.25) and am-DMSO-GAA (pH 9.4) solvent systems over 24 minutes.

Because of the low levels of dithranol anticipated and the known instability of dithranol in alkaline media a pH restricted non-aqueous solvent system was required. Dithranol rapidly degraded in DMF. Dimethylsulphoxide (DMSO) was found to produce, at equivalent concentration, a similar fluorescence response to DMF. Dithranol decomposition kinetics in DMSO have already been studied

(see Chapter 3). As this solvent forms part of the hydrogel loading formulation and has a wide miscibility range with the other solvents used, the sensitivity of the determination and the stability of dithranol in extracts diluted in DMSO was further investigated. The hydrogel and stratum corneum extract in glacial acetic acid was added to DMSO. The fluorescence response for dithranol in this DMSO-glacial acetic acid solution (illustrated in Fig. 2.5) though high enough for the sensitivity required was insufficiently stable. It was found that addition of concentrated ammonia solution into the DMSO-glacial acetic acid solution neutralised the glacial acetic acid, promoted ionisation (as seen by an increase in the absorption at 360 nm), resulting in an increase in the pH and response and improved stability, as shown in Fig. 2.5.

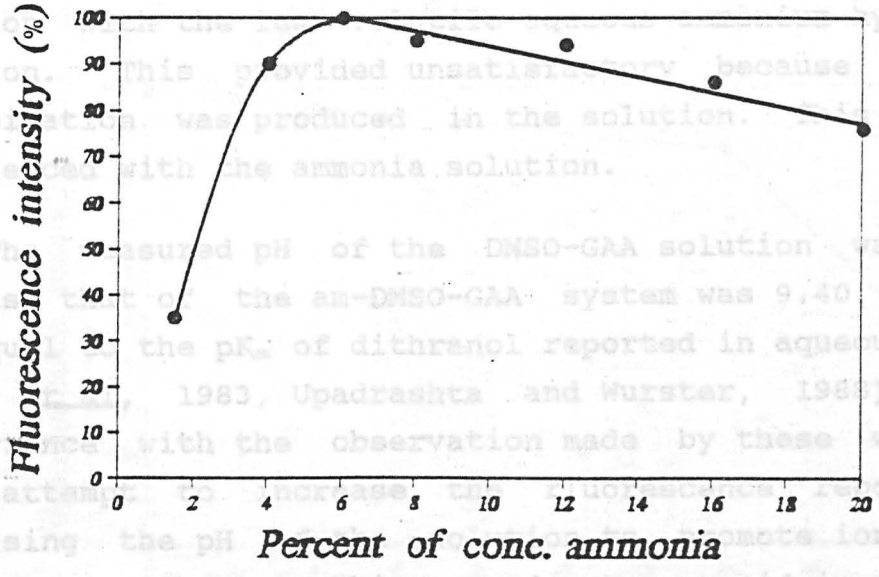


Fig.2.6 Percent of fluorescence intensity of dithranol (8 ng/ml) with varying percent of conc. ammonia solution present in am-DMSO-GAA solvent system.

The acid-base ratio, i.e. ammonia to glacial acetic acid added to the DMSO was found to be of importance in terms of maximising the fluorescence response and the stability of the reading. As the concentrated ammonia solution added was increased from 2 to 4%, an increase in

the fluorescence intensity of dithranol using wavelength pair 1 (WP1, excitation and emission wavelengths of 390 and 500 nm, respectively) was obtained, as shown in Fig. 2.6.

It is noted that those at WP2, WP3 and WP4 followed the same pattern. All showed a plateau in the 4-12% region. The similarity in the pattern of response using all the wavelength pairs indicated that no distortion of the spectrum for the monoanionic form was occurring. Based on these results, it was decided for assay purposes to use 8% of concentrated ammonia solution and 2% of glacial acetic acid in DMSO, i.e. base-acid ratio of 4. By using this ratio, the fluorescence intensity of dithranol was both maximised and stabilised.

Attempts were made to replace the volatile ammonia solution with the less volatile aqueous ammonium hydroxide solution. This provided unsatisfactory because a fine precipitation was produced in the solution. This was not experienced with the ammonia solution.

The measured pH of the DMSO-GAA solution was 7.25 whereas that of the am-DMSO-GAA system was 9.40. The pH is equal to the pK_a of dithranol reported in aqueous media (Melo et al, 1983, Upadrashta and Wurster, 1988). In concurrence with the observation made by these workers, any attempt to increase the fluorescence reponse by increasing the pH of the solution to promote ionisation was not successful. This was due to a rapid increase in the rate of dithranol decomposition. The "am-DMSO-GAA" solvents sytem developed was shown to have the necessary stability (see Fig. 2.5), sensitivity and specificity to determine the dithranol content in a variety of media.

Assay development for aqueous receptor media

As described above, the poor fluorescence characteristic of dithranol in water requires a solvent system which would enhance this property. The resulting higher

sensitivity of detection would then allow the determination of the low levels of dithranol in the aqueous receptor media used in the release studies (see Chapter 5). To achieve this end, the effect of the presence of varying amount of the aqueous media on the response and stability of dithranol in the "am-DMSO-GAA" system was evaluated. An aqueous acetate buffer (pH 2.8) was used in this study because it is the solvent of choice in <u>in vitro</u> release studies. The effect of the addition of the buffer on fluorescence intensity at WP1 of dithranol is shown in Fig. 2.7. It shows a decrease of the fluorescence intensities when the concentration of the buffer added was higher than 8%.

The stability of the fluorescence intensity of dithranol in the "am-DMSO-GAA" system with up to 8% of the buffer present was observed to be the same as that without buffer as previously mentioned.

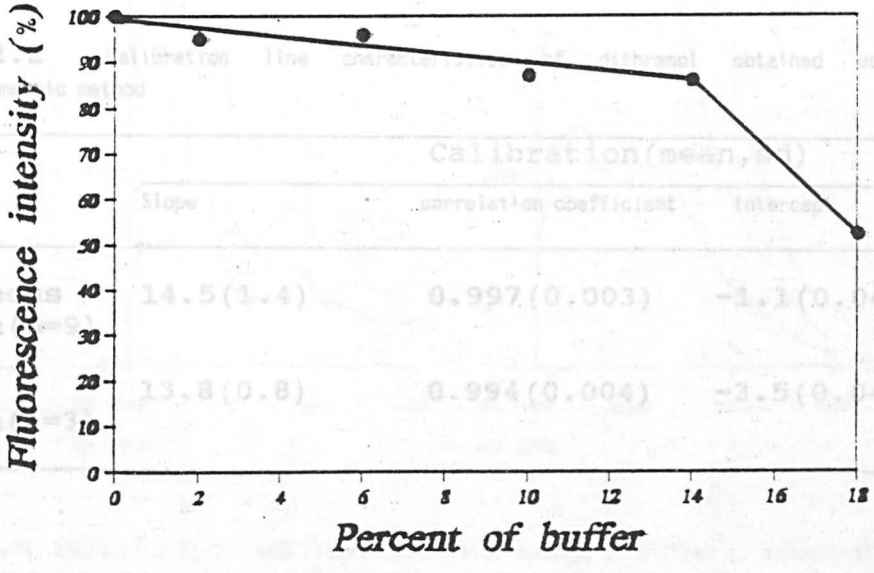


Fig.2.7 Percent of fluorescence intensity of dithranol (8 ng/ml) with varying percent of acetate buffer at pH 2.8 added to am-DMSO-GAA solvent system.

The measured pH of the solution containing between 1% to 8% of acetate buffer (pH 2.8) did not affect the pH of the solution, i.e. remaining at 9.4. The spectra of dithranol in the presence of not more than 8% of the

buffer in the solution are the same as that of the standard shown in Fig.2.8. There is no significant difference ($p>0.05$) between the fluorescence intensity ratios obtained from the four wavelength pairs of the solutions with and without the buffer upto 8%. This indicated that no distortion was evident in the spectra for the monoanion dithranol. This percentage was applicable to any analysis involving aqueous media.

Calibration graphs and Assay validation

Table 2.2 shows the calibration graphs of dithranol classified into two categories according to the types of samples used. Those samples which are non-aqueous solutions are the stratum corneum extracts and the hydrogel solution in glacial acetic acid. Aqueous samples are the aqueous receptor media (detailed in Chapter 5).

Table 2.2 Calibration line characteristics of dithranol obtained using the spectrofluorimetric method.

Sample	Calibration(mean,sd)		
	Slope	correlation coefficient	intercept
non-aqueous solution(n=9)	14.5(1.4)	0.997(0.003)	-1.1(0.04)
aqueous solution(n=3)	13.8(0.8)	0.994(0.004)	-3.5(0.04)

Despite these differences, the calibration graphs obtained during a period of 6 months were linear and reproducible in the range 0 to 9 ng/ml using the settings specified. Typical excitation and fluorescence spectra of dithranol standards are shown in Fig 2.8. This figure illustrates the relationship between fluorescence

intensity and concentration of dithranol over the working range, 0 - 8 ng/ml, used in the analysis.

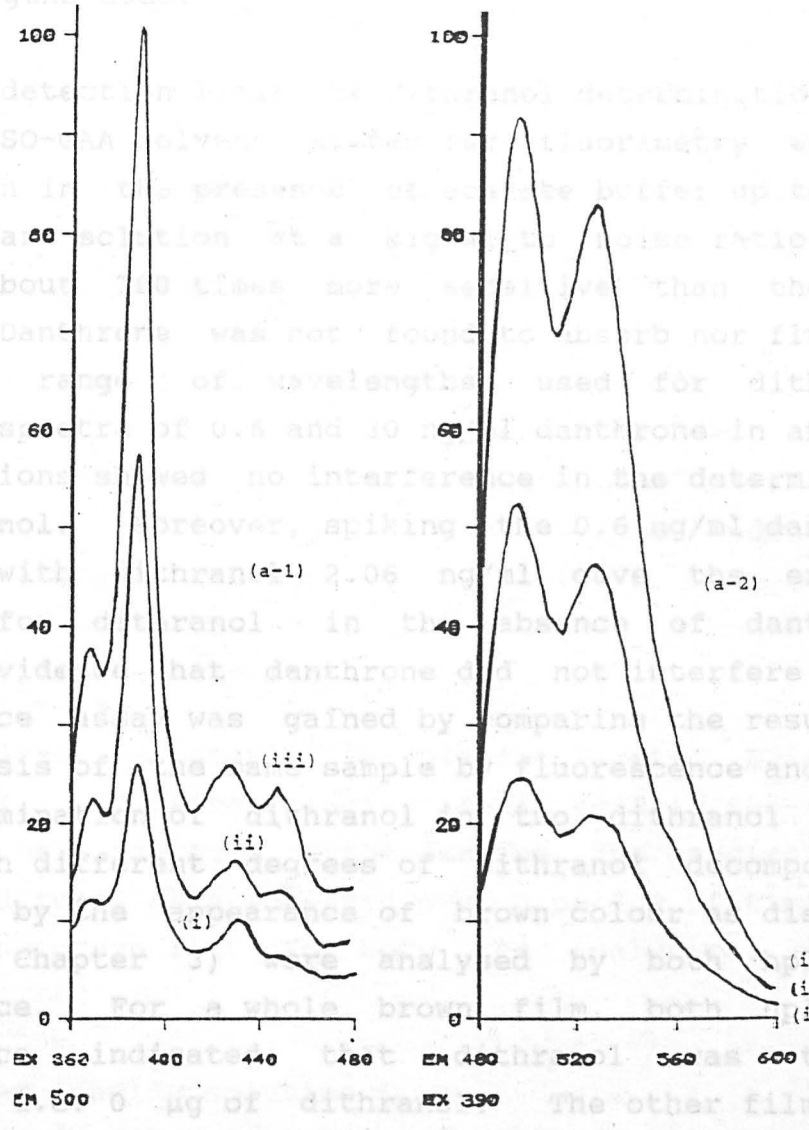


Fig. 2.8 Excitation (a-1) and fluorescence (a-2) spectra of dithranol, concentration :(i) 1, (ii) 4 and (iii) 8 ng/ml, in the am-DMSO-GAA solvent system, fixing the λ_{exc} and λ_{ems} at 390 and 500 nms. NB: The scale used for the 8 ng/ml solution is 70% of the 1 and 4 ng/ml.

The ratios of fluorescence intensity of WP1, WP2, WP3 to that of WP4 (4.5 : 4.1 : 1.1 : 1, respectively) used to monitor spectral purity of dithranol in the fresh standards were found to be constant over 6 months. Those obtained from samples analysis were not significantly

different ($p>0.05$) from those obtained using a fresh dithranol standard solution prepared under the same conditions. This approach also served to indicate the presence or absence of "blank" interference over the range of wavelengths used.

The detection limit of dithranol determination using the am-DMSO-GAA solvent system for fluorimetry was 0.2 ng/ml both in the presence of acetate buffer up to 8% or the standard solution at a signal to noise ratio of 3. This is about 100 times more sensitive than the hplc method. Danthrone was not found to absorb nor fluoresce over the range of wavelengths used for dithranol. Recorded spectra of 0.6 and 30 ng/ml danthrone in am-DMSO-GAA solutions showed no interference in the determination of dithranol. Moreover, spiking the 0.6 ng/ml danthrone solution with dithranol 2.06 ng/ml gave the expected spectra for dithranol in the absence of danthrone. Further evidence that danthrone did not interfere in the fluorescence assay was gained by comparing the results of the analysis of the same sample by fluorescence and hplc. The determination of dithranol in two dithranol loaded films with different degrees of dithranol decomposition (observed by the appearance of brown colour as discussed later in Chapter 3) were analysed by both hplc and fluorescence. For a whole brown film, both hplc and fluorescence indicated that dithranol was totally degraded, i.e. 0 µg of dithranol. The other film which was two-thirds brown was shown to contain 10% dithranol by both hplc and fluorescence.

Dimer was found to fluoresce under the conditions used. The excitation and fluorescence spectra of dimer were similar to that of dithranol but the λ_{max} of the excitation and emission spectra were 396 and 509 nm which were about 7 and 9 nm deviated from those of dithranol. Additionally, the concentration of dimer had to be about 10 times higher than that of dithranol to gain a

fluorescence response equal to that of dithranol. Because of the possibility that the dimer may interfere in dithranol determination, the method was used when the sensitivity of the hplc analysis was not sufficient. The spectral purity of the dithranol was checked as described above: any significant contribution by the dimer to the observed fluorescence would lead to a change in the anticipated ratios. The similarity in the pattern in which both dithranol and dimer absorb and emit light indicates that the dimer still possesses the ionisable hydroxyl groups characteristic of dithranol.

2.3.3 SAMPLE ANALYSIS

Generally, samples with high concentrations of dithranol such as loading solution, cream, loaded hydrogel or stability studies were assayed by hplc. Samples with dithranol concentration of lower than 30 ng/ml were assayed spectrofluorimetrically.

Dithranol is stable in glacial acetic acid (see Chapter 3). By using glacial acetic acid as the solvent for the final dilution of the samples, the samples can be stored and repeatedly analysed over a period of time. The solvent was convenient for both the analytical methods used.

Assay of the loading solution

Hplc was used. The solution was diluted 1 in 1000 prior to analysis. No interference in the hplc chromatogram of dithranol, danthrone and dimer was observed throughout the study. The presence of blank loading solution did not alter the expected quantitative response for all three compounds.

Assay of hydrogel

The analysis of dithranol in the hydrogel was possible after the hydrogel containing dithranol dissolved in

glacial acetic acid. It was found that the optimum volume of glacial acetic acid which would dissolve a piece of dry hydrogel, 1-cm diameter, was 2 ml. The use of glacial acetic acid made it possible to carry out dithranol analysis using the am-DMSO-GAA system and fluorimetric analysis. The presence of the hydrogel was shown not to interfere with nor alter the expected quantitative response for the compound being determined in both hplc and fluorescence analysis.

Since the loaded hydrogel is essentially transparent, an attempt was made to quantify the amount of dithranol present in the gel by direct spectrofluorimetry measurement. This would have had the advantage of avoiding the destruction of the film. The hydrogel swells to reach its highest degree at 5°C (see Chapter 4). At 20°C, the loaded films which were attached onto the surface of the cuvette were found to shrink slightly because of the higher temperature. After a period of about 10 minutes in the cuvette with light exposure due to spectral recording, liquid drops were found scattered on the suface of the films. Because of this, no correlation was observed between the measured response and the concentration of dithranol present.

Assay of Dithrocream

The same method previously described was used thoroughout the study. There was no detectable danthrone or dimer in the sample of the cream applied to the stratum corneum. The presence of stratum corneum in the extract did not significantly interfere with nor affect the percentage recovery of dithranol ($p<0.05$).

Assay of stratum corneum

A single extraction of dithranol from the stratum corneum using glacial acetic acid was found to be sufficient to recover all the dithranol present. The spectra of blank extracts of stratum corneum showed no fluorescence over

the wavelengths of measurement. Spiked samples of the blank extract showed the expected dithranol responses.

2.3.4 CORRELATION BETWEEN HPLC AND SPECTROFLUORIMETRY

The determination of dithranol obtained by analysis of the same samples using the hplc and the spectrofluorimetric methods were compared statistically using paired-sample t-test. No significant difference ($p>0.05$) was found in 11 samples of the freshly-prepared dithranol loading solution, 4 freshly-loaded hydrogel films and 4 aqueous receptor media sampling at different release times. This shows that the results obtained from the determination of dithranol using the hplc and spectrofluorimetry are identical. Using the F-test to analyse the results obtained by both hplc and spectrofluorimetric assay demonstrated that there was no significant difference ($p > 0.05$) in precision between the two methods.

CHAPTER 3

STABILITY OF DITHRANOL

3.1 INTRODUCTION

Preliminary studies indicated that dithranol decomposition in solutions was a rapid process. Using the hplc methods discussed in Chapter 2, the stability of dithranol was monitored in aqueous or organic solvents, the loading solution, the hydrogel film, and in stratum corneum. Other factors including temperature, protection from light and humidity were investigated. Additionally, the antioxidants isoascorbic acid, salicylic acid, sodium metabisulphite and d-α-tocopherol acetate were investigated as potential stabilisers in an attempt to select an appropriate candidate for the development of dithranol loading solution. The extent of ionisation of dithranol in solution has been suggested as one of the crucial properties affecting its decomposition, and so the effect of pH on stability was studied.

The aim of this part of the project was to develop a system which would stabilise dithranol in the hydrogel, this being a prerequisite to gaining knowledge about the release characteristics and clinical effect of the dithranol hydrogel.

3.2 EXPERIMENTAL METHODS

3.2.1 STABILITY STUDIES IN SOLUTIONS

In aqueous systems

EFFECT OF PH - Acetate buffer at pH 2.8 or pH 5.0, distilled water (measured pH 5.50 at 20°C) or 0.2M mixed phosphate buffer pH 7.0 (B.P.) were used. Dithranol solution (approximately 4 µg/ml) in 5% glacial acetic acid in each of the solvents was prepared and stored at 20°±2°C. The experiment using pH 2.8 acetate buffer was carried out unstirred or stirred (using a magnetic stirrer). All these were repeated 4 times. Samples were taken, diluted with hplc mobile phase (the internal standard included) and analysed using hplc (system 2, see Chapter 2, p 38). Results are shown in Table 3.1 as an average of concentration and percentage of dithranol remaining in each set of conditions.

EFFECT OF ANTIOXIDANT - A dithranol solution (about 4 µg/ml) was prepared using 5% glacial acetic acid in 0.1% sodium metabisulphite in distilled water (measured pH 2.2, at 20°C). The solution was stored and assayed as described previously. Results are shown in Table 3.2.

In non - aqueous systems

METHANOL - A dithranol solution in 2% glacial acetic acid in methanol (approximately 40 µg/ml) was prepared and stored at 20°C. Samples were taken, diluted with hplc mobile phase (the internal standard included) and analysed using hplc (system 2, as above). This was repeated twice. Average results are shown in Table 3.3.

ACETONE - The test was performed as for Methanol but using acetone as a solvent. Samples were taken, diluted with hplc mobile phase (the internal standard included)

and analysed using hplc (system 2, as above). This was repeated twice. Results are shown in Table 3.3.

GLACIAL ACETIC ACID/HEXANE - A dithranol solution in 40% glacial acetic acid in hexane (about 0.3 mg/ml) was prepared and stored at 20°C for 5 days. (A preliminary attempt to store the solution at 5°C caused the separation of the liquids, possibly because of the difference in the freezing points). 0.1 ml of the solution was sampled, dried and re-dissolved using 1 ml glacial acetic acid. Samples were taken, diluted with hplc mobile phase (the internal standard included) and analysed using hplc (system 2, as above). Results are shown in Table 3.3.

GLACIAL ACETIC ACID - A dithranol solution in glacial acetic acid (about 2 mg/ml) was prepared and stored at -21°C (±2°C). Samples were taken, initially and after 1, 7, 9 and 12 months, for analysis by melting to 20°C using an ultrasonic bath and a vortex mixer. 0.1 ml of the solution was diluted to 5 ml of glacial acetic acid and further diluted 1 in 50 using an appropriate hplc mobile phase (the internal standard included) for hplc analysis. Results are shown in Table 3.4.

The stability of a dithranol solution in glacial acetic acid (about 40 µg/ml, which was prepared monthly and stored at -21°C for the preparation of standard solution for analysis) was randomly assessed 5 times. The amount of dithranol remaining in the solution from each analysis was compared with a fresh dithranol solution prepared and analysed using the same procedures. This was found to be 100 ± 0.2 % (n= 5).

DMSO AND PEG 400 - A dithranol solution (about 80 µg/ml) in 4% glacial acetic acid in DMSO or PEG400 was prepared and stored at 20°C. After 24 hours, there were 2 layers in the PEG solution: the upper was dark brown-coloured("top") and lower light brown-coloured("lower"). The layers were assayed separately and then together after mixed using a vortex mixer and an ultrasonic bath to

remove the bubbles formed. 0.05 ml samples were taken, diluted with hplc mobile phase (the internal standard included) and analysed using hplc (system 2, as above). These were repeated twice. The results are shown in Table 3.5.

EFFECT OF ANTIOXIDANTS - The tests were performed as for Methanol but incorporating 0.1 % of each antioxidants in methanol. The antioxidants used were isoascorbic acid, salicylic acid and d-α-tocopherol acetate. Results are shown in Table 3.6.

In dithranol loading solution

STABILITY UPON STORAGE - Using the conditions used for loading (see Chapter 4, p 120), i.e. 5°C, light-protected, with less than 2% of air present in the container, the stability of dithranol in the loading solution was investigated. 100 ml of dithranol loading solution was prepared, cooled down to 5°C and then filled into four 25-ml bottles. Each one was stored at 5°C for up to 7 days. This was repeated twice. The solution was analysed using hplc system 1 (method in Chapter 2, p 42).

EFFECT OF LIGHT, TEMPERATURE AND OXYGEN ON STORAGE - 200 ml of dithranol solution was prepared and filled into eight bottles. The volume of air in the head space of four of the bottles was less than 2 % of total volume, while the others being about 80%. One from the four bottles of each set was stored under one of the conditions as follows: a) in the dark (P) at 5°C (C); b) in the dark (P) at 20°C (R); c) exposed to light (N) at 5°C (C); d) exposed to light (N) at 20°C (R).

The solution was assayed, using hplc (system 1, as above), initially and after 2 days. The percentage of dithranol remaining in the solution was calculated by comparing with the initial concentration. Results are shown in Fig. 3.2.

3.2.2. STABILITY STUDIES IN DITHRANOL LOADED FILM

In each experiment, the films were loaded using the standard procedures, i.e. using 500 µg/ml solution at 5°C (detailed in Chapter 4, p120), exceptions are those being investigated. The dry hydrogel films, used as received, were cut to form a circular disc 1 cm in diameter for loading. Analysis of the films, where applicable, was performed using hplc (system 1, as above)

Effect of temperature used in the loading process

The films were loaded in a solution at 20°C. The other techniques used to treat and store the films were those described under "bottle" below. The films were randomly sampled and analysed by hplc (system 1, as above) at predetermined times. The results are shown in Table 3.8 and Fig.3.3.

Effect of storage conditions of loaded film

Two types of containers used for storage of the dithranol loaded film were investigated as follows:

BOTTLE - The film was placed in a 25-ml light-protected glass bottle and the air inside the bottle was displaced with nitrogen gas. The bottle was tightly closed and stored at 5°C. The films were randomly sampled and analysed at predetermined times. Results are shown in Table 3.9 and Fig. 3.3.

LAMINATED PAD - Two sheets of Vistafoil which consisted in a paper sheet and a sticky plastic sheet were used for each film. The plastic sheet was cut to form a 2x2 inches square shape and the paper sheet to 1.7x1.7 inches square. The loaded film was placed on the waxed side of the paper sheet and the non-waxed side of the paper sheet was stuck onto the plastic sheet. Both sides of the loaded film were treated in the same manner, care being taken to keep air trapped inside the paper sheets to a minimum. The 2

plastic sheets were then tightly sealed, avoiding the penetration of air or moisture. The pads were stored in an amber glass jar at 5°C. The films were randomly sampled and analysed using hplc (system 1, as above) at predetermined times. Results shown in Table 3.10 were obtained using single wrapped pads, and in Table 3.11 where the original pad were further wrapped in larger sheets of Vistafoil.

Effect of pre-washed films

A trace amount of ferric chloride had to be incorporated in the production of the hydrogel [Graham,N.B.,personal communication]. The presence of ferric ion was shown to accelerate dithranol decomposition in methanol [Smith, 1989]. Thus, the quantification and elimination of ferric ions from the hydrogel was investigated. A "washing" technique was developed to "clean" the hydrogel of ferric ions was investigated and the stability of dithranol in this pre-washed film was then studied.

DEVELOPMENT OF THE TECHNIQUE USED IN ELIMINATING FERRIC ION IN THE HYDROGEL - An atomic absorption analysis for the total Fe^{++} was employed to monitor the technique used for eliminating the ferric ions from the hydrogel. For analysis purpose, the films had to be dissolved and analysed using the method described for viscous sample solutions [Beckett and Stenlake, 1988]. The total ferric ions present in 1 g of hydrogel was found to be 118 µg.

The extraction of ferric ion was initially investigated by soaking dry hydrogel film in an excess volume of deionised water at 5°C for 3 hours. No detectable amount of ferric ion was found in the water. This indicates an insufficient degree of swelling of the hydrogel which requires longer period in water (discussed later in Chapter 4, p 107). Thus a longer period (2 days) of soaking was used plus additional boiling for 1 hour before the gel was removed and solution was analysed.

This method provided a maximum of 82 µg of Fe^{+++} extracted per 1 g of hydrogel. The method was used although only 69% recovery has been achieved. The extraction method was performed using heat, excess volume of solvent and adequate time scale which should provide maximum hydrogel swelling. This suggests that the maximum extraction was obtained in regard to the method used.

STORAGE OF LOADED FILM - The pre-washed films using the previously described method were loaded with dithranol (at 5°C) and stored using double-wrapped laminated pads. These were randomly sampled and analysed by hplc (system 1, as above) at predetermined times. Results are shown in Table 3.12.

3.2.3 STABILITY OF DITHRANOL APPLIED TO STRATUM CORNEUM

PREPARATION OF STRATUM CORNEUM - A dry piece of neonatal rat stratum corneum was cut into a circular shape (diameter 1.5 cm), and hydrated by soaking in water at 20°C for 1 hour, blotted dry, placed on a piece of wax paper and left for 1 hour before use.

Effect of Preparations: Hydrogel, Dithrocream, and control

HYDROGEL - A loaded film was placed on the hydrated stratum corneum and wrapped in the laminated pad as previously described, the trapped air being kept to a minimum. At predetermined times, the stratum corneum and hydrogel were separated and dissolved in glacial acetic acid. Additionally, the wax paper in contact with the stratum corneum or hydrogel was also added to the same glacial acetic acid to dissolve any dithranol which was adhering to it. (Preliminary experiments had indicated that failure to do this did not give 100% recovery.) The solution was diluted with hplc solvent (containing internal standard) for hplc (system 2) analysis.

DITHROCREAM (0.1%) - 0.0250 g of Dithrocream, accurately weighed, was evenly applied to the stratum corneum and then treated as above. At predetermined times, the residual cream, stratum corneum and paper were extracted and assayed using the method described in Chapter 2.

DITHRANOL ALONE (CONTROL) - A dithranol solution (about 0.3 mg/ml) in 40% glacial acetic acid in hexane was prepared. 0.05 ml of the solution was slowly dropped onto the prepared skin and allowed to dry. The skin was sealed using the method described for the hydrogel above and kept in the dark. The wax paper and the skin were dispersed in 2 ml glacial acetic acid: the extract was diluted with hplc mobile phase (with internal standard) and analysed using hplc (system 2, as above).

All these experiments were performed at 20±2°C. The results are shown in Table 3.13.

Effect of the stratum corneum and humidity

The effect of humidity on stability was investigated by performing a test using the same method as the "control" described previously. A blank was performed by dropping the same volume of the dithranol solution in glacial acetic acid and hexane on a piece of wax paper (size equal to that used in the "control").

The test and blank were stored in a laboratory dessicator exposed to the atmosphere of 93% relative humidity (above a saturated solution of potassium nitrate at 20°C) and kept in the dark for 5 days. Extraction and hplc analysis were performed as previously described. The results are shown in Table 3.14.

