DEVELOPMENT AND EVALUATION OF ORAL SOLID DOSAGE FORMS FOR COLONIC DELIVERY OF DRUGS FOR THE TREATMENT OF CYSTINOSIS

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ABSTRACT

Modified release multiparticulate pellets have been produced by the process of extrusion and spheronisation for colon-targeted delivery of cystamine, a cysteamine derivative. Orally ingested cysteamine formulations used in the treatment of cystinosis, are observed to cause gastrointestinal mucosa irritation leading to nausea, vomiting and ulceration in patients on such therapies.

Spheronised pellets were prepared varying the type and concentration of the polymer used as an extrusion aid in the formulation. Classes of polymeric materials tested include starches, natural gums and cellulose derivatives. The properties of each wet mass formulation, extrudate and spheronisation product obtained were subsequently evaluated and compared to that obtained from a standard extrusion aid, microcrystalline cellulose. Viable pellets produced were subjected to detailed analytical procedures that include: pellet size analysis, qualitative sphericity determination, friability testing, bulk density measurements, optical and scanning electron microscopy.

Amongst the polymers tested, blends of 60/40% and 65/35% low/high acyl gellan gum (Kelcogel®) incorporated into lactose-based wet masses produced good pellets with relatively high sphericity (percentage sphericity: 65-70%).

Cystamine was subsequently incorporated into gellan and microcrystalline cellulose pellets and efforts made to tailor the release profile of the drug

by coating pellets with Eudragit® FS30D, a pH-sensitive polymer. The dosage forms described should selectively deliver the drug to the colon through pH-dependent dissolution of the polymer coating at colonic pH conditions (pH 7.4) thereby minimising the incidence of any gastrointestinal side effects. Dissolution results showed that Eudragit® FS30D coated cystamine pellets released < 3% of incorporated drug in 0.01M HCl over 1 hour at gastric pH of 1.2, while > 90% of the drug was released in phosphate buffer at pH 7.4. Indicating the suitability of these coated pellets as potential colon-targeted dosage forms for delivery of cysteamine.

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1. INTRODUCTION

1.1 Cystinosis

This is a rare genetic metabolic disorder characterised by an unusual accumulation of the amino acid cystine within the lysosomes of human cells. This condition is due to a defect in the normal lysosomal transport mechanism for cystine caused by genetic mutation. Continued cystine crystallisation within the cells of organs like the kidneys, pancreas, and eyes, would eventually result in organ damage and end-organ failure in such patients [1].

The condition can be treated pharmacologically, by the administration of the drug cysteamine (available commercially as the bitartrate salt, Cystagon ®). Cysteamine works by interacting with excess intracellular cystine to form a cysteamine-cysteine disulphide complex spatially identical in structure to the amino acid lysine, which means this complex is subsequently transported out of the cell by the transport mechanism for lysine. Unfortunately, oral cysteamine therapy is affected by poor patient compliance due to various side effects caused by its ingestion, as well as the offensive taste and smell of the drug. On oral administration, cysteamine irritates the gastrointestinal mucosa causing nausea, vomiting and ulceration; its metabolites are also excreted in sweat and breath resulting in body odour and halitosis.

Scientists have attempted to deliver the drug as suppositories in a bid

to overcome gastrointestinal (GIT) mucosa irritation [2]. But, the inconvenience and unpredictability of drug absorption through the rectal route are issues that limit the use of suppositories [3]. Intrarectal drug administration has been associated with clinically significant interpatient variations in peak blood levels [4]. This is undoubtedly a consequence of the irregular pharmacokinetics of drugs delivered through the rectal route.

Rectal drug absorption can be delayed or as rapid and complete as intravenous bolus doses depending on factors like pH of the absorption site, presence of stool in the rectal vault and formulation composition [4]. Also, post-absorptive drug distribution and metabolism is not well-defined because rectal venous drainage is different within the rectosigmoid region [5]. Drugs administered high in the rectum are drained by the superior rectal veins which carry them to the liver where they are metabolised [5]. While, drugs administered further down the rectum are delivered systemically via the inferior and middle rectal veins prior to hepatic distribution [5]. Patients could also expel an unestimated amount of the drug, making it difficult to optimise the dose of the drug required for each patient or disease state [4].

Much research has also gone into eliminating the halitosis and body odour caused by the drug through the use of pro-drugs which are pharmacologically inactive, but are subsequently converted to the active compound in-vivo [6].

The cysteamine molecule lacks a chromophore and is hence UV transparent. Therefore for the purpose of this research, cystamine, a disulphide of cysteamine was tagged with a phenyalanine conjugate to enable quantitative analysis/detection of the release of the active pharmaceutical ingredient (API) by ultraviolet/visible spectroscopy.

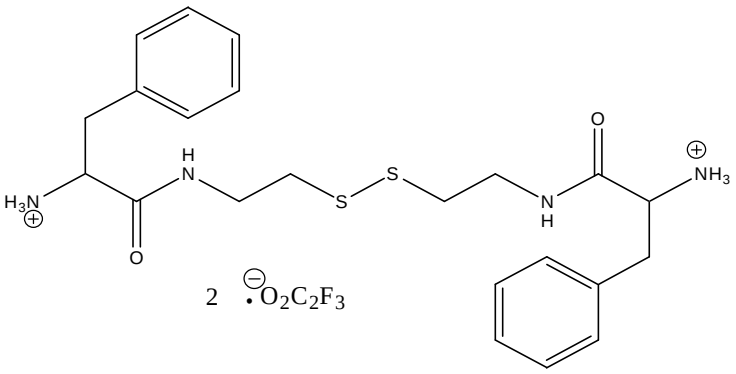

Figure 1: cystamine –phenylalanine conjugate

1.2 Oral Modified Release Dosage Forms (OMRDFS)

Oral modified release dosage forms (OMRDFS) are defined by the USP as dosage forms whose drug release profiles of time course and/or location are designed to facilitate therapeutic or convenience goals not offered ordinarily by conventional dosage forms [7]. OMRDFs are formulated to consist of an in-built chemical or physical drug release barrier, responsible for regulating the release of the active pharmaceutical ingredient (API) from the dosage form.

Based on the nature of this barrier, they are grouped as monolithic/matrix systems where active drug particles are dispersed in

a soluble or insoluble matrix, or reservoir and osmotic pump delivery systems made up of drug units coated with a release-controlling membrane.

OMRDFs can also be formulated as single unit, whole, non-disintegrating dosage forms e.g. tablets and some capsules or as mutiparticulates / multiple unit systems. Oral modified release dosage forms lead to greater patient compliance as well as a reduction in dosing frequency. Modified release dosage forms also significantly reduce the risks of local irritation and side effects associated with dose dumping. Thereby offering an overall enhancement in safety profile and peak therapeutic performance of administered medication.

1.3 Multiparticulate Systems and Drug Delivery

Multiparticulate OMRDFs are spherical, free-flowing granules with a narrow size distribution and mean diameter ranging between 0.7 and 2mm e.g. pellets, beads, and microspheres.

The in-vivo behaviour of orally ingested multiparticulate dosage forms, have been extensively investigated by researchers using reliable techniques like gamma scintigraphy based imaging technology [8, 9] and multiparticulates have been shown to perform better as oral modified release dosage forms than single–unit dosage forms. Abrahamsson and co-workers found that multiparticulate pellets had shorter mean gastric emptying times and longer mean colonic residence times than tablets [10]. They also observed that they were

more slowly and widely dispersed in the gastrointestinal tract, having longer total transit times than tablets.

Mutiparticulates offer superb pharmacological and technological advantages over conventional single unit dosage systems. Therapeutically, drug absorption is optimised when dense, small-sized multiparticulates are used in drug delivery, because they are observed to have free passage and easy distribution along the GIT, facilitating uniform drug absorption from the gut. The consequences of therapeutic failure of orally administered medication due to failure of the dosage form to release the drug are also significantly less. This is because the failure of a single pellet to release the API will not result in drastically compromised plasma concentrations of the drug, when compared to if that was a single unit dosage form. Patient compliance is also improved due to reduced systemic and local side effects.

In processing, pelletisation offers enhanced opportunities for technological creativity. This technique has been used as a way of limiting drug migration for low dose actives [11] and improving the flowability and density of low density, finely divided APIs and excipients to limit dust formation [12]. Good flow properties, improved strength and density exhibited by pellets promote reproducible die and capsule filling and ensure adequate content uniformity.

Also, pellets with differing release profiles/rates can be incorporated in the same dosage unit by coating them in sub-batches with different

materials; the fill weights of pellets can be adjusted to produce formulations of different doses from the same batch [13] and several actives or incompatible drugs can be combined in a single dosage unit.

Finally, coating is facilitated by the spherical shape, narrow size distribution and low friability of pellets.

1.4 Colonic Drug Targeting

The colon has become an important site for the delivery and systemic absorption of drugs that exercise their pharmacological effects at other sites in the body. The most critical objective of a colon-targeted formulation is ensuring that the drug is protected from release in the upper portions of the GIT, while effecting abrupt release of the API on reaching the colon.

In 1985, Hardy et al demonstrated that multiparticulate dosage forms enabled the API to get to the colon faster than conventional single unit dosage forms because their small sizes enable easy passage through the pylorus reaching the colon faster [14]. Their smooth spherical morphology also facilitates easy application of functional coatings, an integral aspect of colonic drug targeting.

Colon-targeted formulations are often designed to take advantage of certain factors called ‘trigger factors’. These factors and their effect on colonic delivery are explained below.

1.4.1 pH-dependent systems

The terminal ileum and colon have a much higher pH than other regions of the GI tract, hence drug delivery systems can be coated with functional pH-sensitive polymeric coatings that will selectively disintegrate and effect drug release at higher pH values of 6.8-7.5 [15].

Commonly used pH-sensitive coatings for colonic drug delivery are the enteric coatings e.g. polyvinyl acetate phthalate and methacrylic acid copolymers (Eudragits). It has been reported however that achieving successful colon-specific drug release with pH-sensitive coatings alone is not feasible. Eudragit S coated rapidly disintegrating tablets have been observed to fail to disintegrate and release the drug due to drops in pH of 7.0 to 6.0 from the terminal ileum to ascending colon [16].This problem can be tackled by employing optimum combinations of Eudragit L100 and S100 to enable selective drug release at desired pH ranges between 6.0-7.0 . New copolymers like Eudragit P4135/FS that offer a reduced risk of premature drug release by dissolving at similar pH values to S100 but at a slower rate[16], and sensitive radiotelemetry capsules that can accurately measure and determine the most favourable pH for colonic drug targeting are new approaches currently being evaluated [17].

1.4.2 Time dependent systems

These are based on timed release of the drug. Since small intestinal transit times are fairly constant (maintained between 3-4hrs), when compared to gastric emptying times and colonic transit times which are

variable. A colon-targeted formulation developed by Gupta et al containing 5-aminosalicylic acid, employed both pH sensitive coatings and timed release to achieve successful colon-specific drug release [18].

1.4.3 Pressure-responsive systems

A pressure controlled colonic delivery system made up of a dispersion of the active drug particles in a suppository base with an ethylcellulose coating has been described in the literature [19,20]. On oral ingestion, the suppository base melts resulting in a fluid filled ethylcellulose capsule that stays intact in the small intestine, but will rupture and release the drug on exposure to the more intense contractions and higher viscosity luminal conditions found in colonic regions.

1.4.4 Bacteria-dependent systems

The human colon is colonised by a large multitude and variety of bacteria that produce enzymes that can be employed in achieving colon-specific drug delivery. The drug is coated with a polymer material that is selectively degraded by bacterial enzymes in the colon to cause drug release. COLAL® technology which consists of a glassy amylase coating resistant to degradation by small intestinal enzymes but susceptible to breakdown by colonic bacteria, mixed with ethyl cellulose to inhibit swelling has been used successfully to deliver prednisolone metasulphobenzoate to the colon of patients suffering from ulcerative colitis [21].

1.5 Pelletisation by Extrusion and Spheronisation

This multi-step process of manufacturing drug-loaded multiparticulate pellets of about 1mm in diameter was introduced in the late 1960s, and is a useful technique for producing robust high-quality pellets with unique properties, often favoured for controlled drug release purposes [22].

Though the process can be labour-intensive, its inherent intensive mixing capabilities enables the incorporation of high concentrations of the active within the granules (up to 90%) without the production of a bulky dosage form. Thus allowing optimum utilisation of excipients and lowering production costs. The pellets obtained can then either be filled into hard gelatine capsules or compacted into tablets for immediate or more often controlled release applications [23].

1.6 Excipients

Excipients are materials that are added to drug substances to aid processing or to achieve formulation objectives like controlled release and drug targeting. It is important to choose excipients that are compatible with the drug, as well as able to confer desired extrusion characteristics like cohesiveness and plasticity to the wet mass.

Excipients required for pelletisation by extrusion and spheronisation include:

1.6.1 Extrusion Aids

These are materials added to the wet mass formulation to improve its

rheological properties, making it suitable for extrusion and spheronisation. Many materials have been evaluated for use as extrusion aids, especially polymers.

Liew et al proposed the following properties as being required of a material to be employed as an extrusion aid: water insolubility, large surface area to foster interaction with other components of the formulation, large water absorption and retention capacity, good binding/cohesive properties and ability to facilitate drug release [24].

Other desirable properties include compatibility with a wide variety of APIs and granulating fluids, preferably water.

Finally, the ideal extrusion aid should facilitate easy processing of the wet mass to give a high yield of robust pellets that can withstand compression and compaction forces required for tablet manufacture. Commonly used extrusion aids include cellulose ethers, natural gums, starches and other pharmaceutical polymers.

Cellulose derivatives

Cellulose derivatives used in dosage form manufacture include hydroxypropylmethylcellulose, methylcellulose, hydroxyethylcellulose, hydroxypropylcelluose and sodiumcarboxymethylcellulose.

