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Small Scale Manufacturing and Scintigraphic Detection of Pellet Formulations

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This thesis is the result of the author’s original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Signed:

Date:

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Abstract

This research was designed to develop radiolabelling methods for pellets or mini-tablets so that their *in vivo* behaviour can be assessed using gamma scintigraphy. Eight different pellet formulation profiles were developed using paracetamol and indomethacin as partially and poorly water soluble model drugs respectively. Four different drug release profiles for each of the two model drugs were achieved by applying a film coat to the pellet formulations, which were manufactured using either extrusion-spheronisation or the drug-layering techniques. Pelletisation methods were developed on a small scale in order to minimise quantities of radioisotope required for manufacturing for scintigraphic studies.

Extrusion-spheronisation used microcrystalline cellulose and lactose as the main components. Thermally controlled spheronisation at elevated temperature produced pellets within a narrow size distribution. It was found that the drug release rate from extrusion-spheronised pellets could be readily manipulated by changing the ratio of microcrystalline cellulose within the pellets. However, the drug-layering technique was required in order to achieve a rapid release of the poorly water soluble drug indomethacin.

Two radiolabelling methods, the "incorporation" and the "soaking" methods, were successfully developed. In the "incorporation" method a ^{99m}Tc radioisotope is mixed into a powder blend prior to small scale extrusion-spheronisation and in the "soaking" method inert pellet cores are soaked in a solution containing a ^{99m}Tc radioisotope, which becomes attached to the pellet core prior to small scale drug-layering.

The use of radiolabelled placebo pellets in gamma scintigraphic studies was validated. It was found that gastrointestinal transit of drug loaded formulations, which may include novel pellet formulations or mini-tablets, can be traced *in vivo* by mixing with radiolabelled placebo pellets.

A faster and easier alternative to the "soaking" method, the "wet and dry" method, was optimised at a later stage.

Publications

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Table of contents

List of abbreviations

^{99m}Tc:	Technetium-99m
^{111}In:	Indium-111
^{153}Sm:	Samarium-153
BP:	British Pharmacopoeia
DPD:	Dicalcium phosphate dihydrate
DTPA:	Diethylene triamine pentaacetic acid
DVB:	Divinyl benzene
EC:	Ethyl cellulose
f1:	Difference factor
f2:	Similarity factors
GI:	Gastrointestinal
HPMC:	Hydroxypropylmethylcellulose
HPC:	Hydroxypropylcellulose
MCC:	Microcrystalline cellulose
MMI:	Magnet moment imaging
PEG:	Polyethylene glycol
PK:	Pharmacokinetic
PVP:	Polyvinylpyrrolidone
ROI:	Region of interest
Sv:	Sievert
TAP:	tartaric acid pellets
Tg:	Glass transition temperature

List of figures

List of tables

Chapter 1: Introduction

A wide range of pellet and mini-tablet formulations exist on the market and various techniques and excipients are employed in order to manufacture such formulations with the desired drug release profile. There is a lack in the capability of radiolabelling these multiparticulate dosage forms which are needed for scintigraphic studies on pellet and mini-tablet formulations. In order to accommodate scintigraphic studies of this wide range of multiparticulate dosage forms various manufacturing techniques and excipients were investigated as part of this project. The initial part of this chapter therefore reviews excipients and manufacturing techniques for the various existing pellet and mini-tablet formulations. The chapter culminates by discussing the current methods employed for radiolabelling of pellet formulations for scintigraphic detection.

1.1. Pellets and mini-tablets

Pellets and mini-tablets are often wrongly referred to as the same type of formulation with different manufacturing methods. This is probably due to their small size which is an important feature for both formulations. Pellets and mini-tablets do have a lot in common but the most distinct difference is their manufacturing methods which make pellets of non-uniform sizes and mini-tablets of uniform sizes. This project mostly concerns pellet formulations but mini-tablets will be described as well, as some scintigraphic detection methods for pellets also can be applied to mini-tablets.

1.1.1. Pellets

Pellets are spherical agglomerates with smooth surfaces and of non-uniform sizes ranging between 0.5-2 mm (Rahman *et al.* 2009). Pellets are primarily produced for the purpose of oral controlled-release dosage forms having gastro resistant or sustained-release properties. These properties are accomplished by the excipients of the pellet core or by the addition of an appropriate film coat material.

1.1.2. Mini-tablets

Mini-tablets are tablets with a diameter equal to or smaller than 3 mm. They are uniform in size, have a smooth surface, low porosity and a high crushing strength (Lennartz and Mielck 1998). Mini-tablets are primarily produced for the same purposes as pellets. Due to their uniformity and small size, mini-tablets are also used as ophthalmic inserts (Gasthuys *et al.* 2007; Weyenberg *et al.* 2003) or as oral administration to paediatrics (Kayumba *et al.* 2007; Stoltenberg and Breitkreutz 2011; Thomson *et al.* 2009). The small size of mini-tablets ensures that the formulation can be placed in the eye and makes it easier for paediatrics to swallow. Pellets cannot be dosed in this way, as single units, due to their non-uniform size which complicates a consistent dose administration. Another advantage of mini-tablets over pellets is their high robustness which can be useful for their coating and dosing (Lennartz and Mielck 1998).

1.1.3. Advantages and disadvantages in pellets and mini-tablets

The use of pellet and mini-tablet formulations has technical and therapeutic advantages as well as disadvantages. These are considered in Section 1.1.3.1-1.1.3.4 below.

1.1.3.1. Technical advantages in pellets and mini-tablets

Technical advantages in the production of pellets and mini-tablets are as follows (Manivannan *et al.* 2010; Schaefer 2004):

- Their free-flowing character makes them easy to handle and pack in a uniform dosage form.
- Dust is avoided when handling and packing the formulations which improves the work environment for the personnel and avoids cross contamination.
- The smooth spherical shape of pellets makes them easier to coat as the coating layer will not have holes due to sharp corners.
- Incompatible drugs formulated into different pellets and mini-tablet formulations can be added together into one formulation without interfering with each other.

1.1.3.2. Therapeutic advantages in pellets and mini-tablets

Therapeutic advantages in using pellets and mini-tablets are as follows (Bodmeier 1997; Ho *et al.* 2000):

- The formulations spread uniformly throughout most of the gastrointestinal (GI) tract which results in consistent bioavailability and a reduced risk of local toxicity.
- Large, unwanted, dose dumping due to crack in a release controlling film coat of a single formulation is avoided.
- Mixing pellets or mini-tablets containing different drugs into one dosage form decreases the number of dosage forms to be taken by a patient.
- Mixing pellets or mini-tablets of different drug release profiles into one dosage form leads to the possibility of obtaining nearly constant rates of drug release.

1.1.3.3. Technical disadvantages in pellets and mini-tablets

Technical disadvantages in the production of pellets and mini-tablets are as follows (Parul and Avinash 2011):

- The drug load is relatively small due to the high amount of excipients required in the manufacturing process.
- There is a lack in the efficacy when pellets are manufactured: resources are wasted when fractions of produced pellets that are outside a desired size range are discarded.
- There are a large number of process variables and formulation steps when pellets are manufactured.
- Advanced technology may be needed in the manufacturing of both types of formulations.
- High tumbling can occur during coating in the coating pan (described in Section 1.4.1), where a high mechanical robustness is necessary for the formulation not to

break. Mini-tablets are usually manufactured with high crushing strengths but this is usually not the case for pellet formulations.

- The production of both types of formulations is expensive.

1.1.3.4. Therapeutic disadvantage in pellets and mini-tablets

The following therapeutic disadvantage exists:

- The non-uniformity in size of pellets complicates administration of a consistent drug dose.

- Bunching of pellets or mini-tablets may occur at the ileocaecal junction, immediately before entry into the caecum (Wilson 2010). This could lead to local toxicity.

1.2. Multiparticulate dosage forms

Multiparticulate dosage forms are systems consisting of multiple mini drug depots which include nanoparticles, microparticles, microcapsules, pellets, mini-tablets or granules (Shukla *et al.* 2011). These drug depots can be administered in a single dose in the form of hard gelatine capsules or disintegrating tablets that rapidly liberate their contents of multiparticulates in the stomach (Bodmeier 1997; Shukla *et al.* 2011). Furthermore, Schmidt and Bodmeier (2001) have described a novel method of incorporating pellets into a carrier to avoid the existing risks of the pellet film coats breaking due to frictions inside the capsule or due to rupture during compression into tablets (Schmidt and Bodmeier 2001). Compressing pellets into tablets is the most modern technique which offers advantages over filling of capsules. The tablets are smaller, and therefore easier to swallow, and they are cheaper to produce (Bodmeier 1997; Rahman *et al.* 2009). However, sometimes the filling of capsules may be the preferred method e.g. if it is desired to open the capsules in order to sprinkle its content onto food for ease of swallowing. Gelatine capsules exist in various sizes which are designated by a capsule number. Capsule size decreases for capsules of number 0-5 and increases with capsule number 0, 00 and 000. The number of pellets that can be filled in each capsule of number 0-4 was calculated by Rowe *et al.* (2005) and is shown in Table 1.1.

Table 1.1. Number of pellets that can fit into each capsule number. Modified from (Rowe *et al.* 2005).

Capsule No.	Pellet diameter		
	0.8 mm	**1.0 mm**	**1.2 mm**
0	381.9	377.3	369.9
1	273.1	269.2	267.4
2	210.2	207.5	205.8
3	152.4	150.7	149.4
4	135.8	134.8	133.9

1.3. Pelletisation and mini-tableting techniques

Pelletisation refers to the process of enlargement of agglomerates into pellets (Rahman *et al.* 2009). The process is controlled, using advanced equipment, so that the agglomerates become spherical (Schaefer 2004).

The most popular pelletisation techniques are the extrusion-spheronisation and drug-layering techniques. These techniques will therefore be described in more detail. Modern pelletisation techniques are also described and may be of special importance if they will be used in the future development of new pellet formulations. Another pelletising technique, which is of less relevance, is the balling technique. This technique is outdated and was the technique which gave the name to the formulation called “pill” which is wrongly interchanged with the modern formulations, “tablet”. In this technique pellets are produced in a continuous rolling and tumbling motion in pans, discs, drums or mixers (Manivannan *et al.* 2010). This technique will not be explained in more detail.

The technique used for mini-tablet manufacturing is very similar to that of conventional tablets and involves compression of powder using a conventional tablet press.

1.3.1. Extrusion-spheronisation

The most popular pelletisation technique is the extrusion-spheronisation (Manivannan *et al.* 2010; Schaefer 2004) and is shown in Figure 1.1. This technique is a multi-step compaction process involving dry mixing of powders, wet granulation, extrusion, spheronising, drying and screening to collect pellets of the correct size (Liew *et al.* 2007; Manivannan *et al.* 2010).

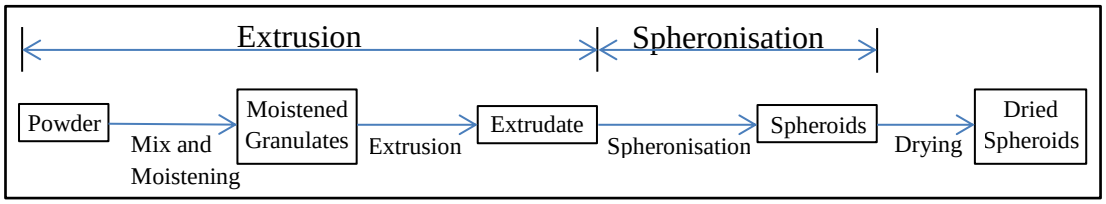


Figure 1.1. Illustration of the extrusion-spheronisation technique. The processing steps involved in the extrusion part and the spheronisation part are indicated. Modified from (Liew *et al.* 2007).

In the extrusion-spheronisation technique the drug is incorporated into the matrix of the pellet formulation. This is an advantage over the drug-layering technique (described in Section 1.3.2) because the drug release can be modified by changing the excipients to accelerate or sustain the drug release, without the necessity of applying a film coat. The disadvantage in the extrusion-spheronisation technique is that the pellets produced have large size variations which create a large waste of pellets outside a desired size range.

1.3.1.1. Extrusion-spheronisation apparatus

The main apparatus used in this technique are the extruder and the spheroniser.

1.3.1.1.1. The extruder

The extruder can be classified as screw, gravity or ram systems, based on the type of feed mechanism (Manivannan *et al.* 2010; Rahman *et al.* 2009).

- The screw extruders include the axial type, dome type and radial type. In the axial type the extrudate is discharged at the end and in the radial type the extrudate is discharged perpendicular to the axis of the screw (Rahman *et al.* 2009).

- The gravity extruders include the rotary cylinder and the rotary gear extruder. In the rotary cylinder extruder one of the two counter rotating cylinders is hollow and perforated and the other one is solid and presses the extrudate through the holes. In the rotary gear extruder both extruders have holes that the extrudate is pressed through when the cylinders are pressed together (Rahman *et al.* 2009).

- The ram extruder, which is probably the oldest type of extruder, is a piston-type extruder. In this apparatus a piston forces the extrudate through a die at the end (Rahman *et al.* 2009).

The existing extrusion apparatus described above are commonly used for the production of larger pellet batches due to the necessity of a large wet mass in the feeder of the extruder. However, for small scale pellet production a simple sieve extrusion may be used whereby the wet mass is forced through a sieve using a spatula.

It is not always possible to transfer a formulation directly from one extrusion system to another due to different properties of the final extrudates produced. Baert *et al.* (1992) compared the gravity extruder with the ram extruder and found that a lower quantity of water was necessary for the process by the ram extruder. This is due to a variation in formulation water movement within the two different apparatus. Formulation water movement is caused by pressure on the wetted powder mixture during extrusion. In the gravity feed extruder water movement is small because of a discontinuous pressure. In the ram extruder formulation water movement is more extensive due to the continuous action and results in non-uniform extrudates (Baert *et al.* 1992).

The non-uniform extrudates produced by the ram extruder result in non-uniform pellet sizes. This was also found by Fielden *et al.* (1992) who showed that the cylinder extruder made better size uniformity. This was explained by the high shear stress at the die wall in the ram extruder which makes extrudates of variable moisture content (Fielden *et al.* 1992).

Baert *et al.* (1993) compared the twin screw extruder with the gravity feed extruder and found that the twin screw extruder produced weaker bound extrudates than the gravity feed extruder when small amounts of moistener were used. This was explained by the shorter length of the extruder die in the twin screw extruder (Baert *et al.* 1993).

Furthermore, different extrusion types with the same extrusion feed mechanism have been shown to make pellets of different properties. Desire *et al.* (2011) showed that the three screw extruders: the axial, dome and radial extruder produce pellets of different properties. In their experiment radial extrusion created smaller pellets of larger size distribution and axial extrusion created pellets which presented a majority

of rods. The axial system produced pellets of highest strength followed by the dome extrusion. This difference was explained by a higher density of extrudates from the axial system. The dome extruder created the most spherical pellets within the most narrow size distribution (Desire *et al.* 2011).

1.3.1.1.2. The spheroniser

The spheroniser makes spheres during centrifugation by forcing the agglomerates to the walls of a container where the particles are spheronised due to a combination of a toroidal flow of the agglomerates and friction between other agglomerates and a rippled friction plate spinning at the bottom of the spheroniser (Schaefer 2004). The friction plate is most often cross-hatched but different types and sizes are available. Spheronisation can also be done by injection moulding of thermoplastic matrix formulations (Quinten *et al.* 2009).

Hellen *et al.* (1993) showed that a greater load, higher speed of friction plate and a longer residence time in the spheroniser reduced the size of pellets (Hellen *et al.* 1993). However, Fielden *et al.* (1993) showed that a longer spheronisation time increased pellet size when the moisture content was above a certain percentage (Fielden *et al.* 1993).

Newton *et al.* (1995) showed that overloading the plate requires a longer process time for making spheres while under loading reduces the efficiency. Low speeds fail to provide the necessary interactions to provide rounding of the particles (Newton *et al.* 1995).

Newton *et al.* (1995) compared the use of a cross-hatched plate to a radial plate and found that the plate design only appears to have marginal influence on the ability to spheronise, but it may have a potential to influence less robust formulations. A similar result was found by Liew *et al.* (2007) who compared a cross-hatched plate to a tear drop plate, which has more round edges. They found that the degree of agglomeration and breakdown of spheroids would be determined by the collisions with the spheroids rather than the design of the plate (Liew *et al.* 2007).