3.3 RESULTS AND DISCUSSION

3.3.1 STABILITY OF DITHRANOL IN SOLUTIONS

Aqueous solutions

Results in Table 3.1 show a decrease in the concentration of dithranol remaining in 5% glacial acetic acid in acetate buffer at pH 2.8 and 5.0, phosphate buffer at pH 7.0 or water at pH 5.5 between 0 and 7 days.

Table 3.1 Dithranol stability in 5% glacial acetic acid acetate buffer pH 2.8 (stirred and unstirred), acetate buffer at pH 5.0, water (pH 5.5) and phosphate buffer pH 7.0; unstirred solutions: expressed as mean (±sd, µg/ml) of concentration and percentage (in bracket) of dithranol remaining at 20°C.

Time	Dithranol (µg/ml)				
	acetate buffer			phosphate buffer	water
(day)	pH2.8 unstirred n=6	pH2.8 stirred n=4	pH 5.0 unstirred n=2	pH 7.0 unstirred n=2	pH 5.5 unstirred n=6
0	3.84(100) ±0.3	4.14(100) ±0.3	3.19(100) ±0.0	3.41(100) ±0.0	4.14(100) ±0.2
1	2.88 (82) ±0.2	3.14 (76) ±0.0	0.92 (29) ±0.3	3.03 (89) ±0.0	2.87(69) ±0.1
2	3.04 (79) ±0.4	3.10 (75) ±0.7	-	2.93(86) ±0.3	2.71(65) ±0.5
3	2.59 (67) ±0.2	3.09 (75) ±0.49	-	2.46(72) ±0.3	1.91(46) ±0.3
4	2.49 (65) ±0.2	-	0.16 (5) ±0.1	2.63(77) ±0.1	-
5	1.76 (46) ±0.3	-	<0.02 (0) ±0.0	2.25(66) ±0.1	-
6	1.52 (40) ±0.2	-	<0.02 (0) ±0.0	1.87 (55) ±0.0	-
7	1.36 (35) ±0.4	-	<0.02 (0) ±0.0	1.65 (48) ±0.0	-

In water, (Table 3.1 and Fig. 3.1), it appears that there is an initial decrease in the percentage of dithranol remaining after 1 day, suggesting that it may be either a zero or first order process. A zero-order plot gave a correlation coefficient of 0.958, a rate constant of 16.6 day^{-1} and a half-life of 3 days, whilst a plot of log % dithranol remaining against time gave a correlation coefficient of 0.9699, a rate constant of 0.24 day^{-1} and a half-life of 2.9 days. Therefore, a half-life of dithranol in water at pH 5.5 of about 3 day is obtained.

Table 3.1 shows that dithranol decomposed faster in water at pH 5.5 than in acetate buffer at pH 2.8 (either stirred or unstirred). A plot, in Fig.3.1 (between percent of dithranol remaining against time), illustrates a linear relationship in acetate buffer at pH 2.8 (correlation coefficient 0.986), giving a rate constant of 9 day^{-1} (half-life 7 days). To confirm this, a solution of acetate buffer at pH 2.8 was stored at 20°C for 12 days and found to contain 11% of dithranol without detectable danthrone or dimer. The rate constant indicates that 13% of dithranol should remain after 12 days of storage in the conditions specified. This finding indicates that this stability investigation has simulated the fate of dithranol in the receptor medium. Dithranol is, thus, more stable at pH 2.8 ($t_{1/2}$ = 7 days) than at pH 5.5 ($t_{1/2}$ = 3 days). This is expected because in a low pH solution, dithranol is not as highly ionised as at a high pH. The unionised form of dithranol undergoes decomposition at a slower rate than the ionised form [Upadrashta & Wurster, 1988 . There is no significant difference ($p > 0.05$) between the percent remaining in the stirred and unstirred solutions of dithranol in acetate buffer at pH 2.8. This finding was used to ensure that stirring or mixing of aqueous receptor media during the release studies would not affect dithranol stability.

Acetate buffer at pH 5.0 was investigated because it was being considered as a possible vehicle for release studies in an attempt to imitate the physiological pH of 5 on the outer layer of the skin. Table 3.1 shows that dithranol decomposed faster in acetate buffer at pH 5.0 than in water at pH 5.5. Fig. 3.1 shows that the decomposition of dithranol in acetate buffer at pH 5.0 is unlikely to follow zero-order kinetics (A zero-order plot gives a correlation coefficient of 0.848). A first order plot between log percent dithranol remaining against time gives a correlation coefficient of 0.983, a rate constant of 0.7 day^{-1} and a half-life of 0.97 day. This confirms that dithranol decomposed faster in acetate buffer at pH 5 ($t_{1/2}$ = 1 day) than that in water at pH 5.5 ($t_{1/2}$ = 3 days).

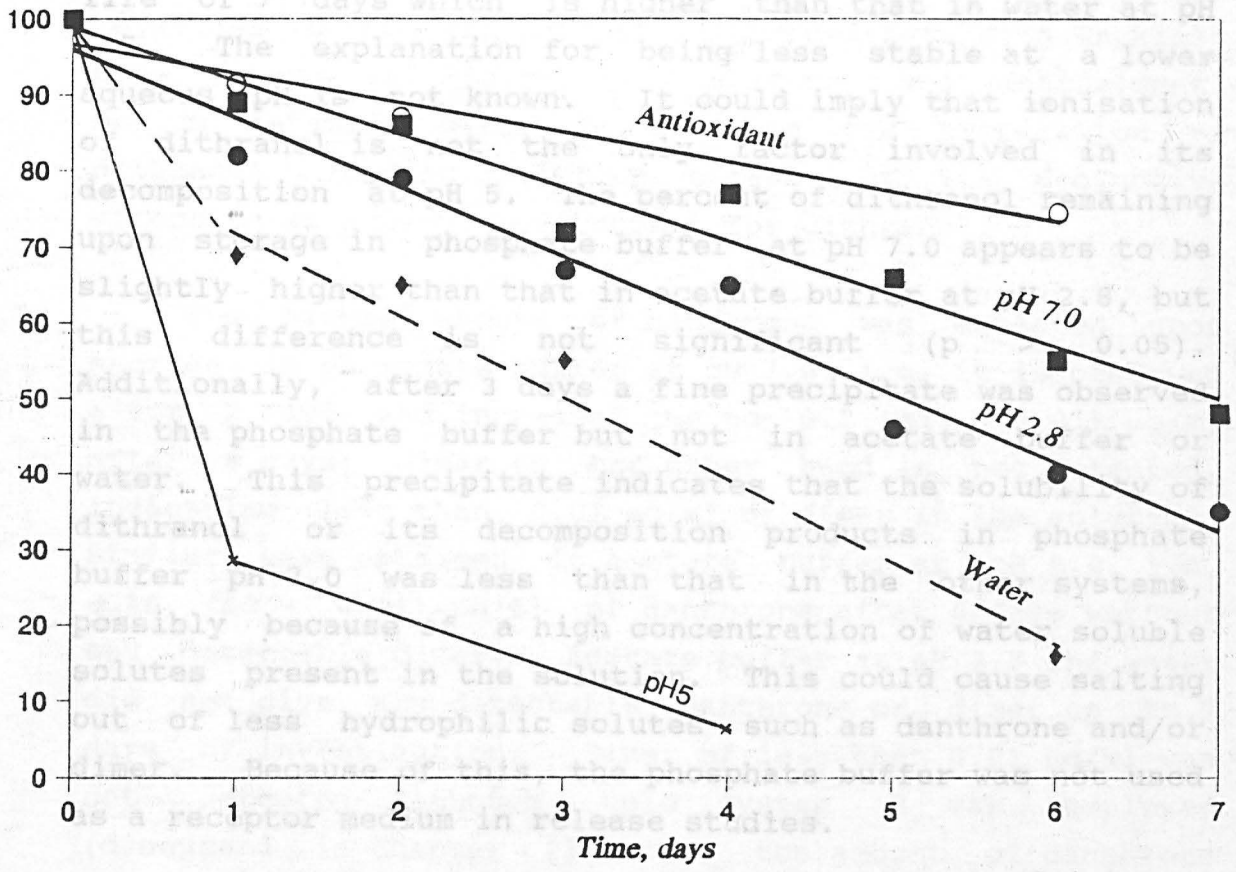


Fig. 3.1 Stability profiles of dithranol solutions (4 µg/ml) in 5% glacial acetic acid in acetate buffer (pH 2.8), acetate buffer (pH 5.0), water (pH 5.5), phosphate buffer (pH 7.0) and 0.1% sodium metabisulphite in water (pH 2.2).

An initial sharp drop in dithranol remaining from 100% to 29% after 1 day of storage in acetate buffer at pH 5 which is not seen in water indicates the presence of some catalyst in the buffer. This could be acetate anion. The concentration of acetate ions in acetate buffer at pH 5 is higher than that at pH 2.8, thus resulting in an expected higher decomposition rate. Therefore, it was decided that acetate buffer at pH 5.0 would not be suitable for use as a receptor medium because of these uncertainties about the decomposition of dithranol.

The plot of percent dithranol remaining in phosphate buffer at pH 7.0 against time, in Fig.3.1, shows a possible linear relationship (correlation coefficients 0.978). This gives a rate constant of 7 day^{-1} and a half-life of 7 days which is higher than that in water at pH 5.5. The explanation for being less stable at a lower aqueous pH is not known. It could imply that ionisation of dithranol is not the only factor involved in its decomposition at pH 5. The percent of dithranol remaining upon storage in phosphate buffer at pH 7.0 appears to be slightly higher than that in acetate buffer at pH 2.8, but this difference is not significant ($p > 0.05$). Additionally, after 3 days a fine precipitate was observed in the phosphate buffer but not in acetate buffer or water. This precipitate indicates that the solubility of dithranol or its decomposition products in phosphate buffer pH 7.0 was less than that in the other systems, possibly because of a high concentration of water soluble solutes present in the solution. This could cause salting out of less hydrophilic solutes such as danthrone and/or dimer. Because of this, the phosphate buffer was not used as a receptor medium in release studies.

Table 3.2 shows the effect of sodium metabisulphite on the stability of dithranol in water.

Table 3.2 Dithranol stability in 0.1% sodium metabisulphite in distilled water: expressed as average (standard deviation) of concentration (4 µg/ml, n=2); and percentage of dithranol remaining.

Time	Dithranol(µg/ml)		Danthrone((µg/ml)	Dimer(µg/ml)
(day)	mean(sd)	% remain	mean(sd)	mean(sd)
0	3.94(0.07)	100	<0.01	<0.01
1	3.60(0.21)	91.2	0.02(0.01)	<0.01
2	3.45(0.16)	87.6	0.03(0.01)	<0.01
6	3.01(0.21)	76.3	0.56(0.06)	<0.01

The addition of 0.1% sodium metabisulphite to the water causes pH to decrease from 5.5 to 2.2, and the rate of dithranol decomposition in water decreased from 16.6 day^{-1} ($t_{1/2}$ 3 days) to 3.6 day^{-1} ($t_{1/2}$ 14 days). This is estimated using zero order kinetics because of the linearity (correlation coefficient of 0.966) in the relationship between percent dithranol remaining against time, shown in Fig.3.1. Therefore, the antioxidant, sodium metabisulphite, is used in the preparation of dithranol loading solution because of its protective effect in an aqueous environment for dithranol.

The highest amount of danthrone was detected upon storage in phosphate buffer at pH 7.0, i.e. it is observed after 2 days and increased up to 0.2 (sd = 0.01) µg/ml after 6 days; dimer on the other hand is not detected, indicating less than 0.01 µg/ml of dimer in the solution. Similar were obtained in acetate buffer at pH 5.0, i.e. 0.16 (sd = 0.01) µg/ml of danthrone after 6 days without any detectable dimer. Acetate buffer at pH 2.8 and water did not give any detectable danthrone or dimer up to 7 days of investigation. Dimer of less than 0.01 µg/ml was not detected although hplc system 2 was employed (discussed in Chapter 2) whilst the amount of danthrone detected was not higher than 0.2 µg/ml. These results indicate clearly that dithranol decomposed in aqueous solutions at pH equal to and lower than 7 which has not been reported previously. In weak alkaline systems,

Schaltegger (1985) identified two pathways of dithranol decomposition, depending on the form of the dithranol, i.e. in solution danthrone was formed, whilst in suspension dimer was produced. This might be applied to present results, where concentration of danthrone was found higher than that of dimer. Nevertheless, the increased amount of danthrone formed does not correlate to the decrease of dithranol, probably because of its low water solubility. This implies that dimer might have been present in the solutions, but not at a high enough concentration to be detected because it further decomposed to form dithranol brown which is also not detectable. The presence of sodium metabisulphite is useful in the prevention dithranol decomposition in water whereas catalysts such as anionic acetate, accelerated this process.

Whitefield (1981a) indicates that the causes of rapid dithranol decomposition are due to the availability of dissolved oxygen and interference with the intramolecular bonding. In this study, the presence of dissolved oxygen in the solutions is recognised. However, each solution was treated using the same method, thus the results can be compared. Apart from difference in type of buffer solution used, several other factors are may interfere with the intramolecular bonding of dithranol causing an discrepancy between these results and the literature. One of these is the organic co-solvents added to water due to poor water solubility of dithranol. Solutions of dithranol had to be prepared by adding a small volume of a concentrated solution of dithranol in a organic solvent to the aqueous medium. Cavey et al (1982) used 1% acetone in Ringer buffer at pH 7.5 (dithranol solubility of 10 µg/ml) and showed 100% dithranol decomposition within 4 hours at 37°C, detecting 40% dimer without detectable danthrone. The organic solvent used in this study was glacial acetic acid (5%) which might delay dithranol decomposition, however, the same amount of glacial acetic acid was added

to each solution, thus the results are comparable. Another factor is temperature which was 20°C in this study. This is lower than that of Cavey (37°C), as a consequence, lower dithranol decomposition rate than that of Cavey is observed. Thus, the different medium and conditions do not allow direct comparison between our results and that reported.

Additionally, the pH-rate profiles of dithranol so far reported [Upadrashta & Wurster, 1988b] were obtained in various buffer solutions at pH higher than 7 (at 25°C). In which cases, the half-life of dithranol decreases as the pH increases from 44 minutes (pH 7.74) to 15 minutes (pH 9.44). A subsequent increase in half-life to 18.4 minutes as pH further elevated to pH 10.02 was observed. For the reasons discussed above, it is not possible to extrapolate this data to the lower pH used in the present work.

Despite the catalyst present in acetate buffer at pH 5.0, this phenomenon could occur with other anionic ions. It is surprising to observe that the behaviour of dithranol at pH 5, which is the physiological and therapeutical pH of dithranol, has not been reported previously. This information could be important in any explanation of the mechanism of action of dithranol.

Non-aqueous solutions

Of the solvents investigated methanol and acetone, were selected for their potential use in loading the hydrogel, and hexane and glacial acetic acid for their use in analysis. Dithranol in the polar solvents methanol and acetone decomposed at a greater rate than in the non-polar (Table 3.3).

Table 3.3 Dithranol stability in 2% glacial acetic acid in acetone, 40% glacial acetic acid in n-hexane, and 2% glacial acetic acid in methanol: expressed as average (standard deviation) of concentration (µg/ml, n=2); and percentage of dithranol remaining at 20°C.

Time	Dithranol (µg/ml)					
(Day)	Acetone		Hexane		Methanol	
	mean(sd)	%remain	mean(sd)	%remain	mean(sd)	%remain
0	48 (4)	100	342 (2)	100	43 (1)	100
1	30 (1)	59	-		-	
3	-		274 (10)	80	-	
4	-		-		18 (7)	43
5	-		259 (12)	76	-	
6	-		-		9 (6)	22

Table 3.3 shows that half-life of dithranol in 2% glacial acetic acid in acetone is about 1 day at 20°C. Cavey *et al* (1982) showed that the half-life in acetone alone is less than 1 hour at 25°C. The combination of the presence of glacial acetic acid (2%) and the lower temperature used in our study explain the dicrepancies between the results.

Linear regression analysis of results in Table 3.3 for hexane and methanol gives correlation coefficients of 0.968 and 0.998, and slopes of 4.95 and 13.18 day^{-1}, respectively. A (zero-order) half-life of dithranol in 40% glacial acetic acid in hexane is calculated to be 10 days, and that in 2% glacial acetic acid in methanol being 3.8 days at 20°C.

Reports on dithranol stability in methanol are numerous with results varying from one laboratory to the other in the same way as previously discussed for aqueous solutions. For example, at 25°C in the dark, 0.2% of dithranol remaining in methanol after 5 hours [Kneczke *et al*, 1989], 83% after 6 hours and 10% after 24 hours [Lee, 1987], and 69% after 8 hours [Burton and Gadde, 1985]. With so much discrepancy between reported findings, it seems reasonable to conclude that the general

decomposition profile of dithranol has not been adequately elucidated.

It is shown in this work that dithranol decomposed faster in acetone than in methanol. The dipole moment of acetone is higher than that of methanol, thus acetone might interfere with the intramolecular bonding of dithranol to a greater extent than methanol. This would cause dithranol to undergo oxidation faster in acetone than in methanol.

Table 3.4 Stability of dithranol in glacial acetic acid: expressed as average (standard deviation) of concentration (µg/ml, n=2); and percentage of dithranol remaining at -21°C.

Time	Dithranol (µg/ml)	
(months)	mean(sd)	%remain
0	2350(1.0)	100
1	2340(7.0)	100
7	2348(10.6)	100
9	2290(12.0)	97
12	2316(35.0)	99

Results in Table 3.4 show that dithranol was stable for up to 1 year in glacial acetic acid when frozen at -21°C and protected from light. This condition provides insufficient thermodynamic energy for the ionisation and decomposition of dithranol. The results emphasise the effect of temperature on dithranol stability. This was useful for storing samples of dithranol, such as loaded hydrogel, by dissolving the hydrogel in the acid and freezing as described.

An investigation by storing dithranol hydrogel samples in glacial acetic acid at -21°C shows that after 2 and 6 days the percentage dithranol remaining was 98% and 80% (n = 3 each). The results are lower than expected, possibly because of the interference of the hydrogel in the frozen glacial acetic acid on the dithranol decomposition. However, the method enables repeat

analysis for the determination of dithranol to be carried out within 1 day after the film was dissolved in the acid. This is particularly useful in the release studies where receptor medium, stratum corneum and hydrogel remaining from the studies are required to be analysed simultaneously, whilst the hplc analysis needs time for each sample.

Table 3.5 Dithranol instability in DMSO or PEG400: expressed as mean (sd) of concentration (µg/ml, n=2); and percentage of dithranol remaining at 20°C.

Time	Dithranol		Danthrone	Dimer
(hour)	(µg/ml)	% remain	(µg/ml)	(µg/ml)
DMSO				
0	81 (1)	100	0.2 (0.0)	< 2
1	79 (2)	97	0.3 (0.1)	< 2
5	63 (1)	78	2.6 (0.3)	< 2
24	5.4 (5)	7	14.3(0.5)	< 2
48	1.6 (1)	2	14.5(0.6)	< 2
PEG400				
0	87 (3)	100	<0.3	< 2
5	59 (1)	67	1 (0.2)	12 (1)
24[a]				
(upper)	3.3 (0.8)	4	5 (0)	14 (3)
(lower)	12 (3)	14	8 (8)	4 (2)
(total)	3.9 (2.4)	5	5 (0)	10 (1)

NB: [a]At 24 hours, the discolouration of the solution was observed and analysed separately as "upper" (the upper darker-coloured layer) and "lower" (the lower lighter-coloured layer); the solution was subsequently mixed and analysed as "total".

Table 3.5 shows the stability of dithranol in DMSO and PEG400, the initial concentration of which was about 80 µg/ml. Both solutions of dithranol gradually discoloured and were obviously brown after 24 hours. Because of its viscosity, the PEG400 solution was dark brown on the surface(~0.2 cm) but the solution underneath was yellowish brown. This phenomenon was not observed in the DMSO solution which were uniformly brown. DMSO, is a basic solvent (Melo et al 1983, Muller 1988) which produces danthrone rather than dimer. Any dithranol dimer which was produced was rapidly oxidised to form dithranol

brown. In the present work, dithranol dimer was not detected in the assays and whilst dithranol brown, which is a polymer, could not be analysed using hplc the brown discolouration was clearly seen.

PEG400, on the other hand, dissolves dithranol by forming hydrogen bonds with the hydrophilic groups of dithranol. An infrared spectrum of dithranol dissolved in PEG400 showed a broader and stronger band appeared nearer 3400 cm^{-1} than that of PEG400 alone, indicating an intermolecular hydrogen bonding. Colwell and Livengodd (1962) also suggested this association reaction. This interferes with the intramolecular hydrogen bonds of dithranol, as a result of that, the C_{10} becomes susceptible to available oxygen and 2 separate molecules of dithranol complex to form dimer [Whitefield, 1981a]. The upper darker layer of the PEG400 solution was found to be rich in both danthrone and dimer, whereas the lower layer, away from the atmosphere contained more dithranol. This indicates the involvement of dissolved oxygen in dithranol decomposition.

Table 3.6 shows the effect of some antioxidants investigated using 2% glacial acetic acid in methanol as the solvent. Calculated half-lives of dithranol in the solvent in the presence of isoascorbic acid, salicylic acid, and tocopherol acetate are 23, 2 and 2 days, respectively. 0.1% isoascorbic acid significantly ($p < 0.05$) protected dithranol in methanol, whereas neither 0.1% of salicylic acid nor d-α-tocopherol acetate gave any protection. A result from one experiment using butylated hydroxy anisole (the same method) shows a half-life of less than 2 days. The similar effects of ascorbic acid and salicylic acid on the stability of dithranol has been reported [Kneczke et al, 1989].

Table 3.6 Dithranol stability in methanol with 0.1% isoascorbic acid, 0.1% salicylic acid or 0.1% d-α-tocopherol: expressed as average (standard deviation) of concentration (μg/ml, n=2); and percentage of dithranol remaining at 20°C.

Day	Dithranol		Danthrone	Dimer
	mean(sd)	% remain	mean(sd)	mean(sd)
0.1%	ISOASCORBIC ACID IN METHANOL			
0	46 (3)	100	<0.2	< 1
4	39 (1)	85	0.3 (0.0)	< 1
6	40 (0)	88	2.4 (1.1)	< 1
12	33 (1)	73	5.6 (1.5)	6.9 (0.1)
0.1%	SALICYLIC ACID IN METHANOL			
0	42.8(1.8)	100	<0.2	<0.8
4	1.6(0.9)	3.6	0.32(0.05)	28.8(0.3)
0.1%	D-α-TOCOPHEROL ACETATE IN METHANOL			
0	43.1(1.4)	100	<0.2	<0.8
4	6.1(8.1)	14.1	0.40(0.2)	29.7(1.63)

The protective effect of salicylic acid is likely to depend on other circumstances, such as the presence of zinc oxide which has been found to stop discolouration in dithranol paste because of the presence of zinc oxide. Ponec-Waelsch and Hulsebosch (1974) reported that this might occur on the surface of zinc oxide due to adsorption. Braun and Wiegrebe (1988) detected no loss of dithranol in vaseline containing 2% zinc oxide and 2% salicylic acid, compared with a 15% loss in that containing 2% zinc oxide alone after 22 days of storage. Thus, it appears that salicylic acid could be effective in protecting dithranol from decomposition due to zinc oxide in ointment bases. Whether in a solution salicylic and zinc oxide react with dithranol in the same way as in semi-solid vehicles is not known.

Despite better stability of dithranol in methanol than in acetone, methanol was not used in the formulation of dithranol loading solution. Isoascorbic acid was not used in the solution either. This is because of their effect on dithranol solubility as discussed later in Chapter 4.

Dithranol loading solution

The stability of dithranol in hydrogel loading solution upon storage (at 5°C, in the dark) up to 7 days is shown in Table 3.7. This indicates that the solution was stable under the storage conditions used during the 3 hours required for loading the hydrogel.

Table 3.7 Dithranol stability in the loading solution; expressed as mean (sd) of concentration (µg/ml) and percentage of dithranol remaining in the solution at 5°C, light-protected and full-filled conditions.

	Dithranol (µg/ml)		
time	mean(sd)	%remain	n
0	486(4.5)	100	5
3 hr.	489(4.9)	100	5
1 d.	480(1.0)	99	2
2 d.	460(0.0)	95	2
7 d	438(0.4)	90	2

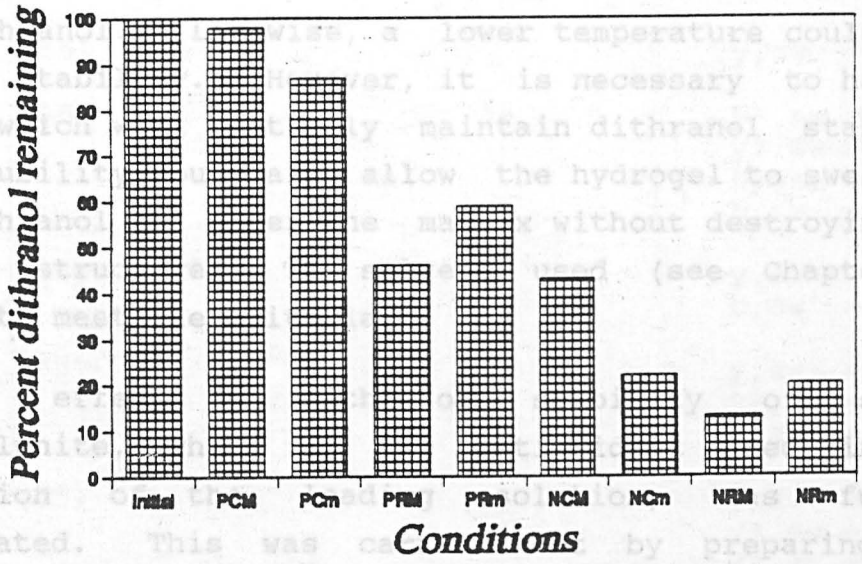


Fig. 3.2 Effect of storage conditions on dithranol stability in loading solution (500 µg/ml): percentage of dithranol remaining after 48 hours of storage (n=2)

Conditions P = light protected, N = light exposed;
C = 5°C, R = 20°C;
M = <2% of air in bottle, m = >80% air in bottle

The effect of storage conditions on dithranol stability in loading solution is shown in Fig. 3.2 as the percent dithranol remaining relative to the initial concentration of the same solution after 2 days of storage. It is seen that maximum concentration of dithranol is obtained in the solution which has been stored in the dark (P) at 5°C (C) and contained less than 2% of air (less oxygen available) in the container. This finding is in line with the previous stability results discussed. Thus, these storage conditions are used throughout the study.

This information is important for carrying out loading of the hydrogel. The data indicates that the solution formulated for loading dithranol into the hydrogel film can stabilize dithranol if the solution is stored in the strictly controlled conditions and the faster the freshly-prepared dithranol solution can be protected, the better will be the stability of dithranol. It is possible that the stability could be further improved by using solvents which would further suppress dithranol decomposition but would still act as solvents for dithranol. Likewise, a lower temperature could also improve stability. However, it is necessary to have a system which will not only maintain dithranol stability and solubility, but also allow the hydrogel to swell and the dithranol to enter the matrix without destroying the hydrogel structure. The solvent used (see Chapter 4) appears to meet the criteria.

The effect on dithranol stability of sodium metabisulphite, which is the antioxidant used in the preparation of the loading solution, was further investigated. This was carried out by preparing the dithranol loading solutions with and without sodium metabisulphite and storing at 5°C for 1 and 2 days. It was found that 65% and 50% of dithranol remaining after 1 and 2 days in the solution without, whilst 99% and 98% remaining in the one with the antioxidant. This suggests

that sodium metabisulphite should be added to reduce dithranol decomposition.

3.3.2 STABILITY STUDIES OF DITHRANOL LOADED HYDROGEL

The development of methods to stabilise dithranol in hydrogel is divided into 3 stages - 1) evaluation of loading conditions in which the temperature used in the loading was studied, 2) investigation of the storage condition effect, and 3) effect of Fe^{+++} in the hydrogel on dithranol stability.

The initial phase involves studying the effect of loading temperature (20°C and 5°C).

Table 3.8 Dithranol stability in film loaded at 20°C; stored in light-protected bottles with N_2 displacement at 5°C; expressed as amount of dithranol (and percent remaining) and danthrone (µg).

Time	dithranol (µg)		danthrone (µg)	film
(day)	mean (sd)	% remain	mean (sd)	description
0	163 (22)	100	< 1	y,sw = 10
1	144 (15)	89	< 1	y,sw = 6 y^2,sk = 1 y^2,sw = 1
2	149 (15)	92	< 1	y,sw = 3
3	20 (26)	12	7 (3)	y^1,sw = 1 y^3,sw = 1 b,sk = 1 b,sw = 1
4	88 (74)	54	3 (4)	y,sw = 2 b,sw = 1
5	52 (65)	32	3 (2)	y,sw = 1 y^1,sw = 1 b,sw = 1
6	58 (55)	36	6 (3)	y,sw = 1 y^2,sw = 1 y^3,sw = 1 b,sw = 1 b,sk = 2

NB: y - light yellow, y^1 - two-third brown, the rest yellow, y^2 - 1 small brown spot at the edge of the film, y^3 - small brown scattering spots, not dissolve in glacial acetic acid, b - all brown, with scattering darker brown spots, sw - film remained swollen, sk - film shrank.

Tables 3.8 and 3.9 show the percent of dithranol remaining in films which were loaded at 20°C and 5°C, respectively. A bottle was used to store these films and air was displaced by N_2 to reduce the amount of oxygen. The films were stored at 5°C for both loading temperatures.