The most effective and widely used cellulose derivative for extrusion and spheronisation purposes is microcrystalline cellulose (MCC). Which consists of a random aggregation of filamentous crystals that create a high internal porosity and large surface area of about 130-270 m^2/g within

its structure [25].

This unique structure as well as the availability of free hydroxyl groups that are able to form hydrogen bonds with water molecules confer high water retention and absorption capacity on MCC and enable it to control the movement of water through the wet mass [26]. Thereby, modifying the rheological properties of other excipients and preventing phase differentiation during extrusion. Water that is retained within the amorphous regions of MCC can act as a plasticiser, enhancing molecular flexibility by breaking up intramolecular hydrogen bonds [26]. Therefore, MCC also imparts plasticity and cohesiveness on the moistened mass enhancing the entire process of pellet manufacture. It is available commercially as Avicel® in a range of grades.

However, MCC is expensive [27] and suffers disadvantages like adsorption of some drugs to the surface of its fibres [28], chemical incompatibility with certain drugs [29], prolonged pellet disintegration when used with poorly soluble drugs (which poses a problem in the formulation of controlled drug release systems) [30] and variability in the properties of pellets produced with microcyrstalline cellulose from different sources. This has prompted the need to investigate the possibility of replacing MCC with other materials in the formulation of pellets by extrusion.

Basit et al (1999), observed that for a certain formulation of ranitidine, the decomposition of the active could be reduced to acceptable levels if

the concentration of microcyrstalline cellulose used in the formulation was lowered and glycerylmonostearate added instead [31]. While, Linder and Kleinebudde (1994) reported higher dissolution rates and porosity for pellets produced with powdered cellulose than microcrystalline cellulose [32].

Natural gums

These are polysaccharides obtained from natural sources like plants, animals or even bacteria. A variety of natural gums have been evaluated for potential application as extrusion aids in the formulation of wet masses for extrusion, the common ones being agar, carrageenan and sodium alginate [33].

Gellan gum, an anionic extracellular polysaccharide discovered by Kaneko and Kang [34] in 1978 has been tested as an extrusion aid in lactose based wet masses. It is obtained as exo-secretions when a carefully formulated fermentation medium is inoculated with Sphingomonas paucimobilis formerly referred to as Pseudomonas elodea, extracted from the elodea plant tissue [35]. Native gellan gum is composed of repeating units of β-D-glucose, L-rhamnose, D-glucuronic acid and two acyl groups (acetate and glycerate) bound to the glucose residues adjacent to glucuronic acid. These acyl components of gellan gum have significant influence on the rheology of gellan gels and can be removed by deacetylation of the gum by alkaline treatments to yield lower acyl gellan gum derivatives [36].

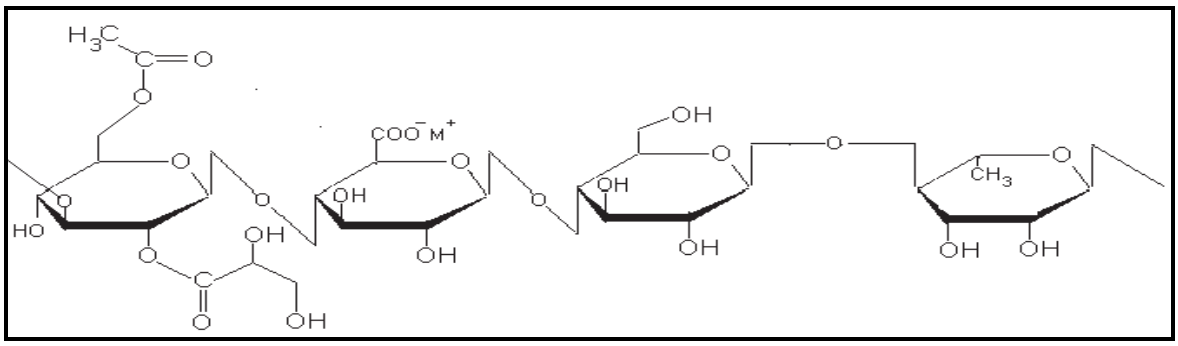

Fig 2: Repeating units of chemical structure of native gellan gum

Gels from native gellan gum are soft, elastic, cohesive thermoreversible gels, while deacetylated gellan gum often yields harder, brittle and more thermostable gels. Gels with intermediate characteristics can be derived by manipulating the degree of deacetylation [36].

There are three types of deacetylated gellan gum viz:

Kelcogel® CGHA: food grade, high acyl gellan gum.

Kelcogel® CGLA: food grade, low acyl gellan gum.

Gelzan™: low acyl gellan gum deproteinised and clarified for use as microbiological media.

Sustained release beads prepared by gelation of gellan gum with calcium ions were used for the delivery of metribuzin (a weed killer) and theophylline. The gum was observed to prolong the release of the API in acidic media, but facilitate rapid drug release in phosphate buffer (pH 7.4) [37]. These properties should enhance successful colon specific drug delivery.

Though research involving the use of gellan gum in pharmaceutical dosage form manufacture is still in its infancy, the polymer has promising

physical properties that can make it a suitable replacement for existing polymers used not only in extrusion and spheronisation, but in a wide variety of industries and applications.

1.6.2 Filler/diluents

These are added to increase the bulk volume or size of the dosage form. Commonly used fillers for extrusion purposes are dibasic calcium phosphate (Emcompress®) and lactose.

1.6.3 Binders

These are added to improve cohesion, increase plasticity and reduce extrudate friability e.g. methylcellulose and sodiumcarboxymethylcellulose. Funck et al (1991) observed that the inclusion of low proportions of common binders enabled the production of high drug loaded microcrystalline-based pellets [38]. It is however crucial to optimise the amount of binders included in the formulation because when high proportions of binders like sodium carboxymethylcellulose are present in wet masses for extrusion, the mass acquires elastic rather than plastic properties, leading to the formation of long coils of extrudate on extrusion that do not deform into proper spheres when spheronised [25].

1.7 Formulation Development and Variables

The quality of the pellets produced at the end of the process is highly formulation dependent and will ultimately be influenced by the physicochemical properties and concentrations of the drug and excipients employed and to a large extent, type and concentration of the granulating

fluid used. Generally, the desired goal is to obtain a wet mass which is cohesive and plastic enough to deform when extruded and round off into spherical granules on spheronisation. The composition of the wet mass is critical in obtaining a high yield of pellets of good quality.

The concentration of water incorporated into the formulation, during the granulation of the wet mass for extrusion, greatly affects the quality of the pellets obtained at the end of the process. Usually, the amount of water required for successful extrusion is about 20-30 % w/w, more than is needed for granulations for other applications; as water is needed to serve as a lubricant to facilitate the smooth entry and passage of the wet mass through the extruder die [25]. Optimum water content can be determined by preparing granulations of varying water content and subjectively assessing which formulation has the water level most suitable for extrusion. This assessment is based on ease of processing, extrudate quality, deformation characteristics and properties of the spheres produced.

Fluffy, insufficiently wetted formulations require high extrusion forces making processing difficult and generating high amounts of friction and pressure within the equipment, which can damage the extruder [11]. Extrudates obtained from such masses will also be observed to have poor surface characteristics, high friability and will likely not deform into good spheres. However, overly wet masses are problematic in that they stick to equipment parts and cluster together to form larger spheres resulting in poor size distribution [11].

The granulating fluid concentration required for a particular drug formulation has been found to decrease linearly as a function of the natural log solubility of the drug in question [39]. Hence, the water content of the formulation will have greater implications in the formulation of drugs with high water solubility.

1.8 Process Outline

Conine and Hardley [40] described the steps involved in the manufacture of spheronised pellets as the following:

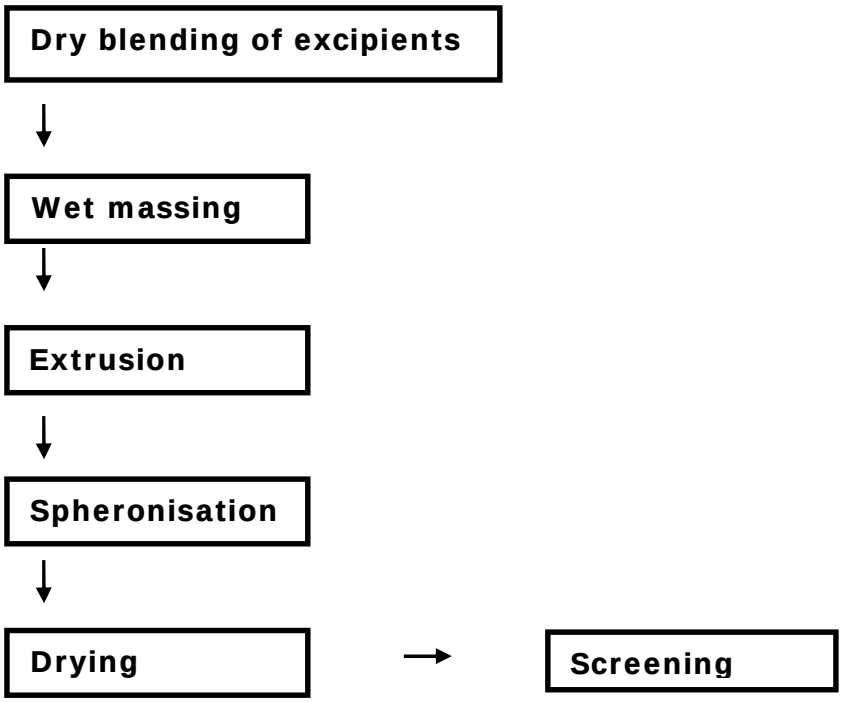

Fig. 3: Process Outline for the Production of Spheronised Pellets.

1.8.1 Dry Mixing

This entails blending the drug and excipients in the dry state to achieve homogeneous powder dispersion prior to granulation.

This is done to prevent localised overwetting that can result from a non-uniform distribution of powders of varying size and solubility resulting in pellets with an uneven size distribution.

1.8.2 Wet Massing

This is the process of mixing the uniform powder blend prepared earlier with an adequate quantity of granulating fluid to obtain a moistened mass that is cohesive and plastic enough for extrusion/spheronisation. The wet mass obtained should also posess sufficient inherent fluidity and self lubricating properties, permitting flow under a constant pressure as the mass enters and passes through the die.

Conventional mixers and granulating equipment are normally used, but it is pertinent to guard against heat build up from such equipment especially the high shear mixers which generate very high temperatures leading to a greater than acceptable degree of evaporation of granulating fluid, or increase in solubility of dispersed solids and an associated reduction in plasticity [41].

1.8.3 Extrusion

This refers to the formation of rod shaped granules of uniform diameter by forcing the mass through an extrusion screen that consists of dies of

uniform diameter. Extruders come in various designs and are grouped into three classes based on their feed mechanism: screwfeed extruders, gravity-feed extruders and piston-feed extruders.

The extrudates produced should have sufficient mechanical strength, smooth surface properties; possess enough plasticity to deform under their own weight on extrusion and form spheres that do not adhere to each other in the spheroniser.

1.8.4 Spheronisation

Here, extrudates are rounded off into spherical granules in the spheroniser which consists of a cylindrical bowl with a fast-rotating friction plate at the base. Spheronisation is brought about by frictional forces generated between the particles and/or the particle and equipment [11]. The bottom plate is grooved to increase these forces and two geometric patterns are available: a cross-hatched pattern with grooves running at right angles to each other and a radial pattern with grooves running from the centre of the disc [23].

Spheronisation occurs in stages and can take between 2-15 minutes depending on the properties of the formulation being processed. The acceptable spheronisation speed required to produce a spherical particle varies widely. Recently more emphasis has been placed on evaluating not just the absolute rotational speed of the friction plate, but the peripheral velocity of the plate which can be derived using a combination of the speed and diameter of the friction plate to assess the performance of a

given formulation [42].

1.8.5 Drying

This is essential to achieve acceptable moisture content of the product. Drying can be done using the tray drier or fluidised bed drier.

1.8.6 Screening

A nest of sieves is used to seperate pellets into different size distributions.

1.9 Pelletisation process variables

Processing factors that influence the properties of pellets obtained from the process of extrusion and spheronisation are stated in the table below:

Table 1: Processing variables

Dry Mixing Equipment type; mixing time
Wet massing Equipment type; type and concentration of granulating fluid.
Extrusion Extruder type; diameter and length of extruder die; extrusion speed; feed rate.
Spheronization Equipment type; product residence time; spheronisation speed; product charge.

1.10 Coating Technologies

Pharmaceutical solid dosage forms are either coated in a bid to alter their taste, improve their appearance or offer protection from light or humidity, termed 'plain coatings' by the USP [43], or to confer modified release characteristics on the dosage form (controlled release coatings). Controlled release coatings include enteric coating and other forms of pH sensitive coatings, non-enteric polymeric coatings and enzyme-degradable coatings. The focus of this research will be on pH-dependent coatings.

1.10.1 Enteric coatings

These can be used to protect susceptible APIs from degradation in the acidic contents of the stomach; prevent local irritation of the GIT mucosal lining by certain drugs; provide a delayed release component for repeat action tablets [43] and/or facilitate drug delivery to distal regions of the gut.

pH-sensitive polymers most commonly used to achieve targeted drug release for oral dosage forms are the methacrylic acid polymers (Eudragits®).

1.10.2 Eudragit® polymers

These are synthetic cationic and anionic copolymers of dimethylaminoethylmethacrylates, methacrylic acid and methacrylic acid esters mixed in varying proportions.

Eudragits used in the formulation of enteric coatings and targeted drug release products includes: Eudragit® L, S, FS and E which have acidic or

alkaline functional groups and selectively effect drug release by salt formation at target pH values at which they are soluble in digestive fluids. Eudragit® RL, RS and Eudragit® NE polymers with alkaline and neutral groups respectively are impermeable in digestive fluids. They are often used for sustained drug release because they offer timed release of the API by pH -independent swelling of the dosage form [44].