1.3.1.1.3. The drier

Drying can be done in a fluid bed dryer, which may also be used for coating, or it can be done in an oven tray. Sousa *et al.* (1996) showed that pellets dried in a tray oven equipped with a fan had higher crushing strengths than the correspondent pellets dried in a fluid bed and that the fluid bed dries the pellets more quickly (Sousa *et al.* 1996). The mechanical strength of a formulation may influence the kinetics of the drug release (Steendam *et al.* 2000). A monograph in the British Pharmacopoeia (BP Online 2013, Appendix XVII G. Friability) describes how pellet friability can be tested using a fluidised bed indicating that pellets may be prone to high physical stress during this drying process (British Pharmacopoeia Online 2013).

1.3.1.2. Extrusion-spheronisation excipients

The most important excipient for extruded and spheronised pellet formulations is the plasticiser. This is due to the importance of plasticising properties during the spheronising process (Schaefer 2004). A moistener is also essential for the process of extrusion-spheronisation. However, for hot-melt extrusion a matrix former, which becomes liquid during heating, is used instead of a moistener. Other basic excipients include fillers and disintegrants.

1.3.1.2.1. Plasticiser

Microcrystalline cellulose (MCC) is a key excipient in pellet production because of its plasticising properties when mixed with a moistener, which is necessary for the process of extruding and spheronising (Kristensen *et al.* 2000; Schaefer 2004). The amount of MCC to be added depends on the other excipients and the type of technique or apparatus being used (Kristensen *et al.* 2000). An amount of at least 15-30% (w/w) of MCC has been reported necessary to produce suitable spheres when spheronising granules into pellets (Kristensen *et al.* 2000).

MCC has the disadvantages of lacking disintegration for immediate release of poorly water soluble drugs. MCC forms an insoluble hydrophilic matrix of low porosity caused by contraction of MCC during drying. Drug release for poorly soluble drugs in particular is thus very slow when MCC is used as less wetting is accomplished (Kleinebudde 1994; Pinto *et al.* 1992).

Ibrahim *et al.* (2011) added the co-solvent polyethylene glycol (PEG) 400 to the granulation liquid used for wet massing during extrusion-spheronisation of MCC pellets containing a poorly water soluble drug, in order to increase drug dissolution rate (Ibrahim *et al.* 2011). Other methods for increasing drug dissolution rate of extrusion-spheronised pellets include increasing porosity of the pellets or making the pellet formulation disintegrate. These methods are detailed below. Disintegrating pellet formulations seem to have the general disadvantage that their mechanical resistance is reduced thereby complicating film coating of the pellets.

The following initiatives have been taken to reduce the low porosity of extrusion-spheronised pellets caused by MCC:

- **Reduce MCC content:** The porosity can be increased by partially replacing the MCC with a filler that easily dissolves, such as lactose (Kleinebudde 1994).

- **Modification of granulation liquid:** Millili and Schwarts (1990) used a water-alcohol mixture as an alternative to distilled water for granulation. This showed to decrease the contraction of MCC during drying and thereby causing higher porosity (Millili and Schwartz 1990).

- **Addition of superdisintegrant:** Souto *et al.* (2005) found that the addition of 2-5% (w/w) of the superdisintegrants, croscarmellose sodium or sodium starch glycolate caused a modest increase in the drug dissolution rate of extrusion-spheronised pellets containing MCC by an increased porosity without causing pellet disintegration (Souto *et al.* 2005).

Following initiatives have been taken to enable disintegration of extrusion-spheronised pellet formulations:

- **Modification of granulation liquid:** Millili and Schwartz (1990) found that a mixture of ethanol and water, in concentrations of less than 30% (v/v) water, produces pellets that disintegrate during dissolution due to weakened bonding of MCC. However, the mechanical strength of these pellets is greatly reduced by the addition of ethanol in the granulation liquid (Millili and Schwartz 1990). A recent study by Chamsai and Sriamornsak (2013) describes the disintegration of

extrusion-spheronised pellets containing MCC when the granulation liquid contains 15% (v/v) ethanol and 10% (v/v) polysorbate 80 or PEG 400. However, they also found a reduced mechanical strength of their disintegrating pellets (Chamsai and Sriamornsak 2013).

- **Addition of superdisintegrant:** Little information is available on making disintegrating extrusion-spheronised pellets containing MCC by incorporating a superdisintegrant. As indicated in the results by Souto *et al.* (2005), 2-5% (w/w) croscarmellose sodium or sodium starch glycolate does not results in disintegrating extrusion-spheronised pellets (Souto *et al.* 2005). However, Chamsai and Sriamornsak (2013) found that the addition of 2-10% (w/w) croscarmellose sodium to their extrusion-spheronised pellets containing MCC and a modified granulation liquid, causes a more rapid increase in the pellet disintegration (Chamsai and Sriamornsak 2013). Chatchawalsaisin *et al.* (2004) added 4-8% (w/w) chitosan to MCC during extrusion-spheronisation and achieved disintegrating pellet formulations. They did not describe the affect that the chitosan had on the mechanical strength of their pellet formulation (Chatchawalsaisin *et al.* 2004).

- **Partial or total replacement of MCC:** Alvarez. L. *et al.* (2003) found that the use of powdered cellulose in place of MCC can produce disintegrating extrusion-spheronised pellets. The particles of powdered cellulose are larger than those of MCC which causes less cohesion of the particles. However, the size distribution of their manufactured pellets was high and the mechanical strength low (Alvarez *et al.* 2003). Thommes and Kleinebudde (2006) produced κ-carrageenan based extrusion-spheronised pellets based on a specific starch grade, which were shown to have a negative effect on the mechanical strength (Thommes and Kleinebudde 2006). Dukic *et al.* (2007) produced disintegrating extrusion-spheronised pellets based on a specific starch which required the addition of a binder during pelletisation due to a reduced mechanical strength (Dukic *et al.* 2007).

1.3.1.2.2. Moistener (and binder)

A moistener acts as a binder during wet massing, a lubricant during extrusion and a plasticiser during spheronisation (Sousa *et al.* 1996).

Furthermore, a moistener has a positive effect on the following properties: reduced dust formation, increased agglomeration and increased crushing strength. The increased crushing strength is probably due to the water-bridges built after partial dissolution of the powder mix. Furthermore Sousa *et al.* (1996) found that the drug release pattern is also influenced by the moistener content.

The amount of moistener necessary in the manufacturing of extrusion-spheronised pellets depends on the excipients used and the desired size of the pellets. Water insoluble excipients and fine powders, which seem to increases the capillary suction of moistener, need larger amounts of moistener (Fielden *et al.* 1993).

Distilled water is usually used for moistening as it gives pellets of high mechanical strengths (Millili and Schwartz 1990). Other moisteners have also been found in the literature and can be beneficial when certain properties are desired. PEG 400 has been added as co-solvent in order to increase the dissolution rate of a poorly water soluble drug (Ibrahim *et al.* 2011). A mixture of water and alcohol can be used in order to decrease the drying time of the pellets (Millili and Schwartz 1990). A binder may be added to the granulation liquid in order to obtain pellets of low friability. The binder may include hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC) or polyvinylpyrrolidone (PVP) (Chitu *et al.* 2011).

1.3.1.2.3. Filler and disintegrant

A filler is a substance that is added to expand the volume of the formulation. It is necessary to expand the volume in order to improve mass variation and handling during manufacturing.

A disintegrant is a substance that is added to a formulation to make it break apart when placed in an aqueous environment. Two main types of disintegrants exist. Those that enhance the action of capillary forces for a rapid uptake of aqueous

liquids and those that swell in contact with water (López-Solís and Villafuerte-Robles 2001).

Lactose is a commonly used excipient for tablets manufacturing. It combines the functions of filling, binding and disintegrating (Kristensen 2000). However, lactose only shows filling and disintegrating properties when used as an extrusion-spheronisation excipient because it has no plasticising effect, which is necessary for binding during extrusion-spheronisation. Sinha *et al.* (2005) showed that the use of dicalcium phosphate dihydrate (DPD) as a filler produces more spherical pellets of higher density (Sinha *et al.* 2005). However, lactose has advantages over DPD because lactose is soluble in water whereas DPD is insoluble in water. Lactose thus acts as a disintegrant as it is water soluble and therefore is able to take up water that aids disintegration through the creation of pores.

1.3.2. Drug-layering

The drug-layering technique is a technique where the drug is deposited on a starter core which may be crystals or granules of the drug itself or an inert material (Manivannan *et al.* 2010). The amount of drug deposited on the starter core depends on the size of the starter core and the drug concentration in the solution, suspension or dry powder mix.

As shown in Figure 1.2 the layering process may include the deposition of drug entities from a solution, suspension or a dry powder (Glatt-Group 2013).

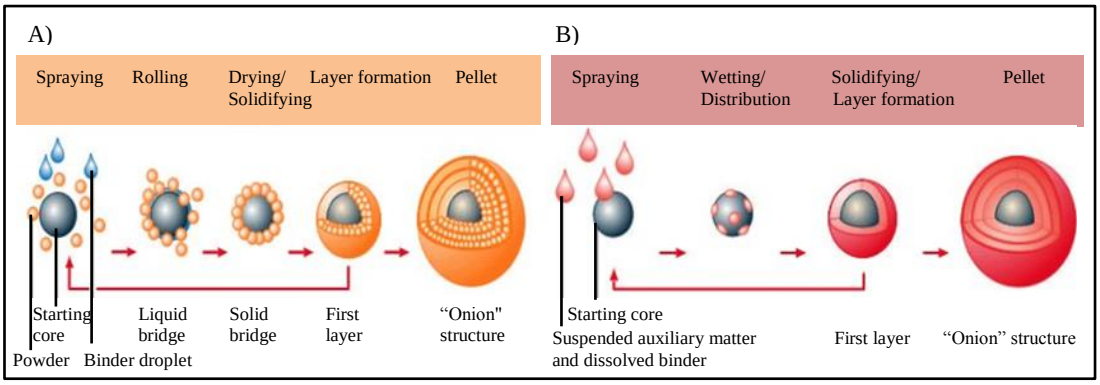


Figure 1.2. Scheme of stages involved in drug-layering using A) dry powder and B) solution/suspension. Modified from (Manivannan *et al.* 2010).

The most common technique is to apply the drug layer from a solution or suspension. If a drug content of only 10% (w/w) in solution leads to a viscosity too high to be readily sprayed then suspension layering is usually the more economic technique (Sellassie 1989). The layer in the solution and suspension technique is usually 60-200% of the weight of the starter core. The average weight gain per processing hour is about 15-20% (Glatt-Group 2013).

Dry powder layering is suitable for layering of poorly soluble drugs or drugs that exhibit stability problems in water as only a little water is sprayed onto the starter core in order to bind the dry powder to its surface. A rolling motion of the starter core strongly assists the layering process, similar to that of a snowball effect, i.e. rolling about a larger particle in a slightly wetted mass. Hourly weight gains of up to 300% are possible so this process is relatively fast. The structure of a powder layer is less compact and smooth than a layer produced from a solution or suspension. It is therefore recommended to apply a thin neutral film layer to these pellets (Glatt-Group 2013).

The advantage in the drug-layering technique is that spraying and drying is accomplished in one closed system. Drawbacks in the layering technique are the non-uniformity in size, the small drug loading and the time consuming process (Rahman *et al.* 2009).

1.3.2.1. Drug-layering apparatus

In the solution or suspension layering the layer is applied by same type of equipment used for coating; a coating pan or a fluidised bed (see Section 1.4) (Bohr *et al.* 2011; Manivannan *et al.* 2010).

Dry powder layering need specialised equipment so that the powder can be applied to the starter core and a rotor insert is usually used for applying a rolling motion to assist the “snowball effect” described above (Section 1.3.2).

1.3.2.2. Drug-layering excipients

1.3.2.2.1. Starter core

Various commercial starter cores of sizes ranging from 0.16-2.00 mm in diameter exist (Umang innovative solutions 2013). The most commonly used starter cores are sugar spheres (nonpareils) and MCC cores (Gryczova *et al.* 2008). Alternative starter cores are composed of tartaric acid, silica, calcium carbonate, mannitol, lactose or starch (Umang innovative solutions 2013).

Nonpareils consist of an initial sucrose crystal which is then layered to various commercially available sizes with sucrose and starch (Gryczova *et al.* 2008). The sugar spheres have the disadvantage that they may dissolve during the drug-layering process forming undesirable agglomerates and giving a lower yield (Gryczova *et al.* 2008). Alternatively, water-insoluble MCC starter cores can be used. They have been reported to offer higher abrasion resistance, lower agglomeration tendencies during aqueous drug layering and less interference by mechanical stress during dissolution compared to nonpareils (Gryczova *et al.* 2008).

It has been demonstrated that the dissolution of drug from water soluble pellet cores can be much faster than that of water insoluble pellet cores, depending on the type of coat the pellets are coated with. This is due to the difference of osmotic pressure created in the outer and inner part of the starter core. This difference in osmotic pressure is only created when liquid is able to penetrate to the core which occurs when the core is water soluble (Kállai *et al.* 2010).

Alternative starter cores will not be described in more details, except the more recent developed tartaric acid pellets (TAP). These are used as pellet cores for drug substances showing poor solubility in high pH-values. TAP act as a pH lowering agent. Thus in the case of a drug which has a low solubility at higher pH-values, TAP is able to enhance drug release by creating an acidic micro-environment (Pharmatrans Sanaq AG 2013).

1.3.2.2.2. Binders

In the drug-layering technique, binders are added to improve drug adherence to the starter cores. For suspension and solution layering the use of low-viscosity binders is highly recommended in order to facilitate the flow in the feed tubing during spraying. These binders have been mentioned in Section 1.3.1.2.2 and include HPMC, HPC and PVP. For suspension layering, the use of binders also helps prevent sedimentation of drug powder whilst in the feed tubing.

1.3.3. Other pelletisation techniques

Other pelletisation techniques include:

- **Hot-melt extrusion** which is similar to the extrusion-spheronisation technique. However, in this technique pellets are produced without the use of solvents. This is useful when a drug exhibits stability problems in solvents. The drug is dispersed in a thermal carrier which also acts as a matrix former. The matrix former typically provides release controlling properties and can be a wax or a polymer such as ethylcellulose (EC) (Quinten *et al.* 2009). When the matrix former is heated during hot-melt extrusion it becomes a liquid which acts as a moistener. The extruder for hot-melt extrusion has a heated barrel containing the rotating screw. The extrudates are cut into uniform cylindrical segments which are spheronised using a spheroniser with a controllable heat source (Rahman *et al.* 2009).

- **Direct pelletisation** is a technique similar to extrusion-spheronisation. The same excipients are used but the apparatus is different. Direct pelletisation is accomplished in a rotary processor which allows pelletisation to be conducted in a single-step process. Powders are mixed, moistened and set into a centrifugal motion in a single apparatus as shown in Figure 1.3 (Kristensen *et al.* 2000; Manivannan *et al.* 2010).

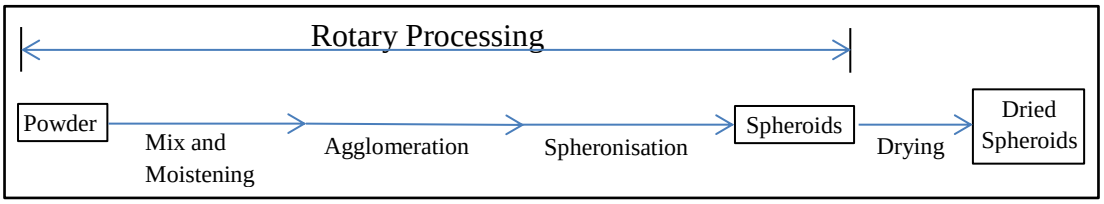


Figure 1.3. Illustration of the direct pelletisation technique. The steps involved in rotary processing by the rotary processer are indicated. Modified from (Liew *et al.* 2007).

The rotary processer has the advantage over the multi-step extrusion-spheronisation technique in that pellets are manufactured in a single step and less water is required in the manufacturing process. Furthermore, Kristensen *et al.* (2000) showed the amount of distilled water added for wet massing can be controlled by the torque increase in the wetted powder blend, measured by the rotary processor, and thereby produce pellets of reproducible size ranges, independent on the compositions of the excipients (Kristensen *et al.* 2000). Liew *et al.* (2007) compared the pellets produced in the direct pelletisation technique with pellets produced in the extrusion-spheronisation technique. They found that the direct pelletisation techniques produces pellets of higher crushing strengths but that pellets produced in the extrusion-spheronisation technique are more spherical in shape and have smaller size distribution (Liew *et al.* 2007).