Table 3.9 Investigation of dithranol stability in the loaded film: loaded at 5°C; stored in light-protected bottles with N_2 displacement; expressed as amount of dithranol and danthrone (µg) and percent of dithranol remaining.

Time	dithranol (µg)		danthrone (µg)	film
(day)	mean (sd)	% remain	mean (sd)	description
0	162 (9)	100	< 1	y,sw = 6
1	173 (7)	107	< 1	y,sw = 6
2	171 (21)	106	< 1	y,sw = 6
3	144 (8)	89	< 1	y,sw = 3
4	150 (6)	93	< 1	y,sw = 3
5	151 (16)	93	< 1	y,sw = 6
6	120 (58)	74	3 (1)	y,sw = 2 y^1,sw = 1
7	146 (24)	90	3 (1)	y,sw = 5 y^2,sw = 1
8	118 (59)	73	3 (2)	y,sw = 3 y^2,sw = 2 b,sw = 1
9	180 (2)	111	< 1	y,sw = 2

NB: y - light yellow
y^1 - two-third brown, the rest yellow
y^2 - 1 small brown spot at the edge of the film
b - all brown, with scattering darker brown spots
sw - film remained swollen

The results in Table 3.8 and 3.9, show high standard deviations, particularly after decomposition occurs, i.e.when danthrone was detected. These figures appear to correlate to some extent with dithranol decomposition. For example, after 5 days (Table 3.8) the standard

deviation, 65 µg, of the amount of dithranol present is higher than the mean, 52 µg, while the colours of the films varies greatly, i.e. from one yellow, two-thirds brown to one whole brown films.

The appearance of the loaded films was found to give an approximate indicator for dithranol stability in hydrogel, although there were some exceptions. A "healthy" loaded film remains swollen and yellow without dark spots. It was possible to observe a range of colours in the films after storage from light yellow through darker yellow to brown, however, it was only recorded as yellow or brown so as to be more reliable, as shown in Tables 3.8, and 3.9 and later in Tables 3.10, 11, and 12. Shrinking of films was found after clear drops of a liquid were observed on the film surface. This was thought to be due to the transfer of liquids from the inner hydrogel structure to its surface. Physical observations such as these were found practically useful in developing optimum conditions.

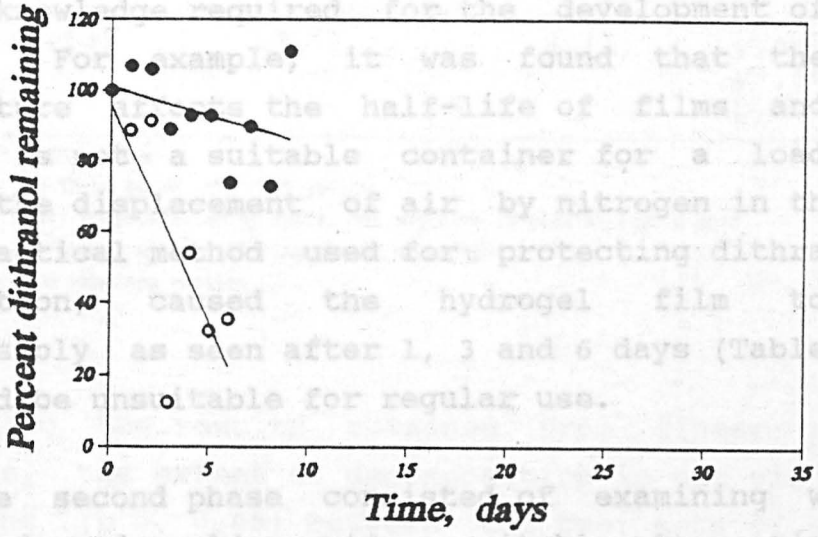


Fig. 3.3 Effect of temperatures used in loading dithranol, 5°C and 20°C, on percent of dithranol remaining in the hydrogel, stored at 5°C in bottle; dark spots depict films loaded at 5°C (Table 3.9), clear spots depict films loaded at 20°C (Table 3.8).

Fig. 3.3 shows that high temperature (20°C) used in loading the hydrogel results in reduced dithranol stability in hydrogel upon storage over 6 days, relative to those loaded at 5°C. It also shows an approximately linear relationship between percent of dithranol remaining in films loaded at 20°C and 5°C against time, correlation coefficients of which are 0.772 and 0.37, respectively. (The decomposition of dithranol in the film was treated as following zero-order kinetics, because of the confirmation from the second phase results shown in Tables 3.10, 3.11, and 3.12.) The rate constants were estimated to be 1.6 and 12.3 day^{-1} and calculated half-lives for dithranol of 4 and 32 days obtained at 20°C and 5°C, respectively. This experiment suggested that the shelf-life of the loaded films could be extended, so the time scale of storage had to be extended in subsequent work. The plot in Fig. 3.3 was thus extended for comparison with the subsequent results in Fig. 3.4. This evidence further confirms the effect of temperature on dithranol stability. Therefore, 5°C was used for loading of the hydrogel.

The problems encountered at this stage provided the basic knowledge required for the development of the next stage. For example, it was found that the loading temperature affects the half-life of films and that a bottle is not a suitable container for a loaded film. Also, the displacement of air by nitrogen in the bottle, the practical method used for protecting dithranol from degradation, caused the hydrogel film to shrink irreversibly as seen after 1, 3 and 6 days (Table 3.8) and so would be unsuitable for regular use.

The second phase consisted of examining whether a laminated pad could provide a suitable alternative storage system. A single loading of 50 separate films was made and divided for storage under two sets of conditions. The data following the degradation in bottles and between laminated pads as shown in Table 3.9 and Table 3.10.

Table 3.10 Investigation of dithranol stability in the loaded film: loaded at 5°C; stored in single-wrapped laminated pads; expressed as mean(sd) of amount of dithranol and danthrone (μg)

time	dithranol(μg)		danthrone(μg)	film
(day)	mean(sd)	% remain	mean(sd)	description
0	175 (19)	100	< 1	y,sw = 14
1	175 (5)	100	< 1	y,sw = 3
2	182 (8)	105	< 1	y,sw = 3
5	163 (12)	93	< 1	y,sw = 15
6	167 (22)	96	< 1	y,sw = 3
7	160 (13)	91	< 1	y,sw = 3
8	162 (9)	93	< 1	y,sw = 3
9	166 (11)	95	< 1	y,sw = 3
10	146 (14)	84	< 1	y,sw = 9
15	131 (3)	75	3 (0)	y^1,sw = 1 y^3,sw = 11
20	136 (11)	78	3 (0)	y^3,sw = 9
21	52 (43)	30	3 (4)	y^3,sw = 2 b,sw = 1
25	24 (36)	14	11(5)	y^3,sw = 3 b,sw = 9
30	38 (13)	22	8 (1)	y,sw = 1 y^3,sw = 10 b,sw = 1

NB: y - light yellow
y^1 - two-third brown, the rest yellow
y^3 - small brown scattering spots, not dissolve in glacial acetic acid
b - all brown, with scattering darker brown spots
sw - film remained swollen

Using the results obtained from linear regression analysis, the extent of decomposition is not significantly different ($p > 0.05$) between the two sets of conditions over the first 9 days, but storage in the bottles resulted in films which gave higher standard deviations after 5, 6, 7, and 8 days without any observed dithranol discolouration. This indicates a lack of reliability in

the method of storage which was not experienced with the laminated pads. The use of laminated pads was therefore further explored and storage times extended to 30 days.

Table 3.10 shows the stability of the batches which were single wrapped whilst Table 3.11 shows corresponding data for films stored in two layers of pading. The results from the single wrapped films showed an unusual pattern in the amount of dithranol remaining with time. For the first 15-20 days, there is only a slow decrease of dithranol, but then there is a sharp drop in quantity by day 25. There is also an increase in standard deviations. It is thought that this was caused by a slight air leak into the packing. At first it would not be sufficient to have a significant effect, but later sufficient oxygen would have gained access to cause rapid oxidation. Thus, double-wrapped films were investigated.

Table 3.11 Investigation of dithranol stability in the loaded film: loaded at 5°C; stored in double-wrapped laminated pads; expressed as mean and standard deviation of amount of dithranol and danthrone (µg) and percent of dithranol remaining.

time	dithranol (µg)		danthrone (µg)	film
(day)	mean (sd)	% remain	mean (sd)	description
0	199 (8)	100	< 1	y,sw = 9
5	187 (11)	94	< 1	y,sw = 9
10	170 (6)	86	< 1	y,sw = 9
15	166 (10)	84	< 1	y^3,sw = 9
20	190 (54)	96	< 1	y^3,sw = 6
21	102 (38)	51	5 (3)	y^1,sw = 1 y^3,sw = 2
25	127 (36)	64	4 (1)	y^3,sw = 9
30	84 (7)	42	5 (1)	y^1,sw = 3
33	128 (40)	64	4 (1)	y^3,sw = 6

NB: y - light yellow
y^1 - two-third brown, the rest yellow
y^3 - small brown scattering spots, not dissolve in glacial acetic acid
sw - film remained swollen

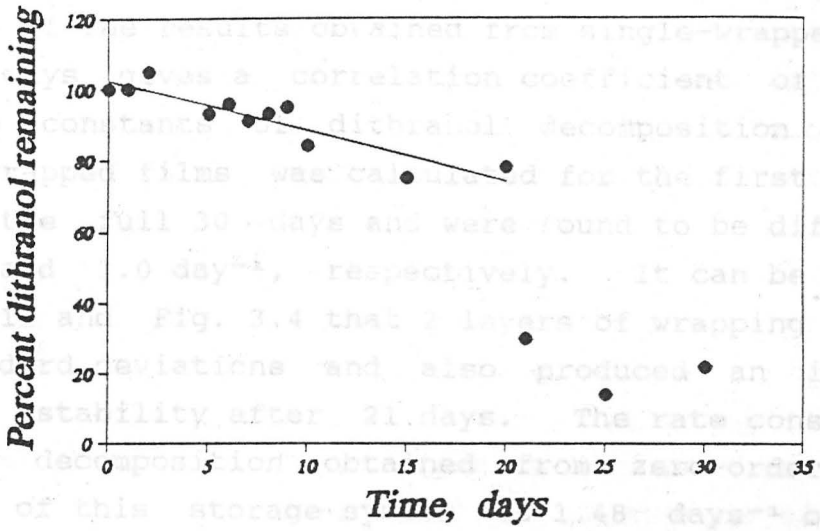


Fig. 3.4 Stability of dithranol in hydrogel stored in single-wrapped laminated pads, in the dark, at 5°C.

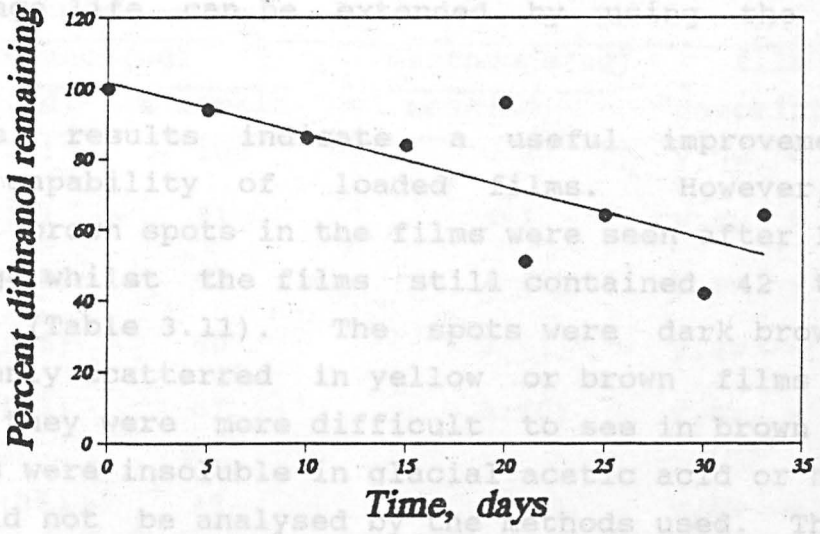


Fig. 3.5 Stability of dithranol in hydrogel stored in double-wrapped laminated pads, in the dark, at 5°C.

Plots (Figs.3.3 and 3.4) using the results of single and double wrapped films (Table 3.10 and 3.11, respectively) show linear relationships between percent of dithranol remaining in films and time. Linear regression analysis of the results obtained from single-wrapped films over 20 days gives a correlation coefficient of 0.909. The rate constants of dithranol decomposition in the single wrapped films was calculated for the first 20 days and for the full 30 days and were found to be different, i.e 1.4 and 3.0 day^{-1}, respectively. It can be seen in Table 3.11 and Fig. 3.4 that 2 layers of wrapping reduced the standard deviations and also produced an improved dithranol stability after 21 days. The rate constant of dithranol decomposition obtained from zero-order plot, Fig.3.4, of this storage system is 1.48 $days^{-1}$ over 33 days, giving a half-life of 35 days. This estimated half-life does not differ much from the result obtained over 20 days of storage of single-wrapped films (37 days) and that obtained over 9 days of storage in bottle (32 days). The similarity in the figures confirms the half-life of the dithranol in the hydrogel loaded and stored using the methods developed in this study, being at least 30 days. The storage life can be extended by using the double wrapping.

These results indicate a useful improvement in storage capability of loaded films. However, some scattered brown spots in the films were seen after 15 days of storage whilst the films still contained 42 to 64% dithranol (Table 3.11). The spots were dark brown and found evenly scatterred in yellow or brown films alike although they were more difficult to see in brown films. The spots were insoluble in glacial acetic acid or hexane, thus could not be analysed by the methods used. This was not clearly understood and was seen as a challenge to improve the appearance (and potential patient acceptability) of the product. This led to the third stage when it was advised [Graham, N. B., personal

communication] that trace amounts of ferric chloride might be present in the film arising from the manufacturing process.

Third stage of study involved the elimination of ferric ion from hydrogel and monitoring the effect on dithranol stability. The pre-washed films were prepared using the method discussed previously. A preliminary experiment was performed to demonstrate that the uptake behaviour and trend of dithranol decomposition were not affected by the washing process. This was carried out initially and after 7 days of storage using six pre-washed films each. The uptake of dithranol by these films was 9.2 (sd =0.0) µg per mg of film in line with previously used unwashed films. Additionally, an average of 87% of dithranol remained in the films after 7 days also in line with previous data. These results were therefore within the expected range. The pre-washed film was used in further stability study (results as shown in Table 3.12.)

Table 3.12 Dithranol stability in the loaded film: loaded from pre-washed films and stored in double-wrapped laminated pads at 5°C; expressed as mean(sd) of amount of dithranol and danthrone (µg) and percent of dithranol remaining.

time	dithranol (µg)		danthrone (µg)	film
(day)	mean (sd)	% remain	mean (sd)	description
0	165 (1)	100	< 1	y,sw = 6
7	133 (5)	81	< 1	y,sw = 6
14	104 (29)	63	4 (1)	y,sw = 6
15	99 (11)	60	3 (1)	y,sw = 6
21	110 (23)	67	3 (0)	y,sw = 4 y^4,sw = 2
23	69 (32)	42	5 (2)	y,sw = 4 y^4,sw = 1 b,sw = 1
28	86 (67)	52	7 (5)	y,sw = 5 b,sw = 1

NB: y - light yellow, y^4 - 1 very small brown spot, the rest light yellow, b - all brown, with some darker brown spots, sw - film remained swollen

The data showed that the kinetics of decomposition resembled those obtained previously during the first 15 days; and that the same pattern of decomposition was followed for the remaining time. Only 7 brown spots developed in 5 films (from a total number of 42 films) when compared to numerous spots in previous studies. Their appearance indicates the inadequate method used to eliminate ferric ions which removed about 70% (see p 70) of ferric ions from the hydrogel. It is not known whether complete elimination of the ferric ions present in the hydrogel would be possible. However, plots of the data of pre-washed films (Fig.3.6) indicates that the dithranol in the loaded films decomposed at a constant rate of 1.79 day^{-1} (and a half-life of 25 days) estimated by assuming a zero-order reaction (correlation coefficient of 0.903). Thus, removal of the trace of ferric ion by pre-washing has improved the appearance of dithranol loaded film.

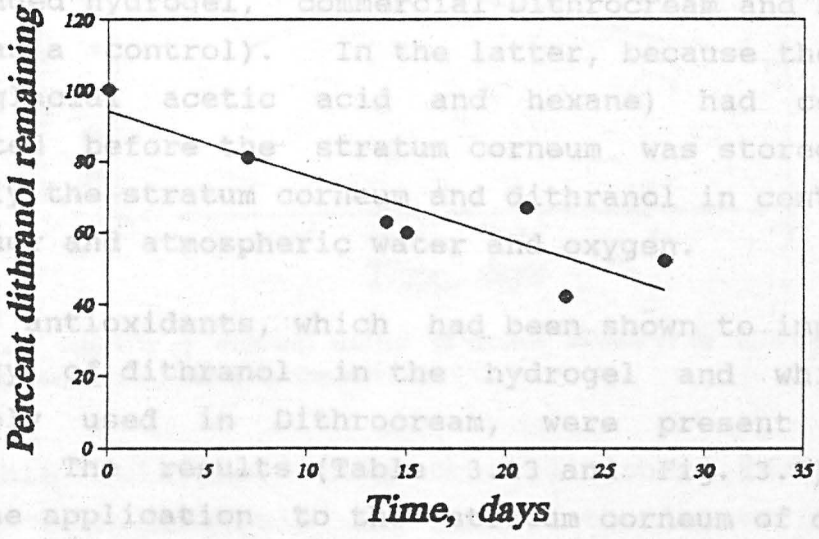


Fig. 3.6 Stability of dithranol in pre-washed hydrogel films stored in double-wrapped laminated pads, in the dark, at 5°C.

The trace amounts of the ferric ion appeared to have been scattered throughout the hydrogel, probably in a discrete domain of the polymer structure where the Fe^{+++} could act as a catalyst for dithranol oxidation. Thus many of the un-washed films became obviously spotted after about 14 to 15 days. This could suggest that the dithranol might takes more than 10 days to reach the "inner" region of the hydrogel where the ferric ions were situated. The reaction between dithranol and Fe^{+++} which leads to dithranol discolouration is a fast process involving superoxide formation [Muller & Kappus, 1988].

Pre-washed films were, therefore, used in the subsequent loading of dithranol in this study. The films would expected to have a shelf-life (not more than 10% loss) of 7 days when stored double wrapped at 5°C.

3.3.3 STABILITY OF DITHRANOL APPLIED TO STRATUM CORNEUM

The study was designed to simulate and compare the effect of dithranol preparations on the stratum corneum, in situ. Table 3.13 presents data on the stability of dithranol on neonatal rat stratum corneum when applied in the form of the loaded hydrogel, commercial Dithrocream and solutions (used as a control). In the latter, because the solvent (40% glacial acetic acid and hexane) had completely evaporated before the stratum corneum was stored, there was only the stratum corneum and dithranol in contact with each other and atmospheric water and oxygen.

No antioxidants, which had been shown to improve the stability of dithranol in the hydrogel and which were presumably used in Dithrocream, were present in this control. The results (Table 3.13 and Fig. 3.7) suggest that the application to the stratum corneum of dithranol in a loaded hydrogel or as Dithrocream produced a slower rate of overall decomposition than that of the control, probably because of antioxidant(s) involvement.

Table 3.13 Dithranol stability on stratum corneum and in stratum corneum with the hydrogel or Dithrocream applied; expressed as mean (sd) of amount of dithranol, danthrone and dimer (μg, n=2).

Time	Dithranol		Danthrone	Dimer
(day)	(μg)	%remain	(μg)	(μg)
HYDROGEL				
0	145 (5)	100	< 1	< 3
1	119 (2)	82	2 (0)	< 3
2	113 (10)	78	5 (2)	19 (1)
DITHROCREAM				
0	24 (2)	100	< 0.2	< 1
3	24 (2)	99	0.4 (0.1)	< 1
Control (dithranol applied using volatile solvent)				
0	16 (1)	100	<0.02	< 0.1
1	6 (0)	40	1 (1)	9 (1)
2	9 (1)	57	1 (1)	9 (2)
5	6 (1)	34	1 (1)	13 (4)

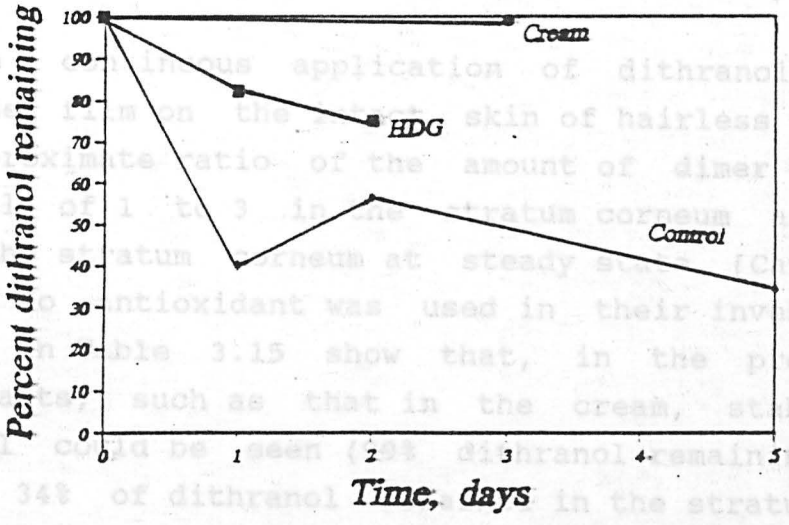


Fig. 3.7 Stability of dithranol applied to stratum corneum using 0.1% Dithrocream (●), hydrogel (■), and control, stored in the dark, at 20°C.

Table 3.13 also shows that dithranol decomposed to produce both danthrone and dimer, the latter being at a higher concentration. Comparisons with hydrogel show that dimer formation in contact with stratum corneum is about five times that with hydrogel alone.

Table 3.14 Dithranol stability in stratum corneum stored at 93% humidity in the dark expressed as mean and standard deviation of amount of dithranol, danthrone and dimer (μg, n=2).

Time (day)	Stratum corneum	Dithranol (μg)	Danthrone (μg)	Dimer (μg)
5	None(control)	13 (1)	0.3 (0.1)	3.2 (1.5)
5	1.8 cm^2	0.6 (0.7)	0.9 (0.2)	10.4 (2.7)

Because dithranol therapy under occlusion is to be expected when the dithranol loaded hydrogel is applied to psoriatic plaques high humidity is to be expected. The effect of humidity on stability is, therefore, of particular interest. The 5 day data for the volatile solvent applied dithranol (Table 3.13) is compared with the corresponding figure for the experiment carried out at 93% relative humidity in Table 3.14. It is interesting to observe that the decomposition of dithranol was faster in the high humidity environment than that in the low humidity.

The continuous application of dithranol using a cellulose film on the intact skin of hairless rat showed the approximate ratio of the amount of dimer to that of dithranol of 1 to 3 in the stratum corneum and 2 to 1 below the stratum corneum at steady state [Cavey et al, 1985]. No antioxidant was used in their investigation. Results in Table 3.15 show that, in the presence of antioxidants, such as that in the cream, stability of dithranol could be seen (99% dithranol remaining after 3 days). 34% of dithranol remained in the stratum corneum after 5 days in the control experiment whereas 78% remained when hydrogel was applied. The ratio of dimer to dithranol was 1 to 1 in the control and 1 to 5 in the hydrogel test, after 2 days. The initial concentration of dithranol applied might be one of the factors concerned. However, it shows that the trend of dithranol decomposition in the stratum corneum was towards dimer rather than danthrone and is in broad agreement with Cavey

et al (1985). This is in contrast to the decomposition in aqueous system (as shown in Table 3.2) where dissolved oxygen was likely to react with ionised dithranol and so danthrone was formed.

CHAPTER 4

LOADING STUDY

4.1 INTRODUCTION

A concentrated solution of dithranol is required for film loading procedures. The relevant properties of the hydrogel and dithranol are the solubility of dithranol and hydrogel in the solvent, the swelling property of the hydrogel in the solvent and factors such as temperature and time scale required in the loading process. The stability of dithranol in the loading solution is also of vital concern in this instance because of its instability as discussed in Chapter 3. Thus, appropriate solvents must be selected to meet all the requirements, namely that the loading solution must not dissolve the gel but must enable it to swell and dissolve dithranol whilst not impairing its stability. Finding a suitable system is the subject of this chapter. In order to achieve maximum loading, the gel must swell in the loading solution to reach highest degree of swelling [Graham et al,1982]. After initial screening on the hydrogel, the solubility and stability of dithranol were investigated. Finally, the techniques and conditions to be used in the loading of the films were developed. Variations in the uptake of dithranol by the hydrogel were analysed statistically.

4.2 EXPERIMENTAL METHODS

4.2.1 PHYSICAL PROPERTIES

Dithranol solubility

The saturation solubility of dithranol in solvents was determined by taking 100 ml of solvent (distilled water, acetate buffer at pH 2.8 and 5.0, phosphate buffer at pH 7.0, methanol, hexane, and acetone) and adding about 1 g of finely ground dithranol powder. The suspensions were agitated in tightly closed bottles, protected from light, at the temperatures shown in Tables 4.1 and 4.2. The amount of dithranol in each solution was determined using hplc (system 1, p 37) by periodically filtering a sample of the supernatant and diluting as appropriate for the analysis. The equilibrium solubility of dithranol in each solvent was achieved when two or more successive samples gave the same concentration. The results are shown in Tables 4.1 and 4.2.

Hydrogel swelling

Each film (1 cm in diameter) was weighed in a tared closed container to an accuracy of ±0.01 mg. The film was soaked in solvents at the appropriate temperature (5, 20 and 30°C). A film was removed at predetermined times, blotted dry and rapidly weighed in the tared closed container. The percentage swelling of the hydrogel (%swelling or pph) was calculted as a ratio of the weights of the swollen and dry hydrogel. Each of these was repeated using six films. The results, shown in Table 4.3, were compared statistically using the student t-test (two-independent group test).

4.2.2 DELVOPMENT OF the "LOADING SOLUTION"

SCREENING TEST - Most organic solvents were found to dissolve the hydrogel but those which did not cause

hydrogel swelling nor dissolve the hydrogel are shown in Table 4.4. Mixed solvents, made from one or two of those which dissolved the hydrogel together with one which did not, were investigated. Those which were found not to dissolve the hydrogel are shown in Table 4.5. An apparent solubility of dithranol in these solvent systems was obtained by adding successive quantities of dithranol to the solvent system at room temperature (using the organic solvents first then adding water second, when appropriate). Butylated hydroxyanisole (BHA), salicylic acid(SA), sodium metabisulphite and sodium sulphite were selected as antioxidants incorporated into the solutions. Those in which precipitates were formed are shown in Table 4.6. Solvent systems which dissolved the dithranol were further investigated by testing hydrogel swelling as shown in Table 4.7. The stability of the solutions (at 20°C) shown in Table 4.7 was assayed using hplc (using hplc system 1, see Chapter 2, p 42).

EFFECT OF DMSO ON DITHRANOL SOLUTION IN ACETONE-WATER SYSTEM - In an attempt to enhance the solubility of dithranol in the solvent, without detracting from other properties, some more complex solvent systems were investigated. For this, 0.01, 0.02, 0.03, 0.04, and 0.05 g of dithranol were dissolved in 10 ml of acetone then titrated by 0.3% sodium metabisulphite in distilled water, the cloud point being the end point of the titration, and indicating saturation of the solution. A similar procedure was followed using 0.02, 0.04, 0.06, 0.08 and 0.1 g of dithranol dissolved in 10 ml of 40% DMSO in acetone. The percentage (volume by volume) of water used in the titration and percent (weight by volume) dithranol dissolved were calculated and plotted as shown in Fig. 4.1. Each ingredient in each solvent system was calculated as a percent weight by weight (%w/w), as shown in Table 4.8 and Fig. 4.2.

4.2.3 FACTORS INVOLVED IN DITHRANOL UPTAKE BY HYDROGEL

Because of the temperature-dependent swelling property of the hydrogel used, the effect of temperature as well as the time scale required for maximum loading were investigated. Additionally, the volume of solution used for loading, which might affect the amount of dithranol uptaken, was also investigated. The hydrogel films used in these studies are dry, circular and 1 cm in diameter (average weight 15 ± 0.5 mg). A "loading solution" (see details on p 120) was adopted for an analysis of these factors the concentration of dithranol in the solution being 500 µg/ml.

EFFECT OF TIME AND TEMPERATURE OF THE LOADING SOLUTION - 24 films were soaked in the loading solution. Six films were loaded for each of 1, 3 or 5 hours at 5°C. Six films were loaded for 3 hours at 20°C. The amount of dithranol in the films were assayed using the hplc described in Chapter 2 (system 1, p 42). The results of the effect of temperature and time are shown in Table 4.9 and 4.10, respectively. These were compared statistically using student t'test (two-independent t'test).

EFFECT OF VOLUME OF THE LOADING SOLUTION - A film was loaded in 2, 3, 12 or 25 ml of the loading solution at 5°C. The loading solution was stored as control using the same conditions. After 3 hours, the concentrations of dithranol in the loading solution and control were assayed using hplc (as above). The results are shown as the volume of the solution per unit area or per weight of dry film and difference between dithranol in the loading solution that in the control in Table 4.11.