These polymers are available from the manufacturer as aqueous dispersions, powders or organic solutions to be applied as coatings to oral dosage forms.

For colonic drug targeting, Eudragit® S and Eudragit® FS30D that dissolve at pH 7.0 can be used. Eudragit® FS30D has good film forming properties, requires no plasticizer and is favoured for coating multiparticulates due to its mechanical flexibility (44).

2. MATERIALS AND METHODOLOGY

2.1 Materials

Cystamine-phenylalanine conjugate was synthesized within RGU laboratories; microcrystalline cellulose (Avicel® PH 101) was obtained from FMC Ltd. (Cork, Ireland). Dibasic calcium phosphate (Emcompress®) was from Penwest GMBH (Germany) and lactose was from Honeywell and Stein (London, England).

Theophylline, gellan gum (Gelzan™), triethylcitrate, rice and corn starch, xanthan, karaya, ghatti, locust bean and guar gum were purchased from Sigma-Aldrich, UK. Wheat starch was from Fisons Ltd. (Loughborough, England), potato starch was obtained from Merck GaA (Dermastadt,Germany), maize starch and glycerol monostearate were from BDH Chemicals limited (Poole, England).

Methylcellulose (MC) (Methocel®) and simethicone emulsion were from The Dow Chemical company (Michigan, U.S.A), hydroxypropylmethylcellulose (HPMC K100M) was from Colorcon Ltd. (Kent, England), hydroxyethylcellulose (HEC) (Natrosol®) and hydroxypropylcellulose (HPC) (Klucel®) were from Aqualon (Detroit, U.S.A). Sodium carboxymethylcellulose (SCMC), croscarmellose sodium (CCS) and Tween® 80 were from Fisher scientific ltd. (Loughborough, England).

Food grade gellan gum (Kelcogel®-CGLA and Kelcogel®-CGHA) and

Eudragit® FS30D were obtained as gifts from Cp Kelco (Atlanta, USA) and Evoniks Industries (Cheshire, UK services) respectively.

2.2 Methods

2.2.1 Preparation of placebo pellets

Required amounts of extrusion aid and filler were weighed out using formulation ratios given in Table 2 and dry-blended in a Kenwood planetary mixer (model: KMC510L, Kenwood Company, USA) at speed 3 for 10 minutes.

Pre-optimisation of the quantity of extrusion aid and distilled water required for optimum wet mass consistency was done by visual assessment of the extrusion properties of the wet mass formulation based on ease of processing, extrudate surface texture, deformation characteristics and properties of the spheronisation product.

Sufficient amount of distilled water was then gradually incorporated into the blend and mixing continued at speed 4 for a length of time needed to yield a homogeneous mass.

The composition of formulations used for subjective comparative evaluation of wet mass properties is highlighted in the table below:

Table 2: Formulations of wet masses used in comparative evaluation studies

	Extrusion aid (g)	Filler (g)		Distilled water (g)	Solids mass fraction (wt %)
		Emcompress®	lactose		
MCC	50	50	-	66	60.2
HPMC	30	50	-	60	57.1
HPC	20	60	-	30	72.7
MC	30	30	-	40	60.0
SCMC	25	50	-	55	57.7
HEC	25	25	-	40	55.6
CCS	40	40	-	80	50.0
Rice starch	30	40	-	35	66.7
Maize starch	40	50	-	37	70.9
	40	-	40	60	57.1
Wheat starch	40	50	-	33	73.1
	40		30	36	66.0
Corn starch	30	40	-	30	70.0
	30	-	40	30	70.0
Potato starch	30	40	-	25	73.7
	30	-	40	25	73.7
Xanthan gum	10	100	-	20	84.6
Karaya gum	8	80	-	30	74.6
Guar gum	7	70	-	45	63.1
Ghatti gum	4	80	-	20	80.8
Locust bean gum	8	80	-	45	66.1
Gellan gum (Gelzan™)	3	-	60	20	75.9

The wet mass was then fed into a laboratory bench-top basket extruder featuring a 1mm extrusion screen (Caleva Process Solutions Ltd., Dorset, UK) and the resultant extrudate loaded into a bench-top 120 spheroniser (Caleva Process Solutions Ltd., Dorset, UK) with a 1mm cross-hatched friction plate fitted at the base of the spheronisation bowl. Spheronisation speed was determined and recorded by the use of a tacometer.

Viable pellets produced were dried in a fluidised bed drier (Copley Retsch TG100, Nottingham, England) at 60^{o}C for 30 minutes and shaken through a nest of sieves (Copley Retsch As200 Sieve Shaker, Rheinische, Germany) (mesh size range from 90µm-1mm) for 10 minutes to separate the pellets into different size distributions. Pellets were then bench-coated and those from the 710-1000µm size range kept for further characterisation.

2.2.2 Optimisation of the formulation of gellan pellets.

Results from the above experiment showed that gellan gum had the most potential for use as an extrusion aid, necessitating the need for optimising gellan formulations. Gelzan^{Tm} was used for prior studies, but this product was taken off the manufacturer's brochure and replaced with Food grade Gellan gum (Kelcogel®-CGLA and Kelcogel®-CGHA).

Blends of Kelcogel®-CGLA (CGLA) and Kelcogel®-CGHA (CGHA) in varying ratios were incorporated into lactose based wet masses as shown in Table 2, to determine if there was a significant potentiation in the desirable rheological properties of the gum.

Batches of spheronised pellets were prepared by mixing a dry pre-blend of low: high acyl gellan gum made using the ratios in Table 3 below with lactose at a ratio of 1:20 and subsequently mixing in a sufficient amount of distilled water to give a homogeneous mass.

Table 3: Composition of gellan formulations used for optimisation studies

Blending ratios (% w/w)	**A** (0:100)		**B** (25:75)		**C** (50:50)	**D** (60:40)		**E** (65:35)	
Formulations	**(i)**	**(ii)**	**(iii)**	**(iv)**	**(v)**	**(vi)**	**(vii)**	**(viii)**	**(ix)**
CGLA (g)	3	-	1	3	2	1.8	1.2	1.95	1.05
CGHA (g)	-	3	3	1	2	1.2	1.8	1.05	1.95
LACTOSE (g)	60	60	80	80	80	60	60	60	60
DISTILLED WATER (ml)	30	18	26	23	18	22	20	20	19

Each wet mass formulation was then processed into pellets as described earlier and the resultant pellets characterised.

2.2.3 Preparation of drug-loaded pellets

Two batches each of theophylline and cystamine pellets were prepared varying the extrusion aid used in the formulation

MCC-based theophylline pellets were prepared by wet massing a pre-blend of 60g dry MCC:Emcompress (1:1) with a solution of 6g of theophylline in 35mls of distilled water in the planetary mixer. This formulation was repeated with cystamine pellets, changing the granulating solution to 6g cystamine phenylalanine conjugate in 40mls of distilled

water and 0.01ml ethanol added as a cosolvent to hasten dissolution of the API.

Gellan-based theophylline pellets were prepared by wet massing a dry mix of 3g CGLA: CGHA blend (65:35 % w/w) and 60g lactose with a solution of 6.3g theophylline in 37mls distilled water in the planetary mixer.

Cystamine in Gellan formulations were also prepared by dry mixing 6g of CGLA: CGHA (65:35 % w/w) blend with 60g of lactose in the planetary mixer, and gradually incorporating a solution of 6.6g of cystamine in 22mls of distilled water and 0.01mls of ethanol until a homogeneous wet mass was obtained.

The wet masses were subsequently extruded and spheronised into pellets as described earlier.

2.2.4 Coating of pellets

To facilitate colonic targeting, cystamine and theophylline pellets (10g) from the 710-1000µm size range were coated in the aeromatic fluidised bed coater (Gea Process, Switzerland) using an aqueous spray suspension of Eudragit® FS30D (available as 30% w/v aqueous dispersion), a pH-sensitive polymer that consists of acidic functional groups which selectively effect drug release by salt formation at alkaline pH values at which they are soluble in digestive fluids.

The spray suspension was prepared by homogenising (Silverson machines, Inc.) the required amounts of triethylcitrate (plasticiser), polysorbate 80 (emulsifier), glycerolmonostearate (glidant) and drops of simethicone emulsion (antifoam) in a portion of the heated diluent for 10

minutes, stirring in the rest of the diluent and pouring this suspension into the Eudragit dispersion with magnetic stirring.

The spray suspension was then passed through a 0.5mm sieve to remove films of the Eudragit polymer that may have been formed while mixing and left to stir continuously on a magnetic stirrer plate. The spray suspension formulation is stated below and was adapted from the manufacturer's current applications brochure [45].

Table 4: Composition of spray suspension for coating pellets

For a 100ml spray suspension:

Ingredient	Quantity*	Weight (g)	Dry Substance
Eudragit® FS30D	-	59.77	17.93
Polysorbate 80(33% aqueous solution)	1.6	0.88	2.90
Triethyl citrate	5.0	0.90	0.90
Glycerylmono Stearate	4.0	0.72	0.72
Distilled water	-	37.73	
Total		100.00	19.84

*expressed as % w/w of dry polymer substance

The pellets were then filled in 10g batches into the coating chamber of the fluidised-bed coater (Aeromatic Strea 1 Fluidised Bed Film Coater, Bubendorf, Switzerland) fitted with a bottom spray, 0.8mm bore injection nozzle.

Coating process parameters are given below:

Atomising air pressure:	1.8 bar
Inlet air temperature:	40°C
Inlet air volume (fan speed):	10-12 revolutions per minute
Height of wurster column:	8cm
Product bed temperature:	25-30°C

Drying process parameters are given below:

Drying temperature:	50°C
Drying time:	60 minutes
Spray rate:	10ml/minute

The pellets were pre-warmed for 10 minutes and then the spray suspension was fed into the coater using a peristaltic pump (HR Flow Inducer, Cornwall, England). The pump was switched off intermittently during the spraying process to prevent overwetting and agglomeration of the pellets. Different batches of pellets were coated with successively increasing amounts of spray suspension (15-30ml) yielding pellets with varied coating thickness.

The effectiveness of the applied coating in terms of retardation of drug release was determined by carrying out dissolution tests in 0.1M HCl. 25ml of spray suspension (equivalent to 4.5g of dry Eudragit® FS30D per 10g of pellets) was found to give an optimum pellet coating thickness that retarded drug release to less than 5% in 0.1M HCl, while ensuring drug release in pH 7.4 phosphate buffer was not inhibited.

After delivering the spray suspension, the pump was switched off and the pellets left to dry at 50°C for one hour in the same equipment.

2.3 Characterisation and evaluation of pellets

2.3.1 Pellet Size Distribution Analysis

The dried pellets were shaken through a 'nest of sieves' with progressively smaller mesh sizes from top to bottom (1mm, 710µm, 500µm, 355µm, 250µm, 180µm, 125µm and 90µm) for 10 minutes at an amplitude of 1.5mm/'g'. Each sieve fraction of pellets was weighed and the value expressed as a percentage of the total weight of pellets before sieving.

The percentage pellet size distribution of the formulation was analysed by plotting the percentage weight of pellets retained on each sieve against the corresponding mesh size.

A good formulation yields pellets with a narrow size distribution. The mesh sizes given above had a modal size distribution of 710-1000µm and pellets from this sieve fraction were retained for further characterisation and coating.

2.3.2 Sphericity evaluation

Microscopic examinations of samples of 20 pellets obtained randomly from the 710µm size distribution of each batch of viable pellets was carried out using a standard laboratory microscope (Leica DFC 420), set to a magnification of x 5.

Pellet shape was then assessed subjectively by allocating a figure to each pellet in an order that correlates with its relative degree of sphericity, on a visual scale of 0-5. Therefore, the most spherical pellets were awarded a '5', while irregular non-spherical ones were furthest down the scale as shown in the diagram below.

5	4	3	2	1	0
perfect sphere	spheroid	irregular spheroid	ellipsoid	dumbbell	rounded cylinder

Fig. 4: Diagrammatic representation of classification of pellets based on their visual appearance

The average sphericity of each sample was calculated by dividing the summation of the various figures allocated to each pellet by the number of pellets in the distribution. The average sphericity value obtained was expressed as a percentage of the value corresponding to a perfectly spherical pellet i.e. '5' to obtain the percentage sphericity of a formulation.

2.3.3 Bulk density and Flowability Determination

This was determined by carrying out tapped density and bulk density measurements. Pellets (10g) were weighed and placed in a measuring cylinder to determine the initial volume and density referred to as the 'poured or fluff density', D_o. The cylinder was then tapped mechanically 1280 times using a volumeter (Copley Tap SVM 22 Volumeter, Nottingham, England) and the volume of pellets after tapping noted and used to determine the 'tapped or consolidated bulk density' D_f.

Hausner's ratio, D_f/D_o was calculated for each batch of pellets and used as a gauge for predicting pellet flow properties [46].

The percentage compressibility of the pellets, also known as Carr's compressibility index (%) was also calculated using the equation below:

$$D_f - D_o / D_f \times 100. \qquad \text{(Equation 1)}$$

Where, D_f = Tapped density

D_o = Bulk density

Values ranging from 5-16% indicate pellets with adequate flow properties, according to Carr [47]. Free-flowing pellets are critical in ensuring uniform and reproducible pellet filling and packaging.

2.3.4 Pellet Friability determination

Pellets (5g) were weighed and rotated for 30 minutes in a friabilator

(Erweka GMBH, Germany). The pellets were then sieved to get rid of broken pieces and re-weighed.

The percentage weight loss of the pellets after agitation in the friabilator was calculated and recorded. Friability values less than 2% are recommended for pellets to be able to withstand coating processes [44].