- **Spray congealing** in which a solution or suspension of a drug in a molten mass is atomised by spraying followed by solidification when in contact with cool air (Qiu *et al.* 2009).

- **High speed shear mixing** in which a drug in a solid mass, typically made of wax, is broken into granules by shear mixing followed by spheronisation (Lee 2003).

- **Cryopelletisation** in which liquids or molten solids are introduced into a cooling fluid as droplets which solidify when exposed to the low temperature. These pellets are then lyophilized to remove water and organic solvents. A major limitation to cryopelletisation is the impact of liquid or semisolid droplets on the surface of the liquid nitrogen which creates surface irregularities in the pellets. Furthermore, pellets produced by freeze-drying become highly porous (Cheboyina *et al.* 2004; Rahman *et al.* 2009).

- **Freeze pelletisation** which is a technique similar to cryopelletisation but with less limitation as there is no freeze-drying or impact to the droplets involved in this process (Cheboyina *et al.* 2004). As shown in Figure 1.4 a molten solid carrier along with dispersed drug and excipient is introduced by a needle to an inert column of liquids. This matrix is introduced as droplets that are immiscible in the liquid. The droplets move up or down in the liquid depending on the density of the droplet and the liquid. The droplets solidify into spherical pellets when they move into freezing areas of the liquid (Cheboyina *et al.* 2004; Cheboyina and Wyandt 2008).

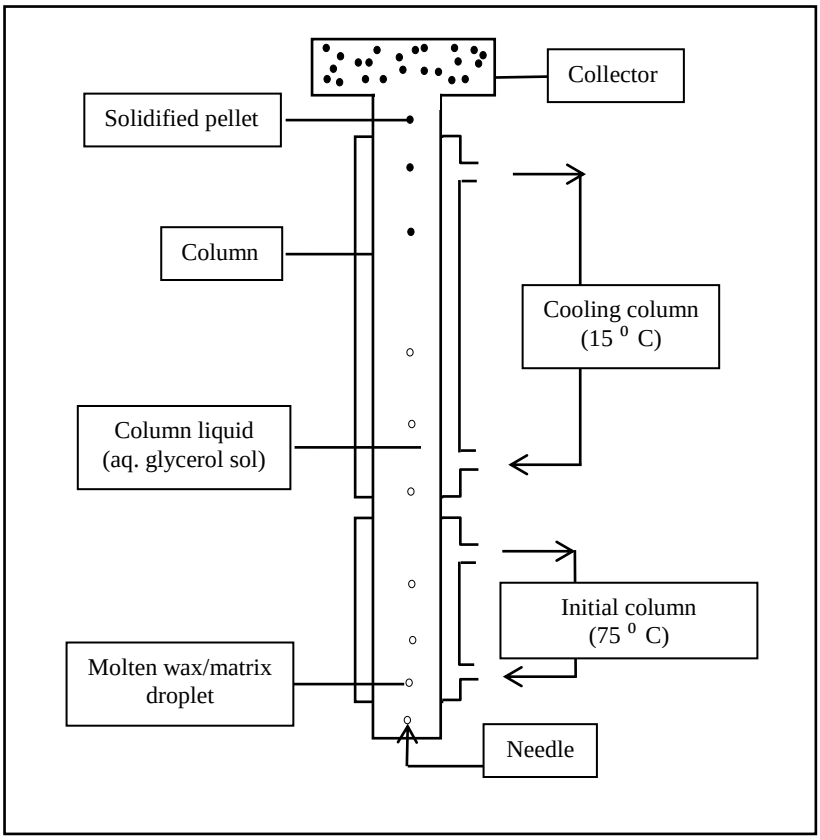


Figure 1.4. Schematics of the freeze pelletisation apparatus II where droplets are introduced from the bottom and solidify at the top. Examples of column liquid and temperatures are illustrated. Modified from (Cheboyina and Wyandt 2008).

This technique is cheap, has fewer process variables than other pelletisation techniques and no drying is necessary as pellets are solid at room temperature (Cheboyina *et al.* 2004; Cheboyina and Wyandt 2008). A drawback in this technique is that it is new and therefore not as safe and well-established as other techniques. Non-established specialised apparatus and excipients, in the form of a carrier, are needed. Also, pellets collected in this apparatus are washed in distilled

water to remove glycerol from the surface, so another drawback in this technique is that fractions of water soluble drug or excipients may be lost during this washing procedure.

1.3.4. Mini-tablet manufacturing

Mini-tablet manufacturing is similar to well-established tablet manufacturing techniques. Because of the manufacturing process, defined sizes and strengths can be easily produced, and the variability within a batch is small (Lennartz and Mielck 1998). However, due to the small size of the mini-tablets the tooling used must meet special requirements.

1.3.4.1. Mini-tablet apparatus

Ordinary reciprocating or rotary tableting machines with small enough punch and die sets to produce pellets of less than 3 mm are used (Lennartz and Mielck 1998). For small scale manufacturing a bench press can be used.

1.3.4.2. Mini-tablet excipients

Basic excipients are similar to those of tablets made by direct compression.

1.3.4.2.1. Filler, disintegrant and binder

Fillers and disintegrants are described in section 1.3.1.2.3. A binder is added to improve the mechanical resistance of the formulation. Examples of binders are described in Section 1.3.1.2.2.

1.3.4.2.2. Glidant, lubricant and antisticking agent

A glidant is added in order to improve the flow properties, lubricants reduce frictions of the powder mix and antisticking agents prevent adhering to the tableting machine. A mixture of magnesium stearate and talcum (10:90% w/w) is typically added in concentrations of up to 5% (w/w) to serve the purpose as glidant, lubricant and antisticking agent (Kristensen 2000).

1.4. Coating

Pellets and mini-tablets can be coated to match any of the following necessary criteria for the final product (Kristensen 2000; Schaefer 2004):

- Protection from light, air or water
- Resistance to acid
- Modified release
- Masking of taste or smell
- Avoiding local effects in mouth
- Facilitate swallowing
- Easy to identify
- Cosmetic properties

Sugar coats used to predominate but it has now been taken over by thin film coats. A sugar coat has a high saccharide content and is 200-500 μm thick (Kristensen 2000; Schaefer 2004). A film coat is a 5-100 μm thick layer surrounding the formulation and usually makes up 3-4% of the entire formulation mass (Kristensen 2000; Schaefer 2004). Coating with a sustained releasing wax has also been carried out (Barthelemy *et al.* 1999; Chen *et al.* 2010).

1.4.1. Coating methods

Dip coating is an early coating method which is simple and involves coating individual dosage forms by dipping them in the coating solution and picking them up to let them dry. Coating thickness generally increases with faster withdrawal speed and is primarily affected by fluid viscosity, fluid density, and surface tension. This method may be particular useful for small scale manufacturing.

The most commonly used coating method nowadays relies on the principle of atomisation and film formation of a coating solution. This principle is similar to that described in the pelletisation method for drug-layering in Section 1.3.2. The apparatus used for large scale manufacture are the pan coater or fluidised bed (Hodges 2005; Kristensen 2000; Schaefer 2004). The fluidised bed is applicable to coating of particles as small as 50 μm, whereas the pan coater is not applicable to

film coating of pellets due to the high mechanical force (Kristensen 2000; Schaefer 2004). A fluidised bed with bottom spray instead of top spray has the advantage that dry coating is completely avoided (Schaefer 2004). Dry coating occurs when the solvent of the film coating liquid evaporates, causing the film polymer to harden, before contact with the formulation (Schaefer 2004). This makes the coating layer less smooth and cracks may occur which leads to a bad functionality of the coat.

Chen *et al.* (2010) and Barthelemy *et al.* (1999) have applied a wax as a hot-melt coating agent to pellet formulations. Chen *et al.* (2010) applied the coating material using a heated coating pan where the wax was gradually added to the coating pan containing the dosage form to be coated. The wax melted and rolled over the dosage form, coating it uniformly (Chen *et al.* 2010). Barthelemy *et al.* (1999) used a fluid bed coating process with a top spray and atomization air temperature of 90 °C to melt the sprayed wax (Barthelemy *et al.* 1999). However, coating with wax will not be described in more detail.

1.4.2. Coating excipients

The three main excipients used in film coating are a polymer, a plasticiser, and a solvent. These are described in more detail below. Other excipients, which are not further detailed, may contribute to further desired properties in the coating process or in the final product. These include surfactants, colourants, hydrophilising agents and sweeteners (Hodges 2005; Kristensen 2000). For coating with a wax no other excipients are necessary than the wax. Chen *et al.* (2010) and Barthelemy *et al.* (1999) applied glyceryl behenate as a wax for coating.

1.4.2.1. Polymers

Some water soluble polymers dissolve independently of pH and do not have any function in controlling the drug release as they dissolve immediately upon contact with the GI fluids. The primary water soluble polymer used is HPMC but sometimes hydroxyl propyl cellulose HPC, methyl cellulose (MC) or (PVP) is used (Hodges 2005; Kristensen 2000).

Water insoluble and pH-independent polymers may function to control the drug release by a sustained action. The primary water insoluble polymer is EC but sometimes acrylic polymers, such as ethyl acrylate or methyl methacrylate, are used. Bodmeier *et al.* (1997) showed that acrylic polymers are more suitable for coating pellets that are to be compressed into tablets, as these polymers are more elastic and can withstand tableting forces without the film being compromised and changing its properties. In order for water to permeate the insoluble film coat, so that drug can be released, a hydrophilic substance is required in the film. This is typically PEG, which also acts as a film coat plasticiser, or HPC which is soluble in both water and ethanol (Kristensen 2000).

pH-dependant soluble polymers are insoluble in environments outside a certain pH range. Polymers that are insoluble in an acidic environment but soluble in an alkaline environment have a function of gastro resistance and are termed enteric polymers. These include hydroxymethyl propyl cellulose phthalate, cellulose acetate phthalate and methacrylic acid copolymers. These dissolve in organic solution and disperse in aqueous solutions (Hodges 2005; Kristensen 2000).

1.4.2.2. Plasticiser

Plasticisers lower the glass transition temperature (T_g) of the film forming polymer making it more elastic and thereby increases its mechanical resistance (Hodges 2005; Kristensen 2000). Water soluble plasticisers have the best interactions with water soluble polymers, and the same counts for water insoluble plasticisers with water insoluble polymers (Hodges 2005).

Propylene glycol or other poly alcohols, such as PEG, are the most common water soluble plasticisers used. Propylene glycol and the lower molecular weight PEGs, which are liquid at room temperature, are commonly used in coating of small particles such as pellets or mini-tablets. This is due to their higher plasticity, which is required for film coating the smaller particles (Hodges 2005).

Various organic esters are commonly used with water insoluble plasticisers (Kristensen 2000). These include tributyl citrate, acetylated monoglyceride, castor oil, dibutyl sebacate, acetyl triethyl citrate and acetyl tributyl citrate (Hodges 2005).

1.4.2.3. Solvents

Organic solvents are the most effective solvents used in film coating as they evaporate quickly and moistening of the formulation is avoided. Often mixed solvent solutions, containing two or three solvents, are used. Solvents include: water, ethanol, methanol, acetone, methylene chloride and isopropanol. However, due to environmental and safety concerns water is now the most commonly used solvent (Hodges 2005; Kristensen 2000).

1.5. Gamma scintigraphy

Since the late 70's gamma scintigraphy has been the gold standard imaging technique in the study of *in vivo* characteristics of orally administered formulations (Burke *et al.* 2007; Goodman *et al.* 2010). In scintigraphy, the GI transit of an administered dosage form is monitored by the incorporation of a radioactive tracer into the formulation. Scintigraphy is therefore unlike a diagnostic X-ray where external radiation is passed through the body to form an image.

Even though scintigraphy appears to be the method of choice, alternative methods to investigate motility and transit parameters do exist. These include magnet moment imaging (MMI), ultrasound, the paracetamol absorption test and 13C-octanoic breath test (Davis *et al.* 1986; Goodman *et al.* 2010; Sunesen *et al.* 2005). However, of the mentioned methods only MMI is adaptable for the study of dosage forms and has the advantage that no radioactive exposure occurs (Goodman *et al.* 2010). Scintigraphy has an advantage over MMI because the formulations being studied using MMI need a large amount of iron oxide to be monitored. This may completely change some of the properties of the formulation such as the density, volume, mass and drug release. The radioactive tracer incorporated in scintigraphy is of smaller magnitude and does therefore not change the properties of the formulation as much.

Scintigraphy has the advantages of being non-invasive, physiologic, easy to perform, quantitative and qualitative (Bennink *et al.* 2003). Drawbacks include radiation exposure to the volunteers, which minimises the number of repeat studies and restricts its use in women and children (Goodman *et al.* 2010). Furthermore, the necessity of incorporating a radioactive tracer to the formulation can be a challenge.

It is especially a challenge when the formulation concerned is composed of small dosage units such as pellet and mini-tablet formulations. In these small dosage forms the simple drill and fill method, which involves drilling a small hole in the dosage form and closing the hole with bone cement, is insufficient due to the size of the hole necessary to be drilled. Furthermore, when small dosage units, such as pellet and mini-tablet formulations are dispersed in the GI tract it may be difficult to distinguish an intact unit from a completely dissolved unit. Thus scintigraphic studies of the disintegration site of pellet formulations have been limited to estimations using a combination of scintigraphy and pharmacokinetic (PK) analysis of blood samples (see Section 1.6.2).

1.5.1. Gamma camera

The radioactive tracer incorporated into a formulation for scintigraphic studies is monitored using a gamma camera. The principles of a gamma camera are as follows: the head of the gamma camera is equipped with a NaI crystal. A collimator surrounds the crystal and filters the stream of rays so that only those travelling parallel to a specific direction are allowed through. When radiation photons from a gamma-emitting nuclide strike the NaI crystal it flashes. The flash is detected by photomultiplier tubes. The detected flash is then displayed in a cathode ray oscilloscope and can be stored electronically (Digenis 1994). Images can then be analysed using an analysing program (McInnes *et al.* 2007).

Assessment of radiolabelled tablets in the GI tract of a subject is typically done in the upright position where anterior and posterior images are taken. External radioactive markers, of approximately 0.01MBq, are taped on the front and the back of the body to align the sequential images in the same position (Hodges *et al.* 2009; McConville *et al.* 2009; McInnes *et al.* 2007). A region of interest (ROI) is then drawn on the images so that radioactive counts within this region can be analysed and give quantitative information on the formulation within this region (Hodges *et al.* 2009; McConville *et al.* 2009; McInnes *et al.* 2007).

1.5.2. Radioactive tracers

Gamma scintigraphy requires a radionuclide of short lived gamma emitting radiation and a photon energy between 100-200 keV (Burke *et al.* 2007). The most commonly used radionuclide is technetium-99m (^{99m}Tc) and to a smaller extent indium-111 (^{111}In) and samarium-153 (^{153}Sm) (Burke *et al.* 2007; Ghimire *et al.* 2011; Ghimire *et al.* 2010; Hodges *et al.* 2009; Kelly *et al.* 2003; McConville *et al.* 2009; McInnes *et al.* 2007; Stevens and Speakman 2006; Wilson *et al.* 2009).

^{99m}Tc is the most commonly used radionuclide used as a radioactive tracer in scintigraphic studies (Ghimire *et al.* 2011; Ghimire *et al.* 2010; Hodges *et al.* 2009; Kelly *et al.* 2003; McConville *et al.* 2009; McInnes *et al.* 2007; Stevens and Speakman 2006; Wilson *et al.* 2009). The "m" indicates that the radionuclide is metastable and therefore has a short half-life of 6.03 h. This is useful for clinical use, as its short half-life keeps the radiation exposure low but for a long enough period to be detected throughout the GI tract (Burke *et al.* 2007; Hodges *et al.* 2009). Furthermore, its monoenergetic gamma emission of photopeak energy 140 keV is readily detectable by the gamma camera (Burke *et al.* 2007; Hodges *et al.* 2009).