4.2.4 EVALUATION OF DITHRANOL UPTAKE

The "pre-washed" films (to eliminate ferric ion, details of method in Chapter 3) as well as films which were used as received ("unwashed") were evaluated. This was aimed at obtaining a parameter which would indicate the "dose" of the loaded hydrogel. Additionally, if a constant parameter was obtained, this could be used as an indicator for quality control in the loading of films with different sizes and shapes. This is expected to be a important parameter for subsequent clinical work.

The amount of dithranol initially taken up by the hydrogel from 15 batches, used in the stability studies on loaded hdyrogel in Chapter 3, are summarised in Table 4.12 (both unwashed and pre-washed films). Two-independent student t'tests were used in the comparison of the means of 2 batches with the same sample size; ANOVA was used when 3 or more batches were compared; F test was used to examine the variability between batches.

4.3 RESULTS AND DISCUSSION

4.3.1 PHYSICAL PROPERTIES

Solubility of dithranol

A number of values for the approximate equilibrium solubility of dithranol important information for the loading and release study have been reported in the literature as follows: 2 µg/ml[Whitefield, 1981a] and 0.1 - 0.2 µg/ml [Kneczke *et al*, 1989] in water; 3 µg/ml in phosphate buffer pH 6.8 [Caron *et al*, 1982]; 1 mg/g in methanol[Kneczke *et al*, 1989]; and 0.001 to 0.05% in hexane[Ponec-Waelsch & Hulsebosch, 1974]. The solubility values in water are variable, and so requires further evaluation.

Table 4.1 Solubility study: The amount of dithranol (μg/ml) dissolved in distilled water, acetate buffer (pH 2.8 and 5.0) and phosphate buffer (pH 7.0) at saturation.

Time (min)	Dithranol (μg/ml)				
	water		pH2.8	pH5.0	pH7.0
(Temp)	20°C	35°C	20°C	20°C	20°C
5	0.2	-	-	-	-
15	0.4	0.4	-	-	-
30	0.5	0.6	-	-	-
60	2.2(0.9)[1]	1.1	13.2	1.2	7.4
90	1.2	1.2	-	-	-
120	1.0	1.0(0.2)[1]	9.9	1.5	8.2
240	1.2(0.3)[1]	-	-	-	-
360	-	-	12.3	1.2	5.2

NB : [1] = mean(sd), n=3

Table 4.1 shows dithranol solubility in water until it reached equilibrium solubility at 20 or 35°C after about 1 to 4 hours. It seems that the solubility of dithranol in distilled water at 20° and 35°C is 1 - 1.5 μg/ml, in acetate buffer at pH 2.8 and 5.0 is 12 and 1 μg/ml, respectively, and 7 μg/ml in phosphate buffer at pH 7.0. Because it was thought that sodium metabisulphite might affect dithranol solubility in water, a test was performed using the same method as that in water. After 1 hour, 0.98 μg/ml of dithranol was detected in 0.3% sodium metabisulphite in water at 20°C, showing that it had only minimal effect. The presence of the polar groups at C_1, C_8 and C_9, whilst the majority of the molecule is the hydrophobic anthracene ring, enables dithranol to be partially soluble in water . The main problem in determination of the saturation of dithranol in water is that a colloidal dispersion is likely to be formed, particularly in the presence of an excess amount of dithranol, in order to reduce the interfacial tension due to its hydrophobicity. As a result, the end point of its solubility is very difficult to detect with any accuracy. Measurements of the surface tension of water in the presence of dithranol has not been carried out to confirm this suggestion.

The solubility in methanol, acetone and hexane at 20°C is 0.8, 7 and 1 mg/ml, respectively, as shown in Table 4.2. Despite the presence of the polar groups at C_1, C_8 and C_9, the dithranol molecule is quite lipophilic in nature. The criteria for selection of a suitable organic solvent were the solubility and relative stability of dithranol, its water miscibility and ability to swell hydrogel. From this, because of the approximately 10 fold difference between dithranol solubility in the aqueous solvents and organic solvents investigated, a loading solution developed should contain organic solvent(s) in order to increase dithranol solubility.

Table 4.2 Solubility study: Amount of dithranol(mg/ml) dissolved at 20°C in methanol, acetone and hexane at saturation.

Time	Dithranol (mg/ml)		
(minutes)	methanol	acetone	hexane
60	0.83	7.15	0.83
120	0.79	5.95	1.05

Swelling property of the hydrogel

The solubility parameter in solvents in relation to the swelling of the PEO hydrogel, including the temperature effect, have been reported by Graham et al (1982).

Table 4.3 shows the means and standard deviations of the percentage swelling of the hydrogel in water. Water caused the PEO hydrogel to swell. The extent of hydrogel swelling is in line with Graham et al (1982). In their work different molecular weights but the same type of polymer chains were used in the hydrogel, therefore, a different degree of swelling was expected. However, the proportion of swelling at each temperature correlates, indicating the same pattern of swelling.

Table 4.3 Swelling tests. The degree of hydrogel swelling (calculated as ratio of weight "swollen" to weight dry, pph) in various solvents, at different times and temperatures.

solvents	temp (°C)	swelling degree (pph) 1 hour mean(sd)	3 hours mean(sd)	5 hours mean(sd)
Water	5	134 (4)	139 (3)	152 (3)
	20	119 (4)	123 (5)	134 (6)
	30	109 (2)	114 (2)	112 (2)
10%acetone in water	5	147 (15)	151 (15)	154 (14)
	20	131 (5)	138 (5)	140 (7)
	30	136 (20)	137 (17)	142 (17)
2%benzyl alcohol. in water	5	172 (8)	180 (9)	183 (7)
	30	152 (11)	155 (11)	158 (11)
acetate buffer pH 2.8	20	314 (3)	327 (3)	327 (1)
acetate buffer pH 5.0	20	135 (2)	135 (0)	140 (3)
phosphate buffer pH 7.0	20	125 (2)	133(1)	135 (2)
loading solution vehicle	20	499 (5)	568 (22)	593 (13)

A comparison between the swelling in water at 5°, 20° and 30°C indicates that the degree of the hydrogel swelling was significantly affected by temperature ($p < 0.05$), confirming that the swelling degree of the hydrogel is directly proportional to negative reciprocal temperature [Graham et al, 1982]. It was also reported that, at 100°C, the ratio of water molecules associated to the polymer chains is 3 per 1 $-O-CH_2CH_2$ unit, indicating high affinity of the water to the poly(ethylene oxide). The swelling behaviour of the hydrogel was observed when the latent heat of fusion for the crystallites is adequately supplied. Water has a strongly negative enthalpy of mixing with poly(ethylene oxide) sufficient to cause melting of the crystallites at 5°C. As the temperature rises, the entropy change is, thus, negative

and reduces the free energy of mixing. Thus, temperature will affect the loading process. The time required for equilibrium swelling was not shown in Graham et al (1982). The hydrogel was removed after 48 hours of soaking which was assumed to be equilibrium. A preliminary study shows that there is no significant difference ($p > 0.05$) in the present work between swelling degree of the hydrogel after 5 and 24 hours in water at 5°C, indicating an approximate equilibrium at 5 hours.

The results in Table 4.2 indicate that the swelling of the hydrogel in water had not reached equilibrium after 5 hours at 20 or 30°C. However, addition of 10% acetone to the water enabled equilibrium to be approached after 3 hours at 5°C. Acetate buffer at pH 2.8 significantly enhanced the swelling of the hydrogel ($p<0.05$) in comparison with that at pH 5.0 and phosphate buffer pH 7.0. Glacial acetic acid has been shown to cause a higher swelling degree of the hydrogel [Graham et al, 1982]. Acetate buffer at pH 2.8 contains about 20% of glacial acetic acid which is sufficient to exert an effect on the hydrogel swelling. This also explains the higher swelling degree in acetate buffer at pH 5.0 than that in phosphate buffer at pH 7.0.

The effect of solvents (with different solubility parameters) on the swelling of hydrogel is well reported [Graham et al, 1982]. The addition of 10% acetone to water did promote the hydrogel swelling at 20 and 30°C, but not at 5°C. Thus, the temperature effect, particularly at 5°C where the melting of the crystallites occurs, are dominant and can be confirmed statistically. The swelling degrees in water and in 10% acetone in water at 5°C after 3 hours are not significantly different ($p > 0.05$), but are significantly different at 20°C ($p < 0.05$). It has been suggested that benzyl alcohol [Gander et al, 1986] might give maximum swelling of a hydrogel. Their work used a different hydrogel but one

which is similar to the one being used in this work. Thus, benzyl alcohol was investigated. 2% benzyl alcohol enhanced the swelling at 5° and 30°C to a significantly greater extent ($p < 0.05$) than 10% acetone. However, benzyl alcohol has poor water miscibility (maximum in 2% as used in this study although 4% is suggested to be its solubility in water in B.P.1988) and it also tended to dissolve the hydrogel, so it is not a particularly good solvent system to use.

The hydrogel swelling in the solvent system or vehicle selected as the loading solution (see later p 120) was the highest among the solvent systems investigated, i.e. as high as 590 pph by weight (150 % by area).

4.3.2 DEVELOPMENT OF the "LOADING SOLUTION"

Table 4.4 Solvent systems which give dithranol solution but dissolve or destroy the hydrogel at 20°C and the approximate dithranol solubility at saturation (µg/ml).

Solvent/solvent system	Dithranol solubility(µg/ml).
acetone	6000
acetonitrile	800
benzyl alcohol	-
chloroform	-
dichloromethane	-
ethyl acetate	-
ethyl alcohol	-
glacial acetic acid	2000
methanol	800
toluene	-
>50% acetone in water	7
10%dichloromethane, 40%acetone, 50% DMSO	550
12%acetonitrile, 44%acetone, 44%hexane	100
50%acetone, 50% hexane	500
50%DMSO, 50%ethyl acetate	-
55%acetone, 20%DMSO, 25%water	8

The hydrogel dissolved in most organic solvents at 20°C such as acetone, acetonitrile, benzyl alcohol, chloroform, dichloromethane, ethyl acetate, ethyl alcohol, glacial acetic acid, methanol and toluene, as shown in Table 4.4. A series of acetone in water solvent system was thus investigated for the combined effects on the hydrogel swelling and dithranol solubility. The upper limit of the concentration of acetone for hydrogel swelling is shown in Table 4.4.

The technique suggested by Cavey et al (1982) that an aqueous solution of dithranol must be prepared by adding a small volume of a concentrated solution of dithranol in an organic solvent to the aqueous medium was used. The preliminary screening (Table 4.4) indicates that a mixed system of less than 50% acetone and 20% DMSO in water might not dissolve the gel and should be further investigated. Table 4.5 shows the solvent systems which give dithranol solubility but caused no change in the hydrogel swelling such as ethyl ether, liquid petrolatum, n-hexane, DMSO, dimethyl formamide, propylene glycol, digol and PEG400. Solvent systems investigated and found not to dissolve the hydrogel at 20°C are also shown in Table 4.5.

Table 4.5 Solvents which give a dithranol solution but cause no change of the hydrogel (0 pph) at 20°C; and the approximate dithranol solubility (µg/ml).

Solvents	Dithranol solubility(µg/ml)
ethyl ether	-
digol	-
dimethylformamide	-
dimethylsulphoxide	3000
n-hexane	1000
liquid petrolatum	-
propylene glycol	-
PEG400	-
8%acetone,20%DMSO, 40%propylene glycol, 32%water	500

Of all water miscible organic solvents investigated, acetone was the most suitable candidate because of the relatively high dithranol solubility. Despite the faster decomposition of dithranol in acetone than that in methanol (see Table 3.3), it was thought that the use of an antioxidant, short loading time and the low temperature which enhanced the hydrogel swelling would compensate this fact. It was expected that the higher the concentration of dithranol, the greater would be the uptake by the hydrogel.

Another option for the loading solution development investigated was mixed organic solvents containing one of the hydrogel dissolving solvents, acetone, and one of the hydrogel non-dissolving solvents, n-hexane. Thus a hydrogel was placed in a mixture in 50% n-hexane and acetone. The hydrogel swelled but did not dissolve in the solution. However, the swollen gel shrank and destructured when it was dried. This was thought to be due to the absence of water in the loading solution which is an essential component for the hydrogel [Roorda et al, 1986] and the use of such organic solvents disturbed the gel structure. Because of these problems with solubility and stability of dithranol and the swelling property of the hydrogel, it seemed unlikely that the loading solution for dithranol could be developed using a single solvent, therefore a combined organic solvent(s) and water system was investigated.

The solvent systems which provide hydrogel swelling but failed to give homogeneous solutions when antioxidants are incorporated are shown in Table 4.6. In effect, these system were unsuitable for use.

Table 4.6 Solvent systems which produce hydrogel swelling but show a precipitation when antioxidants are incorporated.

Dithranol	antioxidant(%)			tween80	solvents (%)						
(µg/ml)	B	SA	SM	(%)	A	DMSO	EA	PG	PEG	H	W
>50	-	0.3	0.1	-	40	-	-	-	-	-	60
100	0.1	0.3	0.1	-	40	-	-	-	-	-	60
700	-	0.1	0.1	-	20	40	-	-	-	-	40
700	-	1	0.1	-	40	40	-	-	-	-	20
70	-	0.1	-	-	-	40	30	-	-	-	30
70	-	0.1	-	-	-	30	50	-	-	-	20
400	-	0.1	-	-	-	-	-	50	20	-	30
400	-	0.1	-	-	-	8	-	50	-	-	42
700	-	-	-	-	80	-	-	-	-	20	-
100	-	0.3	0.1	0.1	40	-	-	-	-	-	60

Abbreviations: antioxidants: B = butylated hydroxyanisole, SA = salicylic acid, SM = sodium metabisulphite,. solvents: A = acetone, DMSO = dimethylsulphoxide, EA = ethyl acetate, PG = propylene glycol, PEG = polyethylene glycol(400), H = n-hexane, W = water.

DMF was found to cause rapid dithranol decomposition, i.e. complete decomposition was found after 4 hr of storage in the dark at room temperature (This is in good agreement with Melo *et al*, 1983). DMSO was the best of these solvents because of the relative stability of dithranol in the solutions relative to PEG400 as discussed in Chapter 3 (Table 3.5, p 81). PEG400 was introduced to replace DMSO because the latter is a skin penetration enhancer (Barry, 1983) might be expected to affect the penetration of dithranol, which may not be acceptable. However, replacement of DMSO by PEG400 accelerated dithranol decomposition in the solution; i.e. 10% of dithranol decomposed in DMSO contained solution after 3 days at 20°C but in PEG 400 containing solution after 5 hours at 20°C, as shown in Table 4.7.

All the solvent systems shown in Table 4.7 caused the gel to swell and dissolved dithranol in a range of 300 to 1000 µg/ml and so are potential loading solvent systems. Two other factors were then used in the attempt to develop a possible working solvent system, namely the appearance of loaded film (being indicative of the stability of the

dithranol in the film) and the extent of swelling (being indicative of the extent of loading achieved) [Graham et al 1982]. Using these criteria, the following can be deduced from the data in Table 4.7.

Table 4.7 Solvent systems which produce the hydrogel swelling (pph) and the approximate dithranol solubility (µg/ml) and stability at 20°C.

Formula	Swell	Dithranol	
		solubility	stability
	(pph)	(µg/ml)	
			10%loss in
20%acetone,50%DMSO, 0.1%SS,30%water	110pph	1400	3 days
2%Tween,40%acetone 0.1%SS,58%water	140pph	300	1 day
20%DMSO,50%acetone 0.1%SS,30%water	150pph	700	> 3 days
50%acetonitrile, 20%PEG 0.1%SS,30%water	170pph	400	6 hours
25%acetone, 25%acetonitrile 20%PEG, 0.1%SS, 30%water	180pph	500	5 hours

Abbreviation : PEG = polyethylene glycol(400), SS = sodium metabisulphite

Rapid decomposition could be seen with solutions contained 20% PEG400, although the degree of swelling of the gel in one of the solutions was the greatest observed in this test. The gel loaded from the formula composed of 50% acetonitrile and 20% PEG400 in water contained long needled-like yellow crystals, shown in Fig 4.3, instead of being the normal clear film. 2% Tween 20 in 40% acetone in water gave a dithranol solution with optimum hydrogel swelling but poorer dithranol solubility than either of the solutions which contained DMSO. 20% acetone and 50% DMSO in water gave poor swelling but good solubility whilst 50% acetone and 20% DMSO in water provided a better

degree of swelling and good stability although with reduced solubility. No system could be considered to be ideal, but 20% DMSO 50% acetone 30% water appears to give the best compromise of factors from the solvent systems examined. Additionally, the maximum amount of water in the system was set at 30% because sodium metabisulphite would be precipitated if less than 30% of water was used whilst dithranol would precipitate if more than 30% of water was used. The amount of DMSO was also maximised at 20% so as to achieve maximum hydrogel swelling. This system was selected for further evaluation.

Investigation of possible antioxidants which would probably be essential in the protection of dithranol from oxidation is shown in Table 4.6. Butylated hydroxyanisole (used in a commercial dithranol stick, Bristol Meyer) and salicylic acid are not suitable because of their limited solubility. Salicylic acid was investigated although its antioxidant effect on dithranol stability in solutions is uncertain as shown and discussed in Chapter 3 (Table 3.6, p 83). 0.1% sodium sulphite was tried but the dithranol in the 20% DMSO and 50% acetone and water system completely decomposed in 1 hour. Thus, sodium metabisulphite at 0.1% is used.

Chelating agents are another technique often used in the prevention of oxidation. However, the efficacy of EDTA as a chelating agent improves in an aqueous media at high pH. Because, in general, dithranol stability decreases with increasing pH [Upadrashta &Wurster, 1988b], this would not be a suitable approach and so EDTA was not included in this investigation. At a later stage in this work, the idea of incorporating EDTA in the formulation was again considered when the presence of trace amounts of Fe^{+++} was discovered in the hydrogel (see Chapter 3) but was not tried because the problem was solved by other means.

Additionally, a solubilising agent, Tween 80 was investigated. Preliminary screening showed that Tween 80 was better than Span 20 both from the point of view of the stability of dithranol and the appearance of the solution. Tween 80, however, was not used in the final formulation chosen because the solubilisation might complicate the physicochemical system by introducing diffusion from the micelles into the polymer network.

The formulation of a dithranol solution (see p 120) was chosen as the best available from the information obtained during the study. However, it might be possible to further develop the formulation. Two possibilities are suggested as follows : (1) by increasing DMSO and decreasing water, thus increasing the maximum concentration of dithranol in solution. This may increase the uptake of dithranol by the hydrogel but may have adverse effects on swelling and stability due to reduction in the use of sodium metabisulphite; (2) by replacing acetone by another organic solvent.

A different approach to preparing loaded hydrogel is to dissolve the hydrogel and dithranol in a volatile organic solvent which must stabilise dithranol. The dithranol film is then obtained after pouring the solution onto a plate and evaporating off of the solvent. It was observed, however, that the replacement of acetone by acetonitrile resulted in the crystallisation of dithranol inside the hydrogel film, so this may not produce any improvement. Time prevented any further experiments on the solvent system.

Bubble formation was found in the solution and on the film during first 15 minutes of loading. The air bubbles would remain in the film causing an unacceptable appearance. This was overcome by gently rolling the bottle containing loading solution and films until bubble formation ceased. Another approach is to standing the

solution in an ultrasonic bath for 15 minutes so as to eliminate the air, after that the films can be immersed.

The calculated solubility parameter of the "loading solution" (see p 120) using the volume ratios of acetone, DMSO and water is 14.5 $(cal/ml)^{0.5}$. Graham et al(1982) has reported a value of 10-12 $(cal/ml)^{0.5}$ for a similar hydrogel with a PEO molecular weight of 6000. The present hydrogel has a PEO molecular weight of 5830. Thus a small discrepancy is expected, but the agreement with published information indicates that a reasonable solvent system for the hydrogel has been developed.

EFFECT OF DMSO AS AN SOLUBILITY ENHANCER FOR DITHRANOL IN ACETONE – WATER SYSTEM – The experiment was divided into 2 groups, one using a constant amount of acetone (A) and the other using DMSO to replace 40% of the acetone (AD), known quantity of dithranol added (and dissolved in the acetone or 40% DMSO in acetone. The solutions were titrated with water until cloudy point was reached indicating dithranol saturation solubility (at 20°C).

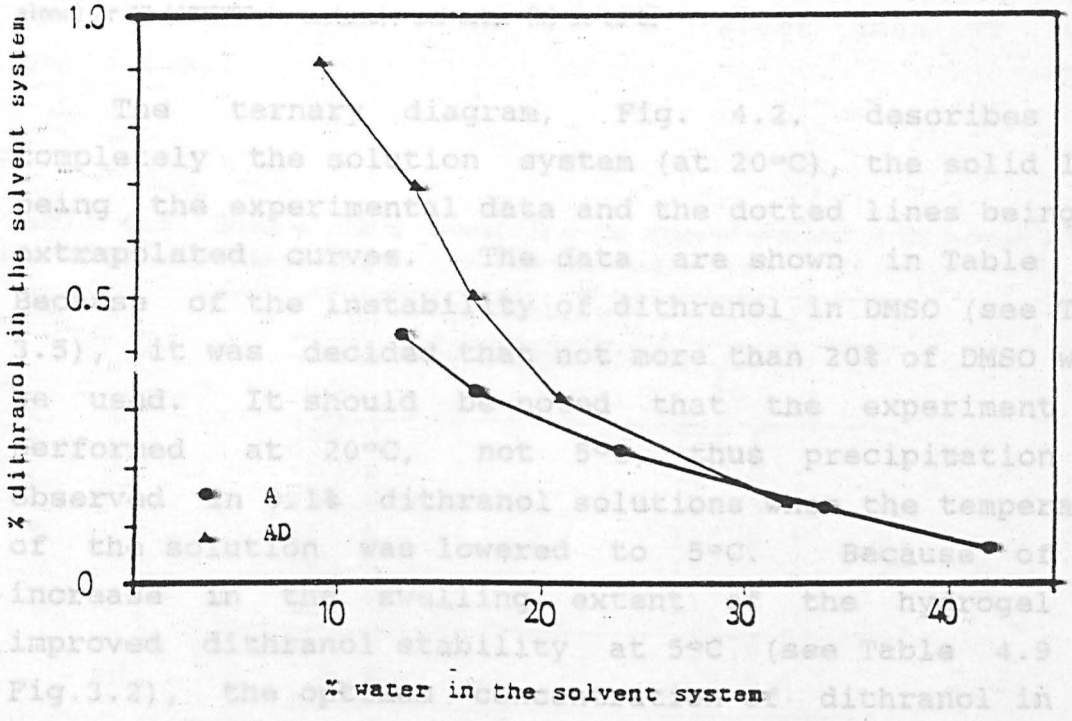


Fig. 4.1 Effect of water content in solvent systems: A (acetone alone) or AD (40%DMSO in acetone) at 20°C.

The plots of the dithranol solubility (%) against percent water in the solvent systems (Fig 4.1) indicate that the solubility of dithranol in DMSO containing systems was higher than that in acetone and water. The concentration of dithranol in the solutions could be increased up to 0.1% at 20°C when 30% water was present.

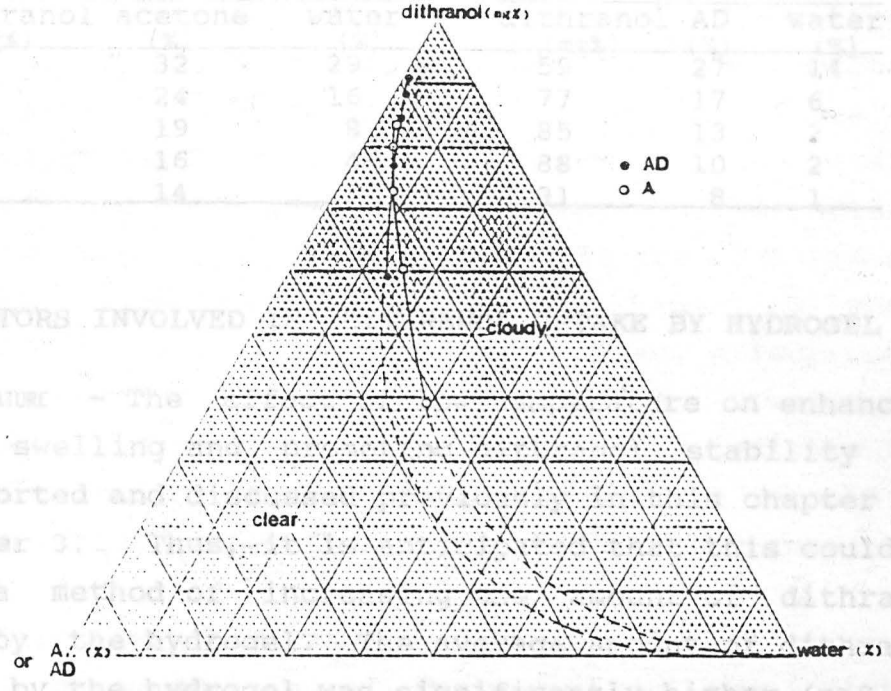


Fig. 4.2 Ternary diagram of dithranol (mg%); non-aqueous solvent systems(%), A (acetone alone) or AD (40%DMSO in acetone); and water (%) at 20°C.

The ternary diagram, Fig. 4.2, describes more completely the solution system (at 20°C), the solid lines being the experimental data and the dotted lines being the extrapolated curves. The data are shown in Table 4.8. Because of the instability of dithranol in DMSO (see Table 3.5), it was decided that not more than 20% of DMSO would be used. It should be noted that the experiment was performed at 20°C, not 5°C, thus precipitation was observed in 0.1% dithranol solutions when the temperature of the solution was lowered to 5°C. Because of the increase in the swelling extent of the hydrogel and improved dithranol stability at 5°C (see Table 4.9 and Fig.3.2), the optimum concentration of dithranol in the

loading solution was found to be 0.05%. This was therefore used subsequently in this study.

Table 4.8 Solubility of dithranol in acetone(A) or 40% DMSO in acetone(AD) in water : expressed as %w/w.

(% w/w)

without DMSO			with 40%DMSO		
dithranol (mg%)	acetone (%)	water (%)	dithranol (mg%)	AD (%)	water (%)
39	32	29	59	27	14
60	24	16	77	17	6
73	19	8	85	13	2
80	16	4	88	10	2
85	14	3	91	8	1

4.3.3 FACTORS INVOLVED IN DITHRANOL UPTAKE BY HYDROGEL

TEMPERATURE - The effect of low temperature on enhancing hydrogel swelling and promoting dithranol stability has been reported and discussed previously in this chapter and in Chapter 3. Thus, it is anticipated that this could be used as a method of increasing the amount of dithranol take up by the hydrogel. The average amount of dithranol taken up by the hydrogel was significantly higher ($p>0.05$) when the film was loaded at 5°C rather than at 20°C, (Table 4.9).

Table 4.9 Effect of soaking temperatures on the uptake of dithranol in the hydrogel after 3 hour loading. Expressed as the quantity of dithranol in µg.

	Dithranol (µg)	
	Soaking temperature(oC)	
	5	20
	149.6	132.1
	160.3	137.0
	136.8	128.5
	141.5	115.5
	143.5	138.7
	155.5	117.4
mean	147.9	128.2
sd	8.9	9.8

Thus, even though only 30% of water is present in the solution, the temperature of the loading solution affects the uptake by the hydrogel. These results correlate with the swelling test performed in water and 10% acetone in water previously discussed. Thus, decreasing the experimental temperature results in an increased swelling and quantity of dithranol loaded. This finding is in agreement with results discussed using the similar type of PEO hydrogel but with a different chain length and loaded with propanolol HCl [Schacht, et al 1987]. The swelling of the polyurethane hydrogels based on PEO lose water as the temperature is raised to the lower critical solution temperature or above. Hence, reducing the experimental temperature results in an increase swelling and a substantial increase the drug load. This effect could perhaps be confirmed using thermal analysis. However, the results obtained in the present work clearly indicate that for optimum loading the loading temperature should be controlled at 5°C rather than normal room temperature.

Additionally, temperature also has an effect on the oxidative decomposition of dithranol in the solution (Chapter 3), therefore, the lower the temperature used, the better the stability of dithranol. Therefore, 5°C was used in the loading process.

Table 4.10 Effect of soaking time on the uptake of dithranol in the hydrogel loaded at 5°C. Expressed as the quantity of dithranol in µg.

	Loading time (hours)		
	1	3	5
	104.5	149.6	118.8
	99.4	160.3	118.1
	81.2	136.8	141.9
	101.7	141.5	144.2
	113.8	143.5	133.9
	93.1	155.5	146.0
mean	99.0	147.9	133.8
sd	11.0	8.9	12.6

TIME - The time scale required for soaking the hydrogel to give equilibrium swelling in various solvents was discussed previously. This could have a profound effect on the amount of dithranol loaded. The experiment was carried out using 6 films loaded for 1, 3, or 5 hour. The results obtained from each individual film at 5°C are shown in Table 4.10. After 1 hour, the hydrogel has already taken up an average of 99 µg of dithranol. This figure increases to 148 and 134 µg after 3 and 5 hours, respectively.

There is a significant difference ($p<0.01$) between the average amount of dithranol loaded in the film at 1 hour and at 3 hours but no significant difference between that at 3 and 5 hours. This indicates that the equilibrium swelling, i.e. maximum loading of dithranol into the hydrogel, has been achieved after 3 hours of loading. This represents the minimum time required to reach a balance between the two opposing forces: namely the volume expansion due to penetration of dithranol loading solution into the glassy polymer and the elastic contraction of the stretched polymer network.