2.3.5 Dissolution testing

A series of in vitro dissolution tests using the USP 2 rotating basket method were carried out on different formulations of coated and uncoated pellets containing both model and test drug. An on-line dissolution apparatus (Sotax dissolution apparatus, Basel, Switzerland) connected to a UV spectrophotometer (Lambda 40 UV/VIS, PerkinElmer Instruments USA) was used to measure the concentration of drug released from the pellets at set times. The pellets were placed in separate rotating baskets positioned within dissolution flasks containing 900ml of the required dissolution medium at 37 +/- 0.5 ^{o}C, and rotating at a speed of 100rpm.

Samples of uncoated and coated cystamine and theophylline pellets (pellet weights equivalent to 300mg cystamine and 30mg theophylline) were analysed at 256nm and 275nm respectively in 0.1N HCl for 1 hour, and subsequently transferred to phosphate buffer pH 7.4 where applicable, to assess their release profile in both media. Drug release profiles were obtained by measuring ‘absorbance’ values every 5 minutes and the percentage of drug dissolved in the dissolution medium at a specific time computed automatically using precalculated A [1%, 1cm] values obtained from the calibration curve.

For each formulation, triplicate values were obtained and the rate of drug release in each medium evaluated by plotting the mean percentage drug dissolution against time. Colon-targeted pellets coated with Eudragit FS30D should be gastro resistant, retarding the release of the drug in acid medium (0.1N HCL), while effecting drug release in a buffer at similar pH conditions to the colon (pH 7.4).

2.3.6 Scanning electron microscopy (SEM) imaging

Scanning electron microscopy (Leo S430 scanning electron microscope) imaging was undertaken on a selection of cystamine pellet samples containing MCC and gellan gum respectively.

Dried samples were mounted using double-sided tape on aluminium stubs and gold sputtered (SC7640 Plasma Magnetron Sputter Coater) prior to imaging.

A magnification of X 55 and X 200 was sufficient to reveal multiparticulate morphology.

3. RESULTS

3.1 Extrusion-spheronisation attributes of polymer formulations

Table 5: *Comparative evaluation of extrusion characteristics of polymers

	Wet mass			**Extrudates**			**pellets**		
	cohesive	fluid	sticky	plastic	friable	deform	round	discrete	hard
MCC	+++	+++	-	+++	-	+++	+++	+++	+++
HPMC	+++	-	+++	-	-		-	-	-
HPC	+++	-	+++	-	-	-	-	-	-
MC	+++	-	+++	-	-	-	-	-	-
SCMC	+++	+++	+	+	-	+	-	-	-
HEC	-	-	-	-	+++	-	-	-	-
CCS	-	-	-	-	+++	-	-	-	-
Rice	++	++	-	+++	+	+++	++	+++	-
Maize	++	++	-	+++	+	+++	++	+++	-
Wheat	++	++	-	+++	+	+++	++	+++	-
Corn	++	++	-	++	+	+++	+	+++	-
Potato	++	++	-	-	-	-	-	-	-
Xanthan	+++	+++	-	+++		+++	++	-	+++
Karaya	+++	+++	-	+++	-	+++	++	-	+++
Ghatti	+++	+++	-	+++	-	+++	++	-	+++
Guar	+++	++	+	-	-	-	-	-	-
Locust bean	+++	++	+	-	-	-	-	-	-
Gelzan^{Tm}	+++	+++	-	+++	---	+++	++	+++	++

***Key:** +++ - excellent; ++ - good; + - average; - - none

3.2 Optical micrograph of spheronisation products

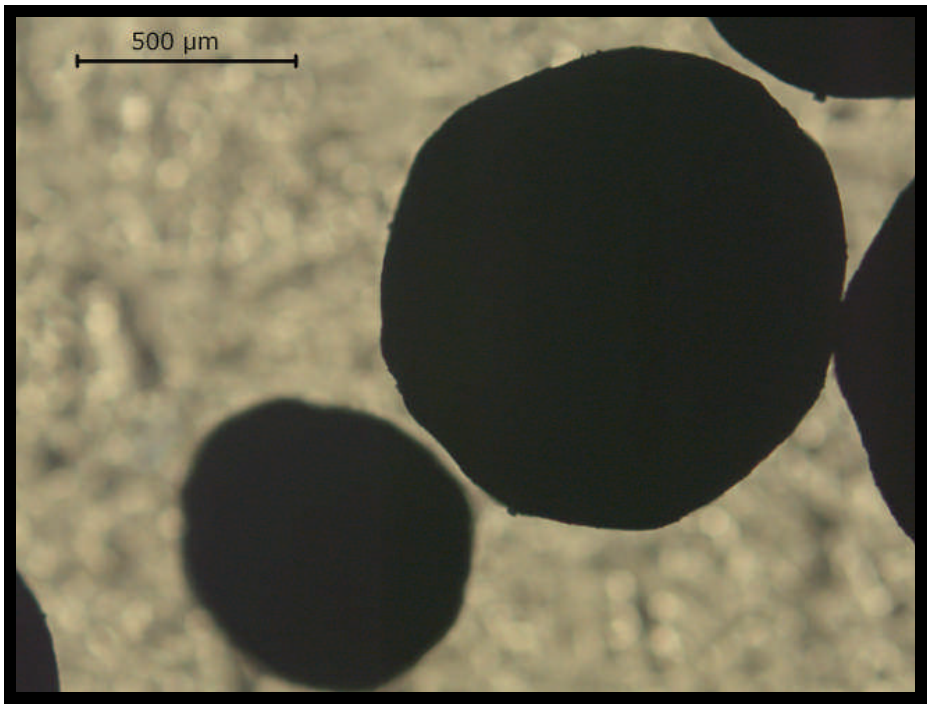

Fig. 5: Spherical pellets from an MCC-based wet mass (x 5).

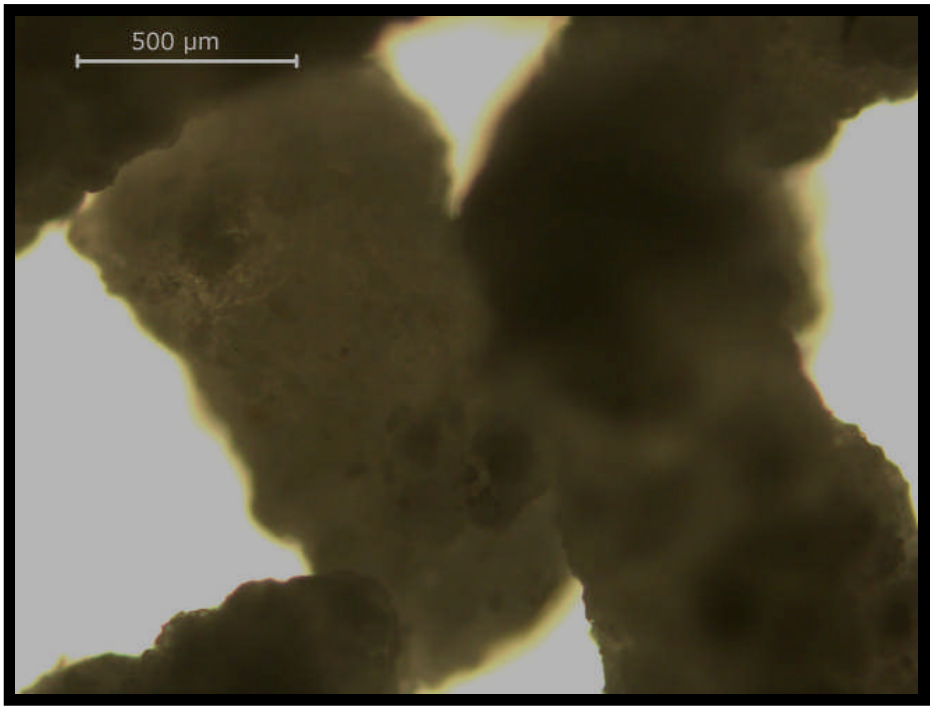

Fig. 6: Rod-like spheronisation product of an HPMC-based wet mass (x 5).

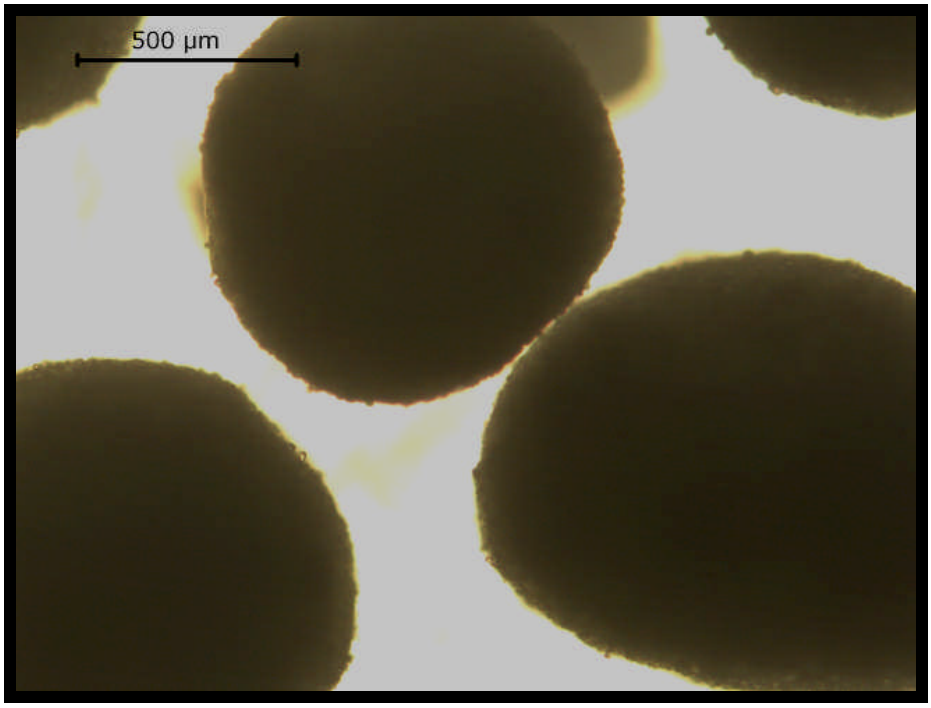

Fig. 7: Spherical but friable wheat starch pellets; note the edges of the pellets appear crumbly (x 5).

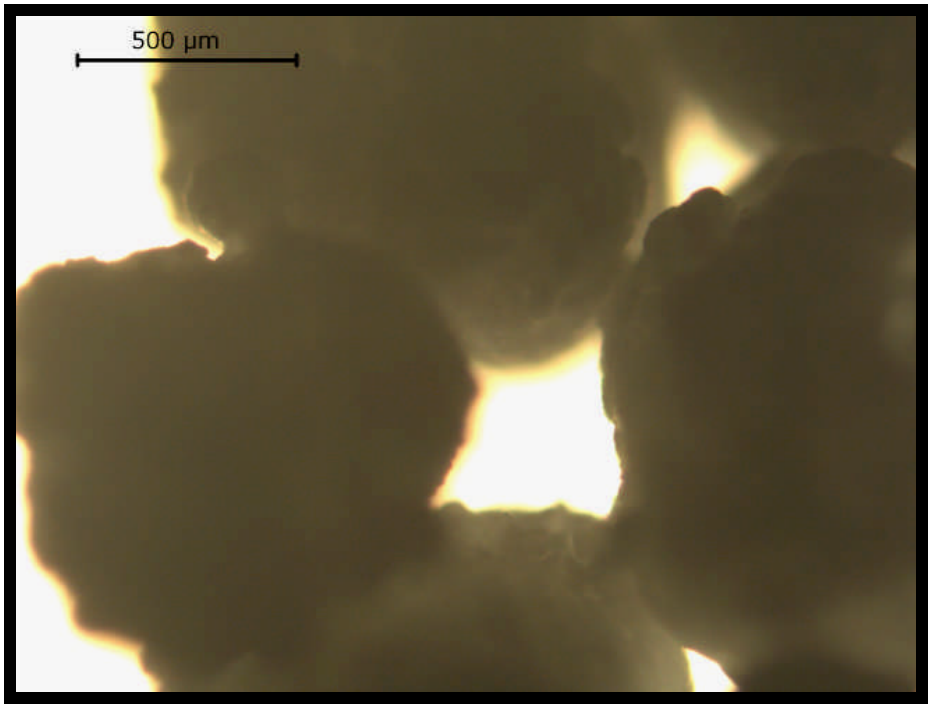

Fig. 8: Aggregates of karaya gum pellets (x 5).

3.3 Characterisation of gellan pellets

Optimum gellan combinations in terms of sphericity and size distribution were identified as Formulation D(vi) containing 60% CGLA/ 40% CGHA and Formulation E(viii) containing 65% CGLA/ 35% CGHA as extrusion aids.

Results of physical characterisation of these pellets were compared with data obtained from characterisation of spheronised pellets containing MCC, CGLA and CGHA separately as sole extrusion aid.

Key : Type and proportion of extrusion aid used

MCC- 100% MCC;

Formulation A (i) – 100% CGLA;

Formulation A (ii) – 100% CGHA;

Formulation D (vi) - 60% CGLA/ 40% CGHA;

Formulation E (viii) – 65% CGLA/ 35% CGHA.