In order to visualise two separate components at the same time two radionuclides with different photopeak energies can be administered at the same time. Clarke *et al.* (1993) used this principle by radiolabelling pellets of two different size and densities with ^{99m}Tc and ^{111}In respectively in order to visualise and compare the GI transit of the different pellets.

1.5.2.1. Retention of radioactive tracers

Premature leakage of the radioactive tracer from an ingested formulation may incorrectly suggest gastric emptying or disintegration. In order to successfully monitor a drug formulation using gamma scintigraphy, the radionuclide needs to be retained within the formulation for the required length of time. A common method of retaining a radionuclide within a formulation is to bind it to an ion exchange resin or by adsorbing it onto activated charcoal.

Most of the radiolabelled pellet formulations found in the literature have a radioisotope bound to an ion exchange resin, which has been incorporated to the formulation (see Section 1.6). A further advantage of binding a radioisotope to an ion exchange resin is that an ingested radioisotope which is bound to an ion exchange resin will not be absorbed systematically, thereby avoiding health and safety issues.

The section below will explain more detail about the functions and properties of ion exchange resins, as they play an important part in the radiolabelling of pellet formulations used for scintigraphic studies.

1.5.2.1.1. Ion exchange resins

When an ion comes into contact with an ion exchange resin it binds to the resin with the release of another ion within the resin. This process is called an ion exchange. The ions within an ion exchange resin have a relatively low charge strength which causes them to be loosely held enabling the ions to be exchanged. The exchange takes place without any physical alteration to the resin (Kammerer *et al.* 2010).

The order of anion exchange resin affinity to some common anions is:
$OH^- \approx F^- < HCO^{3-} < Cl^- < Br^- < NO^{3-} < HSO^{4-} < PO_4^{3-} < CrO_4^{2-} < SO_4^{2-}$ (Alchin 2013).

It is noted that phosphate ions have relatively strong affinity to anion exchange resins, suggesting a low risk of anions being displaced from an anion exchange resin during *in vitro* dissolution in phosphate buffered dissolution media.

Various papers describe how radioactive tracers can be taken up by a pellet formulation containing an ion exchange resin by soaking the formulation in a solution containing the radioisotope (Section 1.6, Table 1.2). When choosing an ion exchange resin for this purpose, various properties have to be considered. These include ionic charge, particle size, crosslinkage and porosity of the ion exchange resin. These properties are described in more detail in the sections below (Sections 1.5.2.1.1.1 to 1.5.2.1.1.4) in relation to incorporating pellets with the commonly used radioactive tracer, pertechnetate. Pertechnetate with the chemical formula TcO_4^- is a water-soluble form of technetium described above (Section 1.5.2).

1.5.2.1.1.1. Ionic charge

Anion exchange resins exchange negatively charged ions and cation exchange resins exchange positively charged ions. Pertechnetate is a monovalent anion which needs an anion exchange resin in order to be exchanged.

Strong base anion exchange resins are classed as Type 1 and Type 2. The Type 1 functional group is the most strongly basic functional group available, it has the greatest affinity for weak acids in both its basic and salt form and it is the most chemically stable (Wołowicz and Hubicki 2009). A Type 1 anion exchange resin is therefore preferred for ion-exchanging pertechnetate for the purpose of scintigraphy. Type 2 ion exchange resins are typically used in the case where the ion exchange resin is washed and reused as their functional group has relatively low affinity for weak acids and can therefore be easily regenerated.

1.5.2.1.1.2. Particle size

The particle size of the ion exchange resin in a pellet formulation should be as small as possible. This will result in:

- A more uniform distribution of the ion exchange resin within the pellet formulation.
- Small changes to the properties of the pellet formulation when compared to similar pellets without ion exchange resins.
- An increase in the rate of the ion exchange process as the contact area of the ion exchange resin increases (Helfferich 1962).

1.5.2.1.1.3. Cross linkage

Ion exchange resins are usually based on cross linked polystyrene, polyacrylate esters or polymethacrylate esters. The crosslink is often achieved by adding divinyl benzene (DVB) at the polymerisation process. The crosslink of an ion exchange resin makes it swell less and makes it more mechanically stable (Helfferich 1962). These are desired properties for an ion exchange resin used in a pellet formulation. A drawback of cross links is the decrease in the ion exchange capacity as sites where the functional group can be attached are reduced (Kammerer *et al.* 2010). Typical

cross linkages are between 8-12% DVB (mole percent of pure DVB in the polymerization process).

1.5.2.1.1.4. Porosity

Ion exchange resins can be of the gel type or the macroporous type. The gel type has small pores with pore structure determined by the distance between the polymer chains and the crosslinks. The macroporous type has additional artificial porosity which is obtained by adding a substance designed for this purpose. The macroporous type has a more advanced manufacturing process and is used for special requirements such as for large molecules and for nonpolar solvents where swelling of the ion exchange resin is important (Helfferich 1962).

1.6. Radiolabelled pellets and mini-tablets for scintigraphic studies

Table 1.2 illustrates scintigraphic studies of pellet formulations found in the literature. These scintigraphic studies are explained in more details in the following sections (Section 1.6.1-1.6.5). No literature was found for scintigraphic studies of mini-tablets but radiolabelling techniques of mini-tablets are dealt with in Section 1.6.6. Table 1.2 gives details of amount of radioactivity and mass of administered dose as well as pellet composition, radiolabelling method and what has been investigated. This table can prove useful prior to initiating a scintigraphic study, as it gives references for the various properties required for scintigraphically detection of pellet formulations.

Table 1.2. Scintigraphic studies of pellet formulations found in the literature. Details of radioactivity and mass of administered dose, composition of pellet formulation, radiolabelling method and investigation are also detailed.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Soaking method to investigate pellets' GI transit behaviour (detailed in Section 1.6.1)					
Not stated	100 mg radiolabelled resin dosed in a size 4 capsule	100% ion exchange resin	Soaking anion exchange resin pellets	Gastric emptying of pellets in capsule	(Hunter *et al.* 1982)
2 MBq	140 mg radiolabelled resin dosed in a size 3 capsule	100% anion exchange resin	Soaking anion exchange resin pellets	Comparison of pellet and tablet GI transit	(Davis *et al.* 1984)
Not stated	Not stated	Not stated	Pellets radiolabelled with ^{99m}Tc and ^{111}In	Evaluation of GI transit of pellets with varying size and density	(Clarke *et al.* 1993)
3.7 MBq	Size 0 capsules	65% MCC, 30% barium sulphate and 5% anion exchange resin coated with ethylcellulose containing 30% PEG4000	Soaking of film coated pellets in ^{99m}Tc for 2 h	Evaluation of GI transit of pellets with varying density	(Clarke *et al.* 1995)

Table 1.2 is continued on next page.

Table 1.2 continued.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Soaking method to investigate pellets' GI transit behaviour (detailed in Section 1.6.1) (continued)					
7.4 MBq	300 mg radiolabelled pellets dosed in a size 0 capsule	MCC, barium sulphate and 5% anion exchange resin Coated with Surelease and Methocel mixture	Soaking of film coated pellets in a sodium ^{99m}Tc-pertechnetate solution	The effect on GI transit of pellets after ingestion of PEG400	(Basit *et al.* 2001)
Not stated	0.28, 0.56, or 1.12 g of 1 mm and 2 mm pellets	100% exchange resin pellets and pellets of polyamide (nylon). Both types were coated with polymethylmethacryl-ates	The exchange resin beads were soaked in ^{113m}In. The polyamide pellets were radiolabelled using a special method in which ^{99m}Tc was added into the coating layer	Determine whether gastric emptying from two pellet formulations of size 1 mm or 2 mm is dose related or related to the presence of oil in a meal	(Meyer *et al.* 2001)
Not dosed	Not dosed	MCC and different amounts of ion exchange resin [0.5%, 1%, 2%, 3%, 4%, 5% and 30% (w/w)]	Soaking of pellets in a ^{18}F solution for different amounts of time (0.1, 5, 10, 15, 20, 40, 60 and 120 min)	Positron emission technology to evaluate the binding of ^{18}F to pellets (GI transit was not investigated).	(Philippe *et al.* 2010)

Table 1.2 is continued on next page.

Table 1.2 continued.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Soaking method to radiolabel GI transit markers to trace drug loaded pellets (detailed in Section 1.6.2)					
4 MBq	250 mg radiolabelled pellets mixed with 490 mg of diltiazem pellets dosed in a size 00 capsule	Not stated	Soaking of pellets in a sodium ^{99m}Tc-pertechnetate solution	GI transit of diltiazem pellets.	(Wilding *et al.* 1991)
1 MBq	70 mg radiolabelled pellets mixed with 190 mg nifedipine pellets dosed in a size 1 capsule	100% ion exchange resin pellets	Soaking in a solution containing ^{111}In	GI transit of nifedipine pellets.	(Wilding *et al.* 1992)
7.4 MBq	Radiolabelled pellets mixed with theophylline pellets dosed in a size 000 capsule	MCC, barium sulphate and 5% anion exchange resin. Coated with Ethocel and Methocel mixture	Soaking of film coated pellets for 0.5 hours in a sodium ^{99m}Tc-pertechnetate solution	GI transit of theophylline pellets.	(Yuen *et al.* 1993)

Table 1.2 is continued on next page.

Table 1.2 continued.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Soaking method to radiolabel GI transit markers to trace drug loaded pellets (detailed in Section 1.6.2) (continued)					
7.4 MBq	300 mg radiolabelled pellets dosed in a size 0 capsule. Administered with a size 0 capsule of non-radiolabelled ranitidine pellets.	MCC, barium sulphate and 5% anion exchange resin. Coated with Surelease	Soaking of film coated pellets in a sodium ^{99m}Tc-pertechnetate solution	GI transit markers of ranitidine pellets.	(Basit *et al.* 2004)
7.4 MBq	240 mg radiolabelled pellets dosed in a size 0 capsule. Administered with a size 0 capsule of non-radiolabelled theophylline pellets	MCC, barium sulphate and 5% anion exchange resin. Coated with Surelease and Methocel mixture	Soaking of film coated pellets in a sodium ^{99m}Tc-pertechnetate solution	GI transit of theophylline pellets.	(McConnell *et al.* 2008)

Table 1.2 is continued on next page.

Table 1.2 continued.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Soaking method applied to drug loaded pellets (detailed in Section 1.6.3)					
Not stated (20 g of pellets had 180-240 MBq)	Two capsules of radiolabelled pellets containing 300 mg tiaprofenic acid	Tiaprofenic acid, ion exchange resin and a film coat	Soaking of film coated pellets in ^{99m}Tc pertechnetate solution for 2 hours	Effect of food on GI transit of their formulation	(Davis *et al.* 1987)
5 to 10 MBq	3-5 g of radiolabelled pellets were administered	Pellets containing Al and Mg hydroxides	Soaking of uncoated pellets for a few seconds in ^{99m}Tc pertechnetate solution containing ethanol followed by coating	Investigation of gastric emptying of their formulation	(Blok *et al.* 1991)
4 MBq	The dose size is unknown but the entire dose was radiolabelled	Inert pellet cores with a ketoprofen-DTPA layer coated with pectin/ethyl cellulose	Pellets with a ketoprofen-DTPA layer were soaked in ^{99m}Tc prior to film coating	GI transit of pellets containing ketoprofen using rabbits	(Subhabrota *et al.* 2011)

Table 1.2 is continued on next page.

Table 1.2 continued.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Neutron bombardment (detailed in Section 1.6.4)					
Not stated	5 radiolabelled pellets mixed with non-radiolabelled pellets	Samarium oxide concentration is not stated. Samarium oxide was layered onto non-pareil beads.	Samarium oxide containing pellets were activated by neutron bombardment	Onsets of enteric coated pellets were registered by dispersal of the radioactive marker	(Ebel *et al.* 1993)
Not stated (2 mg of $^{152}Sm_2O_3$)	Entire dose containing 500 mg mesalazine was radiolabelled	Samarium oxide concentration is not stated. Pellets contained 500 mg mesalazine and 2 mg samarium oxide	Samarium oxide containing pellets were activated by neutron bombardment	Comparison of GI transit of pellets and tablets	(Brunner *et al.* 2003)
1.85 MBq (50 μCi)	5 radiolabelled pellets mixed with 45 non-radiolabelled pellets dosed in a size 0 capsule	Radiolabelled pellets replicated the non-radiolabelled pellets but also contained 13% samarium oxide	Samarium oxide containing pellets were activated by neutron bombardment	Comparison of arrival of paracetamol in the blood and colonic delivery of radiolabelled pellets	(Ahmed and Ayres 2011)

Table 1.2 is continued on next page.

Table 1.2 continued.

Radioactivity of dose at time of dosing	Administered dose	Composition of radiolabelled pellets	Radiolabelling method	Investigation	Reference
Radiolabelling during the pelletisation process (detailed in Section 1.6.5)					
18.5 MBq	700 mg radiolabelled pellets dosed in a size 00 capsule.	Starch based pellets with ^{111}In-DTPA prepared. Batch size was 250 g (Dukic *et al.* 2007).	Pellets were extruded and spheronised with ^{111}In-DTPA in the granulation liquid.	The distributions of pellets and cream in the vagina were compared using sheep.	(Mehta *et al.* 2012)

It is seen from Table 1.2 that the most common radiolabelling method is soaking of placebo pellets in a radioactive solution. The placebo pellets contain an ion exchange resin to bind to the radioisotope during soaking. These radiolabelled placebo pellets have been used for investigations of the general GI transit of pellet formulations, which is described in more detail in Section 1.6.1, or as marker pellets for GI transit of drug loaded pellets described in Section 1.6.2.

Some drug loaded pellet formulations have also been radiolabelled using a method of soaking the pellets. This is however complicated to accomplish as the drug and excipients may easily be lost due to dissolution during soaking. This method is therefore not widely used, and is described in more detail in Section 1.6.3.

Another way to radiolabel drug loaded pellet formulations is using a more advanced method involving irradiation of the drug loaded pellet formulation using neutron bombardment of samarium. This method is described in more detail in Section 1.6.4.

Finally, an alternative method of radiolabelling drug loaded pellet formulations involves the incorporation of the radioactive tracer during the pellet manufacturing. Such method may involve high exposures to radioactivity and should therefore be carried out using a small scale manufacturing method to minimise the amount of radioactivity required. More detail of such radiolabelling method is described in Section 1.6.5.

1.6.1. Soaking method to investigate pellet GI transit behaviour

As seen from Table 1.2, investigations on the general pellet behaviour have been carried out in order to give useful information on factors such as GI transit of pellets compared to tablets, the importance of pellet size and density during GI transit, and food effects (Basit *et al.* 2001; Clarke *et al.* 1995; Clarke *et al.* 1993; Davis *et al.* 1984; Meyer *et al.* 2001)).

Scintigraphic investigations of general pellet behaviour is a fairly easy approach as the pellet formulations do not need to contain a drug or mimic the physical properties of a specific pellet formulation. The only challenge in this investigation is to radiolabel the pellets so they can be traced *in vivo* using scintigraphy. This is done

simply by soaking a water insoluble pellet formulation containing an ion exchange resin that binds the radioactive tracer tightly to the formulation.

1.6.2. Soaking method to radiolabel GI transit markers to trace drug loaded pellets

GI marker pellets are non-disintegrating radiolabelled placebo pellets that act as GI transit markers for drug loaded pellets, when both formulations are ingested simultaneously. The site of drug release can be investigated using scintigraphic methods that combine PK analysis of blood samples. Scintigraphic data give information on location and duration of residence of the pellets, and PK analysis of blood samples provides information on drug kinetics in the main systemic compartment.

Table 1.2 illustrates that various amounts of radiolabelled pellets have been dosed together with drug loaded pellets in either the same capsule (Wilding *et al.* 1991; Wilding *et al.* 1992; Yuen *et al.* 1993) or in individual capsules (Basit *et al.* 2004; McConnell *et al.* 2008). The advantage of dosing the radiolabelled placebo pellets in the same capsule as the drug loaded pellets is that there is higher possibility that the placebo marker pellets indicate the exact position of the drug loaded pellet formulations.

Further to the addition of MCC and an ion exchange resin, some placebo pellets also contain barium sulphate as a component (Basit *et al.* 2004; McConnell *et al.* 2008; Yuen *et al.* 1993). This high density compound is used to manipulate pellet density to ensure that the density of the drug loaded pellet formulation of interest is matched. However, matching the density of drug loaded pellet formulation may not be necessary, as long as both the placebo and the drug loaded pellets have densities below 2.4 g/cm^3. This was suggested by Clarke *et al.* (1993, 1995), who investigated the effect of pellet density on subsequent GI transit behaviour by using radiolabelled placebo pellets. The addition of barium sulphate to increase the density of the placebo pellets may therefore not be required.