FINAL LOADING SOLUTION - As a result of all these investigations, the formula developed and used as the loading solution was:

Dithranol	0.050%
Acetone	50 %
DMSO	20 %
Sod. metabisulphite	0.1 %
Distilled water to	100 %

with a loading time of 3 hours at a temperature of 5°C

VOLUME OF LOADING SOLUTION - The volume of the loading solution is directly associated with the amount of dithranol present in the solution. There might be a reduction in the amount of dithranol uptake if too much hydrogel film was immersed in the solution. An experiment was carried

out by loading film with various volumes of the same loading solution and analysing the solution remaining after loading. The volume of the loading solution per area of dry film and percentage of dithranol remaining in the solution after use are shown in Table 4.11.

Table 4.11 Volume of dithranol loading solution per area of dry film and the deviation of dithranol concentration in the solution after loading at 5°C 3 hours.

Volume/area (ml/cm^2)	% remain mean (sd)	n
2.55	99.2 (4)	3
3.82	99.0 (2)	4
12.29	104.5(5)	2
31.85	105.0(5)	2

Table 4.11 shows that the percentage of dithranol remaining in the loading solution after loading was found to be 99 to 105% using 2.55 to 31.85 ml of the solution per 1 cm^2 of dry film. The standard deviations of dithranol remaining in the solution varied from 4 to 6%. These figures indicate that there is a significant ($p<0.05$) drop of concentration of dithranol in the solution after loading by using a low proportion of solution to film, 2 - 4 ml/cm^2. This indicates a caution to ensure that the solution is not overloaded with films. A further study is needed to prove this effect on the uptake of the hydrogel because the results shown could not indicate clearly whether the decrease is due to dithranol decomposition or depletion of the supply due to uptake. This method cannot be used as an indirect determination dithranol taken up by the film due to its unreliability and insensitivity. For example, if 99% of dithranol remains in the solution (initially 500 µg/ml), only 50 µg must have been taken up by one film (0.785cm^2) in 3 ml (or 3.82 ml/cm^2). This is not the case as shown later in Table 4.12. However, this method indicates that the quantitative analysis of dithranol taken up by one film

could not be performed without irreversible dissolution of the hydrogel.

Caution was taken to optimise the volume of dithranol solution used in the loading of the hydrogel, i.e. the minimum of 5 ml of the loading solution was required for the loading of a dry hydrogel of 1 cm^2. The uptake of dithranol in different size of hydrogel was, however, checked by the method given in Chapter 2 as a precaution.

4.3.4 EVALUATION OF DITHRANOL UPTAKE

The standard method and loading solution established have been used to load about 400 films in 18 batches for stability studies over 18 months. The cumulative data of the amount of dithranol uptake obtained from random sampling of the freshly loaded films, which ensures no interference from dithranol decomposition, should give an approximate dose for the subsequent clinical study. The results are shown in Table 4.12. The average concentration of dithranol used in the loading of these films was 480 µg/ml (sd 4, n = 15).

Variation in the actual amount of dithranol in each film is likely to arise from variations in weight and composition of films as well as inconsistencies in loading procedure technique. There is no significant difference ($p > 0.05$) between the amount of dithranol taken up by films of the same weight. For example, average uptake of 163 and 162 µg from 2 batches into 15 mg film (n = 3 each); 175, 186, 166, 165, 168 µg into 18 mg films in 5 batches (n = 3 each); or 187, 199, 201, and 212 µg into 20 mg films from 4 batches (n = 3 each) was obtained.

This can be highlighted by comparing the ratio of the amount of dithranol per dry weight of film which is independent of film weight. The standard deviations of

the ratio of dithranol to dry hydrogel weight is 0.3 and 0.1, obtained from unwashed and pre-washed films, are 3% and 1% of the corresponding means, respectively. These are less than the quantity of dithranol uptaken per film (unwashed film) shown in Table 3.8 (sd is 13% of mean), Table 3.9 (6%), Table 3.10 (11%) and Table 3.11 (4%) and equal to the pre-washed films results in Table 3.12 (1%).

Table 4.12 Summary of the quantity of dithranol taken up by dry hydrogel (1 cm in diameter) using the loading solution at 5°C for 3 hours.

	unwashed film			pre-washed film		
Wt film (mg)	**Q/wt** (μg/mg)	**Q/A** (μg/cm²)	**n**	**Q/wt** (μg/mg)	**Q/A** (μg/cm²)	**n**
15	10.4	198.6	13	-		
18	9.6	219.9	11	9.5	210.0	6
20	10.0	255.7	12	-		
23	-			9.3	270.9	3
mean	**10.0**	**224.7**		**9.4**	**240.5**	
sd	0.3	23.6		0.1	30.5	

abbreviation : Q/wt = quantity of dithranol uptake per weight of dry film and Q/A = quantity of dithranol uptake per area of dry film.

There is no direct evidence of variation in film structure, although differences in "clearness" of film, presence of bubbles, differences in thickness and pliability of film were noticed. In the stability work, brown spots appeared in some films and not others. This was found to be due to the presence of Fe^{+++}, also indicating some lack of uniformity. Given the nature of the film and its method of production, some small variations are to be expected.

Additionally, the differences between the amount of dithranol taken up by these films of different weights were significant ($p < 0.05$). It is interesting to note that the ratio of uptake of dithranol to weight of film for films pre-washed to eliminate Fe^{+++} was not different

from that for untreated films. Variations in detail on the loading methods occur and were were investigated and discussed in detail in Chapter 3, where the changes in technique to optimise dithranol stability. It should be noted that some batches of films had to be excluded from the statistical analysis discussed above because the sample size from those batches of films were different from the others. However, those excluded results appears to be in line with the results treated.

The variance of the results pooled from the 18 mg films and the 20 mg films were not significantly different ($p > 0.05$). Furthermore, the ratio between the amount of dithranol taken up and the weight of the film shows a small standard deviations and can be used as a guideline for the estimation of dose of dithranol in different size of the film, namely 9.4 µg dithranol per mg of hydrogel or 240 µg/cm² dry hydrogel, in the future development of dithranol hydrogel. It is , therefore, concluded that the loading method is reliable and reproducible.

CHAPTER 5

RELEASE STUDY

5.1 INTRODUCTION

A knowledge of the rate of passage of dithranol from a dosage form into the skin is important in the development of a delivery system. *In vitro* release of dithranol from conventional dosage forms has been investigated recently by a number of workers using organic solvents as receptor media: Kneczke *et al* (1989) used an ointment and an o/w cream with methanol (containing 0.05% ascorbic acid as an antioxidant) as the receptor medium whilst Martin *et al* (1989) used a cream and n-octanol as the receptor medium.

Neither of these arrangements using organic receptor systems can approach the aqueous conditions encountered *in vivo*. In order to do this, a method for measuring the release of dithranol from the loaded hydrogel had firstly to be established because of the differences in the nature of the formulations and the desire to use an aqueous solution as a receptor medium to assist in predicting in vivo behaviour. The choice of the solutions to be used had to take account of the stability and the solubility of dithranol and the swelling properties of the hydrogel. Factors affecting the swelling of the hydrogel such as the type and temperature of solvents and also the physicochemical properties of solutes will also play vital roles in the controlled release of solutes in the hydrogel [Graham *et al* 1980, 1982, Gander *et al* 1986] and so require investigation and control. The types of solute(s)

and the hydrogel are the constant factors in this study. Because the mechanism of action of dithranol is not known (Chapter 1), neither is the target polarity or pH of the "site of action". With administration to the skin this is additionally complicated by the diverse nature of the stratum corneum and other parts of the epidermis. Thus a reasonably wide range of receptor systems needs to be investigated, partly to predict likely *in vivo* release, but also with the anticipation that one may correlate the result with *in vivo* behaviour and give an indication of the target site in the skin. This chapter reports the establishment of *in vitro* release methods using aqueous and non aqueous media, the results and an evaluation of the release characteristics obtained.

5.2 EXPERIMENTAL METHODS

5.2.1 PASSAGE IN AQUEOUS AND NON-AQUEOUS MEDIA

The hydrogel was cut into 1-cm diameter discs from dry film. The preparation of dithranol loaded films was carried out using the standard method previously described (Chapter 4). All films were freshly prepared so that instability of dithranol was minimised. The dithranol was released from the films directly into a liquid receptor phase which was stirred with a magnetic stirrer at about 500 rpm. All experiments were performed in the dark at a controlled temperature (±1°C) using a circulating water bath. Two types of receptor media were investigated and used as follows.

Aqueous media

The hydrogel used in this study was new film used as received within 1 month.

0.1% SODIUM METABISULPHITE IN WATER (pH 2.2) - Release of dithranol from one loaded film was performed in 10 ml of the medium at 5°C. At predetermined times, 4.9 ml of the medium was

withdrawn from the receptor phase and replaced by the same amount of fresh 0.1% sodium metabisulphite in water. 0.05 ml of the internal standard solution was added to the sample and the solution was assayed using hplc (system 1, Chapter 2, p 37). The results are shown in Table 5.1.

ACETATE BUFFER (pH 5.0) - The release from a loaded film was performed in 6 ml of the buffer at 20°C. At predetermined times, a 1 ml sample was withdrawn from the receptor phase, 0.05 ml of the internal standard solution added, and made up to 5 ml with hplc mobile phase for assay using hplc (system 1, as above). The receptor medium was replaced by the same amount of fresh buffer. At the end of the experiment, the film was blotted dry, dissolved and assayed using hplc method (p 42). The results are shown in Table 5.2.

PHOSPHATE BUFFER (pH 7.0) - The release was carried out as before using 6 ml of receptor and 4.9 ml sample volume. 0.05 ml of the internal standard solution was added and the solution assayed using hplc as before. The film was treated as described above. The results are shown in Table 5.3.

ACETATE BUFFER (pH 2.8) - The release was carried out as above. Below 2 hours, 0.2 ml samples were taken and diluted to 5 ml using the am-DMSO-GAA system (see Chapter 2, p 44) and assayed using spectrofluorimetry. After 2 hours, 0.5 ml samples were taken and assayed by hplc as for acetate buffer pH 5. Hydrogel films were treated and analysed as above. The results are shown in Table 5.6.

Non - aqueous medium

23 ml of the loading solution vehicle (see Chapter 4) at 5°C was used as the receptor medium for a dithranol loaded film. Samples were taken at 4, 5, 7, 10, 15, 30, 45, 60, 90, 105, and 120 minutes and assayed as follows: 0.1 ml of the sample was diluted with 2 ml of glacial acetic acid

and analysed using the spectrofluorimetric procedure (p 44).

The film remaining after the release was blotted dry and dissolved in 4 ml of glacial acetic acid. Dithranol was assayed using the spectrofluorimetry (p 44). The results are shown in Table 5.5.

5.2.2 OTHER FACTORS AFFECTING RELEASE

An aqueous receptor medium (acetate buffer at pH 2.8) was used for these further investigations. The loading and releasing procedures were the same as previously. The factors investigated are as follows.

EFFECT OF HYDROGEL STORAGE ON RELEASE - Preliminary release studies (in acetate buffer at pH 2.8) showed that the release of dithranol from a batch of hydrogel which had been stored dessicated for about 1 year was different from the results above. This effect was reproducible. Thus, the release of dithranol from the stored films were investigated. The hydrogel, stored as a whole piece in a laboratory dessicator for about 1 year was cut to size as previously described prior to use. The method used in the release of dithranol in acetate buffer at pH 2.8 previously described was used. Samples were taken as appropriate and assayed as above.

SINK CONDITIONS IN THE RELEASING MEDIUM - The new hydrogel film and acetate buffer at pH 2.8 were used to monitor the effect of using fresh receptor medium after some release had taken place. The method under "acetate buffer pH 2.8" was employed. Samples of the first receptor medium were taken at 1, 2, 3, 4 and 5 hours. At the end of 5 hours, the film was recovered, blotted dry and stored for 16 hours at 5°C in laminated pads for maintaining dithranol stability (discussed in Chapter 3). The film was then used for a

second release experiment using the same procedure as for the first one. Samples were taken at 1, 2, and 3 hours, following which the dithranol remaining in the film was determined using hplc as before. The results obtained are presented in Table 5.7.

EFFECT OF THE DRYING PROCESS ON THE LOADED FILM - For storage, loaded hydrogel had to be dried. In order to find out if the method of drying had any effect the following experiment was carried out using four pieces of hydrogel film and acetate buffer at pH 2.8. Two of the loaded films were blotted and then vacuum dried for 10 minutes and the others were blotted dry but not vacuum dried as a control. Release of dithranol from the films was performed as described under "acetate buffer pH 2.8" above and the results are presented in Table 5.8..

5.3 RESULTS AND DISCUSSION

5.3.1 PASSAGE IN AQUEOUS AND NON-AQUEOUS MEDIA

Aqueous media

The first part of the development of the release method was to select an appropriate aqueous medium. Tables 5.1, 5.2 and 5.3 show the release of dithranol from the hydrogel into three aqueous candidates but which were not used in the subsequent study, 0.1% sodium metabisulphite in water, acetate buffer at pH 5.0 and phosphate buffer at pH 7.0, respectively.

The amount of dithranol released into these media was low (not more than 5 µg) making detection difficult. The solubility of dithranol and hydrogel swelling in these solvents (detailed in Chapter 4, p 107) together with this lack of solubility indicates that these media are not ideal for the release study of dithranol from the hydrogel.

Table 5.1 Release of dithranol from hydrogel film into 0.1% sodium metabisulphite in water (pH 2.2), at 20°C, light-protected, expressed as mean (sd) of the amount of dithranol (Q_{dith}, µg) and danthrone (Q_{dant}, x 10 ^{2}µg)

Time (hour)	Q_{dith} (µg)	Q_{dant} (x10 ^{2}µg)	F	n
0.25	1.5	4	0.816	1
0.50	1.7	4	0.944	1
1	1.5 (0.4)	3 (2)	0.816	11
2	1.8 (0.5)	3 (2)	1.000	2
3	1.5 (0.5)	3 (2)	0.844	2
20	1.4 (0.5)	10(5)	0.793	3
21	1.4 (0.3)	9 (6)	0.771	2
22	1.2	15	0.670	1
48	0.09	63	0.050	1
240	0.06	83	0.034	1

NB : F = fraction of dithranol release = amount in medium at time / maximum dithranol release (1.79 µg in this case).

Table 5.2 Release of dithranol from hydrogel film into acetate buffer (pH 5.0) at 20°C, light-protected, and that remaining in the film (µg): expressed as mean (sd) of the amount of dithranol (Q_{dith}, µg) and danthrone (Q_{dant}, x10 ^{2}µg). (n= 3)

Time	receptor medium		film remaining		parameter	
	Q_{dith}	Q_{dant}	Q_{dith}	Q_{dant}	P	F
(hr)	(µg)	(x10 ^{2}µg)	(µg)	(x10 ^{2}µg)	(x10 ^{3})	
2	2.4 (1)	18 (6)	127 (25)	<1	19	0.50
4	3.2 (1)	18 (1)	120 (21)	<1	27	0.67
6	4.8 (2)	20 (1)	104 (8)	<1	46	1.00
8	2.4 (1)	20 (10)	111 (25)	<1	22	0.50
10	3.7 (2)	33 (2)	113 (30)	<1	33	0.77
12	2.5 (1)	32 (3)	72 (6)	3 (1)	34	0.52
14	1.0 (0)	32 (3)	88 (8)	4 (1)	12	0.21

NB : P = partition ratio of dithranol = dithranol in receptor medium to that remaining in film, F = fraction of dithranol release = dithranol in medium to maximum dithranol in medium (4.8 µg in this case).

Table 5.3 Release of dithranol from hydrogel film into phosphate buffer (pH 7.0), at 20°C, light-protected; and that remaining in the film: expressed as mean (sd) of the amount of dithranol (Q_{dith}, µg) and danthrone (Q_{dant}, x10 ^{2}µg). (n = 4)

Time	receptor medium		film remain		parameter
	Q_{dith}	Q_{dant}	Q_{dith}	Q_{dant}	P
(hr)	(µg)	(x10 ^{2}µg)	(µg)	(x10 ^{2}µg)	(x10 ^{3})
2	<0.04	<20	-	-	-
6	1.8 (0.5)	20 (1)	77 (7)	< 1	23
16	1.2 (0.5)	36 (2)	53 (26)	3 (1)	23
24	0.4 (0.1)	32 (2)	41 (16)	5 (2)	10
26	0.1 (0.2)	53 (2)	10 (13)	6 (1)	1

NB : P = partition ratio of dithranol = dithranol in receptor medium to that remaining in film.

Although dithranol solubility in phosphate buffer at pH 7 was higher than that in acetate buffer at pH 5, the hydrogel swelled to a greater extent in the latter than in the former. This emphasises the combined effect of dithranol solubility and hydrogel swelling properties of the medium used on the release of dithranol from the hydrogel. The next concern was dithranol stability in these solvents in the presence of the hydrogel. The results in Table 5.1 demonstrate a detectable amount of danthrone due to dithranol decomposition in 0.1% sodium metabisulphite in water almost immediately after dithranol was released. Danthrone was readily detectable after 2 hours in acetate buffer at pH 5.0 (Table 5.2) but neither dithranol nor danthrone could be detected in phosphate buffer at pH 7.0 after 2 hours of release. This indicates that dithranol decomposition in all these receptor media will occur once dithranol has been released from the hydrogel. The amount of dithranol remaining in the film after the release indicates that the release process was slow. This can be explained by the hydrophobic nature of dithranol.

The decomposition of dithranol in the film, on the other hand, could be accelerated when the film was immersed in a liquid. Danthrone was detected after 6 hours in phosphate buffer at pH 7.0 (Table 5.3), but such rapid decomposition as this was not seen when the film was stored in laminated pads (see Chapter 3). It should be noted that detectable amounts of danthrone were not formed in the film until 6 hours of release in phosphate buffer at pH 7.0 and before 14 hours in acetate buffer at pH 5.0. Acetate buffer was further investigated for a potential use as the receptor medium because of this.

Danthrone was detectable in these studies whilst dimer was not detected. However, the total amount of dithranol and danthrone recovered, i.e the amount released and remaining in the film, was not 100%, indicating a loss due

to undetectable decomposition compound(s). For example, after 14 hours in acetate buffer at pH 5, the total amount of dithranol and danthrone is 89 μg which is about 60% recovery from total initial dithranol. It is likely that dithranol dimer is formed and rapidly undergoes further decomposition, because the amount of dithranol dimer would have been less than 0.2 μg in the media and 1 μg in the hydrogel. This indicates that a full description of fate of dithranol passage from hydrogel into water can only be achieved if the determination of danthrone and dimer is improved.

A ratio (P) of amount of dithranol detected in the receptor media to that in the hydrogel has been adopted to assisst in data evaluation by allowing for this instability of dithranol in aqueous media. This ratio is based on the assumption that the process of dithranol passage from the fully swollen hydrogel into water is a diffusion process in which a steady state would be achieved and so is, in effect the partition coefficient of dithranol from hydrogel to receptor medium. Table 5.2 shows that the ratio of dithranol in the receptor medium to that in film for acetate buffer at pH 5.0 rises steadily reaching about 0.03 after 10 - 12 hours, after 12 hours a sudden drop to 0.01 was observed with an increase in total danthrone. Values for phosphate buffer at pH 7.0 suggest a similar pattern of increase and decrease over about 16 hours. This ratio, therefore, can be used as an indicator for dithranol decomposition in the media. Thus, the partition coefficient of dithranol between the buffers and hydrogel is between 0.02 and 0.03 at 20°C, indicating that dithranol prefers the hydrogel to the media. This result was expected because of the hydrophobicity of dithranol.

The effective area of release from the hydrogel was 3.5 cm^2, the diameter of the swollen hydrogel in the releasing medium being 1.5 cm. A release constant calculated using the amount released from the film was thus

0.4 μg/cm²/hr for both acetate buffer at pH 5.0 and phosphate buffer at pH 7.0.

The release of dithranol from freshly prepared hydrogel into ACETATE BUFFER AT PH 2.8, shown in Table 5.4, demonstrates a fast process which reaches steady state after about 1-2 hours.

Table 5.4 Release of dithranol from unstored hydrogel film into acetate buffer (pH 2.8), at 20°C, light-protected and that of remaining in the film: expressed as mean (sd) of the amount of dithranol (Q_{dith}, μg) and danthrone (Q_{dant}, x 10^{-2} μg).

Time	receptor medium		film remain	parameters		
(hr)	Q_{dith} (μg)	Q_{dant} (x10 ²μg)	Q_{dith} (μg)	P	F	%
0.25	38 (6)	<1	-	-	0.468	-
0.50	53 (10)	<1	-	-	0.654	-
0.75	43 (1)	<1	-	-	0.526	-
1	64 (18)	<1	-	-	0.781	-
2	68 (1)	<1	87 (13)	0.78	0.829	44
3	57 (4)	<1	-	-	0.698	-
4	68 (3)	1.2 (0.1)	70 (11)	0.97	0.829	49
5	62 (4)	1.2 (0.3)	71 (5)	0.87	0.760	-
6	76 (2)	1.4 (0.2)	71 (12)	1.07	0.933	52
7	59 (1)	1.3 (0.1)	-	-	0.717	-
8	82 (8)	1.0 (0.1)	62 (6)	1.32	1.000	57
9	60 (0)	1.4 (0.1)	76 (5)	0.79	0.709	-
10	67 (16)	1.5 (0.2)	54 (3)	1.24	0.820	55
12	49 (4)	2.0 (0.1)	97 (20)	0.51	0.586	33

NB : P = partition ratio of dithranol = dithranol in receptor medium to that remaining in film,
F = fraction relased = dithranol in medium to maximum dithranol released (82 μg in this case),
% = percentage of dithranol released from total dithranol.

The plot between the amount of dithranol released against time (Fig. 4.1) indicates a fast process reaching equilibrium within 2 hours. The results shown in the table also demonstrate a good reproducibility with low standard deviations. They were obtained from 3 different batches of film, thus confirming reproducibility of the film from batch to batch.

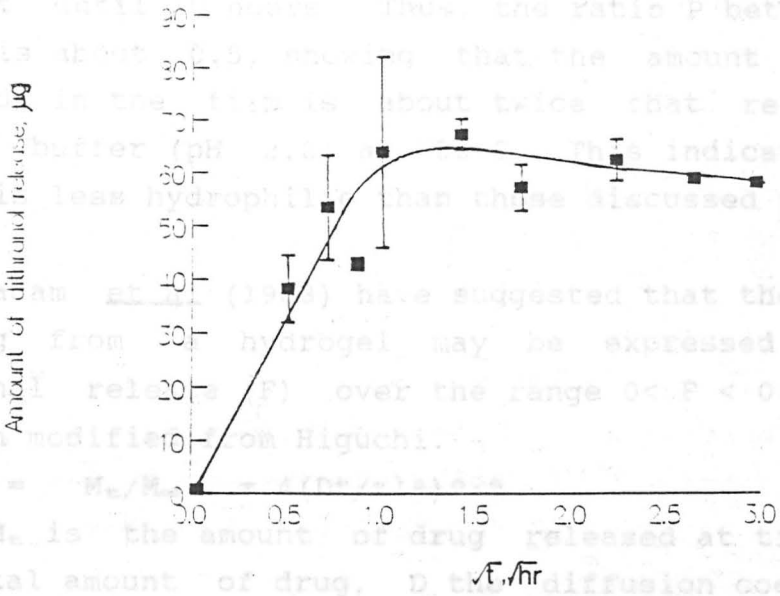


Fig. 5.1 Passage of dithranol from hydrogel and into acetate buffer at pH 2.8 at 20°C, light-protected, showing amount of dithranol released.

No detectable amounts of danthrone occur in the remaining films, i.e. < 1 µg, after 12 hours of study. Although a trace amount of danthrone is produced in acetate buffer at pH 2.8, it is less than that shown in the other aqueous media discussed above. Thus, after 12 hours, 0.02 and 0.3 µg are seen in acetate buffer at pH 2.8 and 5.0, respectively. This suggests that acetate buffer at pH 2.8 is more appropriate than acetate buffer at pH 5.0 and phosphate buffer at pH 7.0 in term of dithranol stability and quantification using the hplc.

The total amount of dithranol can be recovered using acetate buffer at pH 2.8. From the results, totals of 155, 138, 133, 147, 144, 134, 121 and 145 µg (mean and s.d. of 140 ±10 µg) of dithranol were obtained after 2, 4, 5, 6, 8, 9, 10, and 12 hours. These figures are in line with the initial concentration of dithranol discussed in Chapter 4. As a consequence, an accurate release rate for dithranol can be measured when using an acetate buffer at pH 2.8.

The partition ratio (P) increased slightly after 2 hours reaching a maximum after 6 hours after which it is

constant until 10 hours. Thus, the ratio P between 2 to 12 hours is about 0.5, showing that the amount of dithranol retained in the film is about twice that released into acetate buffer (pH 2.8) at 20°C. This indicates that the medium is less hydrophilic than those discussed previously.

Graham et al (1988) have suggested that the release of a drug from a hydrogel may be expressed using the fractional release (F) over the range $0 < F < 0.6$ using the equation modified from Higuchi.

$$F = M_t/M_\infty = 4(Dt/\pi l^2)^{0.5}$$

where M_t is the amount of drug released at time t, M_∞ is the total amount of drug, D the diffusion coefficient of the drug in the gel, and l is the thickness of the gel. Thus, a straight-line relationship is expected from a plot of fraction released (for values of M_t/M_∞ up to approximately 0.5-0.6) against $t^{0.5}$.

The thickness of dry hydrogel is 0.0222 cm (sd = 0.0003 cm, n = 16). After 2 hours in the release medium, the film had increased 1.6 times (this will be discussed in Chapter 8), to give a calculated thickeness of 0.03552 cm. Using the results for 0.25 hours (F=0.468) and 0.75 hours (F=0.526) in Table 5.4, gives diffusion coefficients of 21.7 and 9.1 ($\times 10^{-5}$) cm/hr$^{0.5}$, respectively, and a mean and sd of $15 \pm 6 \times 10^{-5}$ cm/hr$^{0.5}$.

This diffusion coefficient obtained is about 5 fold less than that of caffiene from a fully swollen PEG 6000 hydrogel where l is 0.19 cm using phosphate buffer at pH 7.4 [Graham, et al, 1988]. However, the solubility of caffeine in water is about 17000 times that of dithranol (caffeine 1 in 60, whereas dithranol 1 in 10^{-6}). This leads to further study using a non-aqueous medium.

This calculation has used the observed maximum amount of dithranol released. Whether this is the actual maximum

amount released at infinite time open to doubt because of the instability of the dithranol in the aqueous solution.

Non-aqueous medium

A non-aqueous receptor medium, the vehicle used as the loading solution (20%DMSO, 50% acetone and water, with an antioxidant) was investigated at 5°C.

Table 5.5 shows the amount of dithranol release from the film into the solution at 5°C. The release process was rapid, reaching completion after 20 minutes. The results are also presented in Fig. 5.2.

Table 5.5 Release of dithranol from hydrogel film (µg) into loading solution vehicle, at 5°C and that remaining in the film(µg), expressed as mean (sd, n=3).

Time (min)	release (µg)		film remain (µg)		Total (µg)	parameter F	%
3	62	(2)	106	(9)	168	0.381	37
5	89	(2)	81	(23)	170	0.546	52
7	91	(43)	82	(33)	173	0.564	53
10	102	(12)	29	(25)	131	0.630	78
15	125	(10)	9.7	(1.1)	129	0.770	93
30	142	(16)	3.9	(1.6)	146	0.879	97
45	162	(30)	2.7	(0.4)	165	1	98
60	113	(28)	2.1	(0.1)	115	0.697	98
75	157	(1)	3.4	(1.9)	160	0.966	98
90	126	(11)	3.3	(2.3)	128	0.775	98
105	120	(18)	2.4	(0.5)	123	0.742	98
120	139	(18)	2.6	(0.5)	142	0.357	98

Using the same conditions as those used in loading the film, the film swelled to reach its maximum, thus the partition of dithranol occurred. This was directed towards the lipophilic vehicle rather than the hydrogel, as a result of which, the hydrogel almost completely released its dithranol to the receptor, i.e. about 10 µg of dithranol remaining in the film and 125 µg were released after 15 minutes. The ratio of amount of dithranol released to the total recovered at steady state was a constant at 0.98. This figure indicates that a small

amount of dithranol was retained in the film despite the release time being prolonged to 120 minutes - almost 10 times that taken to reach equilibrium. Whilst an explanation for this phenomenon was not investigated as part of this study, it is suggested that the remaining dithranol is trapped in the crystalline part of the hydrogel. The spectrofluorimetric analysis (detail in Chapter 2) was used for this study because of its sensitivity and so the analysis did not quantify dithranol decomposition. However, it is known from Chapter 3 that dithranol is stable in this solvent system.