3.3.1 Pellet size distribution analysis

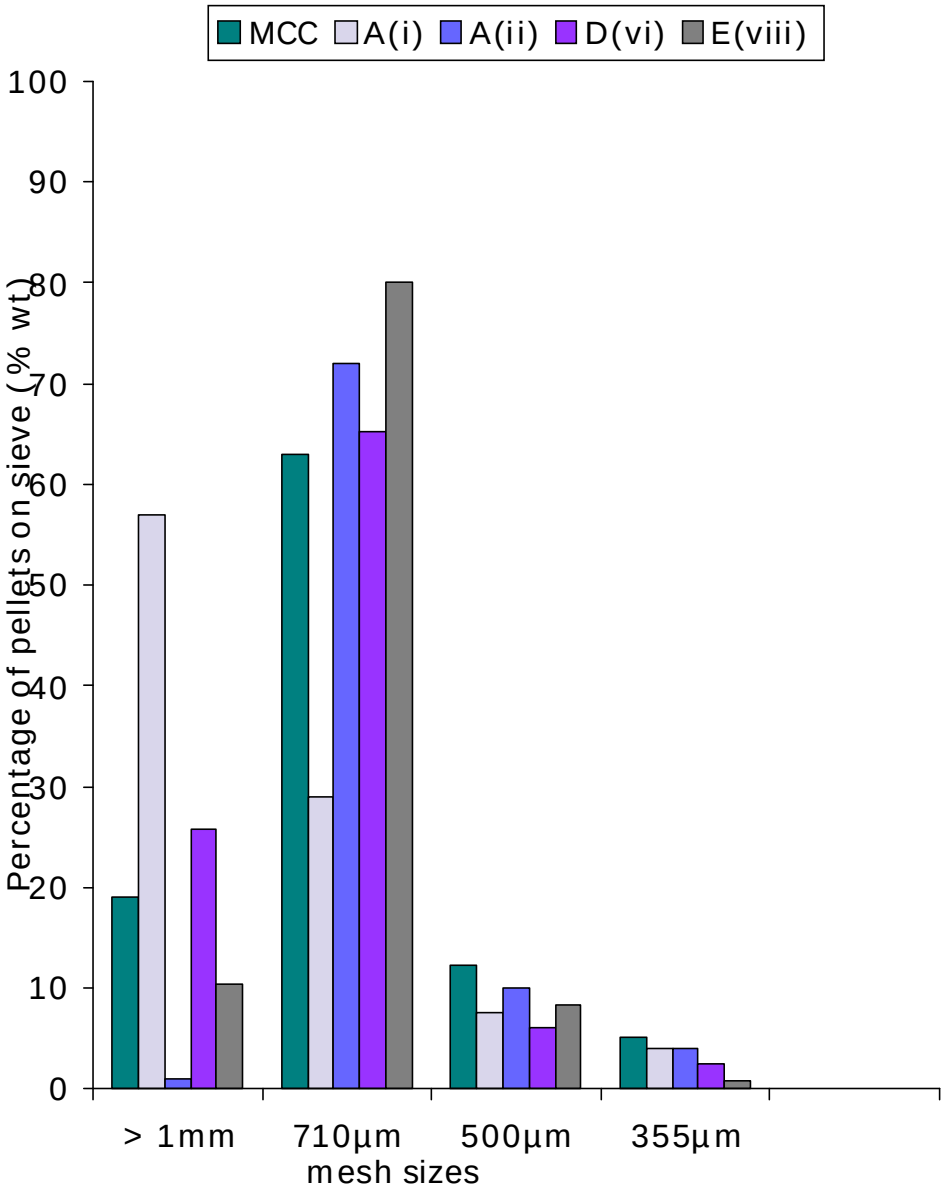

Fig. 9: Pellet size distribution analysis: the size distribution of CGLA pellets (A(i)) was improved by combining it with CGHA, with a large proportion of the pellets obtained being retained within a modal size range (710-1000µm mesh size).

3.3.2 Sphericity evaluation

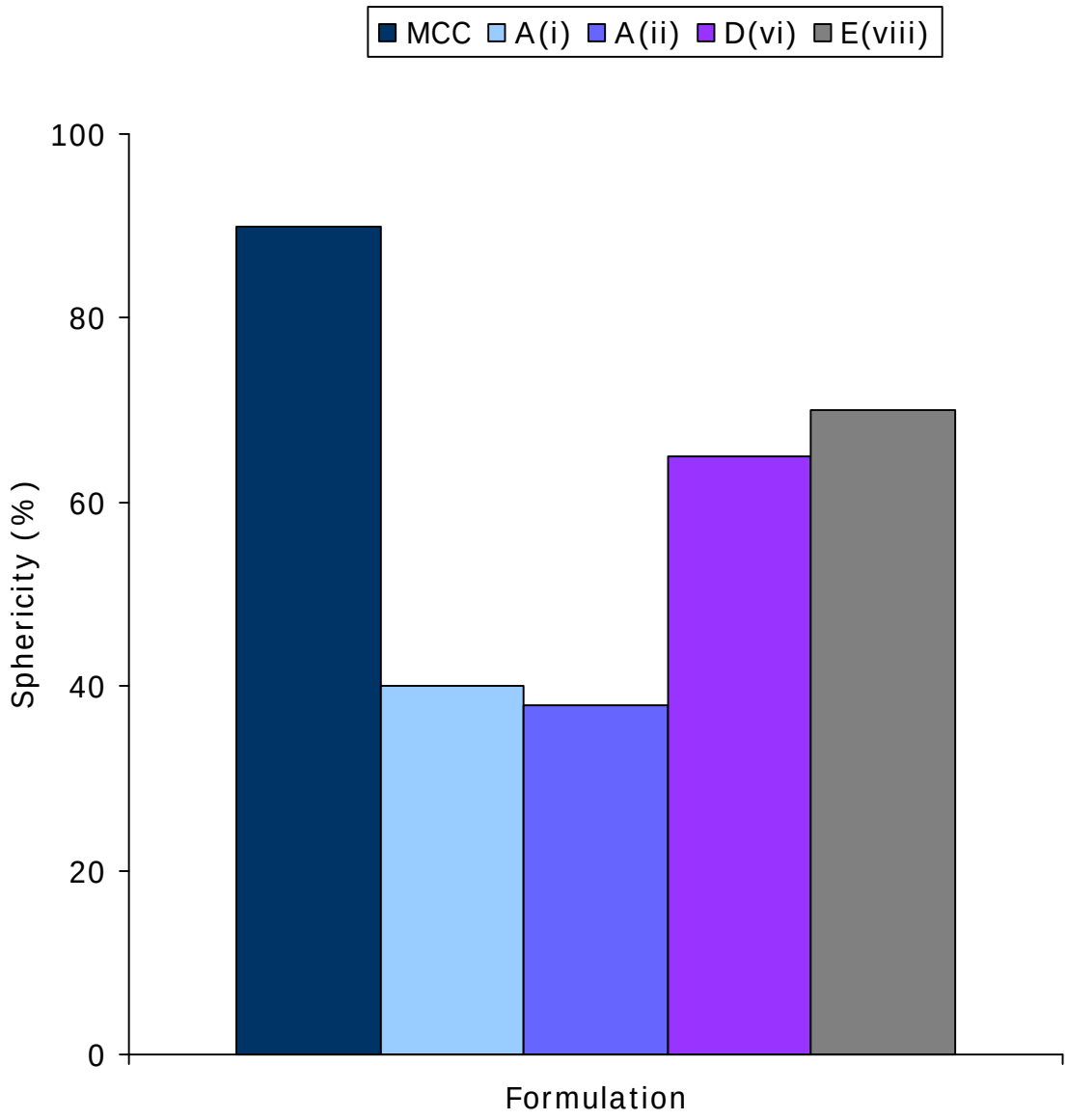

Fig. 10: Pellet sphericity evaluation: Formulations of gellan pellets composed of a combination of CGLA and CGHA (D(vi) and E(viii)) contained a higher percentage of spherical pellets than was observed with formulations containing either CGLA or CGHA alone (A(i)or A(ii)).

3.3.3 Optical micrograph of gellan pellets

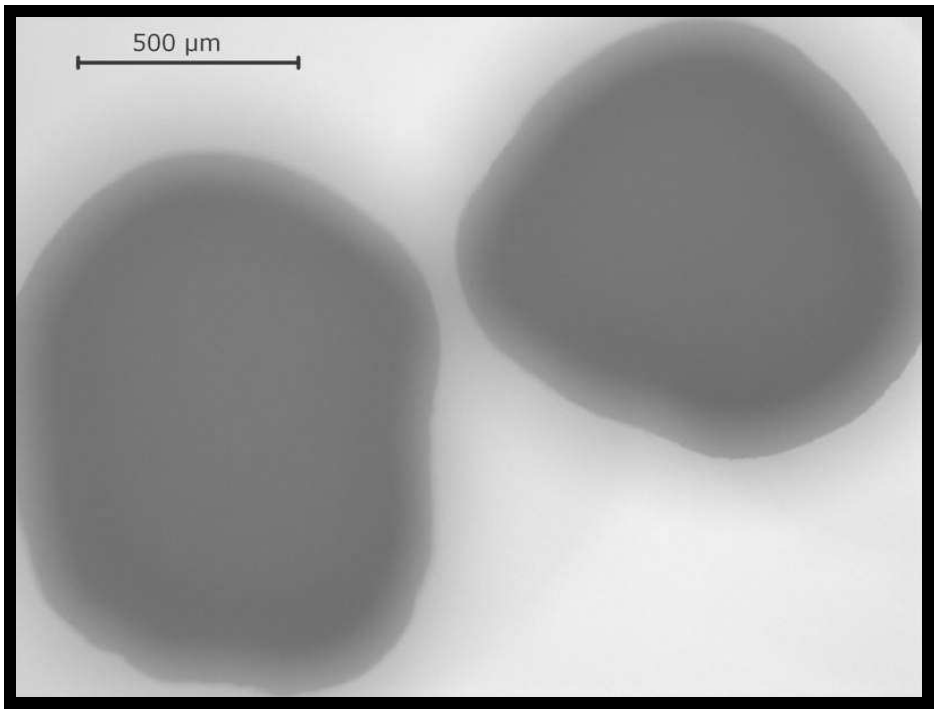

Fig. 11: Pellets from Formulation A(i) (100% CGLA) (x 5)

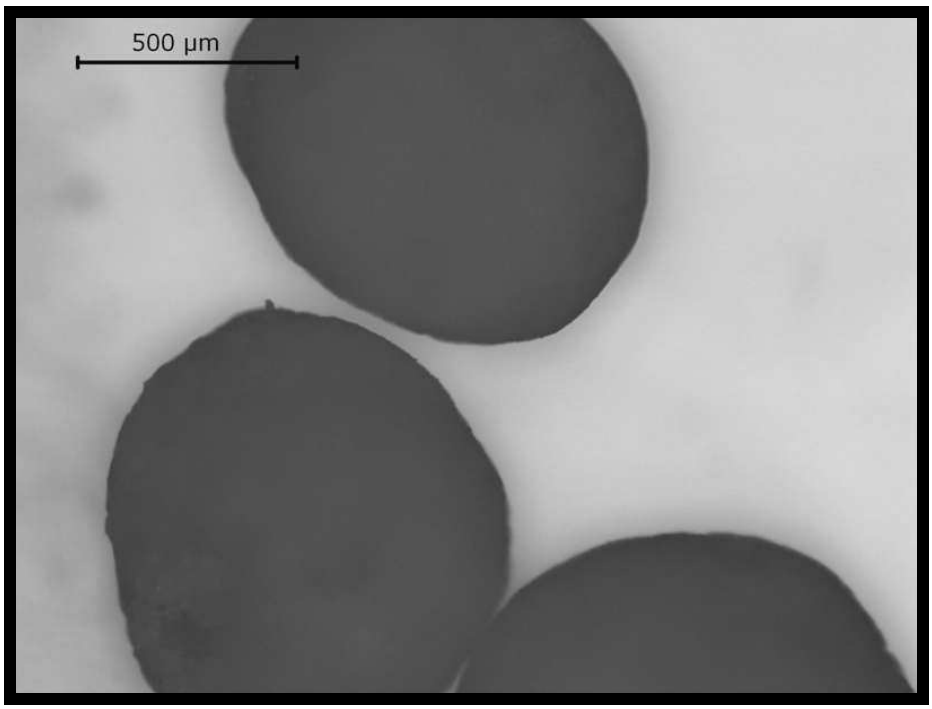

Fig. 12: Pellets from Formulation E(viii) (65% CGLA/ 35% CGHA) (x 5)

3.3.4 Pellet Friability and Flowability Determination

Table 6: Pellet friability and flowability indices including poured density (D_o), tapped density (D_f), compressibility (comp) and Hausner's Ratio (HR).