Further to the density, Clarke *et al.* (1993) also found that pellet size does not influence the rate at which pellets empty from the gastric compartment. This was

tested by comparing the GI transit of radiolabelled placebo pellets of diameter 0.5 mm and 4.75 mm (Clarke *et al.* 1993). This was backed up in a comparative study by Davis *et al.* (1986), which stated that radiolabelled placebo pellets of sizes between 0.3-2.0 mm have identical rates of gastric emptying (Davis *et al.* 1986). However, Clarke *et al.* (1993) also found that transit through the small intestine was prolonged by an increase in pellet diameter.

Pellets are defined as being within size diameter range 0.5-2.0 mm (Rahman *et al.* 2009) so it can be assumed that pellet size does not influence the rate of gastric emptying. However, as some drug loaded pellet formulations disintegrate as a consequence of drug release, some drug loaded pellets may empty from the gastric compartment at a different rate than non-disintegrating placebo pellets. Clarke *et al.* (1993) has stated that there exists an often quoted concept that small micro pellets empty from the gastric compartment like a liquid rather than a solid (Clarke *et al.* 1993). It is uncertain where the threshold of this size is but results of the comparative study by Davis *et al.* (1986) indicated that it must be somewhere below 0.3 mm. It may be postulated that this threshold size goes as low as 0.15 mm as particles smaller than this empty from the gastric compartment independent of its peristaltic-like contractions, which occur every three minutes (Clark *et al.* 1993). The method of using GI transit markers for drug loaded pellet formulations is therefore only adaptable for tracing pellet formulations in the gastric compartment that remain above 0.3 mm during drug release, and there is only evidence that a full GI transit can be mimicked when the pellet density remains below 2.4 g/cm^3 and the size range of the pellets remain the same.

Some placebo pellet formulations have been film coated prior to radiolabelling in order to further mimic the drug loaded pellet formulation (Basit *et al.* 2004; McConnell *et al.* 2008; Yuen *et al.* 1993). Yuen *et al.* (1993) suggested that mimicking the film coat of drug loaded pellets is important as it may influence the hydrophilicity of the pellet surface, especially when the surface has high concentrations of exchange resin, and thereby the ability of the pellets dispersing throughout the GI tract (Yuen *et al.* 1993). Further to this, the addition of a film coat

may aid in retaining the radiolabel so it is not lost from the pellet formulation during GI transit.

1.6.3. Soaking method applied to drug loaded pellets

Soaking of pellet formulations in a radioactive solution requires pellet components that are insoluble in the solution in which they are immersed. As shown in Table 1.2, radiolabelling of drug loaded pellets using the soaking method has been carried out by Blok *et al.* (1991), Davis *et al.* (1987) and Subhabrota *et al.* (2011). However, these papers do not state the existing problem that the drug and soluble components may dissolve during soaking in a radioactive solution, and they do not describe in detail how they have accomplished the radiolabelling without losing the drug component.

Blok *et al.* (1991) radiolabelled the drug loaded pellet formulation by soaking in an ethanol solution for a few seconds followed by simple dip coating in an insoluble polymer (Blok *et al.* 1991). The soaking was carried out for a short time which probably has prevented drug dissolution from the formulation. The addition of an insoluble film coat may have prevented any loosely bound radiolabel to come off the pellets after ingestion but it may also have prevented disintegration and release of drug from the pellets.

Davis *et al.* (1987) film coated the drug loaded pellet formulation prior to soaking for 2 hours. The long soaking time ensured that the radiolabel remained attached to the pellets after ingestion and the addition of the film coat prior to soaking may have ensured that pellet components did not dissolve during soaking (Davis *et al.* 1987).

Both Blok *et al.* (1991) and Davis *et al.* (1987) prepared non-disintegrating radiolabelled pellet formulations. It was assumed that the GI transit behaviour would be similar to that of the pellet formulation being investigated due to the similarity of the pellet components. However, as both formulations contained an insoluble film coat they did not mimic the properties of the original drug loaded pellet formulations after ingestion. Furthermore, the film coat prevented drug release so this type of pellet formulation cannot be used for combining scintigraphic studies with PK analysis to find the site of drug release.

As opposed to Davis *et al.* (1987) and Blok *et al.* (1991), Subhabrota *et al.* (2011) managed to produce drug releasing pellets that contained the radiolabel. Instead of incorporating an ion exchange resin to the pellet formulation they incorporated diethylene triamine pentaacetic acid (DTPA), which was adsorbed to the drug. DTPA was used for complexing to ^{99m}Tc ions after soaking but did not aid in retaining the radiolabel to the pellet formulations (Subhabrota *et al.* 2011). The ^{99m}Tc-DTPA complex cannot be absorbed systemically after ingestion, and the ingestion is therefore considered safe. This complex is thus commonly used in scintigraphy (Ghimire *et al.* 2011; Ghimire *et al.* 2010; Hodges *et al.* 2009; Kelly *et al.* 2003; McConville *et al.* 2009; McInnes *et al.* 2007; Stevens and Speakman 2006; Wilson *et al.* 2009). Subhabrota *et al.* (2011) observed drug release from the pellets scintigraphically by observing the release of drug adsorbed to the ^{99m}Tc-DTPA complex. However, the scintigraphic images shown in their paper do not illustrate how the release of radiolabelled drug is distinguished from the transit of the radiolabelled pellet formulations. Furthermore, their results show that a small percentage of ^{99m}Tc ions attached to the pellet formulation did not complex to DTPA. This can cause health and safety issue due to potential systemic absorption of the radiolabel, which is probably why they administered the radiolabelled pellets to rabbits instead of humans. Furthermore, and more importantly, the paper does not explain how drug dissolution was avoided during the soaking.

1.6.4. Neutron bombardment

Neutron bombardment is the most optimal way of radiolabelling drug loaded pellet formulations without being exposed to high doses of radioactivity during manufacturing. Samarium oxide is always used as a radioactive tracer when this technique is employed. Non-radioactive Samarium oxide is incorporated into the pellet formulation during pelletisation. The pellet formulation is then irradiated by a process known as neutron bombardment, in which the stable ^{152}Sm isotope is transformed into the radioactive ^{153}Sm isotope (Ahmed and Ayres 2011; Brunner *et al.* 2003; Ebel *et al.* 1993). Some examples of neutron bombardment used for radiolabelling of pellet formulations have been found in the literature and are

described in Table 1.2. The only drawback with this method is that it requires advanced equipment.

1.6.5. Radiolabelling during the pelletisation process

A simple radiolabelling technique is the addition of the radioactive tracer during the pelletisation process. However, as pelletisation is often limited to the production of large batches, this method may result in high radioactive exposure to the personnel during manufacture. Suggestions on how to carry out the radiolabelling of pellets prepared by the two most common pelletisation techniques, the extrusion-spheronisation and the drug-layering techniques, are suggested below (Section 1.6.5.1 and Section 1.6.5.2).

1.6.5.1. Extrusion- spheronisation

As shown in Table 1.2, Mehta *et al.* (2012) produced pellets containing ^{111}In-DTPA as a radioactive tracer during pelletisation using the extrusion-spheronisation technique. The batch size of the powder blend used was 250 g (Dukic *et al.* 2007) and every 700 mg produced pellets contained 18.5 MBq of radioactivity at time of dose (Mehta *et al.* 2012). The radioactivity of the powder blend must therefore have been at least 6.6 GBq at the time of manufacturing. This radiolabelling method therefore requires a very high radioactive dose compared to the radioactive dose used in the soaking methods. In the soaking method, only the dosed pellet formulations are radiolabelled as opposed to the entire batch of manufactured pellets.

When working with high radioactive doses there is a risk of being overexposed to radioactivity, causing health and safety issues. The unit referring to the amount of radioactive exposure is called the Sievert (Sv). The yearly dose limit for a member of the public is 1 mSv while the dose limit for a radiation worker >18 years of age is 20 mSv. The dose-rate at 30 cm from a point source of ^{111}In is 9.9 x 10^{-4} mSv/h/MBq (Delacroix *et al.* 2002). The radioactive exposure during a pellet study, which utilises this pelletisation method described by Mehta *et al.* (2012), which requires 6.6 GBq during each manufacturing, can thus be calculated using following assumptions:

- Distance during manufacturing is 50 cm

- Number of manufactures per session of a study is 9
- Exposure during manufacturing is 20 minutes

The calculation gives: 9.9×10^{-4} mSv h^{-1} MBq^{-1} x 6,600 MBq x 9 x $(30/50)^2$ x 20/60 = 7.1 mSv.

This calculation shows that a radioactive dose of 6.6 GBq, which is required for each manufacturing session when using the radiolabelling method described by Mehta *et al.* (2012), causes a radioactive exposure of 7.1 mSv during a study session. This exposure highly exceeds the yearly dose limit for a member of the public but is within the limit for a radiation worker. However, a radiation worker is likely to be exposed from other sources over a yearly period, and will therefore easily exceed the yearly limit of 20 mSv. A major drawback in the radiolabelling method described by Mehta *et al.* (2012) is therefore the high radioactive exposure encountered during manufacturing, which is caused by the large scale of manufactured pellet formulations.

Mehta *et al.* (2012) used the radioisotope, ^{111}In which has a half-life of 2.8 days. A radioisotope of shorter half-life, such as ^{99m}Tc with a half-life of 6.0 hours, will require much higher radioactive dose at time of manufacturing due to the rapid decay. The use of ^{99m}Tc in a study similar to Mehta *et al.* (2012) will presumably result in a radioactive overexposure of radiation workers if manufacturing is carried out 24 hours prior to time of dose. This may explain why ^{111}In was used by Mehta *et al.* (2012). Drawbacks in the use of this radioisotope are the risks of exposure of radioactive contamination due to the slow decay of the radioisotope, and the fact that the dose-rate at 30 cm from a point source of ^{111}In is four times higher than that of ^{99m}Tc.

In order to reduce the high radioactive exposure and being able to incorporate ^{99m}Tc instead of ^{111}In to pellet formulations during extrusion-spheronisation, a scale down of the manufactured batch is required. Most small scale pelletisation equipment is designed for batches of scales comparable to that used by Mehta *et al.* (2012). However, recently a spheroniser that works for batch sizes down to 1 g has been developed and an extruder that works for batches down to 10 g already exists

(Caleva Process Solutions 2013). However, in situations where this small scale equipment is not available, a scale down may require alternative equipment or change of process parameters of existing laboratory scale equipment.

1.6.5.2. Drug-layering

In theory, a radioactive tracer can be added during pelletisation of drug-layered pellets. However, such a method has not been described in the literature.

As described in the section for the drug-layering technique (Section 1.3.2.2.1), inert starter cores used for drug-layering may consist of MCC. If these cores contain an ion exchange resin they can be radiolabelled using the soaking method explained in the sections above (Section 1.6.1-1.6.3). A small batch of radiolabelled pellets can thus be manufactured by drug-layering a small amount of these radiolabelled inert starter cores. However, as with extrusion-spheronised pellet formulations, drug-layered pellets of small scales require special equipment. A small scale fluidised bed coater, which can be used for drug-layering, requires a minimum of between 300 g and 500 g of pellets or tablets to work efficiently (Caleva Process Solutions 2013). This method may therefore require optimisations of process parameters in order to produce radiolabelled pellet batches of safer scales, such as smaller than 10 g.

1.6.6. Radiolabelling of mini-tablets

No literature describes how mini-tablets can be radiolabelled and traced *in vivo* using scintigraphy. However, the soaking methods described in the sections above (Section 1.6.1-1.6.3) can potentially be used for radiolabelling of mini-tablets. As mini-tablets are described as being of size typically below 3 mm (Lennartz and Mielck 1998), it can be assumed that they have same GI transit behaviour as pellet formulations. Thus mini-tablets within the size range of 0.5-5 mm and density below 2.4 g/cm^3 may also travel through the GI tract at same rate, as suggested for pellet formulations by Clarke et al (1993).

A mini-tablet press is required for the manufacturing of mini-tablets, which may therefore be necessary to outsource. Gamlet Tableting Ltd (UK) and Halopharma (US) are in possession of the required mini-tablet press and have shown willingness to produce mini-tablets that can withstand the radiolabelling procedure.

1.7. Aims and objective of research

The aim of this project is to develop scintigraphic methods to study the GI transit of pellet and mini-tablet formulations. This will be done by exploring and validating the following radiolabelling methods:

- Radiolabelling during the pelletisation process
- Radiolabelling by soaking

Small scale pelletisation techniques will be developed in order to accomplish the radiolabelling of pellet formulations during the pelletisation process. This will be done using the two most popular pelletisation techniques:

- Extrusion-spheronisation
- Drug-layering

Pellet formulations of four different drug release profiles will be manufactured using two drug types. This is to ensure that the small scale pelletisation techniques can be utilised for a wide range of pellets and drug types. The four drug release profiles are illustrated in Figure 1.5 and include immediate release, sustained release, time delayed pulsatile release and time delayed sustained release. Paracetamol will be used as a partially water soluble model drug and indomethacin will be used as a poorly water soluble model drug.

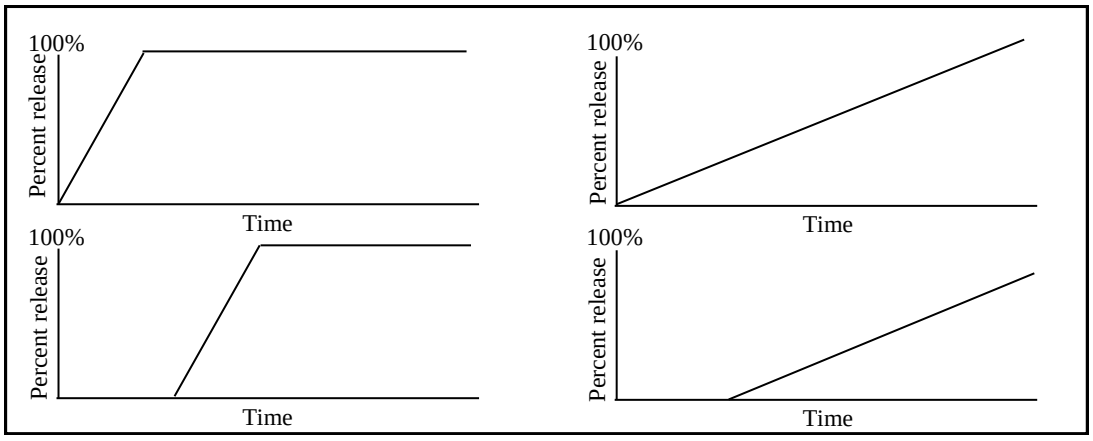


Figure 1.5. Illustration of the four different drug release profiles developed for the partially and poorly water soluble model drugs paracetamol and indomethacin.

The soaking method will be explored and validated using pellet formulations so no mini-tablets will be manufactured in this project.

Chapter 2: Materials and methods

2.1. Materials

2.1.1. Chemicals

Distilled water, paracetamol (637510V426, Covidien, Dublin, Ireland), indomethacin (0102023, Medex, Naseby, UK), lactose (96EXC117, Lactose Standard, Pfizer Ltd, Sandwich, UK), microcrystalline cellulose (MCC) (97EXC032, Avicel PH101, Pfizer Ltd, Sandwich, UK), sodium starch glycolate (E6571X, Explotab®, Mendell Surrey, UK), polyethylene glycol 400 (PEG 400) (ZA2663279 902, BDH Laboratory Supplies, Poole, UK), inert MCC pellet cores (10L014, Cellets 700®, size range 700-1000 μm, kind gift from Pharmatrans Sanaq AG, Basel, Switzerland), anion exchange resin (MKBF9108, Dowex® 1X8 chloride form, strongly basic, 200-400 mesh, Sigma-Aldrich), povidone K30 (PVP K30) (73300675LO, Kollidon 30, BASF, Ludwigshafen, Germany), aqueous ethylcellulose dispersion (E-7-19040, Surelease®, Clear, kind gift from Colorcon®, Dartford, UK), aqueous acrylic enteric system (93A18597, Acryl-EZE®, white, kind gift from Colorcon®, Dartford, UK), simethicone (0005943372, Q7-2243 LVA, Dow Corning, Michigan, USA), triethyl citrate (TEC) (BCBF0400V, ≥98.0% (GC), Sigma-Aldrich), sodium hydroxide (10L150036, VWR, Leuven, Belgium), potassium dihydrogen phosphate (10K080007, VWR, Leuven, Belgium) and hydrochloric acid 37% (121240520, AnalaR Normapur®, VWR, Leuven Belgium).