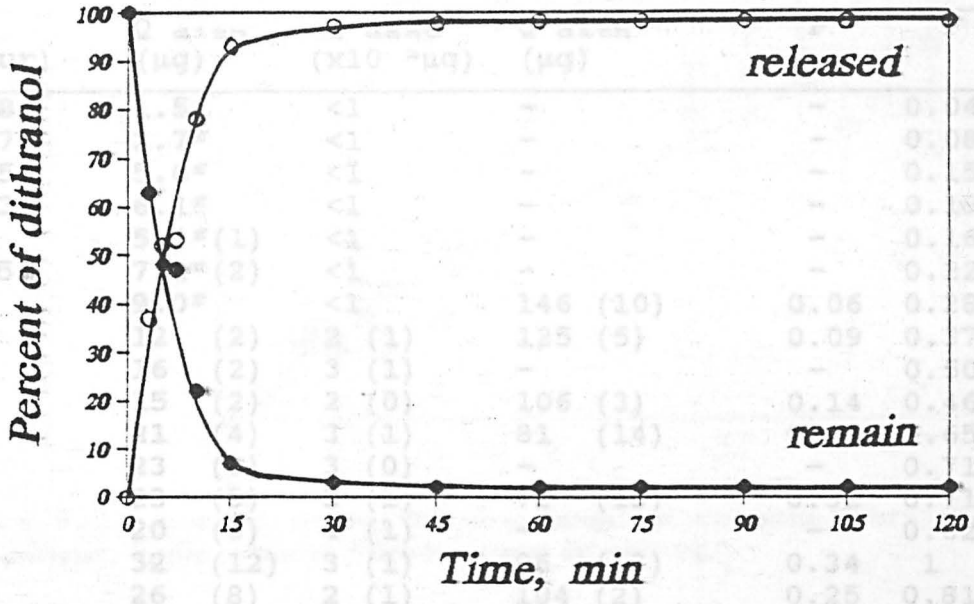


Fig. 5.2 Passage of dithranol from hydrogel into the non-aqueous receptor at 5°C, light-protected, showing percent of dithranol released and remaining.

Using the equation described previously for $F < 0.6$, the diffusion coefficient of dithranol in non-aqueous medium was calculated. The observed thickness of hydrogel was 0.03996 cm. Using F values from 3 mins (0.368), 5 mins (0.522) and 7 mins (0.528) gave diffusion coefficients of 2, 3 and 1 x 10^{-10} cm^2/min, respectively (mean and sd of 2 and 0.4 x 10^{-10} cm^2/min).

5.3.2 OTHER FACTORS AFFECTING DITHRANOL PASSAGE

Effect of storage

The release of dithranol from hydrogel stored in a dessicated atmosphere for about 1 year, into acetate buffer at pH 2.8, is shown in Table 5.6. The results demonstrate a slow release process which reaches steady state after about 10 hours.

Table 5.6 Release of dithranol from stored hydrogel film into acetate buffer (pH 2.8), at 20°C, light-protected; and amount of dithranol remaining in the film: expressed as mean (sd) of amount of dithranol (Q_{dith}, µg) and danthrone (Q_{dant}, x10 $^{-2}$ µg). (n=3)

Time	receptor medium		film remain	parameter		
(hour)	Q_{dith} (µg)	Q_{dant} (x10 $^{-2}$µg)	Q_{dith} (µg)	P	F	%
0.08	1.5^f	<1	-	-	0.047	-
0.17	2.7^f	<1	-	-	0.084	-
0.25	5.0^f	<1	-	-	0.156	-
0.33	6.1^f	<1	-	-	0.191	-
0.5	5.2^f (1)	<1	-	-	0.163	-
0.75	7.2^f (2)	<1	-	-	0.225	-
1	9.0^f	<1	146 (10)	0.06	0.281	6
2	12 (2)	2 (1)	125 (5)	0.09	0.375	9
4	16 (2)	3 (1)	-	-	0.500	-
6	15 (2)	2 (0)	106 (3)	0.14	0.469	12
8	21 (4)	3 (1)	81 (14)	0.26	0.656	20
10	23 (3)	3 (0)	-	-	0.719	-
12	23 (8)	3 (1)	72 (15)	0.32	0.719	24
14	20 (5)	4 (1)	-	-	0.625	-
16	32 (12)	3 (1)	95 (13)	0.34	1	25
18	26 (8)	2 (1)	104 (2)	0.25	0.813	20
22	28 (8)	6 (1)	-	-	0.875	-
24	28 (10)	6 (1)	63 (16)	0.44	0.875	31

Abbreviations : ^{f} = results obtained using fluorimetry.

About 25 µg of dithranol was released into the receptor medium at equilibrium. This differs from the data obtained using newly prepared hydrogel films (Table 5.4 and Fig. 5.3). Only 40% of the equilibrium amount of dithranol was released from the stored film when compared with the new film and the time taken to reach its steady state was 4-5 times longer.

Using the equation described previously for $F < 0.6$, the diffusion coefficient of dithranol was calculated. The observed thickness of hydrogel was 0.0355 cm and the estimated diffusion coefficients (F values from 0.08 to 6 hours) are shown in Table 5.7. Therefore, mean diffusion coefficient is 2.3×10^{-10} cm²/hr (sd 0.4×10^{-10} cm²/hr). That of unstored film (2.3×10^{-8} cm²/hr) is, thus, 100 times greater.

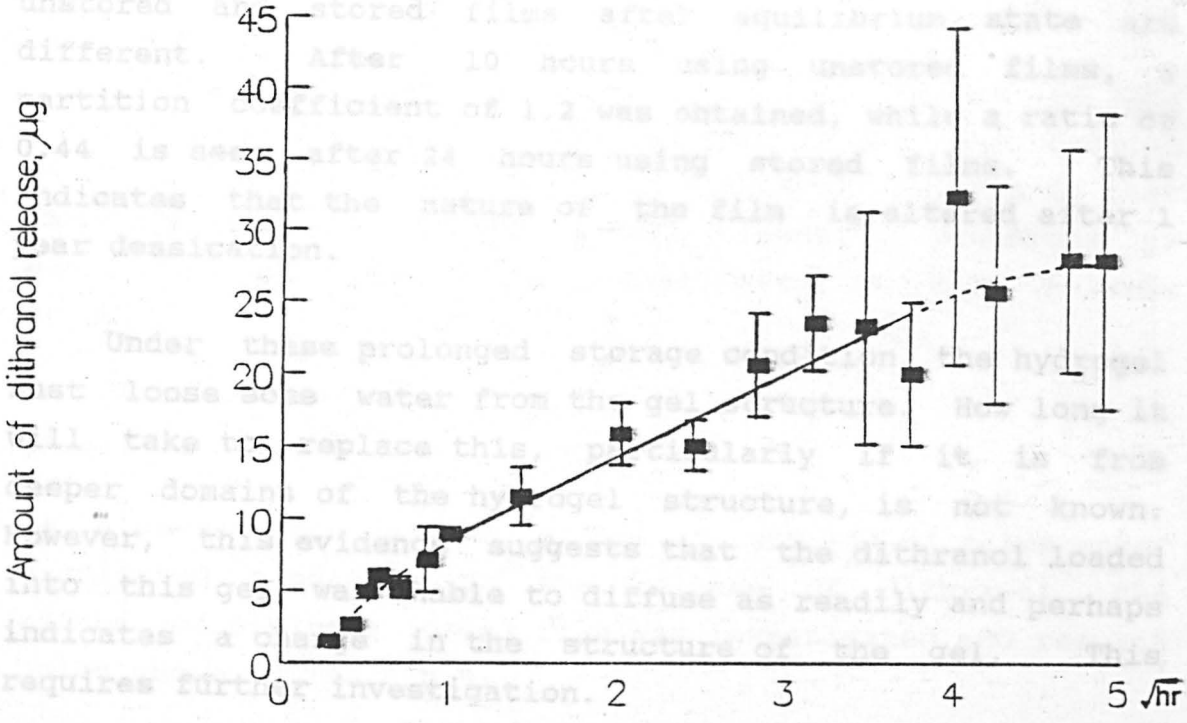


Fig. 5.3 Passage of dithranol from stored hydrogel and into acetate buffer (pH 2.8) at 20°C, light-protected, showing percent of dithranol released and remaining.

Table 5.7 Diffusion coefficient of dithranol in stored films.

time (hours)	F	D (10^{10}) (cm²/hr)
0.08	0.047	0.5
0.17	0.084	1.0
0.25	0.156	5.5
0.33	0.191	7.0
0.5	0.163	1.7
0.75	0.225	2.6
1	0.281	3.6
2	0.375	2.5
4	0.500	2.2
6	0.469	0.8

Linear regression between F of less than 0.5 (as discussed above) and $t^{0.5}$ gives a correlation coefficient of 0.9596 and slope of 0.02 $hr^{-0.5}$ the slope gives a calculated diffusion coefficient of 1.1 x 10^{-10} cm^2/hr, which is similar to the above result.

Similarly, partition ratios (P) obtained from both unstored and stored films after equilibrium state are different. After 10 hours using unstored films, a partition coefficient of 1.2 was obtained, while a ratio of 0.44 is seen after 24 hours using stored films. This indicates that the nature of the film is altered after 1 year dessication.

Under these prolonged storage condition, the hydrogel must loose some water from the gel structure. How long it will take to replace this, particularly if it is from deeper domains of the hydrogel structure, is not known. However, this evidence suggests that the dithranol loaded into this gel was unable to diffuse as readily and perhaps indicates a change in the structure of the gel. This requires further investigation.

Effect of sink conditions

Table 5.8 Amount of dithranol released (µg) from the same hydrogel film into first and second portion of acetate buffer (pH 2.8), at 20°C, light-protected, expressed as mean (sd, n = 4).

Time	Dithranol (µg)	
(hr)	First release	Second release
1	58 (4)	20 (2)
2	69 (4)	21 (2)
3	74 (6)	20 (2)
4	74 (4)	-
5	70 (4)	-

NB Mean(sd) of dithranol remaining in the film after second release (3 hours) was 11 (2) µg.

The ratio of dithranol released and retained by hydrogel shows that half the dithranol was not released, as previously discussed. To indicate whether the volume of the 6 ml receptor medium used provided sink conditions, a test was performed using a second fresh receptor medium, the results of which are shown in Table 5.8. After the first 5 hours of the release, a second release of the same films gave a constant amount of dithranol in the receptor medium of about 20 μg over 3 hours.

Therefore, it was found that the volume of the releasing medium used in the experiment did not provide sink conditions. The ratio of dithranol release to that recovered is 0.64 in the second release. Danthrone or dimer was not detected in either phase of this release study.

Effect of dryness

As a consequence of the effect of storage of films under dessicated condition on the release of dithranol, the effect of drying a loaded film was investigated and results being shown in Table 5.9.

Table 5.9 Effect of dryness of loaded hydrogel on release of dithranol from hydrogel film into acetate buffer (pH 2.8), at 20°C, light-protected: expressed as mean (sd) of amount of dithranol (μg, n = 2).

Time	Dithranol (μg)	
(hour)	vacuum dried	control
1	61 (4)	56 (5)
2	72 (1)	66 (4)
3	78 (4)	70 (4)
4	77 (4)	72 (4)
5	73 (4)	67 (2)

It was found that there is no significant different ($p<0.05$) between the results obtained from dithranol loaded

film which was vaccuum dried and the control (a loaded film which had not subjected to any form of drying except being blotted dry).

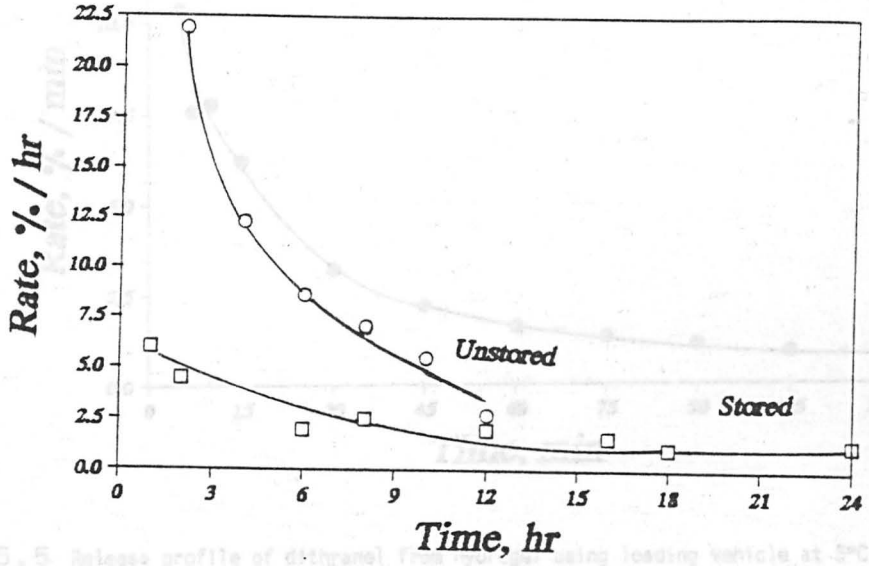


Fig. 5.4 Release profile of dithranol from unstored (○) and stored (□) hydrogel using acetate buffer(pH 2.8) at 20°C.

Fig. 5.4 shows graphs of the release rate (% hr^{-1}) against time using acetate buffer at pH 2.8 (20°C), for both unstored and stored films. It is seen that unstored hydrogel gives an initial non-zero-order release (probably first order) whilst the stored hydrogel gave a constant release of about 2.5 % per hour. However, after about 12 hours these two appear to be releasing at the same rate. The kinetics of the situation requires further investigation.

Fig. 5.5 is a graph of the release rate (% min^{-1}) against time using loading vehicle (5°C) as receptor and shows a similar pattern of release to that into acetate buffer (pH 2.8) at 20°C. These characteristics of release

is similar to caffeine discussed previously [Graham et al, 1988].

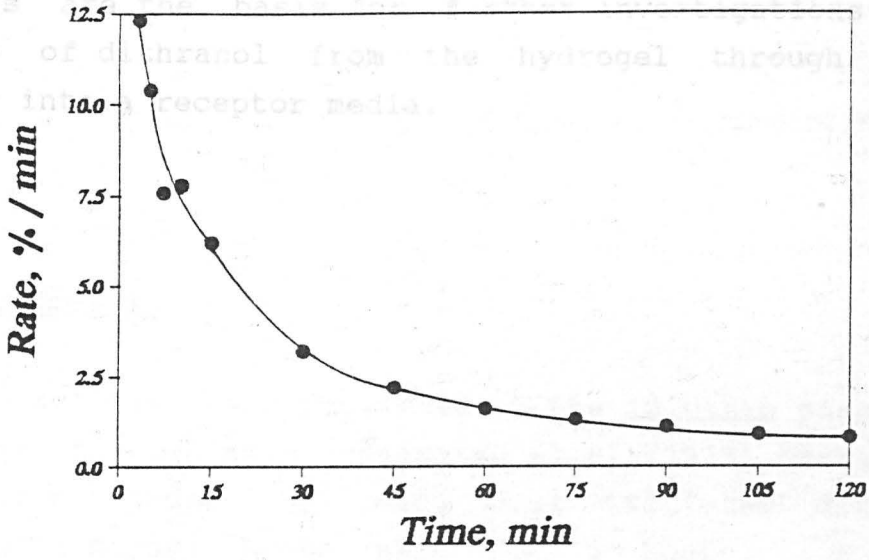


Fig. 5.5 Release profile of dithranol from hydrogel using loading vehicle at 5°C.

The release rate constants of 0.04% and 0.2% dithranol cream (based on white petrolatum, cetomacrogol1000 and cetostearyl alcohol) (Kneczke et al, 1989) through a cellulose membrane into methanol were 0.4 and 2 $\mu g/cm^2/hr$, respectively. An apparent release constant of dithranol from 1% Dithrocreme (American Dermal Corp.) through a silicone rubber membrane into n-octanol reported by Martin et al 1989 was 16.7 $\mu g/cm^2/min^{1/2}$. These results could not be comparing with the results obtained in this study because of the differences in the nature of the preparations, receptor media, temperatures used, and the membrane used in the reported cases. Despite this, the values are similar to the figure of 0.4 $\mu g/cm^2/hr$ reported for the present work.

It can be concluded that dithranol was released from the hydrogel into an aqueous solvent. Factors involved in the release *in vitro* are similar to those involved in the loading process. These include factors affecting hydrogel swelling, dithranol solubility and stability. These findings are the basis for further investigations on the release of dithranol from the hydrogel through stratum corneum into a receptor media.

CHAPTER 6

In vitro RELEASE STUDY IN NEONATAL RAT STRATUM CORNEUM

6.1 INTRODUCTION

Little work has been published on the *in vitro* passage of dithranol through skin. Kammarau *et al* (1975) carried out penetration studies by using 0.1% tritiated dithranol applied on normal human skin for 5 hours. A large accumulation of radioactivity was found in the epidermis but only small amounts of tritiated material were located in the stratum corneum. The penetration was influenced by the types of vehicles used, for example vaseline, a lipophilic base, allowed a better penetration of dithranol into the epidermis than polyethylene glycol, a hydrophilic base. Schalla *et al*(1981) investigated an *in vitro* penetration of dithranol after a short contact application using cadaver skin and tritium-labelled dithranol to compare with the *in vivo* results obtained by Kammerau *et al* (1975). They reported a rapid penetration of dithranol reaching maximum after about 2 hours, both in the epidermis and dermis and sustained until completion of the studies(16.7 hours). In experiments on rat skin (Cavey *et al*, 1985) obtained a similar pattern and also demonstrated the transformation of dithranol and that its ether-insoluble derivatives accumulated in the living tissue of the skin whether or not stratum corneum was present. Only in skin stripped of its stratum corneum did danthrone appear. Dimer accumulation was approximately equal to that of dithranol in normal skin after 24 hours.

In this present work, an attempt was made to monitor the passage of dithranol into and through stratum corneum in vitro in order to provide information which could indicate whether the loaded hydrogels might deliver suitable amounts of dithranol to warrent clinical evaluation. Additionally, a knowledge of the role of stratum corneum as one of the compartments in the release process could contribute important information about the therapeutic activity of dithranol. The model used required the determination of dithranol in 3 compartments, hydrogel, stratum corneum and a receptor medium. The method used for assay of dithranol in skin had to be developed whilst those for assay in hydrogel and the liquid systems has been discussed earlier. It was intended to dissolve the skin in a suitable solvent system for analysis. The trial release studies may be subdivided into 3 situations - with no receptor medium, with a non-aqueous medium and with an aqueous medium.

6.2 EXPERIMENTAL METHODS and APPARATUS

Stratum corneum

Neonatal rat stratum corneum was prepared using the method described by Kligman and Christophers (1963). The sheets were stored dry until used. Prior to use stratum corneum was soaked in distilled water at room temperature for 24 hours and dabbed dry with a tissue paper. The weight increase was from 5 mg to 20 mg($\pm$2, n=2).

Assay of dithranol in stratum corneum

An assay procedure in stratum corneum was initially performed by dissolving the stratum corneum in a range of solvents. Approximately 3 mg of dry stratum corneum was dissolved in 3 ml of each of the solvents listed in Table 6.1 using an ultrasonic bath for 20-40 minutes. Those which gave solution of stratum corneum were further investigated for dithranol stability. This was performed

by mixing 0.03 ml of dithranol solution in glacial acetic acid(40ug/ml) with the stratum corneum solution and the time taken until dithranol discolouration was observed and recorded in Table 6.1.

Because of a failure to obtain a stratum corneum solution which give reasonable dithranol stability, an extraction procedure was used and the validation of this assay of dithranol was discussed in Chapter 2 (pps 42 and 44).

Release without a receptor medium

A freshly loaded hydrogel film was placed on a piece of the stratum corneum. They were then held together by a laminated pad and stored in the dark at 20°C for 2 days. The stratum corneum and the hydrogel were separated and analysed for dithranol be the methods already detailed in Chapter 2 (pps 42 and 43).

Release with a receptor medium

The first diffusion cell used was a 10-ml amber glass container (An eye-drop bottle whose dropper was removed). A magnetic follower (1.2 cm in length) was used throughout the study. The cell was filled with 10 ml of the blank loading solution, used as a receptor fluid, and covered with a piece of neonatal rat stratum corneum (2 cm in diameter). The stratum corneum used in this study had not been pretreated by soaking in water as previously described. A loaded hydrogel film was placed onto the dry stratum corneum. A polyvinyl sheet was used to cover the hydrogel and the skin to keep them in place and the bottle was then tightly closed with its lid. The assembly was placed horizontally in a water bath (5°C) on a magnetic stirrer. At predetermined time intervals, the experiments were stoppped and dithranol assayed in the hydrogel using hplc, stratum corneum and receptor solution using fluorimetry, as described in Chapter 2. The results are shown in Table 6.2.

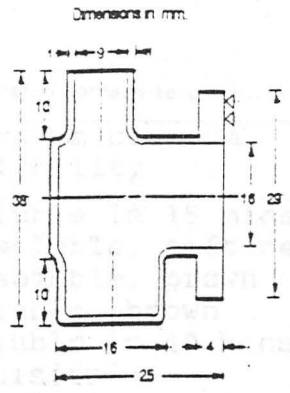


Fig.6.1 Diffusion cell

The method was not successful, so the amber glass bottle was replaced by a transparent diffusion cell, a schematic diagram of which is shown in Fig. 6.1. The volume of the receptor medium filled in the cell was 6 ml. The study was carried out at 20 ± 2°C. The stratum corneum was sealed to the cell using silicone grease to avoid leakage of the receptor medium. A polyvinyl sheet was used to cover the hydrogel and the skin to keep them in place. A teflon tape was used to seal the edge of the vinyl sheet and the whole cell was covered by aluminium foil to protect the contents from light. The experiments in which leakage ocurred were rejected. A magnetic follower was used to stir the receptor media during the experiment. At predetermined time intervals, the experiments were stopped and dithranol assayed in the hydrogel using hplc, and in stratum corneum and receptor solution using fluorimetry, as described in Chapter 2. The determination of danthrone and/or dimer in receptor media was performed using the hplc method described in Chapter 5. The receptor media investigated were acetate buffer at pH 0.5, 2.8, and 5.0, distilled water (pH 6.5) and blank loading solution.

6.3 RESULTS AND DISCUSSION

The establishment of the method to be used in the determination of dithranol in the stratum corneum was first attempted using solvents shown in Table 6.1.

Table 6.1 Screening test for stratum corneum solution.

Solvent(ratio)	stratum corneum solubility	dithranol discolouration
SL	soluble in 15 mins	after 10 mins
SL:GAA(1:2)	insoluble, softened	after 1 hours
SL:DMSO(1:2)	insoluble, brown	not applicable
SL:IPP(1:1)	soluble, brown	after 10 mins
SL:Hex(1:2)	soluble in 40 mins	after 30 mins
SL:$CHCl_3$(1:3)	emulsion	not applicable
TL	insoluble	not applicable
TL:Hex(1:1)	insoluble	not applicable
TL:LA(1:2)	insoluble,immiscible liquids	not applicable
TL:EE(1:1)	insoluble	not applicable
LA	insoluble	not applicable
GAA	insoluble, softened	see Chapter 3
LA:GAA(1:0.1)	insoluble, softened	not applicable

Key solvents : SL = soluene, GAA = glacial acetic acid, DMSO = dimethyl sulphoxide, IPP = isopropanol, Hex = n-hexane, $CHCl_3$ = chloroform, TL = toluene, LA = lactic acid, and EE = ethyl ether.

Soluene can be used in the solution of the stratum corneum, the apparent solubility of which is about 1 mg/ml. However, it was not found to dissolve easily even with the aid of an ultrasonic bath when about 20 minutes was required. It was found that the solution of dithranol in soluene discoloured in 10 minutes, indicating that it was highly unstable. This is because soluene contained 0.5 N quaternary ammonium hydroxide in toluene, the high pH causing rapid oxidation of dithranol. The use of soluene mixed with the other solvents known to have little effect on dithranol stability, for example glacial acetic acid (see Chapter 3), indicated that they were not able to dissolve the stratum corneum but did adversely affect dithranol stability as shown in Table 6.1. A mixture of 1:1 soluene and isopropanol or 1:2 soluene and hexane did dissolve the stratum corneum, although the time in the ultrasonic bath had to be extended to 40 minutes in the latter case. These solutions were slightly brown even in the absence of dithranol, thus, detection of dithranol discolouration was difficult. However, these are not suitable since dithranol brown appeared after 10 to 30 minutes. Glacial acetic acid was found to soften but not dissolve the stratum corneum. It was, therefore, decided

that dithranol would have to be extracted from the stratum corneum for assay.

The single extraction was satisfactory for use in this study as discussed in Chapter 2 (p 61). It had been expected that the extraction would not be as satisfactory as would dissolving the stratum corneum as had been originally intended. Because it relies on the partitioning of the solute between 2 phases some dithranol might remain in the stratum corneum after a single extraction. However, as reported earlier, this can be regarded as negligible because of the failure to detect any dithranol in the second extract. This might not be the case if higher concentration of dithranol were found in the stratum corneum than at these test levels. Tests were, therefore, carried out using a second extract and confirmed that it was not necessary and that dithranol concentrations were within expected levels.

The amount of dithranol uptake from dithranol loading solution or loaded hydrogel by the stratum corneum was investigated in order that the maximum capacity of stratum corneum for dithranol was known. After 3 hours in 500 µg/ml dithranol loading solution, one mg of the stratum corneum absorbed 1.5 (±0.6, n = 3) µg of dithranol at 20°C. Different results ($p < 0.1$) of 2.4 (±1.3, n =3) and 1.3 (±0.3, n = 4) µg of dithranol per one mg of the skin at 5°C were obtained after 3 hours and 2 days, respectively. However, this indicates that approximate 1 - 2 µg of dithranol was taken up by each 1 mg of stratum corneum from the loading solution and that this is not affected by temperature ($p > 0.1$). Equilibrium was achieved in 3 hours and remained constant for at least 2 days.

In duplicated experiments at 20°C, after 24 hours contact between stratum corneum and dithranol loaded hydrogel (area of 1.77cm²), 77 and 115 µg of dithranol

remaining in the gel and 2.7 and 4.5 µg were detected in the stratum corneum. This corresponds to average 0.7 µg/mg of dry stratum corneum and indicates an average partition coefficient between dithranol gel and stratum corneum of 27. It was noted that the amount of dithranol absorbed into stratum corneum per unit area was higher from loaded hydrogel than from soaking in the loading solution. This is not understood, but may be related to the swelling of the stratum corneum in the solution, increasing the area for absorption. Time prevented further investigation of the observation.

Non-aqueous receptor medium

The loading vehicle was used as a receptor medium on the assumption that it would give a fast passage through the stratum corneum by providing good solubility in the receptor. The results are shown in Table 6.2.

It is shown that the passage of dithranol through stratum corneum into the lipophilic solvent system is slow. This is different from the release without stratum corneum, shown in Chapter 5 (Table 5.5) and thus provides evidence that stratum corneum is the rate limiting step in the system.

Table 6.2 Passage of dithranol from loaded hydrogel, through stratum corneum into loading solution receptor medium at 5°C. (n=1).

Time	Hydrogel		Stratum corneum*	receptor
	dithranol	danthrone	dithranol	dithranol
hour	(µg)	(µg)	(µg)	(µg)
4	180	< 1	1.4	< 0.5
24	148	< 1	1.5	< 0.5

NB : * - Prior to use , the stratum corneum had not been soaked in water for 2 days as mentioned in the general method.

Some observations made during this work were used for subsequent improvement of methods. Firstly, the use of an amber glass bottle in this initial part of the study was

an attempt to provide light protection whilst allowing the experiment to be observed. However, the arrangement was found not to give an even mixing of the receptor fluid and so a diffusion cell, shown in Fig.6.1 was adopted for subsequent work.

Secondly, it was difficult to operate at 5°C, particularly due to the temperature-dependent properties of the hydrogel in contact with the stratum corneum. Leakage of the liquids from inside or outside the cell was found. For this reason, a temperature of 20°C was used. The rate of oxidation of the dithranol in the aqueous media at this temperature has been quantified earlier in Chapter 3.

Thirdly, concentration level of dithranol detected in samples of stratum corneum is low. This can only be detected by using the spectrofluorimetric analysis (described in Chapter 2). As a consequence, there is no method for detection of danthrone and dimer, because both require high concentration levels for analysis using hplc. Similarly, the receptor medium was analysed using spectrofluorimetry only, thus danthrone and/or dimer cannot be measured. It was decided that both analytical methods would be used to gain a better understanding of dithranol passage and decomposition.

Finally, it was found that fully hydrated stratum corneum must be used in order to obtain a close contact between the skin and hydrogel. The time and type of solution used to achieve this had to be adjusted according to the condition of the stratum corneum. For example, it was found that soaking the skin for 30 minutes did not allow complete hydration so that the skin swelled further when acetate buffer (pH 2.8) was used as the receptor. The problem was solved by soaking stratum corneum in water for 2 days. Acetate buffer (pH 2.8) had been tried for hydration as it was used as the receptor medium. Leakage

of stratum corneum which has been soaked in the buffer was found during the release experiments. Therefore, water was used throughout the subsequent studies.

Aqueous receptor media

The results showing the passage of dithranol from hydrogel through stratum corneum into aqueous acetate buffer at pH 2.8, are shown in Table 6.3. This buffer was employed because of the results of the direct release studies from hydrogel into the media had shown good stability as detailed in Chapter 5.