Formulation	**Friability**	**Flowability indices**			
		D_o	**D_f**	**Comp (%)**	**HR**
MCC	0.01	0.88	0.99	11.10	1.13
A(i)	0.63	0.66	0.70	5.71	1.17
A(ii)	0.42	0.67	0.69	2.90	1.03
D(vi)	0.92	0.69	0.73	5.50	1.06
E(viii)	0.98	0.69	0.74	6.80	1.07

3.4 Drug release profile of pellets

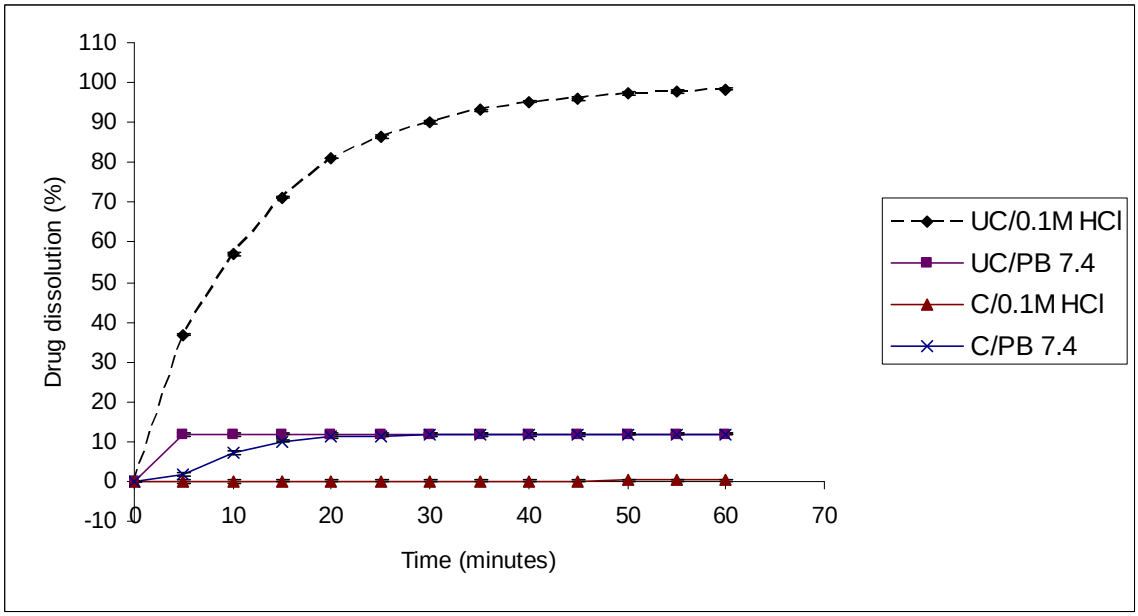

Fig. 13: Dissolution of MCC-based theophylline pellets where C denotes coated pellets; UC – uncoated pellets; PB 7.4 – phosphate buffer pH 7.4

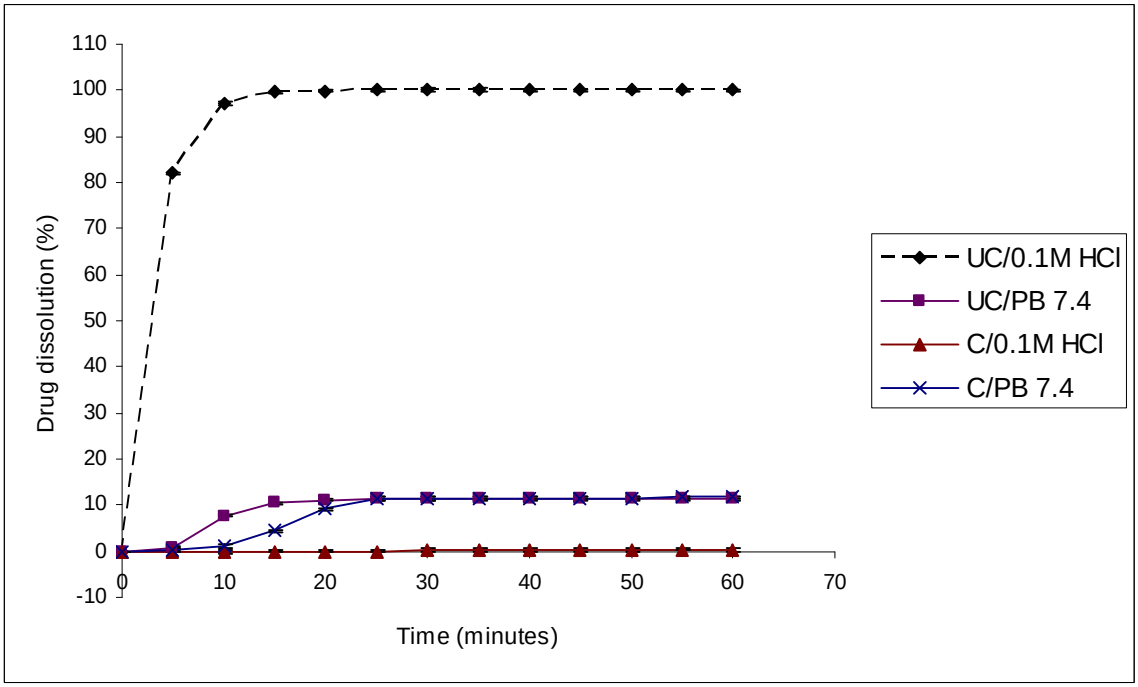

Fig. 14: Dissolution of gellan-based theophylline pellets where C denotes coated pellets; UC – uncoated pellets; PB 7.4 – phosphate buffer pH 7.4

Figure 13 and 14 shows the dissolution profile of coated and uncoated theophylline pellets containing MCC (Fig 13) and gellan (Fig 14) in 0.1M HCl and phosphate buffer.

In 0.1M HCl, all uncoated pellets showed an almost complete release of the drug (>90%). However, while MCC-based pellets exhibited a steady but gradual drug release pattern (Fig 13), gellan pellets demonstrated a more rapid and immediate release of the drug (Fig 14). The ability of the Eudragit coating to limit drug release in acidic media was evident from the dissolution curves of coated MCC and gellan based theophylline pellets in 0.1M HCl, which are almost level with the baseline.

The dissolution profiles obtained for theophylline pellets containing MCC and gellan in phosphate buffer were similar. Drug release was minimal (< 12%) and incomplete for uncoated and coated pellets.

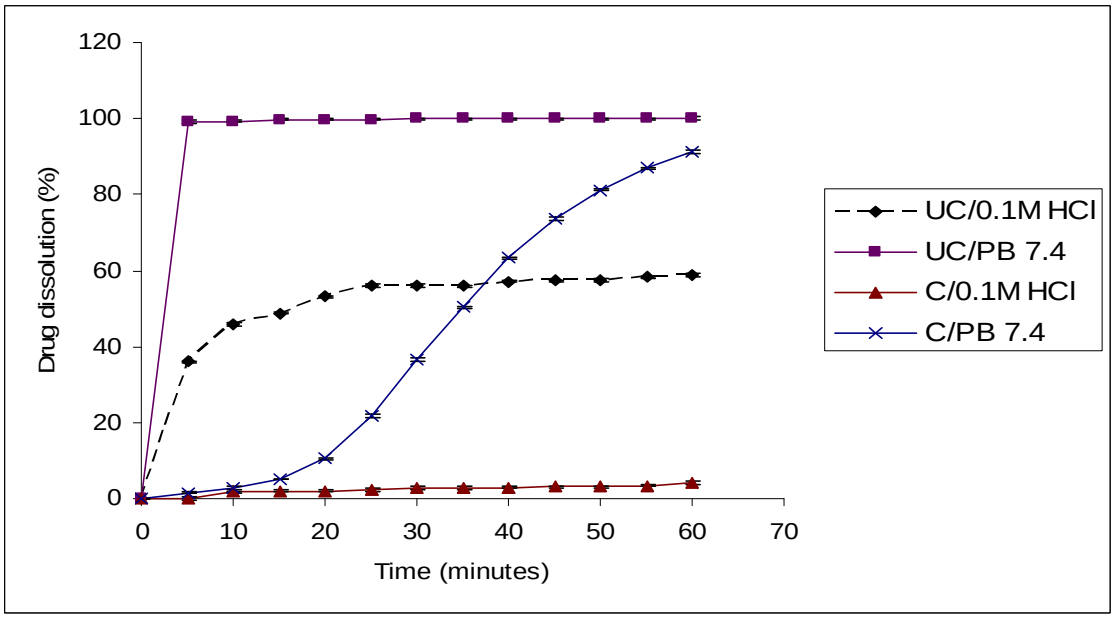

Fig. 15: Dissolution of MCC-based cystamine pellets where C denotes coated pellets; UC – uncoated pellets; PB 7.4 – phosphate buffer pH 7.4

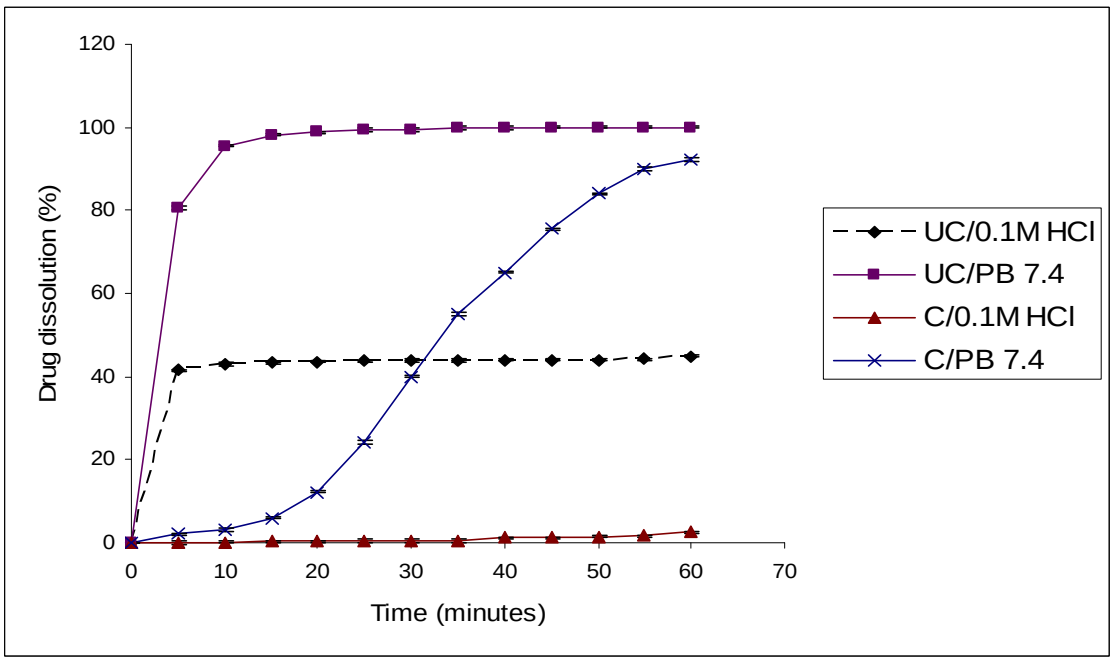

Fig. 16: Dissolution of gellan-based cystamine pellets where C denotes coated pellets; UC – uncoated pellets; PB 7.4 – phosphate buffer pH 7.4

Figure 15 and 16 shows the dissolution profile of coated and uncoated cystamine pellets containing MCC (Fig 15) and gellan (Fig 16) in 0.1M HCl and phosphate buffer.

In acidic media, uncoated cystamine pellets containing MCC showed a steady but gradual increase in dissolution rate (Fig 15), while pellets containing gellan exhibited a rapid and immediate drug release in 0.1M HCl (Fig 16). However, for both pellet formulations drug release was incomplete in 0.1M HCl, as only 40-50% of the drug was released from the pellets within one hour. Drug release from coated pellets was negligible in 0.1M HCl, with the Eudragit coating reducing the amount of drug dissolved to less than 5% in one hour.

In phosphate buffer, both MCC and gellan based uncoated cystamine pellets exhibited almost instantaneous and total release of the drug. Coated pellets in phosphate buffer showed a steady and gradual increase in the amount of drug dissolved, till almost the entire drug (>80%) was released at the end of one hour.

3.5 Scanning electron microscope images of cystamine pellets

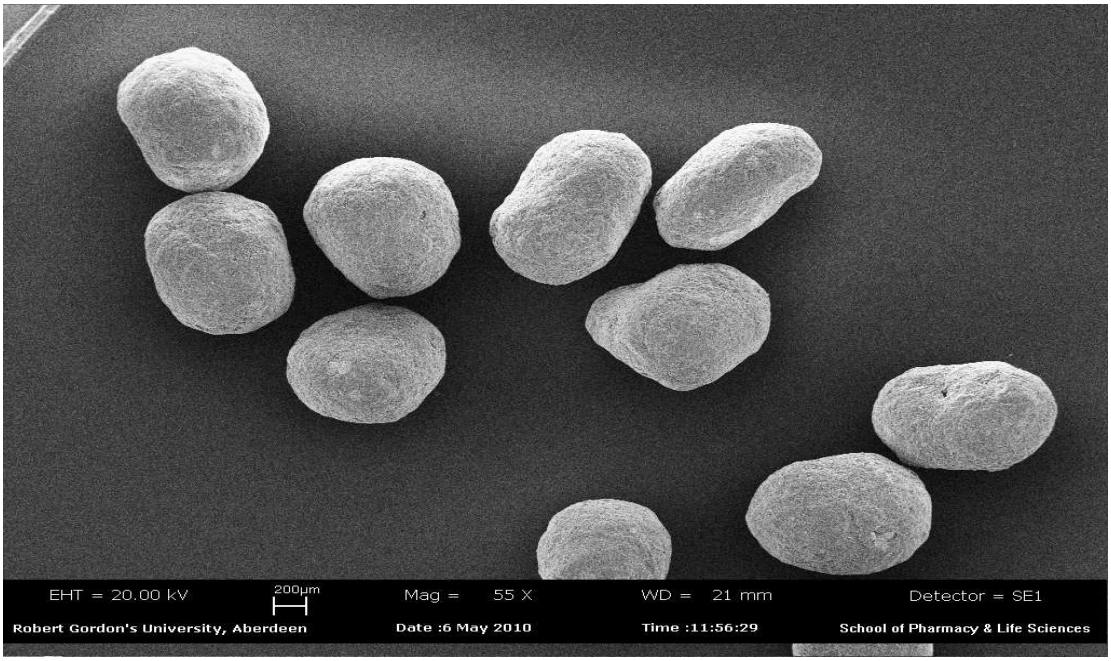

Fig. 17: Uncoated MCC-based cystamine pellets (x 55).