2.1.2. Equipment

Analytical balances (Mettler Toledo, AB204-S and Mettler Toledo, XS205), magnetic stirrer (IKA, RH-KT/C), spheroniser (Caleva, Model 120), fluidised bed coater and dryer (Caleva, Mini Coater/Drier 2), pH meter (Mettler Toledo, FE20), dissolution system (Lab Systems, ADT8), dissolution apparatus (Pharma Test, PTW S III), gamma camera (Siemens, e.cam LR52894), dose calibrator (Southern Scientific, Capintec, CRC-15R), laser diffraction instrument (Malver, Mastersizer 2000) and texture analyser (Exponent Stable Micro Systems, TA-XT2i).

2.2. Pelletisation of eight different non-radiolabelled pellet formulations

Eight different non-radiolabelled pellet batches (batch A-H) were prepared using a small scale extrusion-spheronisation technique or drug-layering technique with or without the application of a functional film coat to modify the drug release. Paracetamol and indomethacin were used as a partially and a poorly water soluble model drug respectively. Each drug was formulated into a pellet formulation of one of the following drug release profiles: immediate release, sustained release, time delayed pulsatile release or time delayed sustained release. Details of these non-radiolabelled pellet batches are summarised in Table 2.1.

Table 2.1. Details of the eight non-radiolabelled pellet formulations.

Batch	Model drug	Drug release profile	Film coat	Pelletisation technique
A	Paracetamol	Immediate release	None (pellet core)	Extrusion spheronisation
B	Paracetamol	Sustained release	Surelease®	Extrusion spheronisation
C	Paracetamol	Time delayed pulsatile release	Acryl-EZE®	Extrusion spheronisation
D	Paracetamol	Time delayed sustained release	Surelease® and Acryl-EZE®	Extrusion spheronisation
E	Indomethacin	Immediate release	None (pellet core)	Drug layering
F	Indomethacin	Sustained release	None (pellet core)	Extrusion spheronisation
G	Indomethacin	Time delayed pulsatile release	Acryl-EZE®	Drug layering
H	Indomethacin	Time delayed sustained release	Acryl-EZE®	Extrusion spheronisation

In vitro dissolution studies, described in Section 2.9.5, were carried out to investigate whether the desired drug release profiles had been achieved.

2.2.1. Pelletisation by extrusion-spheronisation[1]

Pellet cores were prepared by mixing the drug and excipients in a mortar using a pestle. The small scale powder blends (10 g) contained paracetamol (1 g) or indomethacin (1 g) with lactose (6 g) and MCC (3 g) as excipients. Batch B was prepared on a different occasion and had a different composition of lactose (4.5 g) and MCC (4.5 g). The powder blend was wet massed with sufficient distilled water (4.5 mL for paracetamol containing blends, 5.0 mL for indomethacin containing blends and 6.0 mL for Batch B). The wet powder blend was compressed hard using a pestle and was then extruded through a 1 mm sieve aperture using a spatula and spheronised for 15 min at speed 5 units at 60 ^{0}C. The spheroids were then dried on a fluidised bed dryer at 40 ^{0}C for 1 hour. Pellet cores of size range 1.00-1.18 mm were sieve collected.

The yield of pellets in size range 1.00-1.18 mm was calculated from the initial mass of the total powder blend. If the pellet yield was below 15% (w/w) the whole pelletisation process was repeated in order to have enough pellets for coating and potential dosing of six volunteers in a clinical trial.

The crushing strength of extruded and spheronised pellet cores of Batch A and G were evaluated as described in Section 2.9.1.

2.2.2. Pelletisation by drug-layering

2.2.2.1. Preparation of 30% (w/w) aqueous suspension of indomethacin

An aqueous suspension (approximately 140 mL) of indomethacin (30% (w/w)) and PVP K30 (2% (w/w)) was prepared. Distilled water (100 mL) was transferred to a beaker placed on a magnetic stirrer. PVP K30 (2 g) was slowly added to the distilled water under continuous stirring and the solution was left stirring for 5 min. Indomethacin (30 g) was slowly added to the PVP K30 solution under continuous stirring and was left stirring for at least 15 min. The aqueous suspension was passed through a 106 micron sieve and transferred to a 250 mL beaker that was kept under continuous stirring. In order to avoid the formation of foam during the stirring

[1] **This section describes how the non-radiolabelled extruded and spheronised pellet cores were manufactured. However, the extrusion and spheronisation method was later optimised in order to get a higher and more reproducible pellet yield. This optimised method is described in Section 2.5.4.**

process small amounts of the coating suspension was poured into a 25 mL beaker, which was stirred during the coating process.

2.2.2.2. Layering of indomethacin

Cellets 700® (1.5 g) were weighed and placed inside the cone of a fluidised bed coater and sprayed with the indomethacin suspension prepared under Section 2.2.2.1. The process parameters are listed in Table 2.2. The following parameters were started prior to the attachment of the cone to the coater: fan, heat, atomising air and distance from nozzle to pellets. The pump and the agitator were started immediately after the attachment of the cone.

Table 2.2. Process parameters for layering of indomethacin onto Cellets 700®.

Process parameter	Value
Agitator	17.5 Hz
Fan	9.0 m/sec
Heat	60 ^{0}C
Pump	1 mL/min
Atomising air	10 psi
Distance from spraying nozzle to pellets	22 cm

The drug layering process was continued until pellets containing approximately 10% (w/w) indomethacin were produced. This was accomplished by aiming for a weight gain of 173.1 mg taking the weight of PVP K 30 into account and assuming that 3% of the weight gain is lost due to water evaporating from the Cellets 700® and the solid content of the drug layering suspension (see Appendix I for calculations). The Cellets 700® were sprayed for approximately 20 min to achieve the desired weight gain. When pellets of the desired weight gain had been produced they were cured by fluidising for another 5 min.

The actual indomethacin content was assayed as described in Section 2.9.4.2 and compared with the estimated content from the weight gain.

2.2.3. Film coating with Surelease®

2.2.3.1. Preparation of 15% (w/w) solid content Surelease® aqueous suspension

Approximately 50 mL 15% (w/w) solid content Surelease® aqueous suspension was prepared by adding distilled water (20 g) to a Surelease® suspension (30 g). The dispersion was left stirring for at least 15 min. In order to avoid the formation of foam during the stirring process small amounts of the coating suspension was poured into a 25 mL beaker, which was used without stirring, during the coating.

2.2.3.2. Spraying of Surelease® suspension

The cone of a fluidised bed coater was coated for one min in order to have a layer of film coat in the cone that would avoid static effects between the pellets and the cone. To ensure that a coating layer was applied to the bottom of the cone it was taken off and the bottom was held close to the spraying nozzle while spraying. 1.5 g of pellet cores similar to batch A (Table 2.1) were weighed and placed inside the cone. The following parameters were started prior to the attachment of the cone to the coater: fan, heat, atomising air and distance from nozzle to pellets. The pump and the agitator were started immediately after the attachment of the cone. The values of the process parameters are listed in Table 2.3

Table 2.3. Process parameters used for film coating pellets with Surelease®.

Process parameter	Value
Agitator	17.5 Hz
Fan	10.5 m/sec
Heat	60 ^{0}C
Pump	1 mL/min
Atomising air pressure	10 psi
Distance from spraying nozzle to pellets	22 cm

The spraying process was continued until pellets of approximately 10% (w/w) weight gain had been produced. The weight gain was measured within 1 min after removal from the coater so any weight gain due to water uptake from the atmosphere was minimised. Batch B was prepared on a different occasion and its weight gain was measured after 10 min of coating, after which the water uptake from the atmosphere

had settled. The pellet cores were sprayed for approximately 30 min to achieve the desired weight gain. The pump was stopped for one min every ten min in order to detach static pellets and pellets sticking to the cone due to the wet coating suspension.

2.2.4. Film coating with Acryl-EZE®

2.2.4.1. Preparation of 20 % (w/w) solid content Acryl-EZE® aqueous suspension

Approximately 220 mL 20% (w/w) solid content Acryl-EZE® aqueous suspension was prepared by adding simethicone (40 mg) and TEC (4.0 g) to a beaker and then adding distilled water (160 mL). After stirring on a magnetic stirrer for at least 5 min Acryl-EZE® powder (40.0 g) was slowly added to the mixture. After stirring for at least 30 min the dispersion was passed through a 250 micron sieve and poured into a 250 mL beaker and left with continuous stirring. In order to avoid the formation of foam during the stirring process small amounts of the coating suspension was poured into a 25 mL beaker, which was used without stirring, during the coating.

2.2.4.2. Spraying of Acryl-EZE® suspension

1.5 g of pellet cores were weighed and placed inside the cone of a fluidised bed coater. The process parameters are described in

Table 2.4 where only the fan speed differed from different batches due to a difference in size and density of each batch. The following parameters were started prior to the attachment of the cone to the coater: fan, heat, atomising air and distance from nozzle to pellets. The pump and the agitator were started immediately after the attachment of the cone.

Table 2.4. Process parameters used for film coating pellets with Acryl-EZE®.

Process parameter	Batch C	Batch D	Batch G	Batch H
Agitator	17.5 Hz	17.5 Hz	17.5 Hz	17.5 Hz
Fan	12.0 m/sec	12.5 m/sec	11.0 m/sec	12.0 m/sec
Heat	60 ^{0}C	60 ^{0}C	60 ^{0}C	60 ^{0}C
Pump	1 mL/min	1 mL/min	1 mL/min	1 mL/min
Atomising air pressure	10 psi	10 psi	10 psi	10 psi
Distance from spraying nozzle to pellets	22 cm	22 cm	22 cm	22 cm

The spraying process was continued until pellets of approximately 40% (w/w) weight gain had been produced. The weight gain was measured within 1 min after removal from the coater so any weight gain due to water uptake from the atmosphere was minimised. The pellet cores were sprayed for approximately 60 min to achieve the desired weight gain. The pump was stopped for one min every 15 min in order to dry and detach pellets sticking to the cone and cleaning the spraying nozzle. After the first 15 min of the coating process of Batch C, G and H the fan speed was increased from 10.5 to 12.5 m/sec as the pellets had become heavier.

2.3. Extrusion-spheronisation using different lactose and MCC compositions

Six different pellet batches were prepared using 10 g powder blend containing indomethacin or paracetamol. The excipients consisted of a composition between lactose and MCC which was varied between different batches and wet massed with different volumes of distilled water according to

Table 2.5. Other parameters for the pelletisation process were as described in the extrusion-spheronisation technique in Section 2.2.1.

Each batch was denoted the letter P or I for paracetamol or indomethacin containing pellets respectively with the lactose:MCC compositions next to the letter.

Table 2.5. Properties of individual pellet batches of 10 g powder blend each.

Batch	Lactose:MCC composition	Drug	Distilled water for wet massing
P0:90	0:90% (w/w)	10% (w/w) paracetamol	12.0 mL
P45:45	45:45% (w/w)	10% (w/w) paracetamol	6.0 mL
P60:30	60:30% (w/w)	10% (w/w) paracetamol	4.5 mL
I0:90	0:90% (w/w)	10% (w/w) indomethacin	13.0 mL
I45:45	45:45% (w/w)	10% (w/w) indomethacin	6.0 mL
I60:30	60:30% (w/w)	10% (w/w) indomethacin	5.0 mL

The effects of varying the compositions of lactose to MCC were evaluated by the pellet yield in the desired size range (1.00-1.18 mm) and an evaluation of the pellets' drug release profiles.

The yield of pellets in size range 1.00-1.18 mm was calculated from the initial mass of the total powder blend. If a pellet yield above 15% (w/w) could not be achieved with the tested excipient composition the batch would be considered undesirable for use in manufacturing for scintigraphic purposes.

The drug release profiles were investigated as described in Section 2.9.5. For paracetamol containing pellets an immediate release, of at least 85% (w/w) drug released after 30 min ($T_{85\%}$ >30 min) was desired, and for indomethacin containing pellets a sustained release, with at least 80% (w/w) drug released after 12 hours ($T_{80\%}$ >720 min) was desired.

2.4. Extrusion-spheronisation using PEG 400 and Explotab® as indomethacin release enhancers

Two different pellet batches containing indomethacin were prepared with the excipients described in

Table 2.6. Other parameters for the pelletisation process were as described in the extrusion and spheronisation technique in Section 2.2.1.

Each batch is denoted the letter I for indomethacin containing pellets with PEG or Exp next to the I, separated by a hyphen, for PEG 400 (33.3% (w/w)) or Explotab® (5% (w/w)) containing pellets.

Table 2.6. Powder blend and wet massing of pellet Batch I-PEG and I-Exp.

Batch	Powder blend	Wet mass
I-PEG	MCC (6.0 g) Indomethacin (1 g)	Mixture of: Distilled water (7.0 mL) PEG 400 (3.5 g)
I-Exp	Lactose (4.5 g) MCC (4.0 g) Indomethacin (1 g) Explotab® (0.5 g)	8.0 mL water

The pellets were evaluated by their drug dissolution profile where an immediate indomethacin release was desired, with at least 85% drug released after 30 min ($T_{85\%}$ >30 min) of dissolution. The *in vitro* drug dissolution was carried out as described in Section 2.9.5. The drug content was assayed as described in Section 2.9.4.1 in order to produce the dissolution profile.

The pellet batches were also evaluated physically for their smoothness and hardness. This evaluation was carried out visually and the crushing strength of Batch I-PEG was measured as described in Section 2.9.1.

2.5. Radiolabelling of drug loaded pellet formulations

Four radioactive pellet batches were prepared (Batch D*-G*) in order to prove the concept of radiolabelling all the eight non-radiolabelled pellet formulations (Table 2.1) so they can potentially be detected *in vivo* using gamma scintigraphy.

Radiolabelled pellet formulations were manufactured using two different radiolabelling methods:

- The "incorporating" method was used for radiolabelling of extruded and spheronised pellet formulation by incorporating radioactivity in the extrusion spheronisation process prior to a possible film coating.
- The "soaking" method was used for radiolabelling of drug layered pellet formulations by soaking inert pellet cores in a radioactive solution prior to drug layering and a possible film coating.

Details of the radiolabelled pellet formulations are summarised in Table 2.7.

Each radioactive batch is denoted a letter corresponding to the non-radioactive pellet batch that is being reproduced and with an * to indicate that the batch is radiolabelled.

Table 2.7. Details of the radiolabelled pellet formulations.

Batch	Radiolabelling method	Pelletisation technique	Film coat	Model drug	Drug release profile
D*	"incorporating"	Extrusion-spheronisation	Surelease® and Acryl-EZE®	Paracetamol	Time delayed sustained release
F*	"incorporating"	Extrusion-spheronisation	None (pellet cores)	Indomethacin	Sustained release
G*	"soaking"	Drug layering	Acryl-EZE®	Indomethacin	Time delayed pulsatile release
E*	"soaking"	Drug layering	None (pellet cores)	Indomethacin	Immediate release

An *in vitro* radiolabel release study was carried out as described in Section 2.9.6 in order to investigate whether the radiolabel stays attached to the pellet formulation during drug dissolution.

Drug release studies were carried out after all radioactivity had decayed as described in Section 2.9.5. Dissolution profiles were compared to similar batches of non-radiolabelled pellets (Batch D, E, F and G). Batches with a sustained drug release were compared using difference (f_1) and similarity (f_2) factors as described in Section 2.9.7.

2.5.1. Radiolabelling of extrusion-spheronised pellets using the "incorporating" method

2.5.1.1. Radiolabelling of anion exchange resin

Anion exchange resin (150 mg for Batch D* and 100 mg for Batch F*) was radiolabelled by mixing in a bijou (small glass container) with a saline solution (0.5 mL) containing ^{99m}Tc-pertechnetate ions (877 MBq in Batch D* and 730 MBq in

Batch F*). All liquid was dried off by placing the bijou on a clamp stand and blow drying underneath it for 8 min using a hairdryer. The dried radiolabelled anion exchange resin, that had lost weight due to high water loss, was weighed in a weighing boat and topped up with fresh non-radiolabelled anion exchange resin to regain 100 mg anion exchange resin, and thereby ensure reproducible results.