Table 6.3 Passage of dithranol from loaded hydrogel, through stratum corneum into acetate buffer at pH 2.8 (receptor medium) at 20°C expressed as mean (sd) of the amount of dithranol and danthrone (n=2)

Time	Hydrogel		Stratum corneum	receptor medium	
	dithranol	danthrone	dithranol	dithranol	danthrone
hour	(μg)	(μg)	(μg)	(μg)	(μg)
1	196 (19)	1.9 (1)	0.7 (0.2)	0.5(0.1)	0.03(0.00)
3	217 (2)	2.9 (0)	1.2 (0.1)	1.2(0.1)	0.06(0.02)
5	179 (5)	2.5 (0)	1.5 (0.1)	2.4(0.7)	0.09(0.02)
7	182 (22)	1.8 (0)	1.1 (0.2)	3.2(0.4)	0.13(0.01)
24	128 (14)	3.4 (1)	1.2 (0.4)	0.5(0.1)	0.10(0.01)
48	48 (1)	14 (0)	0.9 (0.2)	0.7(0.1)	0.16(0.02)

The results shown in Table 6.3 indicate that the passage of dithranol from the hydrogel through the stratum corneum into the buffer increased from 0.5 μg after 1 hour to 3.2 μg after 7 hours. This is, therefore, a slow process. The amount of dithranol in the aqueous receptor then decreased from 3.2 μg (7 hours) to 0.5 μg (24 hours) to 0.7 μg after 48 hours, whilst danthrone had changed slightly from 0.13 μg (7 hours) to 0.099 μg (24 hours) to 0.16 μg (48 hours). This indicates a simultaneous process of release and decomposition of dithranol. The total amount of dithranol released could have been calculated if the total quantity of dithranol and its derivatives is known. This could not be achieved in this study because of the limitation of the analytical procedure.

Other aqueous sovents were investigated in order to see the effect of pH. Phosphate buffer at pH 7.0, acetate buffer at pH 0.5, 5.0, and distilled water (measured pH of 5.5) were used and the results are shown in Tables 6.4, 6.5 and 6.6.

Because of the instability of dithranol after 24 hours, shown previously in Table 6.3, it was anticipated that lowering the pH of the buffer would assist the stability of dithranol. The results using acetate buffer at pH 0.5 and 5.0, shown in Tables 6.4 and 6.5, respectively, indicate the similarity in the passage of dithranol into acetate buffer at pH 0.5 after 24 and 48 hours, compared to that at pH 2.8 (Table 6.3). Using the buffer at pH 5.0 as the receptor (Table 6.5) gave slightly different results from those obtained using the buffer at pH 2.8 (Table 6.3). After 2 hours, no dithranol was detected in the pH 5.0 receptor whereas after 1 hour 0.5 µg of dithranol and 0.26 µg of danthrone were seen in the pH 2.8 medium.

Table 6.4 Passage of dithranol from loaded hydrogel, through pretreated stratum corneum into acetate buffer at pH 0.5 (receptor medium) at 20°C expressed as mean(sd) of the amount of dithranol and danthrone (n=2)

Time	Hydrogel		Stratum corneum	receptor medium	
	dithranol	danthrone	dithranol	dithranol	danthrone
hour	(µg)	(µg)	(µg)	(µg)	(µg)
24	**144** (4)	**3.5** (1)	**1.2** (0.3)	**0.5**(0.1)	**0.1** (0.0)
48	**74** (3)	**14** (0)	**0.9** (0.3)	**0.7**(0.1)	**0.1** (0.0)

Table 6.5 Passage of dithranol from loaded hydrogel, through stratum corneum into acetate buffer at pH 5.0 (receptor medium) at 20°C expressed as the amount of dithranol and danthrone (n=1 at 2 hours and n = 2 at 24 hours)

Time	Hydrogel		Stratum corneum	receptor medium	
	dithranol	danthrone	dithranol	dithranol	danthrone
hour	(µg)	(µg)	(µg)	(µg)	(µg)
2	**197**	< 1	-	< 0.003	**0.1** (0.0)
24	**99** (0)	< 1	**2.3** (0.6)	**0.3**(0.1)	**0.2** (0.0)

The results in Table 6.6 using distilled water (pH 5.5) as the receptor give similar results from those obtained using the buffer at pH 2.8 (Table 6.3). After 24 and 48 hours, dithranol and danthrone could be detected in both media and stratum corneum at about the same concentration. The amounts of dithranol remaining in the films are different.

Table 6.6 Passage of dithranol from loaded hydrogel, through stratum corneum into distilled water at measured pH 5.5 (receptor medium) at 20°C expressed as mean(sd) of the amount of dithranol and danthrone (n=2)

Time	Hydrogel		Stratum corneum	receptor medium	
	dithranol	danthrone	dithranol	dithranol	danthrone
hour	(µg)	(µg)	(µg)	(µg)	(µg)
24	98 (0)	4 (1)	1 (0.1)	0.1(0.2)	0.1 (0.0)
48	14 (8)	6 (3)	0.8 (0.2)	0.7(0.1)	0.1 (0.0)

Table 6.7 Passage of dithranol from loaded hydrogel, through stratum corneum into loading solution receptor medium at 20°C expressed as mean(sd) of the amount of dithranol and danthrone (n=2)

Time	Hydrogel		Stratum corneum	receptor medium	
	dithranol	danthrone	dithranol	dithranol	danthrone
hour	(µg)	(µg)	(µg)	(µg)	(µg)
1	181 (3)	< 1	0.7 (0.3)	< 0.1	< 0.5
3	195 (2)	< 1	0.6 (0.1)	< 0.1	< 0.5
24	135 (5)	< 1	1.9 (0.0)	1.0 (0.2)	< 0.5
96	17 (7)	10 (4)	0.7 (0.3)	1.3 (0.4)	0.5 (0.06)
120	< 1	12 (4)	-	< 0.1	0.8 (0.02)

Results shown in Table 6.7 are obtained by using loading solution vehicle which is less hydrophilic than the buffers and water discussed. The release of dithranol from the hydrogel into this solvent system was discussed in Chapter 5 (Table 5.5). It was shown that during those studies release from the hydrogel into the medium was complete within 20 minutes and the ratio of amount of dithranol detected in the medium to that remaining in the hydrogel was 49 to 1. The results shown in Table 6.7 are comparable to those in Table 6.2, although different in

some details. These results were obtained using the same medium but different types of cells, temperatures and volume of the medium. This suggests that the passage of dithranol from the hydrogel through stratum corneum into the blank vehicle of loading solution was a complicated process and that the changes have cancelled each other out. These may include the effect of the solvent system on the passage of dithranol or an alteration of the barrier structure of the stratum corneum. These were not experienced when aqueous solvent systems were used and were not investigated further.

However, the results do confirm that the passage of dithranol from the hydrogel through stratum corneum was limited by the skin. In these release studies, the amount of dithranol present in the stratum corneum varied from 0.5 to 2 μg, in good agreement with that taken up from the loading solution. In comparison with the amount of dithranol present in the hydrogel, that in the stratum corneum was very small, the ratio being approximately 150 to 1. This effect occured with both aqueous and non-aqueous receptor solutions and so indicates that the stratum corneum of normal skin may be the rate-limiting step in the percutaneous absorption of dithranol.

The release study in acetate buffer at pH 2.8 produced a linear relationship (correlation coefficient of 0.991) between the amount of dithranol in the receptor solution per unit area against time over a 7 hour time period (Fig. 6.2). The results obtained from 24 and 48 hours are not used because of dithranol decomposition as discussed above. The area used in the evaluation is the open area of the diffusion cell which is 2.01 cm^2. The area of the hydrogel which is in contact with the stratum corneum is 1.77 cm^2. The diffusion of dithranol from the hydrogel in this series of studies was performed by close contact between the skin, which takes up dithranol to an extent, and the liquid media. There is only a slight

contact between the skin, which takes up dithranol to an extent, and the liquid media. There is only a slight difference between the diameter of the hydrogel and the open face of the cell, 0.1 cm. The effective area of release should therefore be considered to be that of the skin to allow for any lateral diffusion of the drug.

Table 6.8 shows the amount of dithranol per unit area each detected in the receptor at each time, the plot of which is shown in Fig.

Table 6.8 Apparent permeation of dithranol from loaded hydrogel, through stratum corneum into acetate buffer at pH 2.8 (receptor medium) at 20°C: expressed as mean of amount of dithranol detected in the medium per area (μg/cm², n=2).

Time (hour)	dithranol (μg/cm²)
1	0.24
3	0.58
5	1.29
7	1.58

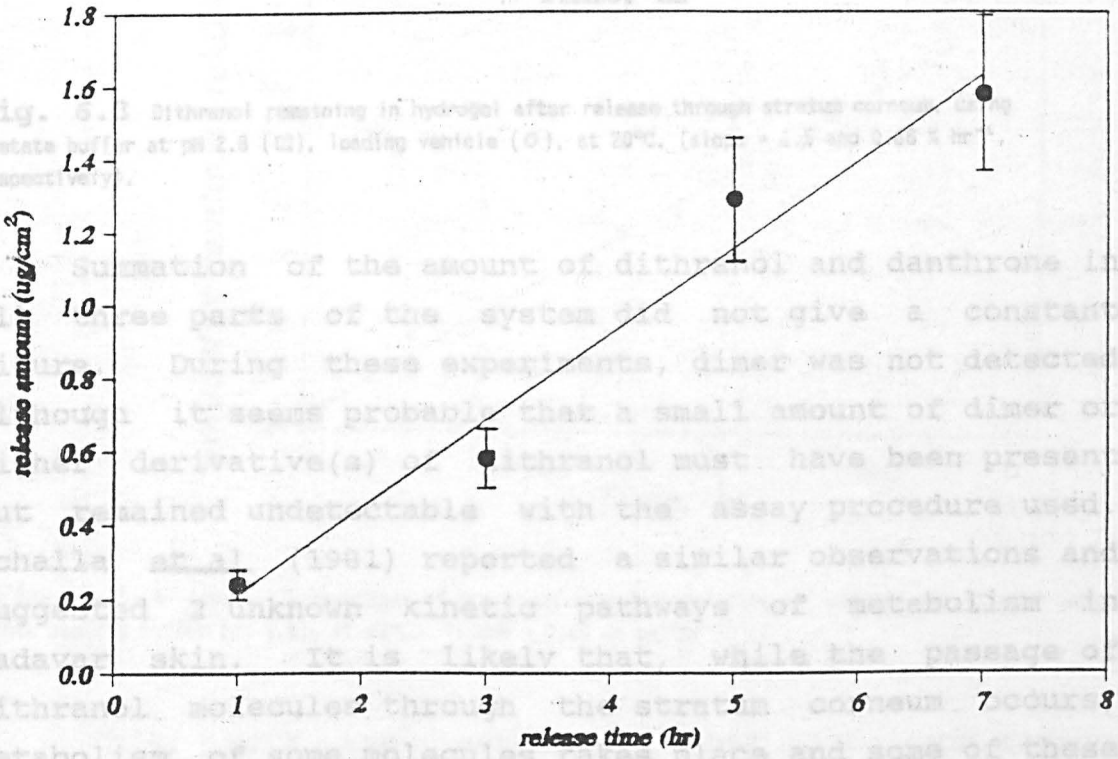


Fig. 6.2 Apparent permeation of dithranol from hydrogel through neonatal rat stratum corneum into acetate buffer (pH 2.8), at 20°C. (slope = 0.234 μg $cm^{-2}hr^{-1}$)

media are shown in Fig. 6.3 (correlation coefficients being 0.978 and 0.973, respectively). Slopes of the plots are used to estimate rates and half-lives of dithranol release from the hydrogel through stratum corneum. These rates are 1.5 and 0.86 % hr^{-1} in aqueous ($t_{1/2}$ = 33 hours) and non-aqueous ($t_{1/2}$ = 58 hours) receptors, respectively.

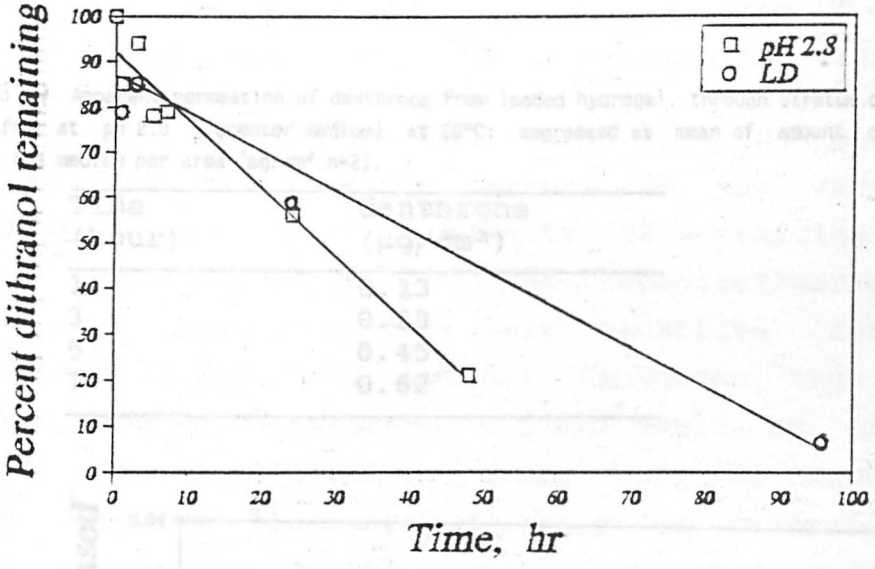


Fig. 6.3 Dithranol remaining in hydrogel after release through stratum corneum, using acetate buffer at pH 2.8 (□), loading vehicle (○), at 20°C. (slope = 1.5 and 0.86 % hr^{-1}, respectively).

Summation of the amount of dithranol and danthrone in all three parts of the system did not give a constant figure. During these experiments, dimer was not detected although it seems probable that a small amount of dimer or higher derivative(s) of dithranol must have been present but remained undetectable with the assay procedure used. Schalla et al (1981) reported a similar observations and suggested 2 unknown kinetic pathways of metabolism in cadavar skin. It is likely that, while the passage of dithranol molecules through the stratum corneum occurs, metabolism of some molecules takes place and some of these metabolites pass through and are detected. This is known

to be the case in the present work because dithranol decomposition in acetate buffer (pH 2.8) has been quantified in Chapter 3, and in the buffer after it has been released from hydrogel in Chapter 5. On this basis, it is possible to treat the results of danthrone (Table 6.2) in the same way as those of dithranol and calculated a rate of passage per unit area. This is shown in Table 6.9 and Fig. 6.4.

Table 6.9 Apparent permeation of danthrone from loaded hydrogel, through stratum corneum into acetate buffer at pH 2.8 (receptor medium) at 20°C: expressed as mean of amount of danthrone detected in the medium per area (μg/cm², n=2).

Time (hour)	danthrone (μg/cm²)
1	0.13
3	0.28
5	0.45
7	0.62

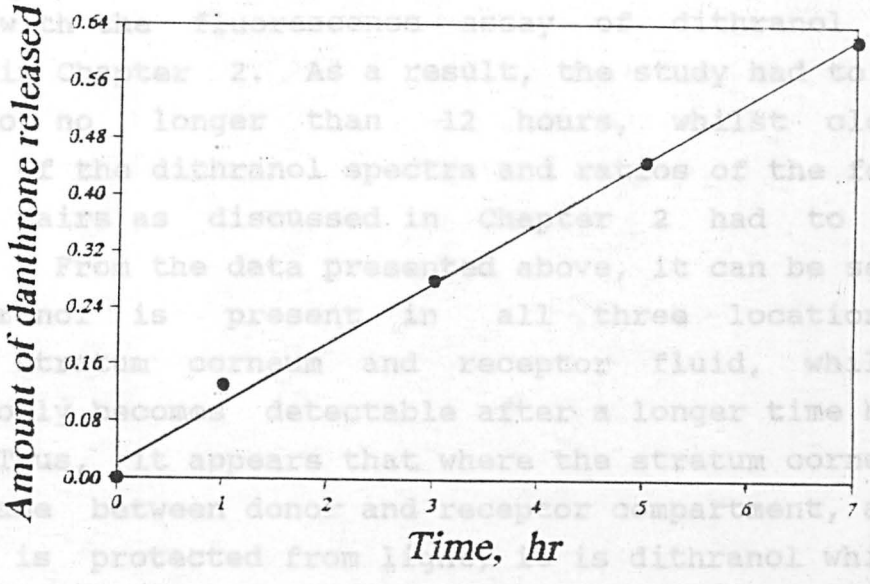


Fig. 6.4 Apparent permeation of danthrone from hydrogel through neonatal rat stratum corneum into acetate buffer (pH 2.8), at 20°C. (slope = 0.09 μg $cm^{-2}hr^{-1}$).

This work has establshed that 0.4 (non-aqueous receptor) to 0.8 (aqueous receptor) $\%cm^{-2}hr^{-1}$ of dithranol has been delivered by the hydrogel into stratum corneum to produce about 0.2 μg dithranol per 1 mg of the stratum

corneum, thus the stratum corneum, not the nature of the receptor, providing the rate limiting factor. The maximum flux achievable is 0.2 μg $cm^{-2}hr^{-1}$. Depending on the pH, the solubility of dithranol is greatly reduced in the aqueous receptor, so that significant oxidation may be anticipated *in vivo*[Schalla *et al* 1981, Cavey *et al* 1985]. Thus, the hydrogel has been demonstrated to deliver dithranol to viable epidermis at a constant rate of over a time period of at least 7 hours in a reproducible manner.

Analytical procedures are one of the problems encountered in this study because of the very low concentrations and the complexity of vehicles and substances being assayed. The spectrofluorimetric analysis was found to be very sensitive for the determination of dithranol but not danthrone, thus hplc had to be used for danthrone. Dimer could be present below the detection limit of hplc but was found to interfere with the fluorescence assay of dithranol as discussed in Chapter 2. As a result, the study had to be limited to no longer than 12 hours, whilst close monitoring of the dithranol spectra and ratios of the four wavelength pairs as discussed in Chapter 2 had to be maintained. From the data presented above, it can be seen that dithranol is present in all three locations: hydrogel, stratum corneum and receptor fluid, whilst danthrone only becomes detectable after a longer time has elapsed. Thus, it appears that where the stratum corneum is a membrane between donor and receptor compartment, and the system is protected from light, it is dithranol which diffuses through without oxidation. The oxidation which did occur in the stratum corneum only appears to be significant at longer contact times. However, it is recognised that the lack of a suitable analytical method to monitor the low concentration of danthrone and dimer in the stratum corneum and receptor limits the ability to fully evaluate the behaviour of dithranol in this *in vitro* model. Despite this, the results are probably valid for

predicting the use of the system *in vivo*. Dithranol in Lassar's Paste or a propietary form is normally left in contact with the skin for no more than 12 hours. The evidence obtained in these studies indicates that the dithranol should maintain its stability during this time. One potential advantage of the hydrogel would be that it may give a more controlled release over a longer time than conventional administration systems. Indeed, this data suggests that instability would not be a problem during the first 24 hours and may not become significant until 4 or 5 days have elapsed.

CHAPTER 7

CLINICAL STUDIES

7.1 INTRODUCTION

Since the beginning of the century, the use of dithranol has been limited by unacceptable staining of the skin and clothing and contact irritation of uninvolved skin. There is, therefore, a strong requirement for the development of an alternative method of applying dithranol preparations which restricts it to the plaque in order to reduce irritation. The two most commonly used methods in clinical practice today are the Ingram regimen [Ingram, 1953] and Short contact therapy [Farber & Nall, 1984], but neither can adequately prevent irritation and often require in-patient treatment.

This study was aimed at assessing the clinical effectiveness and performance of the dithranol hydrogel developed in this work including a comparison with a currently used Dithrocream. Additionally, the potential benefits for use in psoriatic patients in confining the area of dithranol application to the affected area, convenience in use and any reduction in staining of clothes were also to be investigated. Because extensive *in vitro* data was available, it was hoped to obtain an indication of the therapeutic effective dose at the site of action. The study was based in the dermatology Out-Patient Department and Ward 29, Aberdeen Royal Infirmary. Although psoriasis can be classified into 2 categories, namely visible and non-visible psoriasis (clinically non-

apparent to latent psoriasis) (Farber & Nall 1984), only the former was used in this investigation.

7.2 EXPERIMENTAL METHODS

A preliminary study involving normal skin was carried out to observe whether dithranol side effects (staining and irritancy) after application of dithranol loaded hydrogel would occur and an approximate time course for these reactions. These were performed in comparison to commercially available Dithrocream of various strengths and Dithrostick on healthy volunteers. The results were used as an indicator for the later protocol. Five volunteers, 4 male and 1 female, participated in this experiment. Only the skin on the inner sides of the lower forearms of each volunteer was involved. A loaded hydrogel (containing 10 µg dithranol per 1 mg dry hydrogel, see p 123), 0.1%, 0.25% and 1% Dithrocream and 0.5% Dithrostick were placed on 1 cm patch test discs which were fastened to the skin by adhesive bandage. Normal activity such as taking a bath, was encouraged. The side effects of dithranol (namely staining and burning) were assessed by a consultant dermatologist after 24 hours and any other changes were noted.

A further <u>in vivo</u> release study and the effect of dithranol hydrogel on psoriatic plaque were investigated in 2 male volunteers (patients no. 6 and 7) with varied levels of initial severity, as shown in Table 7.1. The loaded hydrogel of 2.5cm x 2.5cm, containing 0.5 mg (allowing 10% deviation) of dithranol and the same size of blank loaded hydrogel were placed on the same plaque. The hydrogels were held in place and protected from the environment by covering with a piece of OpSite I.V. (6cm x 8.5 cm, #4290, Smith T.J. & Nephew, Ltd., Hull). After 1, 3, 5, 14, 24, 48, and 96 hours, the films were removed and analysed using the hplc method (details in Chapter 2,

p 42). The clinical evaluation, see later (p 161) was performed by the same consultant dermatologist. The individual results obtained from each patient are shown in Table 7.1. The average of percentage of dithranol remaining and the percentage of score (% score) and staining are shown in Table 7.2.

Comparative studies on the clinical effectiveness of the dithranol hydrogel, blank hydrogel and Dithrocream were further investigated with 6 inpatients. A plaque, 1 inch or more in diameter and well separated from other affected areas was used for the study.

Freshly loaded hydrogel films (5cm x 5cm, containing 1 mg of dithranol) and identical films loaded with blank loading solution were prepared, transported and stored at 5°C (±1°C) as discussed in Chapter 4 for use up to 7 days after preparation. (Dithranol stability during storage time in Ward 29 has been monitored and showed to be in line ($p<0.05$) with previous results in Chapter 3.) Care had been taken not to use films which were found defective, for example brown, spots or shrinking.

A loaded hydrogel and the blank loaded hydrogel were placed on the selected plaque. Dithrocream (0.1%) was applied to the same plaque using an amount of the cream (1 cm in length) which contained the same quantity of dithranol as the hydrogel. The hydrogels were held in place and protected from the environment by covering with OpSite. Both dithranol and blank patches were changed and additional applications of the cream were made at the same time for each patient.

Intervals between these changes varied. The protocol aimed at changing dithranol on a once a day basis. However, because of the possible clinical side effects, the need to respond to clinical changes had to be available. Thus, the initial application was for 2 days.

Depending on the reaction of the patient, subsequent applications were made daily, every second day or twice weekly. This was continued for 14 days except with one patient who showed burning after 7 days (at which point the treatment was stopped). The mean frequency of application was 1.5 days. Wilcoxon signed rank statistics were used and 95% confidence limits calculated. The protocol was approved by the Joint Ethical Committee Grampian Health Board and the University of Aberdeen.

Clinical assessment - The treated and control areas were clinically assessed for erythema, thickness and scaling, as well as staining and irritancy. Each modality was graded on a 0-4 scale of increasing severity (0 = absent, 1 = trace, 2 = mild, 3 = moderate, 4 = severe). These were assessed at the start and finish of the treatment by the same consultant dermatologist. Fig. 7.2 illustrates an example of the clinical observation after 3 days of treatment.

Quantitative assessment - Dithranol loaded hydrogel patches after use were randomly sampled and assayed for dithranol and its derivatives by hplc (system 2 on p 37 and method p 44).

7.2 RESULTS AND DISCUSSION

The application of dithranol hydrogel to normal skin of healthy volunteers in the preliminary study indicates that dithranol was released into the skin. After about 5-6 hours, discolouration on the sites of some applications was observed. No severe irritancy due to dithranol hydrogel was found. After 24 hours, erythema and staining occurred where the hydrogel and each Dithrocream were administered but not from 0.5% Dithrostick. The degrees of staining were found to be approximately the same for the hydrogel and 0.1% Dithrocream, suggesting possible

bioequivalence of the two. It appears that little dithranol reached the skin from Dithrostick. This may be due to poor release characteristics or problems of ensuring adequate contact in the test procedure used. The degree of erythema and staining of dithranol from the cream with various strengths varied with the concentration of dithranol. It has been reported that dithranol erythema appears after 24 hours and is maximised 48-72 hours after application using chloroform solution on normal skin [Juhlin, 1981, Misch et al, 1981]. Thus, the results obtained in this study are in good agreement with the literature reports. Because of the obvious staining of the skin by dithranol, double blind investigations could not be used for the clinical studies.

The detailed quantitative assessment of dithranol in the hydrogel and clinical evaluation was used in an attempt to establish a therapeutically effective dose of dithranol on psoriatic skin. Two volunteers, patients no. 6 and 7, were involved in this study, results of which are shown in Table 7.1 From the clinical assessment results shown in the table, the total initial scores of patients no. 6 and 7 were 12 and 7, respectively. This indicates that the initial degree of severity of psoriasis was not the same. However, the amount of dithranol and danthrone present in the films over 96 hours of investigation was not significantly different ($p > 0.05$), indicating similar behaviour of dithranol in the film. This implies that the difference in the initial severity of psoriasis did not affect the release pattern or stability of dithranol.

Although the total scores from both patients assessed at each time interval were significant difference ($p > 0.05$), staining due to dithranol was not different ($p < 0.05$). As a result of this, the results shown in Table 7.1 were averaged during the processing detailed below.

Table 7.1 Quantitative assessment of dithranol and danthrone, (average of 2 analysis), μg, detected in the films after application and the total score and staining from clinical assessment in patients no 6 and no 7.

Time	patient no. 6				patient no. 7			
	Quantity		Clinical		Quantity		Clinical	
	Dithranol	Danthrone	Score[1]	Staining	Dithranol	Danthrone	Score[1]	Staining
(hr)	(μg)	(μg)			(μg)	(μg)		
1	307	<1	12	0	450	<1	7	0
3	263	<1	12	1	293	<1	7	1
5	124	<1	12	1	140	<1	7	2
14	32	25	12	1	25	22	6	3
24	<1	27	10	3	<1	28	5	4
48	<1	27	10	4	<1	22	5	4
96	<1	32	9	4	<1	26	3	4

Key Score[1]= total score = erythema + scaling + thickness (maximum = 12).

Clinical assessment was made on a 5 point scale so that clinical judgement could be made without confusion (Omerod, personal communication). However, for the purpose of this analysis, the scales were converted to percentage in order to allow comparison with the quantitative analysis. The percentages of total score and staining were, therefore, calculated from the maximum total score and staining of 12 and 4, respectively, and a percentage "improvement" is obtained by subtracting this from 100, i.e. 100 - (% total score). The results, shown in Table 7.2, indicate that the percentage of dithranol remaining in the film decreased rapidly, reaching 6% in 14 hours.

The relationship between the percentage of dithranol remaining in the films and time is linear over 14 hours (correlation coefficient = 0.91 and slope = -4.97). This is also the cases for percentages of total score (from 5 to 96 hours) and staining (from 0 to 48 hours) against time (correlation coefficients = 0.933 and 0.955, respectively). This indicates the possibility of relating the three variables evaluated, namely %dithranol remaining in the films, %total score and %staining.

Table 7.2 The mean (sd) of the percentage of the amount of dithranol remaining in the film, the total score and staining degree from the results in Table 7.1.

Time	%Dithranol	% Clinical		
hour	remain	Total scores	staining	improvement
0	100	100	0	0
1	77 (20)	100 (0)	0 (0)	0
3	56 (4)	100 (0)	25 (0)	0
5	27 (2)	100 (0)	38 (18)	0
14	6 (1)	93 (10)	50 (35)	7
24	<0.2	77 (8)	88 (18)	23
48	<0.2	77 (8)	100(0)	23
96	<0.2	59 (23)	100(0)	41

Key Score[1] = total scores (= erythema + scaling + thickness)

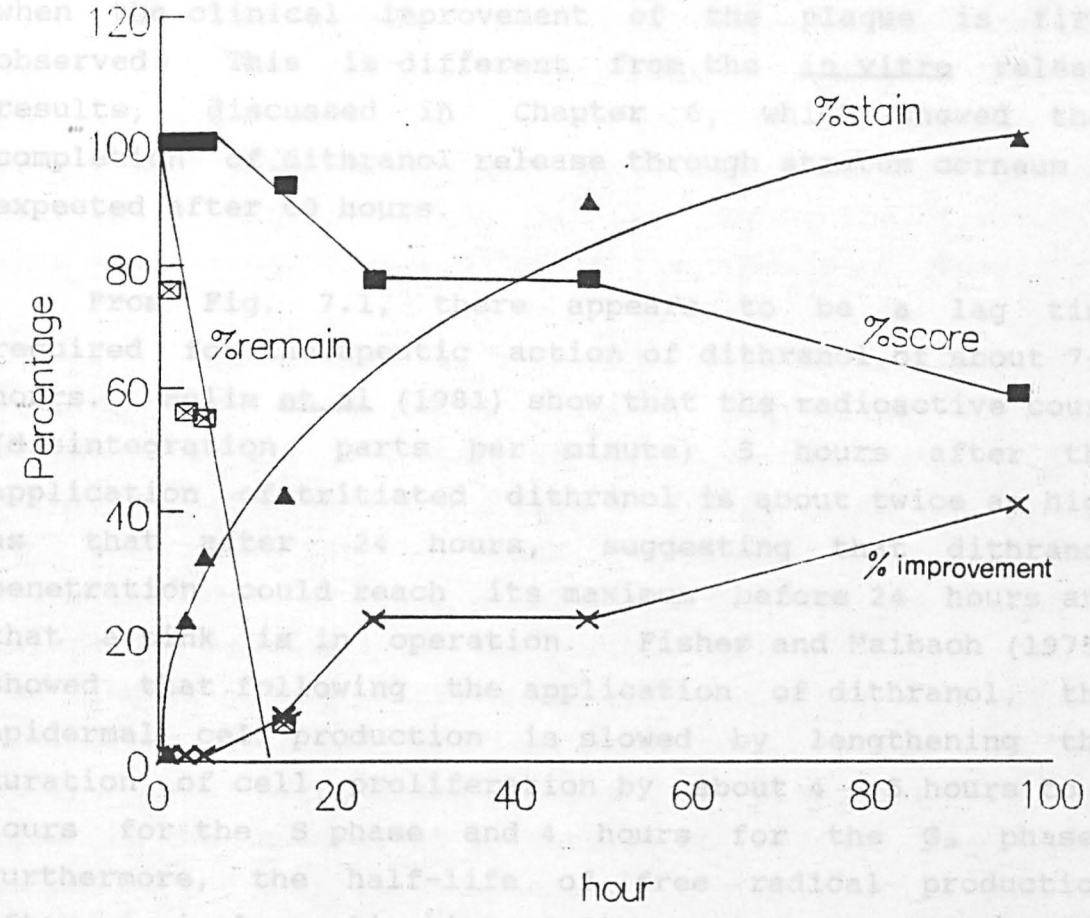


Fig. 7.1 Time course of percentage of total scores, clinical improvement, staining and amount of dithranol remaining in the hydrogel.