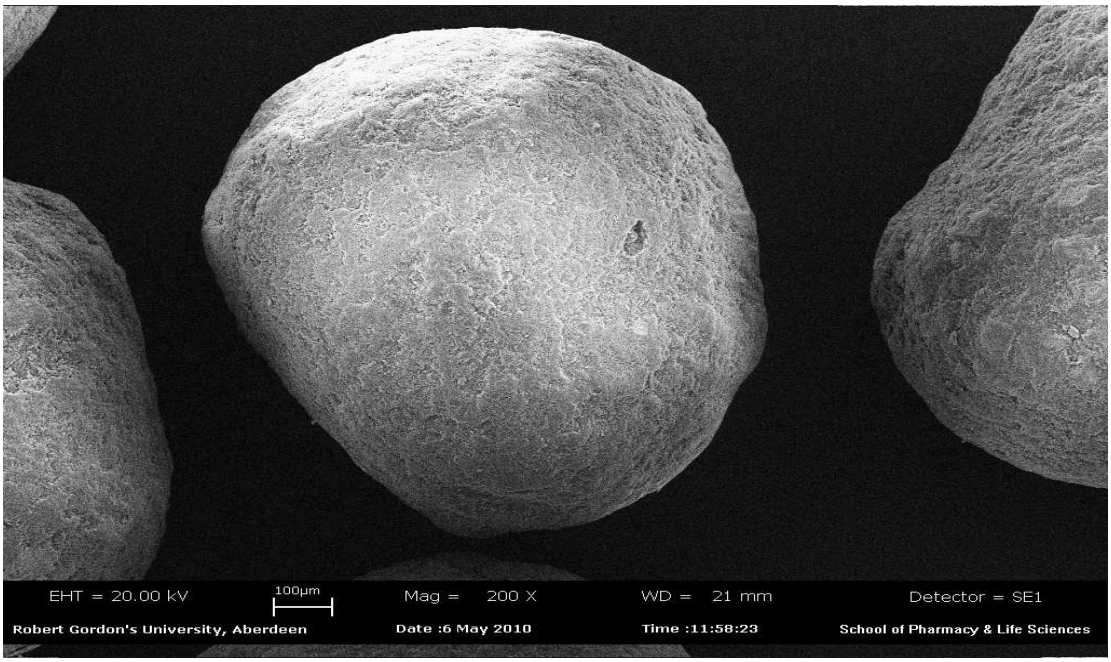

Fig. 18: An uncoated MCC-based cystamine pellet (x 200).

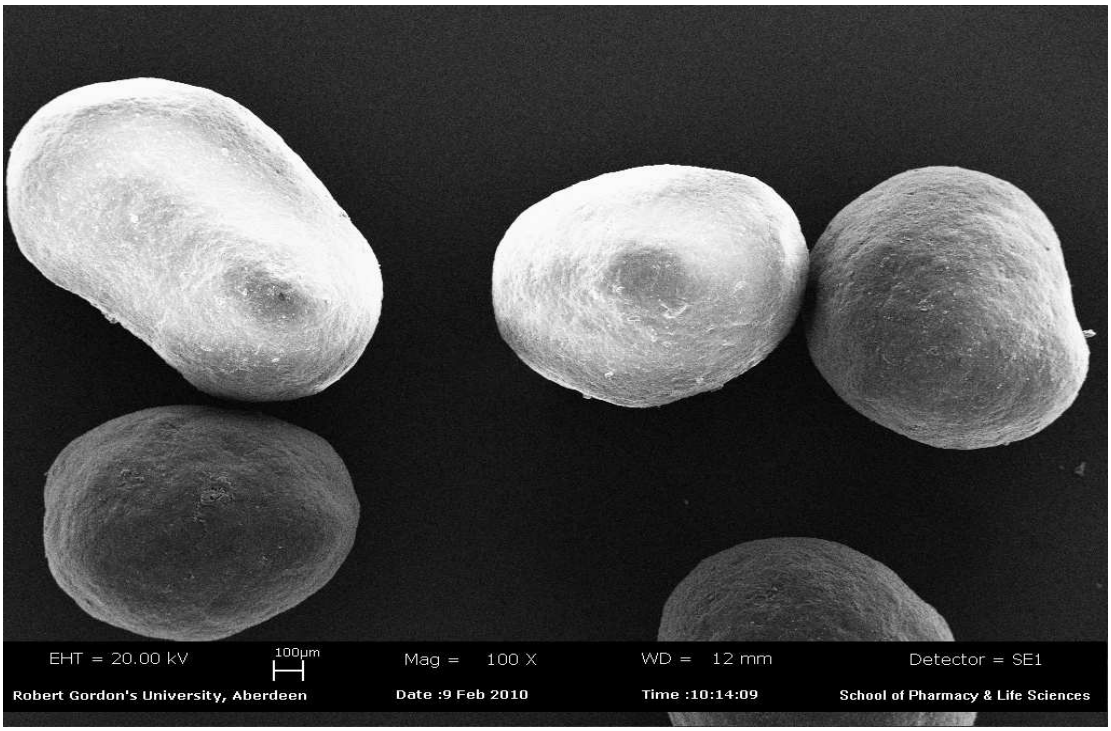

Fig. 19: Coated MCC-based cystamine pellets (x 100), note the surface smoothening effect of the coating on the pellets.

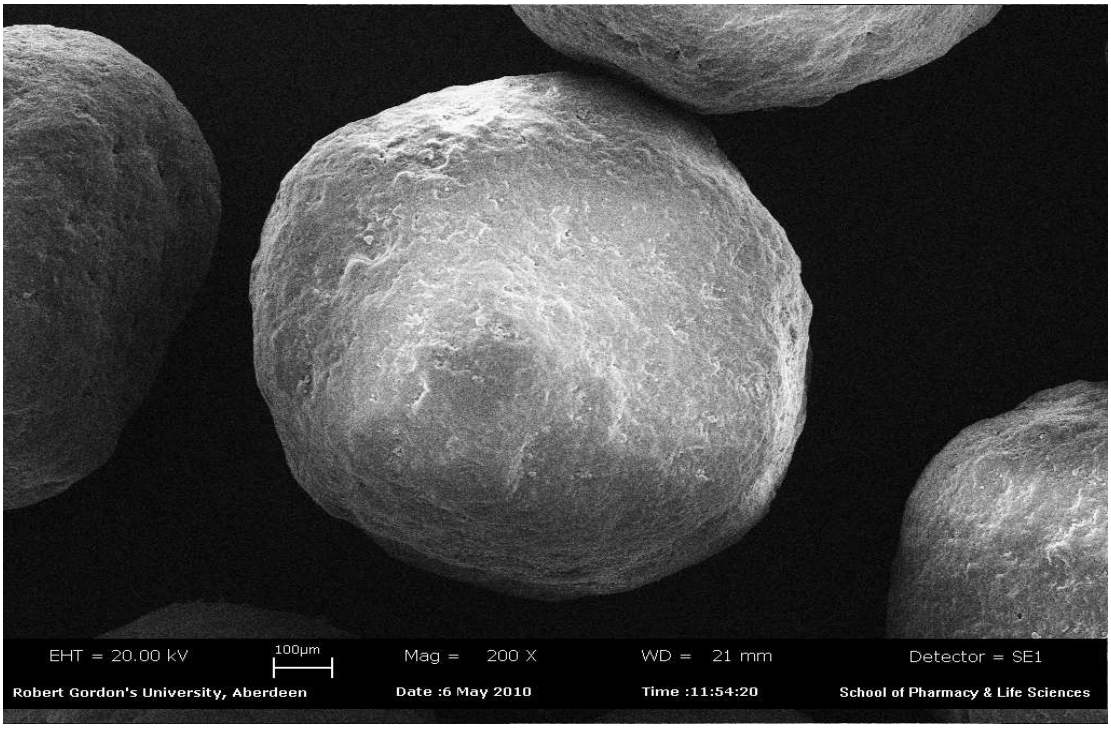

Fig. 20: A coated MCC-based cystamine pellet (x 200).

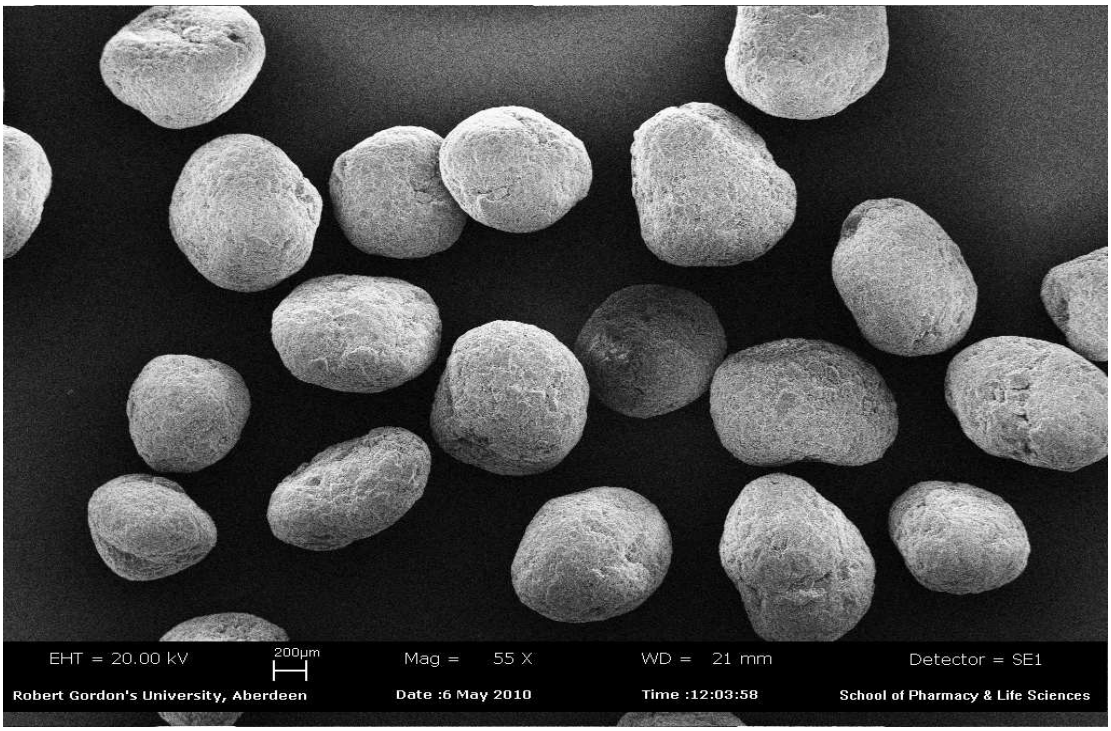

Fig. 21: Uncoated gellan-based cystamine pellets (Formulation Eviii) (x 55), note their high degree of sphericity.

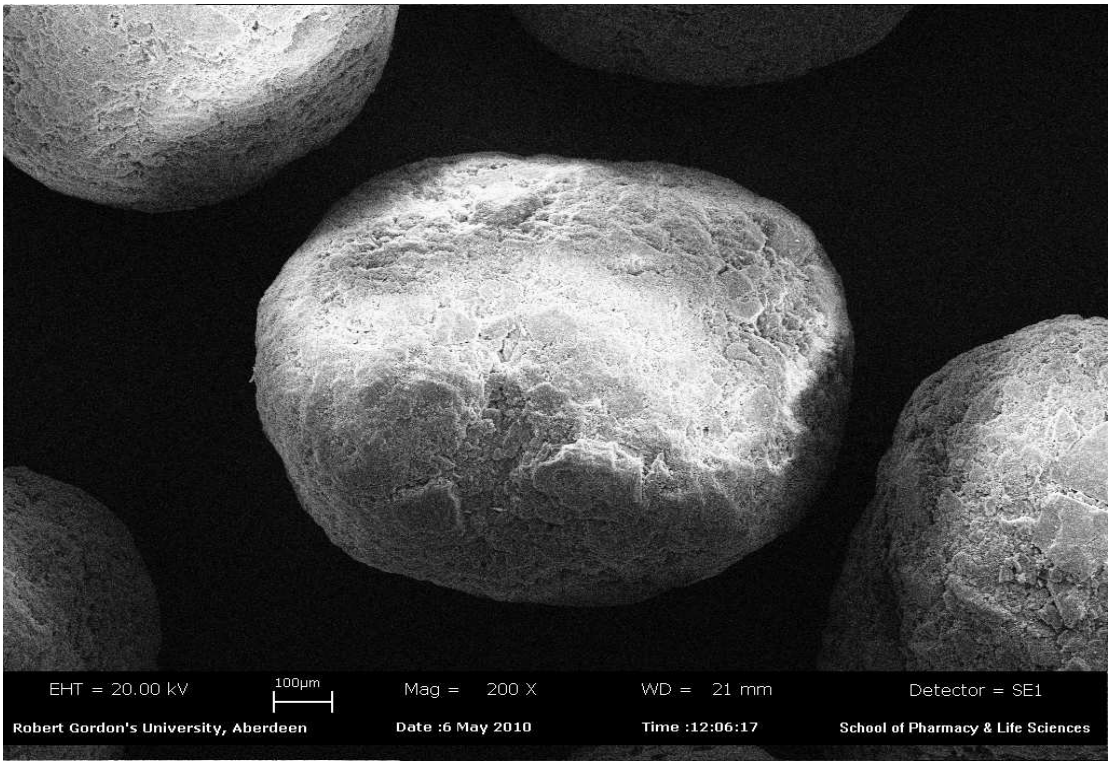

Fig. 22: An uncoated gellan-based cystamine pellet (x 200).