2.5.1.2. Extrusion spheronisation

Pellet powder blends corresponding to Batch D and Batch F were prepared by mixing the model drug (1 g) with MCC (3 g), lactose (5.9 g) and radiolabelled anion exchange resin (100 mg) in a mortar by using a pestle.

The remaining extrusion-spheronisation method for Batch F* was similar to the method for non-radiolabelled pellets described in Section 2.2.1. However, 4.0 mL water was used for wet massing instead of 5.0 mL and the pellet batch was re-granulated with 4.0 mL distilled water.

The extrusion-spheronisation parameters for Batch D* had been optimised as described in Section 2.5.4. The extrusion-spheronisation method therefore differed slightly from the method for non-radiolabelled pellets described in Section 2.2.1.

2.5.1.3. Film coating of radiolabelled pellets

Radiolabelled pellet cores of Batch D* were coated until 10% (w/w) weight gain of Surelease® and approximately 40% (w/w) weight gain of Acryl-EZE® as described in Sections 2.2.3 and 2.2.4 respectively.

2.5.2. Radiolabelling of drug-layered pellets using the "soaking" method

2.5.2.1. Manufacturing of inert pellet cores

Inert pellet cores containing 1% (w/w) anion exchange resin (manufactured beads) were prepared using the extrusion spheronisation technique. The small scale powder blend (10 g) was prepared by mixing 99% (w/w) MCC with 1% (w/w) anion exchange resin in a mortar using a pestle. The powder blend was wet massed with distilled water (12.5 mL), extruded through a 1 mm sieve aperture and spheronised for 15 min at speed 5 units in a spheroniser operated at 75 ^{0}C. The spheroids were

then dried on a fluidised bed dryer at 40 ^{0}C for 1 hour and inert pellet cores of size range 710-1000 μm were sieve collected.

The size distribution, density and crushing strength of the manufactured beads were compared to the commercially available inert MCC pellet cores, Cellets 700® (700-1000 μm, ≥85%). Size distribution was measured using laser diffraction as detailed in Section 2.9, density was measured by the calculation described in Section 2.9.3 and crushing strength was analysed using a texture analyser as described in Section 2.9.1.

2.5.2.2. Radiolabelling of inert pellet cores

Manufactured beads (200 mg) were soaked in a saline solution containing ^{99m}Tc pertechnetate (580 MBq in Batch E* and 618 MBq in Batch G*) and diluted with distilled water (0.75 mL in an eppendorf for Batch E* and 10 mL in a bijou for Batch G*). The inert pellet cores were soaked by rotating on a tube rotator for 60 min and washed three times by replacing half of the liquid content with distilled water and turning the container twice. The radiolabelled inert pellet cores were dried on a fluidised bed at 40 °C for 60 min.

2.5.2.3. Drug layering onto radioactive inert pellet cores

Radiolabelled manufactured beads (200 mg) were mixed with Cellets 700 to make up a total of 1.5 g of inert pellet cores. These were drug layered as described in Section 3.2.1.2.

2.5.2.4. Film coating of radiolabelled pellets

Radiolabelled pellet cores of Batch G* were film coated until 40% (w/w) weight gain of Acryl-EZE® as described in Section 2.2.4.

2.5.3. Dummy experiments

Four dummy batches (Batch Dd-Gd) were prepared using dummy experiments that replicated the radioactive experiments of Batch D*-G*. These experiments were carried out without radioactivity in order to reduce the radioactive exposure. The batches were prepared in order to investigate if any procedure in the methods of

radiolabelling the pellet formulations could cause a significant change in the drug release profile and in order to prepare for the radioactive experiments.

Each batch is denoted a letter corresponding to the radioactive pellet batch that is being replicated and with the letter d to indicate that the batch is from a dummy experiment.

The dummy experiments were carried out in a similar way as the radioactive experiments described under Section 2.5.1 for extruded and spheronised pellets and Section 2.5.2 for drug layered pellets. The experiments differed only in that a saline solution without ^{99m}Tc pertechnetate was used instead of a radioactive solution and all inert pellet cores to be drug layered contained an anion exchange resin that had been soaked in saline prior to drug layering.

Drug release studies of dummy pellets (Batch Dd, Ed, Fd and Gd) were carried out as described Section 2.9.5. Their dissolution profiles were compared to similar batches of non-radiolabelled pellets (Batch D, E, F and G). Batches with a sustained drug release were compared using an f_1 and f_2 test as described in Section 2.9.7.

2.5.4. Dose preparation

Doses of radiolabelled pellets manufactured using the "incorporating" method or the "soaking" method were prepared by aiming at a radioactive dose between 2-4 MBq. These doses were subjected to *in vitro* radiolabel release studies described in Section 2.9.6. The radioactive doses were measured using a dose calibrator and decay corrected to 09.00 a.m. on the morning after manufacturing. The drug content of the radiolabelled dose was calculated using the labelled drug content, which was assayed as described in Section 2.9.4, and subtracting the weight gain from any applied film coat. It was investigated which capsule size the dose would fit into for potential administration to humans by filling dummy batches (Batch Dd, Ed, Fd and Gd) into capsules of different sizes.

2.6. Optimisation and reproducibility of the small scale extrusion-spheronisation technique

Preliminary experiments identified the optimised settings for the different extrusion-spheronisation parameters, which are listed in Table 2.8.

Table 2.8. Comments on the previous settings and optimised settings for different parameters in the extrusion and spheronisation of pellets.

Parameter	Comment on previous settings	Optimised settings
Rotation speed of spheroniser	The rotation speed of 5 units was found to be inconsistent as the knob was loose	The knob was secured at a fixed rotation speed
Temperature of spheroniser	The spheroniser temperature was increasing for a period of time after the water bath had reached 60 °C	The temperature inside the spheroniser was measured until constant at 51-52 °C
Volume of distilled water for wet massing	The volume of distilled water for paracetamol containing pellets (4.5 mL) and indomethacin containing pellets (5.0 mL) had only been optimised using intervals of 0.5 mL	The optimal volumes of distilled water were found as follows for paracetamol containing pellets to be 4.35 mL and for indomethacin containing pellets to be 4.80 mL

The reproducibility of the optimised extrusion-spheronisation was investigated by reproducing five batches containing non-radiolabelled anion exchange resin (150 mg) for paracetamol (Batch P1-P5) and indomethacin (Batch I1-I5) containing pellets, using the optimised extrusion-spheronisation conditions. Furthermore, an extra batch containing no anion exchange resin was made for indomethacin containing pellets (Ires) in order to investigate if the use of an anion exchange resin has an effect on the pellet yield and the sustained indomethacin release profile.

Each batch used for testing the reproducibility of the extrusion-spheronisation is denoted the letter P or I for paracetamol or indomethacin containing pellets respectively followed by the number 1 to 5 for replicated batches or res for the batch containing no anion exchange resin.

The reproducibility in the yield of pellets in size range 1.00-1.18 mm was evaluated using the standard deviation of the average pellet yield between Batch P1-P5 and between Batch I1-I5. Furthermore, the pellet yield of Batch Ires was compared to the average pellet yield of Batch I1-I5 using a one-sample t-test described in Section 2.9.7. Pellet yield within size range 1.00-1.18 mm was calculated from the initial mass of the total powder blend.

The reproducibility of the drug content was evaluated using the standard deviation of the average drug content between Batch P1-P4 and between Batch I1-I3 and Ires. Furthermore, the assayed drug content was compared to the estimated drug content, defined as the amount of drug initially added to the powder blends. The drug content was assayed as described in Section 2.9.4.1.

The sustained indomethacin release profiles of pellet cores of Batch I1-I3 and Ires were compared to each other and to a similar non-radiolabelled pellet batch prepared without using the optimised extrusion and spheronisation procedure (Batch F) using an f_1 and f_2 test as described in Section 2.9.7. The drug indomethacin release profiles were produced as described in Section 2.9.5.

2.7. Radiolabelled GI transit markers for drug loaded pellets

Batch B* was an inert pellet formulation that was radiolabelled using the "soaking" method. The batch is denoted the letter B as the size and film coat of Batch B* replicated that of Batch B, followed by an * as the batch is radiolabelled.

It was investigated whether pellets of Batch B* could be used as scintigraphic marker pellets of Batch B by comparing their size and density during an *in vitro* dissolution study described in Section 2.7.4.

An *in vitro* radiolabel release study was carried out as described in Section 2.9.6 in order to investigate whether the radiolabel stayed attached to the pellet formulation during drug dissolution.

2.7.1. Manufacturing of inert pellet cores

Inert pellet cores containing anion exchange resin [1% (w/w)] were produced so that they had similar size to the pellet cores of Batch B (1.00-1.18 mm), by using the extrusion spheronisation technique. The powder blend contained no water soluble components and was produced by mixing MCC (9.9g) and anion exchange resin (100 mg) in a mortar using a pestle. The blend was wet massed with distilled water (13.5 mL), extruded through a 1 mm sieve aperture and spheronised for 15 min at speed 5 units in a spheroniser connected to a water bath at 75 ^{0}C. The spheroids were then dried on a fluidised bed dryer at 40 ^{0}C for 1 hour. Pellet cores of size range 1.00-1.18 mm were sieve collected.

2.7.2. Radiolabelling of inert pellet cores

Inert pellet cores (1.5g) containing anion exchange resin [1% (w/w)]were soaked in a bijou containing a diluted saline solution (5 mL) with ^{99m}Tc-pertechnetate (88.8 MBq) and rotated on a test tube rotator for 60 min. The pellets were washed three times by replacing 1 mL soaking solution with 1 mL distilled water three times and turning the bijou twice between after each wash. The pellets were dried for 1 hour at 40 ^{o}C on a fluidised bed dryer.

2.7.3. Film coating of radiolabelled inert pellet cores

The dried radioactive pellets were film coated with Surelease®, as described in Section 2.2.3, with a weight gain similar to Batch B.

2.7.4. Comparison of size and density during *in vitro* dissolution of drug loaded pellets and radiolabelled GI marker pellets

Size and density of Batch B* and B were measured before and after a USP I dissolution (900 mL water, 37 ^{o}C, 100 rpm) for 18 hours with scintigraphic monitoring of Batch B* as described in Section 2.7.5. Additional measurements were taken at 2, 4 and 12 h of dissolution for drug loaded pellets, by running three extra drug dissolution experiments without analysis of drug release. Pellet size was measured using laser diffraction described in Section 2.9 and density was calculated as described in Section 2.9.3.

2.7.5. Investigations of imaging and loading of GI transit marker

Simulated chyme was prepared by mixing 150 g of porridge oats with 500 mL of water and microwaving for 3 min at its highest setting. This was repeated twice in order to have sufficient porridge (1L). Loading of ten radiolabelled (0.5 MBq) GI transit markers of Batch W* (see Section 2.8) was investigated by mixing into a plastic bowl containing the simulated chyme and transferring to a 1 L plastic bag with seal. The bag was placed approximately 25 cm away from the gamma camera and a scintigraphic image was acquired.

2.8. Radiolabelling using the "wet and dry" method

Batch W* was an inert pellet formulation that was radiolabelled using the "wet and dry" method. The batch is denoted the letter W for "wet and dry method" and an * because it is radiolabelled.

An *in vitro* radiolabel release study was carried out as described in Section 2.9.6 in order to investigate whether the radiolabel stayed attached to the pellet formulation during drug dissolution.

2.8.1. Manufacturing of inert pellet cores

The inert pellet cores used were the same as those used for radiolabelling of drug layered pellets. The manufacturing of the pellet cores is described in Section 2.5.2.1.

2.8.2. Radiolabelling of inert pellet cores

200 mg manufactured beads were placed in a bijou and wetted in 0.5 mL of a 520 MBq ^{99m}Tc-pertechnetate solution. Immediately after wetting the liquid was evaporated by placing the bijou on a clamp stand and blow drying from underneath for 10 min using a hairdryer. The radiolabelled pellet cores were not washed after drying.

2.9. Analytical techniques

2.9.1. Measurements of crushing strengths

Pellet crushing strength (n=3) was measured using a texture analyser. The initial peak registered by the texture analyser was used as the crushing strength of the pellets. Test speed was set to 0.03 mm/sec, the distance was set to 0.3 mm and the trigger force was set to 1.0 N, except for Batch I-PEG which had a trigger force set to 0.1 N. A force-displacement graph was generated by the texture analyser software.

2.9.2. Laser diffraction for measurements of size distributions

The size distributions of pellet batches were measured using laser diffraction. Distilled water was used as dispersant. The refractive index and absorption index for the sample material were unknown and therefore set as default, with values of 1.52 and 0.1 respectively. The measurement time was 10 seconds with three (n=3) cycles of 0 seconds delay.

2.9.3. Calculation of pellet density

The pellet density was measured using the median pellet size and the number of pellets contained within approximately 200 mg (n=3) of pellets, which was counted by hand. Median pellet size was within the size range 710-1000 μm (855 μm) for manufactured beads, within the size range of 700-1000 μm (850 μm) for Cellets 700® and within the size range of 1.00-1.18 mm (1.09 μm) for Batch B and B*.

2.9.4. Assay of drug content

2.9.4.1. Drug content in extrusion-spheronised pellets

Uncoated extrusion-spheronised pellets (approximately 11.1 mg) containing paracetamol (n=4) or indomethacin (n=4) were placed in a 100 mL volumetric flask filled with distilled water for the quantification of paracetamol or with phosphate buffer pH 6.8 for the quantification of indomethacin. The volumetric flask was sonicated for 10 min and shaken prior to analysis by UV at 257 nm for paracetamol and 320 nm for indomethacin. The drug contents were quantified using calibration

curves (Appendix II and III). Batch I-Exp and I-PEG were only measured in single (n=1).

The drug content assayed for Batch P1-P4 and Batch I1-I3 and Ires was used as the labelled drug content for all extrusion-spheronised pellets containing paracetamol and indomethacin respectively, except for Batch I-PEG and I-Exp.

2.9.4.2. Drug content in drug layered pellets

The dose of all manufactured drug layered pellet batches (n=3) was quantified using the data from each dissolution experiment. A dissolution experiment was carried out as described in Section 2.9.5. The UV absorbance measured at 2 hours of dissolution was used for quantifying the drug dose for uncoated pellets. The UV absorbance measured at 4 hours of dissolution was used for quantifying the drug dose for pellets coated with Acryl-EZE®. The UV absorbance from a dissolution profile of inert pellets coated with Acryl-EZE® was subtracted for drug layered pellets coated with Acryl-EZE® (Appendix IV). The drug contents were calculated using calibration curves (Appendix II and III).

The individual drug content assayed for all drug layered pellet formulations was used as their labelled drug content.

2.9.5. *In vitro* drug dissolution studies

Pellets of approximately 100 mg cores (n=3) were subjected to USP I dissolution (900 mL media, 37 °C, 100 rpm) for up to 18 hours with samples automatically drawn through filters (20 μm pores) analysed every 5 min.

Pellets containing paracetamol underwent dissolution testing in water as the media with detection at 257 nm.

Pellets containing indomethacin underwent dissolution testing in phosphate buffer pH 6.8 as the media with detection at 320 nm.

Pellets with a time delayed drug release underwent dissolution testing in hydrochloric acid pH 1.2 for 2 hours prior to dissolution in phosphate buffer pH 6.8.

Drug release profiles were drawn from the measured UV absorbance. The UV absorbance of 100% drug release was calculated using calibration curves (Appendix II and III) and the drug content assayed as described in Section 2.9.

The drug release profiles of pellets containing an Acryl-EZE® film coat were drawn by subtracting the UV absorbance of blank pellets coated with Acryl-EZE® from the measured UV absorbance (see Appendix IV).

2.9.5.1. Preparation of dissolution media

2.9.5.1.1. Phosphate buffer pH 6.8:

Potassium dihydrogen phosphate (74.8 g) and sodium hydroxide (9.85 g) were transferred to a Duran bottle. The Duran bottle was filled with distilled water (11 L) and left for stirring on a magnetic stirring for at least 10 min. pH was adjusted to 6.8 with a 0.1 M sodium hydroxide solution.