Fig. 7.1 illustrates the linear relationships between application time and the percentage of dithranol remaining in the film, score, staining and improvement. Dithranol staining could be observed 3 hours after application and increased at a similar rate to clinical improvement although the time lag for dithranol staining was shorter (about 2 hours) than for clinical improvement (7 hours). From this evidence (and the knowledge about the behaviour of the film reported in earlier chapter 5), it can be concluded that the decrease of the amount of dithranol remaining in the film was due to dithranol release from the film into the skin. However, the amount of dithranol released could not be obtained by simple subtraction from that remaining because of the possibility of dithranol decomposition. It could be concluded, however, that the release of dithranol is almost complete after 14 hours when the clinical improvement of the plaque is first observed. This is different from the *in vitro* release results, discussed in Chapter 6, which showed that completion of dithranol release through stratum corneum is expected after 60 hours.

From Fig. 7.1, there appears to be a lag time required for therapeutic action of dithranol of about 7-8 hours. Selim *et al* (1981) show that the radioactive count (disintegration parts per minute) 5 hours after the application of tritiated dithranol is about twice as high as that after 24 hours, suggesting that dithranol penetration could reach its maximum before 24 hours and that a sink is in operation. Fisher and Maibach (1975) showed that following the application of dithranol, the epidermal cell production is slowed by lengthening the duration of cell proliferation by about 4 - 5 hours to 6 hours for the S phase and 4 hours for the G_2 phase. Furthermore, the half-life of free radical production after a single application of dithranol to both intact and epidermis-free skin was between 3-5 hours [Shroot & Brown, 1986]. These pieces of evidence indicate that, after

dithranol is applied to the skin, a reaction should be observed between 3 to 6 hours if either of these mechanism is involved. The results obtained from this study are not far from this. These reports, therefore, suggest that after its passage into the skin, transformation of dithranol can be expected, via a free radicals route. After 24 hours, elimination would be expected, indicating a need to reapply the drug.

Additionally, a preliminary test of the solvent and the occlusive effects of the hydrogel was made at this stage. One hydrogel, loaded with a blank loading solution (without dithranol), was applied to a psoriatic plaque of patient no 6 for 24 hours. It was found that the clinical assessment before and after the application was identical. This was not conclusive because the result was obtained from only one experiment and it is possible that the time course for any clinical effect may be longer than 24 hours. However, it indicates that the solvents do not have short term effect and that any occlusion is not clinically significant on its own during the first day. This was further investigated in the subsequent study.

The time course of dithranol erythema was studied by Juhlin(1981) who showed that a degree of dithranol erythema of 83% was observed after 72 hours when 0.1% of dithranol in methacrylate film on a paper disc was applied to the skin for 24 hours. Neither this, nor the investigations of Misch et al (1981), showed the amount of dithranol passing into the normal skin. The time course and degree of the reaction reported, nevertheless indicate similarity with the clinical assessment obtained in this study. The burning and staining of dithranol were not used in the subsequent investigation because the total score of the clinical assessment was sufficient to provide information on the comparative therapeutic effect. However, it is noted from the results in Table 7.2 that there is a possibility of the correlation of staining and

clinical improvement. This will be discussed later in Chapter 8.

So far, it has been shown that the dithranol hydrogel is therapeutically effective to an extent. The experience can then be used in the design of a study to compare the new dosage form with a conventional one. A comparative study was thus established between the dithranol hydrogel, blank hydrogel and Dithrocream at 0.1%. Dithrocream was chosen because it is a recently developed product [Seville et al, 1979] which has gained better patient tolerance and is more convenient than traditional ointment and paste preparations [Shroot et al, 1981]. The concentration of 0.1% Dithrocream was chosen because of the results obtained from the preliminary study in 5 normal volunteers mentioned earlier.

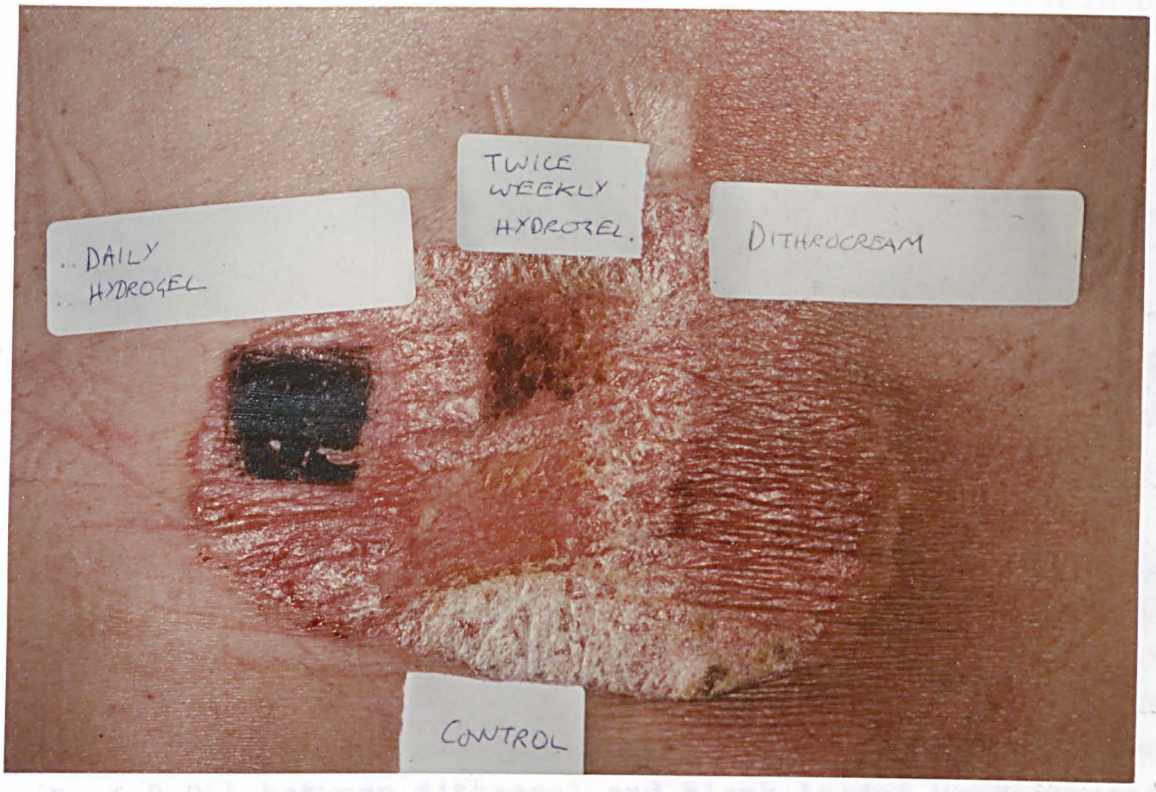


Fig. 7.2 A plaque treated with dithranol loaded hydrogel re-applied daily (DAILY HYDROGEL), twice weekly (TWICE WEEKLY HYDROGEL), 0.1% Dithrocream (DITHROCREAM), and blank loaded hydrogel (CONTROL).

The typical appearance of a plaque is illustrated in Fig. 7.2 which was taken after 14 days of treatment. On the left of the plaque, the dithranol hydrogel patch had been replaced daily, whilst that in the upper middle of the plaque were replaced twice weekly. On the right, Dithrocream 0.1% was applied daily. In the lower middle, blank loaded hydrogel was replaced twice weekly as a control. It shows dithranol staining on the application sites of dithranol hydrogel and Dithrocream. The spreading of the cream to the normal skin is also shown by the darker shade of the skin to the right of the plaque, whereas there is a sharp edge to the hydrogel stained areas. No clinical effect from the blank hydrogel is observed in this figure. The stained area of the plaque where dithranol was applied, either by the hydrogel or the cream, is softer and smoother than the other areas, including that where blank hydrogel was applied, indicating the lower degree of scaling when dithranol was used. The darkest area, where dithranol hydrogel had been replaced daily, was the softest area in this plaque and the twice weekly application hydrogel was smoother than the Dithrocream.

The results of the comparative study between the dithranol loaded hydrogel, blank loaded hydrogel and Dithrocream(0.1%) are shown in Table 7.3. Table 7.3 shows the individual and mean of the total scores before and after the use of dithranol hydrogel, blank hydrogel and Dithrocream (1%) in the treatment of 6 inpatients.

Significant improvement ($p < 0.05$) of psoriasis using dithranol hydrogel or Dithrocream was observed, whilst blank hydrogel produced a less significant improvement at the level of $p < 0.1$. Comparisons between treatments using different regimens, showed a significant difference ($p < 0.05$) between dithranol and blank loaded hydrogel and no significant different ($p = 1$) between dithranol

hydrogel and Dithrocream. Therefore, the clinical effectiveness of the hydrogel was observed.

Table 7.3 Clinical assessment of psoriatic plaques after 14 days treatment recorded as total score (maximum = 12) of psoriatic plaques before and after treatment using dithranol loaded hydrogel, blank loaded hydrogel and 0.1% Dithrocream.

Patient	Hydrogel				0.1%Dithrocream	
	dithranol loaded		blank loaded			
no.	before	after	before	after	before	after
8	6	3	6	5	6	1
9	9	3	9	5	9	3
10*	9	7	9	8	9	4
11	12	2	12	5	12	5
12	5	3	5	5	5	3
13	12	6	12	9	12	6
mean	8.4	4	8.8	6	8.8	3.7
sd	2.7	1.8	2.7	1.7	2.7	1.6
%improvement						
mean	52		32		59	
sd	21		22		15	

NB * stopped after 7 days.

The combined effect of dithranol and any occlusion or solvent effect of the hydrogel was more effective than the latter alone. There is a similarity in the therapeutic improvement when either dithranol hydrogel or Dithrocream are used. However, the spreading of dithranol to the nearby normal skin, which would cause erythema and inflammation, illustrated in Fig. 7.4, was not observed when dithranol hydrogel was used. The hydrogel could be cut to the size and shape of the lesions and left in place until it requires replacement. This may be regarded as the ultimate benefit for the patients in the development of this preparation.

It has been reported that occlusion affects psoriasis by reformation of granular cell layer and reduction of mitotic activity of cells [Baxter & Stoughton, 1970]. The

precise mechanisms of occlusion on psoriasis are not known but were postulated [Friedman, 1987] as follows: Occlusion would trap moisture for the hydration of the stratum corneum, thus preventing development of abnormal cells or occlusion could behave like the normal water barrier or insulator for the skin which is lacking in psoriasis. Furthermore, the efficacy of localised hyperthermia in the regression of psoriasis has been studied [Farber and Nall, 1984]. Occlusion by hydrogels may increase temperature locally in the skin and thus produced the same effect. The mechanism of this effect is not known at the present but may be related to a reduced cooling from evaporation of transepidermal water.

Chemical analysis of the dithranol films used in the comparative study was performed by random sampling from both unused and used films. All used films were brown when the samples were collected. Some patches were removed after 24 hours and some after 72 hours. There is no significant difference between the amount of danthrone in the films removed after 24 and 72 hours ($p < 0.05$), therefore the results were averaged and are shown in Table 7.4. The average concentration of loading solution used to load these films was 485 ± 5 µg/ml (n=24).

Table 7.4 Amount per unit area of dry hydrogel of dithranol, danthrone and dimer present in hydrogels before and after used in the comparative clinical study, expressed as mean(sd).

unused (n=12)	used (n=21)		
dithranol (µg/cm²)	dithranol (µg/cm²)	danthrone (µg/cm²)	dimer (µg/cm²)
202(15)	<0.9	3.5 (0.4)	< 1

The amount of dithranol per unit area of freshly prepared films shown in Table 7.4 is lower than those reported (between 225 and 240 µg/cm²) in Chapter 4

(p 123), but this is not significantly different ($p > 0.05$). This indicates that the production of dithranol hydrogel films could be adopted to meet the requirement of dermatologists, i.e. loading films previously cut or for cutting to the shape and size of a specific psoriatic plaque.

The tendency of dithranol decomposition in the films when in contact with the skin is seen to be toward danthrone rather than dimer. However, by the time of collection, all films were brown, suggesting that decomposition products other than danthrone and dimer, such as dithranol brown, had already formed. The standard deviation of the level of danthrone detected was low thus the amount of danthrone remained constant throughout the investigation.

CHAPTER 8

CONCLUSION

There is no doubt regarding the efficacy of topically applied dithranol for the treatment of psoriasis. The limitation of its use has been the incidence of side effects, indicating the need of a controlled release preparation. It was hoped that the loaded hydrogel developed in this study would be better than the conventional preparations in that it would confine the dithranol to the affected area of skin so that there would be no adverse effects on normal skin, and also provide a controlled delivery rate of dithranol. The main problems encountered in any product development of dithranol, including this hydrogel film, are dithranol stability and solubility [Whitefield, 1981b].

The investigation of drug stability, in general, involves the development of analytical methods which are shown to possess the necessary precision and sensitivity, for both the parent compound and its anticipated decomposition product(s). In this study, the hplc used was shown to be suitable for the determination of dithranol, danthrone and dimer. Danthrone and dimer are two of the three (or more) probable decomposition products. This method was used to assay the loading solution, hydrogel, and <u>in vitro</u> release (without stratum corneum). The hplc detection limits for dithranol, danthrone and dimer are 15, 1.5, 8 ng/ml (at a signal to noise ratio of 3). A spectrofluorimetric method was

devised to replace the hplc method for dithranol assay at lower than 15 ng/ml such as in the in vitro release where stratum corneum was involved. The detection limit of the spectrofluorimetry for dithranol is 0.2 ng/ml (at a signal to noise ratio of 3). Therefore, it was shown to have a sensitivity 75 times higher than hplc for dithranol assay.

The solubility and chemical instability of dithranol were significant in the loading, storage and release studies. The solubility of dithranol in aqueous media is about one thousandth of that in non-aqueous solvents. Basic polar solvents generally interfere with the intramolecular bonding so that ionisation of dithranol molecules occurs in and leading to chemical instability via a free radical chain reaction. Temperature and light have also been found to affect dithranol stability. The lower the temperature, the higher the stability. At -21°C, in an acid non-aqueous media with light protection, dithranol has been shown to be relatively stable. The hydrogel dissolves in most non-aqueous solvents investigated. It swells to a greater extent in aqueous media at lower temperatures than at higher temperatures. Thus, about 40 solvent systems have been investigated in the development of a dithranol solution which gave hydrogel swelling. A low loading temperature (5°C) was used because it provided the advantages of reducing dithranol decomposition and enhancing hydrogel swelling.

The swelling of the hydrogel is a three-dimensional process. An increase in the observed area and degree (%) swelling of hydrogel from its dry state was shown to correspond to an increase in its microvolume. For example, a 1-cm circular disc (0.022 cm thick and 0.017 cm^3) was shown to increase to 1.8 cm (0.04 cm thick and 0.10 cm^3). Thus, an approximate increase in diameter of 1.8 results in a 6 times increase in microvolume and the

film swelling to a degree of 593 pph (Table 4.3). This is also the case for 1.5 and 1.6 times increase in diameter with 130 and 320 pph, respectively. Therefore, by observation of area expansion, it was possible to estimate an approximate degree of swelling.

The resulting hydrogel contained 10 µg of dithranol per mg dry hydrogel or 230 µg/cm² dry hydrogel or 102 µg/cm² swollen hydrogel (twice increase in area from dry to swollen state). The uptake of dithranol was constant despite varying in size and shape of the hydrogel.

The presence of ferric ions affected the appearance of the dithranol hydrogel. A half-life of dithranol and a shelf-life (10% loss of dithranol) of 30 and 7 days, respectively, have been obtained. Chemical interactions between dithranol and polyethylene glycol are suggested [Kammareu et al, 1975] because of the unilaterally projecting oxygen atoms in the dithranol molecules producing a high dipole moment. A similar bonding might happen with acetone, water or dimethylsulphoxide, the solvents used in the loading solution. Any such hydrogen bond formation would result in the molecules being easily ionised and so liable to free radical chain reaction and decomposition.

The receptor media used in the in vitro release studies were chosen from a knowledge of dithranol stability and solubility. Because acetate buffer (pH2.8) gave the best combination of release and stability profiles from the direct in vitro release measurement, it was later used in the release through stratum corneum study. In vitro passage of dithranol was found to be a fast process, reaching its equilibrium within 1-2 hours indicating a partition coefficient between the hydrogel

and acetate buffer (pH 2.8) of about 1 , at 20°C. Hydrogel which have been subjected to prolonged dessication showed no significant difference in dithranol uptake but a slower *in vitro* release rate.

A non-aqueous receptor medium was used for some *in vitro* release rate determination, with and without stratum corneum to observe the effect of hydrophobic medium. The results are not useful for explaining the clinical work because human viable epidermis is an aqueous environment but did confirm that stratum corneum is acting as a rate-limiting membrane.

The effect of the receptor media had on the release from the hydrogel was seen when dithranol was released directly into the media, but not when released through stratum corneum. Neonatal rat stratum corneum was used for the *in vitro* release studies because it was not possible to obtain psoriatic scales in a sheet form or of sufficient size to allow permeability measurements. The rate of dithranol passage from the hydrogel through stratum corneum to the receptor medium (acetate buffer at pH 2.8) at 20°C was 0.234 $\mu g\ cm^{-2}\ hr^{-1}$.

From the quantitative data available, an attempt may be made to define the kinetics of dithranol in the skin. which is turn may be related to the clinical response.

Schalla *et al* (1981) used stripped skin to simulate the lower resistance to permeation found in parakeratosis. They reported an *in vitro* penetration rate of dithranol which was 2.5 times higher than that through normal human skin. Using the rate of depletion of dithranol from hydrogel as the source of data (Fig. 6.3 p 158 and Fig.7.1

p 168), a similar calculation can be made from the results obtained in this study. The rate of dithranol depletion through stratum corneum *in vitro* (neonatal rat) was 1.5 % hr^{-1} (p 158) whilst *in vivo* (psoriatic patients, p 168) it was 4.97 % hr^{-1}. Thus, there is a 3.36 times faster depletion rate with psoriatic patients than through the normal skin model. This figure is similar to that found by Schalla *et al* (1981) although direct comparison is probably not valid due to differences in methods and conditions. In particular neonatal rat stratum corneum will not behave in the same way as human skin. Additionally, Schalla *et al* did not report the temperature of their measurements (presumably at body temperature) whilst in the present work the *in vitro* data was obtained at 20°C whilst the *in vivo* data was at skin temperature, perhaps 35°C due to the occlusion. Temperature is known to affect the degree of swelling of the hydrogel (refer to p 107) and so is also likely to alter the diffusion rate of dithranol in the hydrogel. Despite these differences in detail, the trend is towards increased dithranol absorption through psoriatic plaques, with the evidence suggesting a 3 - fold increase in the flux.

This ratio of 3.36 can then be used to estimate the amount of dithranol being delivered into a psoriatic plaque. The *in vitro* release rate through neonatal rat stratum corneum was found to be 0.234 $\mu g\ cm^{-2}\ hr^{-1}$ (see Fig. 6.2, p 157). Therefore, by applying the factor of 3.36, the rate of delivery of dithranol to the psoriatic plaque viable epidermis would be $0.234 \times 3.36 = 0.79$ $\mu g\ cm^{-2}\ hr^{-1}$ for a 102 $\mu g\ cm^{-2}$ dithranol hydrogel.

There are now two sets of data derived from the present work which attempt to describe the amount of dithranol being delivered to the skin. Using the depletion of dithranol from the hydrogel *in vitro* there is

a depletion rate of 1.48 % hr^{-1} which, for an average 102 $\mu g\ cm^{-2}$ dithranol hydrogel, gives a depletion rate of 1.5 $\mu g\ cm^{-2}hr^{-1}$. However, the experimentally determined value into aqueous receptor is 0.234 $\mu g\ cm^{-2}hr^{-1}$. This suggests that about 85% of dithranol leaving the hydrogel is not being accounted for in the receptor solution. Because of the interplay of the data, the corresponding calculation made on the in vivo depletion and calculated flux, also indicates a loss of 85% dithranol.

A finite quantity of dithranol will be held in the stratum corneum to saturate its binding sites. From the results reported earlier (p 152), this will be 0.6 $\mu g\ cm^{-2}$ and so cannot explain the discrepancies being discussed.

Earlier work (Chapter 3) has established the instability of dithranol. These have shown the formation of the relatively stable danthrone and the dimer which is an intermediate towards the formation of the polymer dithranol brown. Overall dithranol was shown to have a half-life of about 3 days in aqueous system at 5.5 (p 73) and of about 1 day in acetone (p 79). The greater the potential for dithranol to enter into dipole interactions or hydrogen bonding with the system, the greater will be the rate of oxidation, particularly towards dimer. Thus, the simple in vitro measurements which have been possible in the present work, do not account for all the missing dithranol. However, Melo et al (1983) and Upadrashta and Wurster (1989) have found that decomposition of dithranol can be much higher in the presence of proteins, in particular albumins, so that the half-life is reduced and pH is altered. It should be that their work is relevant to the present discussion, although extensive additional experimental would be required to confirm this, which was not possible as part of this study. Another possibility is that large quantities of dithranol brown are formed.

Since no assay was available this cannot be checked. The discolouration of the solution *in vitro* (p 149), and skin *in vivo* (p 168), indicates that dithranol brown is present within two hours and continues to be formed over the normal time course of the experiments.

A discrepancy exists between the two sets of data on the rate of delivery can be explained qualitatively, but not yet quantitatively and so the accuracy of the figures cannot be confirmed. However, for an understanding of the clinical response, it is the amount of dithranol being delivered to the epidermis of psoriatic skin which is of importance. This can only be arrived at by using a derived figure as discussed above. The figure of 0.79 $\mu g\ cm^{-2}hr^{-1}$ was obtained from the direct measurement of dithranol appearance in an aqueous receptor having passed from the hydrogel and through stratum corneum (p 157). In that respect the figure is reliable, but had to be modified by the factor 3.36, this being the ratio of depletion rates of dithranol from hydrogel *in vitro* and *in vivo*. This ratio also is reliable up to the dithranol leaving the hydrogel. Any error would, therefore, arise between leaving the hydrogel and reaching the viable epidermis. The chemical composition of stratum corneum is known to be different in psoriasis, with changes to lipids, keratins, phosphate residue and water content [Hodgson, 1962, Rothberg, 1960, Mier & Cotton, 1976, Serup & Blichmann, 1987]. Any of these may affect the rate of oxidation of dithranol and so invalidate the figure of 0.79 $\mu g\ cm^{-2}hr^{-1}$. However, in the absence of any specific data to enable calculation of these effects, an approximate value of 0.8 $\mu g\ cm^{-2}hr^{-1}$ is the best figure for the delivery of dithranol to the viable epidermis of psoriatic plaque which can be obtained at present. It is likely to represent the maximum actual flux.

The clinical response to the dithranol hydrogel (Fig. 7.1, p 168) shows that these was a lag time of about 7.5 hours before any improvement could be seen. From the flux discussed above, it follows that a maximum of 5 - 6 $\mu g\ cm^{-2}$ of dithranol is required to bring about this clinical response. On the other hand, the *in vitro* studies have shown that dithranol passed through neonatal rat stratum corneum without any measurable lag time (Fig. 6.2, p 157). Since the permeability of psoriatic skin is likely to be higher [Schaefer *et al*, 1980, Schalla *et al*, 1981], there is no reason to expect that there is a lag time *in vivo* in the delivery of dithranol to the viable epidermis. The observed lag time in clinical response, must, therefore, arise from the time scale of action of the dithranol at its site of action. A relatively short time scale is involved. The various theories on the mode of action of dithranol were reviewed in Chapter 1. Of these, the best candidate to explain this observation, is an effect on the S and G_2 phases of cell proliferation which have been reported to change over 6 -10 hours following dithranol application [Fisher & Maibach, 1975].

In vivo release studies have indicated that there will be no further release from the hydrogel 24 hours after it has been applied (Table 7.2, p 168). Despite this, there continued to be an improvement in the clinical assessment until the end of the 96 hours observation period. Since no further dithranol was being supplied to the epidermis, another explanation must be found, of which a number of possibilities arise. The response observed could come from a rapid cellular change (during the time of dithranol delivery) which takes time to manifest itself in clinically observable symptoms, perhaps because it occurs at a lower level of the epidermis or dermis. Alternatively, there could be an ongoing effect of residual dithranol or its metabolites, or it could be a combination of both. If the initial response is due to

changes in proliferation rate, these are occurring in the lowest layer of the epidermis and yet are visible in 8 hours. It seems less likely, therefore, that the longer term effect is due to a lack of visibility, but rather that a second, slower mechanism of action occurs. Whitefield (1981ab) proposed that unless decomposition of dithranol occurred in the skin, it could not exert its therapeutic effect. Neither danthrone nor dimer have any activity [Shroot et al, 1981]. In both intact and stripped skin, Cavey et al (1985) showed that dimer was present in the viable epidermis after 2 hours of applying dithranol to a greater extent than dithranol and danthrone. Metabolism to the dimer and further decomposition to dithranol brown are both via free radical mechanism [Whitefield, 1981a] and Mustakallio (1980a) and have suggested that free radical formation may be the mode of action of dithranol. Reichert et al (1985) indicated that mitochondria respiration is the primary target of dithranol action and Fusch et al (1990) recently showed that the redox potential properties of mitochondrial membrane are affected by this process and that the dimer is too large to pass through the membranes. The fact that dithranol dissolves to a greater extent in non-aqueous solvents than in aqueous solvents leads to the suggestion that dithranol would preferentially dissolve in mitochondrial lipid membranes where it will undergo oxidation. This means that the dimer will also tend to accumulate at these membranes and will continue to produce free radicals as it moves towards dithranol brown. thus, free radicals will be produced in or close to the membrane where they will have an effect on the integrity and function of the membrane. It is possible that this is the second, slower action of dithranol seen in this study.

Other factors could also affect the clinical effectiveness of the dithranol hydrogel. In particular these could be occlusive and/or solvent effects. Because

of the restricted time and small number of patients available, no assessment of these was possible in this work.

The discussion of the results obtained during this study indicates that further research is required in five main areas. These may be summarised as follows.

1. An hplc analysis employing a fluorescence detector for dithranol and dimer coupled with a UV detector for danthrone determination would improve the quantification of the three compounds simultaneously in all parts of the work. An assay for dithranol brown is also required.

2. The use of a hydrogel which would no form hydrogen bonds with dithranol should be investigated to see if improved stability, and hence increased shelf-life, of the loaded film is possible.

3. Ways of increasing the hydrogel load of dithranol need to be investigated. One way may be to simultaneously cast and load the hydrogel to avoid the need for solvents. Colloidal dispersions of dithranol or solubilised solutions, may also achieve a higher loading. They might also improve dithranol stability and prolong half-life in the hydrogel.

4. Alternatives to the <u>in vitro</u> model using neonatal rat stratum corneum for use in release studies should be investigated in order to provide a better understanding of the clinical data.

5. Clinical assessments are required over prolonged times until the staining have faded. Likewise, the *in vivo* release profiles need to be repeated with longer observation times to gain a better understanding of the fate of dithranol in psoriatic skin. A more accurate assessment of the "therapeutically active dose" of dithranol could be obtained by applying the hydrogel to psoriatic plaques for short times only. In this way a small known amount of dithranol can be delivered and the clinical effect monitored. Extension into histopathological examination would produce further evidence about the site(s) of action of dithranol.

It can be concluded that dithranol can be loaded into a hydrogel which is effective in the treatment of chronic stable plaque psoriasis. The hydrogel patch has been shown to have the advantage of limiting application to the psoriatic plaque and preventing it spreading to uninvolved skin. If the stability of the dithranol in the hydrogels can be further improved and a self-adhesive system devised, these hydrogels could represent an improvement in the treatment of psoriasis and also provide a useful tool for further investigations into the site and mode of action of dithranol and its effective concentration.

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