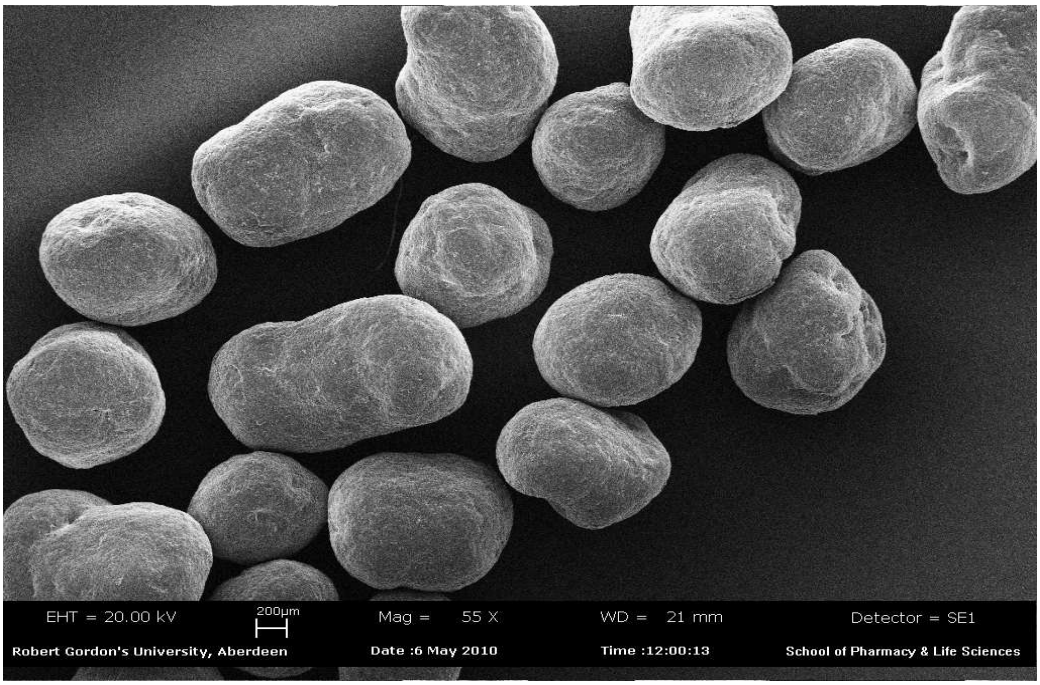

Fig. 23: Coated gellan-based cystamine pellets (x 55).

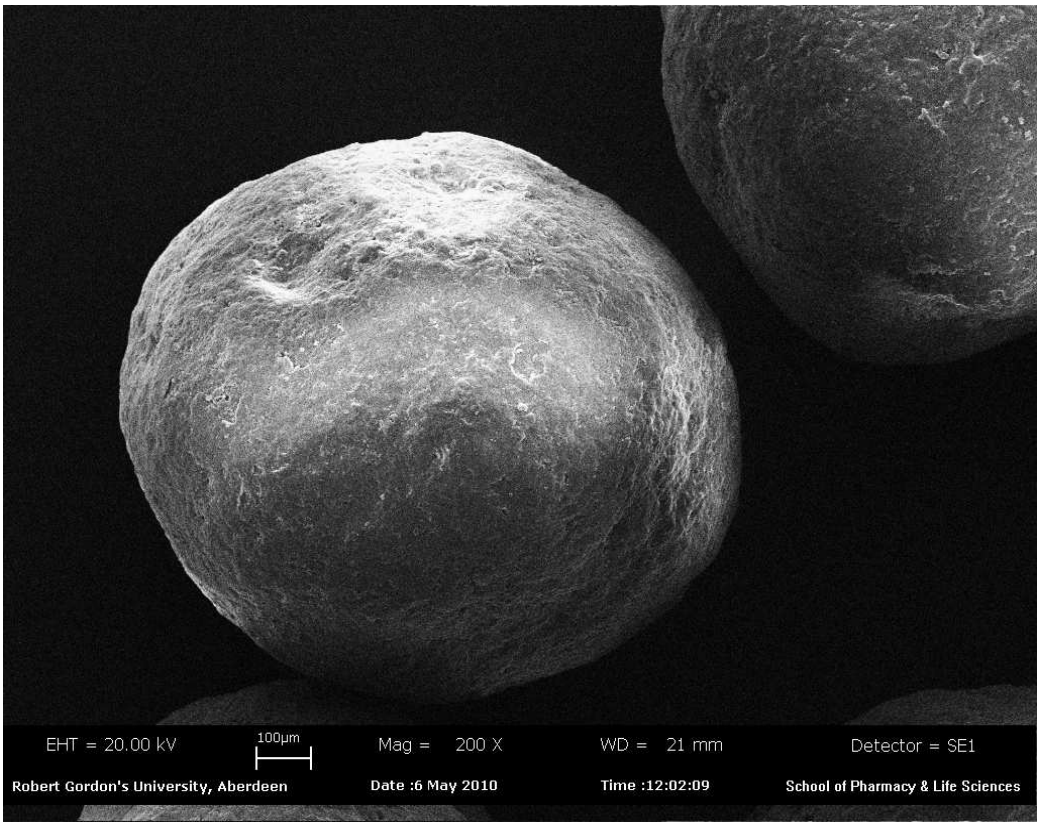

Fig. 24: A coated gellan-based cystamine pellet (x 200).

4. DISCUSSION

4.1 Evaluation of extrusion-spheronisation properties of polymers

In the formulation and manufacture of spheronised pellets, the polymer chosen to function as an extrusion aid in the formulation determines to a great extent the physical properties of pellets produced. Based on the peculiar rheology of the extrusion aid and its interaction with that of other components of the formulation, the process of extrusion and spheronisation could be adequately facilitated to the point where perfect pellets are produced, or no pellets are obtained at all after spheronisation.

Essentially, a polymer intended for use as an extrusion aid should be able to impart the properties of plasticity, fluidity and cohesiveness to a wet mass to facilitate and maintain sequential shape changes that are critical in its extrusion and spheronisation into pellets. So far, microcrystalline cellulose has been widely used as a standard extrusion aid due to its ability to confer a high degree of plasticity to wet mass formulations. In combination with equal proportions of Emcompress®, MCC-based wet masses at optimum water concentration (40% w/w) where found to yield hard spherical pellets within minimal spheronisation times of 5-8 minutes.

Obtaining pellets of similar quality from optimal formulations of other cellulosic polymers like HPMC, MC, HPC and SCMC was not possible, as was highlighted in Table 5.

This was essentially due to the production of smooth coils of very elastic extrudate that are unable to deform into pellets after prolonged spheronisation. Other cellulose derivatives like HEC and CCS impart little or no cohesive forces and fluidity to the wet mass, making processing difficult and leading to the formation of severely shark-skinned, friable extrudates that disintegrate in the spheroniser.

Starches (maize, wheat, rice and waxy corn starch) were observed to be easily spheronised into round pellets with smooth surfaces but very low mechanical strength, fragmenting when handled (pellet friability values: wheat -7.92% ; rice – 8.4%; maize -10.57%). Natural gums such as xanthan, karaya and ghatti gum yielded smooth extrudates, plastic enough to deform into fairly round pellets after spheronising for 10-12 minutes. However, the sticky nature of the gums caused the pellets obtained to clump together into aggregates during spheronisation, making it impossible to collect pellets as discrete units even after drying in the fluidised bed drier. Potato starch, locust bean and guar gum wet masses were found to produce elastic extrudate that barely deforms on spheronisation.

Gellan blends incorporated into lactose-based wet masses at ratios of 1:20 (gellan:lactose) produced round, discrete pellets of acceptable mechanical strength within spheronisation times of 8-10 minutes. Surface texture of gellan pellets was observed to be smoother with lactose being used as the filler rather than Emcompress®. On evaluation of the aforementioned observations, gellan gum was found to show the most

potential for being a suitable extrusion aid, and subsequent work was focused on optimising the properties and yield of pellets obtained from gellan based wet mass formulations.

4.2 Evaluation of the properties of optimised gellan formulations

Optimisation of the extrusion properties of gellan formulation was geared towards obtaining a wet mass that would support the production of a high proportion of spherical pellets within a narrow size range.

Batches of pellets prepared using CGLA (Formulation A(i)) as the sole extrusion aid were seen to have a wide size distribution, with a high percentage of dumbbells retained on the 1mm sieve and low percentage of very spherical pellets on the 710 µm and 500 µm sieves. This is presumably because CGLA forms brittle gels when wetted due to the low levels of acylation of the gum [36]. Therefore, spheronisation times are long (18-20 minutes) and results in breakage of the extrudates into longer rods partially deformed into dumbbells and short rods that are spheronised into pellets.

On the other hand, high acyl gellan gum CGHA characteristically forms soft elastic gels when wetted [36].Hence, pellets containing only CGLA show a reduced tendency to deformation as is often encountered with elastic materials and hence have poor sphericity.

Optimisation studies involved identifying the desirable properties of either grade and combining both grades of the gum in different ratios to

potentiate these extrusion properties and provide a favourable gel texture for successful extrusion and spheronisation. All formulations containing blends of CGLA and CGHA showed improved pellet size distribution. Formulation D(vi) and E(viii): 60% CGLA/ 40% CGHA; 65% CGLA/35% CGHA respectively yielded high proportion of spherical pellets on the 710 - 1000µm mesh as shown in Figure 9 and were retained for further characterisation.

These formulations were also found to possess sufficiently low friability to withstand normal handling stresses and comply with the Eudragit manufacturer's friability limits for pellets intended for coating purposes (< 2% friability) [43]. The results from the flowability determinations of pellet Formulations D(vi) and E(viii) as highlighted by the Hausner's Ratio and Carr's Compressibilty Index were desirable. For Hausner's Ratio, values below 1.25 indicate acceptable flowability [45] (Formulation D(vi) - 1.06; Formulation E(viii) – 1.07), while Carr's indices between 5-15% indicate excellent flow properties [45].

However, it is important to note that the following results are obtained from the assessment of placebo pellets and the incorporation of an API significantly affects the physical properties of the resultant drug-loaded pellets, especially in cases where a high dose or concentration of the API is required. Thus extrusion aids need to be sufficiently robust to withstand to an appreciable extent, fluctuations in wet mass properties caused by changes in formulation excipients. The results show that the physical properties of pellets from Gellan formulation D(vi) were not as good as

was observed with MCC. However, gellan Formulation E(viii), closely replicated the properties of MCC formulations and can be used as a suitable extrusion aid for the production of spheronised pellets.

The suitability of gellan gum as an extrusion aid may likely be due to the presence of several free hydroxyl groups within its structure, enabling the formation of intermolecular hydrogen bonds with water molecules. MCC has been observed to have about 3% of absorbed water bound to hydroxyl groups within its amorphous parts [26]. This sort of linkages with water molecules enables these polymers to entrap water within their structure thereby improving the rheology of the wet mass and facilitating the pelletisation process.

Reducing production costs by replacing high quantities of the costly microcrystalline cellulose with low quantities of gellan gum as an extrusion aid will still produce spheronised pellets of acceptable quality and efficacy. Therefore, future development work in the field of extrusion and spheronisation should focus on the use of gellan gum as a much needed alternative to MCC.

4.3 Evaluation of the effectiveness of coated pellets in facilitating colon-specific drug delivery

For the purpose of this work, the application of a pH-dependent polymer coating on the pellets was chosen as the method of achieving colon targeted drug delivery due to its inherent simplicity, ease of application and high degree of effectiveness. Eudragit® FS30D was selected as the

pH-dependent polymer of choice, based on the manufacturers literature and specifications [43].The application of an optimum coating of Eudragit® FS30D on drug loaded pellets should ensure the release of less than 5% of the drug in 0.1M HCl within one hour while also ensuring that greater than 80% of the drug is released in phosphate buffer pH 7.4, similar to pH conditions found in the colon [43].

The results of dissolution tests on both coated and uncoated pellets indicated the effectiveness of sufficiently thick and uniform coatings of Eudragit® FS30D in retarding drug release in 0.1M HCl. However, theophylline pellets were observed to be unable to release greater than 12% of the total concentration of the drug incorporated in phosphate buffer pH 7.4. Although, 100% of the drug was released from uncoated theophylline pellets in 0.1M HCl.

The reason for this anomalous dissolution behaviour of theophylline pellets in phosphate buffer is unclear. But, a possible explanation could be solid-state transformation of anhydrous theophylline on absorption of water into its lattices to form the less soluble theophylline monohydrate [48]. This sort of phase transformation has been well documented for theophylline, and can be induced by the presence of a supersaturated solution and aggravated by the presence of certain excipients in the formulation [48, 49, 50, 51]. Transformation of anhydrous theophylline to theophylline monohydrate reduces the dissolution rate of the drug considerably, as the dissolution rate changes to the lower dissolution rates of the monohydrate [49].

Solid-state transformation may occur during processing (especially with wet granulation processes that involve the use of high quantities of water), during storage under humid conditions or during dissolution [50, 51, 52].

The formation of theophylline monohydrate has been found to be aggravated by the presence of MCC due to its high water retaining capacity and the presence of water-soluble fillers like lactose that increase the volume of the liquid phase [51]. Also, solution-mediated transformation which causes slowing down of dissolution rates during a dissolution process due to recrystallization of drugs into the less soluble crystalline forms have been found to be dissolution-media dependent and can lead to abnormal dissolution behaviour [52,53]. In-process monitoring of pseudopolymorphic transitions of drugs can be done using near-infrared spectroscopy and Raman spectroscopy [54].

On the other hand, uncoated cystamine pellets showed slow but consistent release of the drug in 0.1M HCl and rapid release of the drug in phosphate buffer pH 7.4.Subsequent coating of cystamine pellets (10g) with 4.5g of Eudragit® FS30D retarded drug release to less than 5% in 0.1M HCl for one hour, while excellent and consistent release of greater than 90% of the drug was observed in phosphate buffer pH 7.4 in an hour. However, gellan gum and microcrystalline cellulose based drug loaded pellets where observed to have similar rates of drug dissolution from the pellet matrix.

Future research focused on optimising the use of effective pH-sensitive polymer coatings like Eudragit® FS30D to produce colon-targeted multiparticulate pellets would be a way forward in combating the unpleasant GIT side effects caused by oral administration of cysteamine.

5. CONCLUSION

The above research findings have highlighted the possibility of replacing high concentrations of microcrystalline cellulose used in the production of spheronised pellets with far lesser quantities of cheaper alternatives like gellan gum, without necessarily compromising the quality and acceptability of pellets produced.

These oral multiparticulate dosage forms have also been shown to be potentially suitable as colon-targeted delivery systems, when coated with pH-sensitive polymers for delivery of cystamine.

Successful colonic delivery of cysteamine will greatly reduce the discomforting and unpleasant gastrointestinal side effects of the drug, thereby facilitating patient compliance and safety.

These beneficial effects will translate eventually into less costly pharmaceutical products, affordable healthcare and an overall enhancement in the quality of life of patients suffering from cystinosis.

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