2.9.5.1.2. Hydrochloric acid pH 1.2:

Distilled water (5.5 L) was transferred to a Duran bottle. Hydrochloric acid (53 mL) was transferred to the distilled water. pH was adjusted to 1.2 with a 10 M hydrochloric acid solution.

2.9.6. *In vitro* radiolabel release study

The dose of radiolabelled pellets subjected to *in vitro* dissolution is described in Section 2.5.4. Doses of radiolabelled pellets were subjected to USP I dissolution 24 hours after manufacturing. The rotation of the dissolution baskets was stopped for 1 min prior to scintigraphic imaging which was acquired for 25 seconds at various time intervals during the radioactive dissolution. No samples were drawn for measuring the drug release and all other dissolution parameters were as described in Section 2.9.5.

Radiolabel associated with the pellet formulation was quantified by drawing a region of interest (ROI) around the pellets to calculate any radioactive loss due to diffusion or erosion of radiolabel into the dissolution media. The radioactivity inside the ROI was decay corrected.

2.9.6.1. Investigation of radiolabel bound to resin

Anion exchange resin (150 mg) was radiolabelled with ^{99m}Tc-pertechnetate (800 MBq) as described in Section 2.5.1.1 but without the addition of fresh anion exchange resin. The radiolabelled anion exchange resin was transferred to a dissolution pot containing phosphate buffer pH 6.8 (900 mL). The dissolution pot was placed in a dissolution apparatus which was switched off and its water bath remained empty. A scintigraphic image of the dissolution pot was acquired for 25 seconds after 20 hours of settling of the anion exchange resin. Immediately after acquiring the scintigraphic image the dissolution media was filtered using a Buchner funnel with a 0.45 μm filter paper. The scintigraphic image was evaluated and the radioactivity of the filtered dissolution media (100 mL) was measured using a dose calibrator and a Geiger counter.

2.9.7. Statistical analysis

Simple statistics were applied to obtain the results. The results were presented by the mean and their standard deviation (±SD).

Size and density of inert pellet cores, gastric markers and drug loaded pellets were compared using one-way analysis of variances (ANOVA). p-values <0.05 or <0.01 were considered statistically significant.

The pellet yields of optimised pellet batches containing indomethacin (Batch I1-I5) were compared to the pellet yield of an optimised pellet batch prepared without use of anion exchange resin (Batch Ires) by using a one-sample t-test. A p-value <0.05 was considered statistically significant.

Sustained drug release profiles were compared using difference (f_1) and similarity (f_2) factors. Calculated values of f_1 lying between 0 and 15 and calculated values of f_2 lying above 50 were interpreted as being similar.

Chapter 3: Small scale manufacturing of eight different non-radiolabelled pellet formulations

3.1. Introduction

The present chapter describes how we have developed small scale manufacturing methods for eight different pellet formulations using the popular extrusion-spheronisation and drug-layering techniques with conventional pelletisation excipients.

Following four different *in vitro* release profiles for a poorly and a partially water soluble drug were manufactured: immediate release, sustained release, time delayed pulsatile release and time delayed sustained release. These eight different pellet batches were chosen in order to simulate a wide range of popular pellet formulations existing on the market, so that in a later stage they can be manufactured with an incorporated radioisotope for scintigraphic purposes on a small scale.

Small scale manufacturing is highly desirable when dealing with scintigraphic studies, which typically only involve dosing of around six subjects (Ghimire *et al.* 2011; Ghimire *et al.* 2010; Goodman *et al.* 2010; Hodges *et al.* 2009; Wilson *et al.* 2009). By manufacturing on a small scale, radioactive exposure can be reduced to a level that is considered safe and less material is wasted.

To our knowledge, there is no available literature describing a pelletisation method for producing extrusion-spheronised pellets or drug-layered pellets on a small scale suitable for scintigraphic studies. Untraditional pelletisation methods have thus been used in the present study, and include sieve extrusion and use of laboratory scale equipment with modifications such as spheronisations under heated conditions and fluidisation under increased dynamic conditions.

3.2. Results and discussion

Small scale manufacturing of eight different non-radiolabelled pellet formulations batches (Batch A-H) was successfully accomplished. Various properties of the manufactured pellet cores were tested, which include pellet yield and crushing

strengths of extrusion-spheronised pellets (Sections 3.2.1.1 and 3.2.1.2) and the drug content of drug-layered pellets (Section 3.2.2). Finally, film coating was evaluated (Section 3.2.3) and an *in vitro* drug dissolution test of the manufactured pellets was carried out to confirm that the desired drug release profile was achieved (Section 3.2.4).

3.2.1. Properties of extrusion-spheronised pellet cores

3.2.1.1. Pellet yield of extrusion-spheronised pellets

Table 3.1 illustrates the pellet yields of the extrusion-spheronised pellet batches (Batch A-D, F and H).

Table 3.1. Pellet yields in size range 1.00-1.18 mm for non-radiolabelled extruded and spheronised pellet batches.

	Batch A	Batch B	Batch C	Batch D	Batch F	Batch H
Pellet yield (% (w/w))	20.3	35.8	15.5	31.0	19.0	N/A (pellet cores of Batch F)

According to Baert *et al.* (1992) and Kristensen *et al.* (2000) an acceptable pellet yield is 90% (w/w) within a size range of 0.71-1.40 mm for pellets manufactured on a laboratory scale (Baert *et al.* 1992; Kristensen *et al.* 2000). This size range is considerably larger than the size range of 1.00-1.18 mm used in this study. The narrow size range used in this study was chosen in order to prove the point that pellet formulations of narrow size ranges can be produced when manufacturing in small scale for scintigraphic purposes. In this study a pellet yield of 15% (w/w) or above is considered acceptable as this will make sufficient pellets to dose six subjects in a clinical trial and will give a sufficient mass for satisfactory fluidisation in a laboratory scale fluidised bed coater.

All extrusion-spheronised pellet batches listed in Table 3.1 had yields above 15% (w/w). However, if a pellet batch had a yield below 15% (w/w) it was discarded and a new batch was produced. This procedure was used because the extrusion-spheronisation process was not fully optimised and standardised to achieve a consistent yield of 15% (w/w) or above.

The reason for the high yield of Batch B is mainly due to its higher ratio of MCC to lactose in the powder blend (45:45% (w/w)) compared to the other pellet batches (60:30% (w/w)). This effect of changing the ratio of lactose to MCC is detailed in Chapter 4.

Batch D was also found to give a relatively high yield (31.0% (w/w)). This high yield shows that it is possible to accomplish a relatively high yield for extrusion-spheronised pellets with high ratios of lactose to MCC (60:30% (w/w)) by optimising the extrusion-spheronisation process.

It is assumed that the large variation in the pellet yield of Batch A, C, D and F is due to a variation in the spheroniser temperature and rotation speed. Lee (2003) showed similar effects caused by changes in the spheroniser temperature and speed during spheronisation of wax based pellets (Lee 2003). The spheroniser parameters were therefore standardised on a later occasion, in order to produce pellets of reproducible yields, with the results discussed in Chapter 7.

3.2.1.2. Crushing strengths of extrusion-spheronised pellet cores

Table 3.2 illustrates the crushing strengths of uncoated extrusion-spheronised pellet batches (Batch A and F).

Table 3.2. Crushing strength of non-radiolabelled uncoated extruded and spheronised pellet (mean (±SD), n=10).

	Batch A	Batch F
Crushing strength	5.60 (±0.71) N	5.21 (±0.93) N

The crushing strengths of the pellets were sufficient to withstand the mechanical force that is applied during film coating, and still allowed water to penetrate the matrix for drug release.

Sousa *et al.* (1996) found that an increased water content during wet massing results in higher crushing strengths of pellet formulations but only when the dry powder blend are the same (Sousa *et al.* 1996). Batch A contained paracetamol and was produced using 10% (v/v) less distilled water than Batch F which contained indomethacin. However, the same crushing strength was expected as the dry powder blend contained different drug and the amount of distilled water added during wet

massing generated approximately same pellet yield using same extrusion-spheronisation parameter settings.

Force-displacement diagrams generated from the texture analyser are shown in Figure 3.1 and Figure 3.2 for Batch A and F respectively.

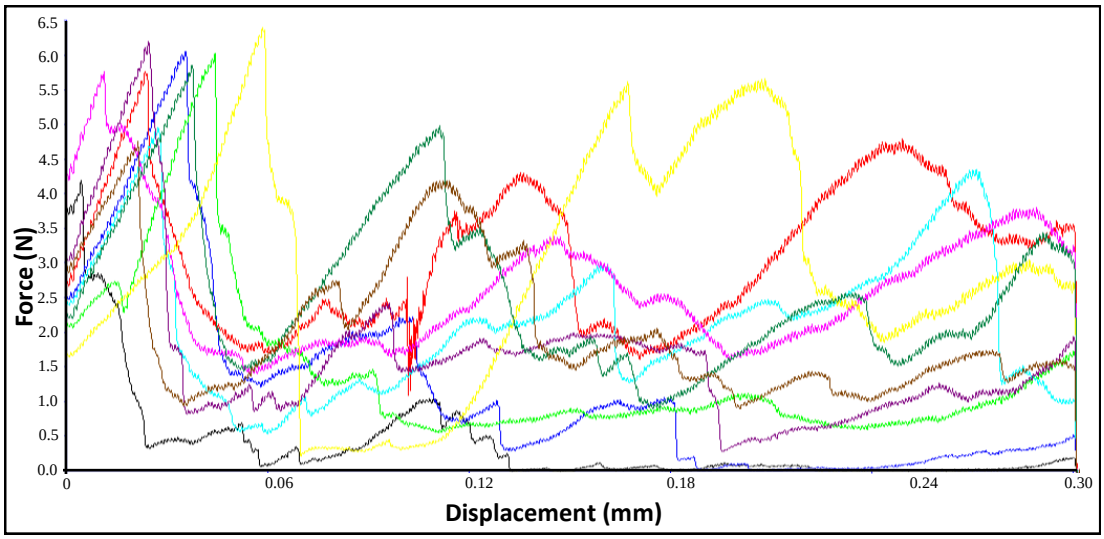


Figure 3.1. Force-displacement diagram for pellets of Batch A (n=10).

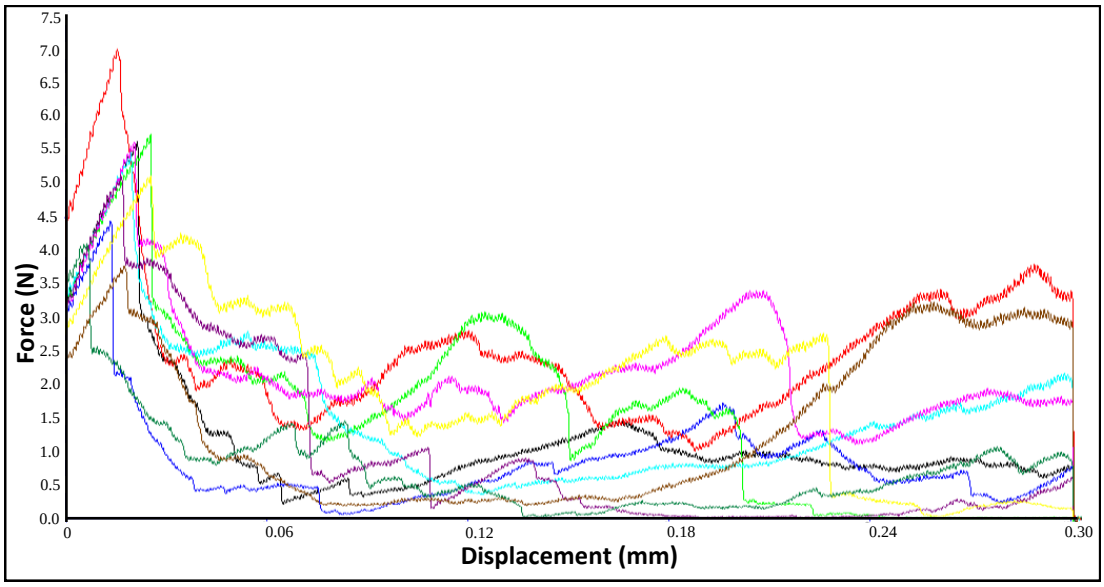


Figure 3.2. Force-displacement diagram for pellets of Batch F (n=10).

The large initial peak on the force-displacement diagrams indicate that both batches show brittle behaviour. A brittle behaviour is usually expected for pellet formulations but can vary from brittle to fully plastic depending on the manufacturing method, excipients used and moisture content (Bika *et al.* 2001).

3.2.2. Properties of drug-layered pellet cores

3.2.2.1. Drug content of drug-layered pellets

Table 3.3 illustrates the estimated and assayed drug contents of the drug-layered pellet batches (Batch E and G).

Table 3.3. Estimated and assayed drug content for non-radiolabelled drug-layered pellets (mean (±SD), n=3).

	Batch E	Batch G
Estimated drug content[a]	12.1% (w/w)	10.6 (±0.1)% (w/w)
Assayed drug content[b]	13.2% (w/w)	10.5 (±0.1)% (w/w)

[a]Estimated content is calculated using the weight gain of the pellet batch after drug-layering.
[b]Assayed content is measured on a fraction (n=3) of the actual batch using UV as described in the method section.

It is seen from Table 3.3 that the estimated drug content of Batch E and G varied from their assayed drug contents. It is thus difficult to estimate the drug content using weight gain.

The difficulty in estimating the deposition of a drug layer using weight gain is commonly known (Pandey *et al.* 2006). Estimations of the drug layer may have been further complicated due to the small manufacturing scale used in the present study, which meant a reduced spraying time, and the poorly water soluble properties of indomethacin causing the drug to precipitate in the container during drug-layering.

In order to correlate for the incorrect estimated drug content it may be suggested to do a quality check by UV analysis to assay the indomethacin content in the drug-layered pellets, after estimations by weight gain, prior to dosing to volunteers. The dose of drug-layered pellets subjected to film coating should be assayed using a sample of uncoated pellets. Such method of dose quantification is more precise due to potential interference of UV absorption by the film coat.

3.2.3. Film coating of pellet cores

Table 3.4 illustrates the achieved weight gains of Surelease® and Acryl-EZE® for film coated pellet batches (Batch B, C, D, G and H).

Table 3.4. Weight gains of Surelease® and Acryl-EZE® for non-radiolabelled film coated pellet batches (Batch B, C, D, G and H).

	Batch B	Batch C	Batch D	Batch G	Batch H
Surelease® weight gain	9.9% (w/w)	N/A	10.3% (w/w)	N/A	N/A
Acryl-EZE® weight gain	N/A	37.8% (w/w)	39.6% (w/w)	40.6% (w/w)	39.6% (w/w)

All batches were successfully film coated with the desired weight gain, which provided the desired *in vitro* drug dissolutions profiles shown in Section 3.2.4.

It was found in a preliminary study that the drug release profiles were reproducible and correlated to film coat weight gains. Both Surelease® and Acryl-EZE® coating systems are manufactured by Colorcon® who suggest a weight gain with Surelease® within the range of 4-16% (w/w) (Scattergood *et al.* 2004) and a weight gain of Acryl-EZE® between 20-30% (w/w) (Young *et al.* 2006). The weight gain of 10% (w/w) with Surelease® therefore correlates well with the weight gain suggested by Colorcon®. The sustained release profile could easily be adjusted by changing the Surelease® weight gain, and it was found that a deviation from a 10.0% (w/w) weigh gain of Surelease® of more than 0.5% (w/w) would result in a significantly different sustained drug release profile. On the other hand, the weight gain of 40% (w/w) with Acryl-EZE® was above the limits suggested by Colorcon®. It was found on a later occasion that a 30% (w/w) weight gain of Acryl-EZE® may be sufficient to its function as a delayed drug release film coat. This was due to the discovery that a titanium dioxide component in the Acryl-EZE® dispersion had interfered with the UV absorption, making it appear as though drug was being released when applying a 30% (w/w) weight gain of Acryl-EZE®. A 30% (w/w) weight gain of Acryl-EZE® may therefore be suggested for future use.

3.2.4. *In vitro* drug dissolution of the eight different non-radiolabelled pellet formulations

The four different release profiles for paracetamol (Batch A-D) are illustrated in Figure 3.3 and the four different release profiles for indomethacin (Batch E-H) are illustrated in Figure 3.4. It is observed that all the desired drug release profiles of Batch A-H were accomplished.

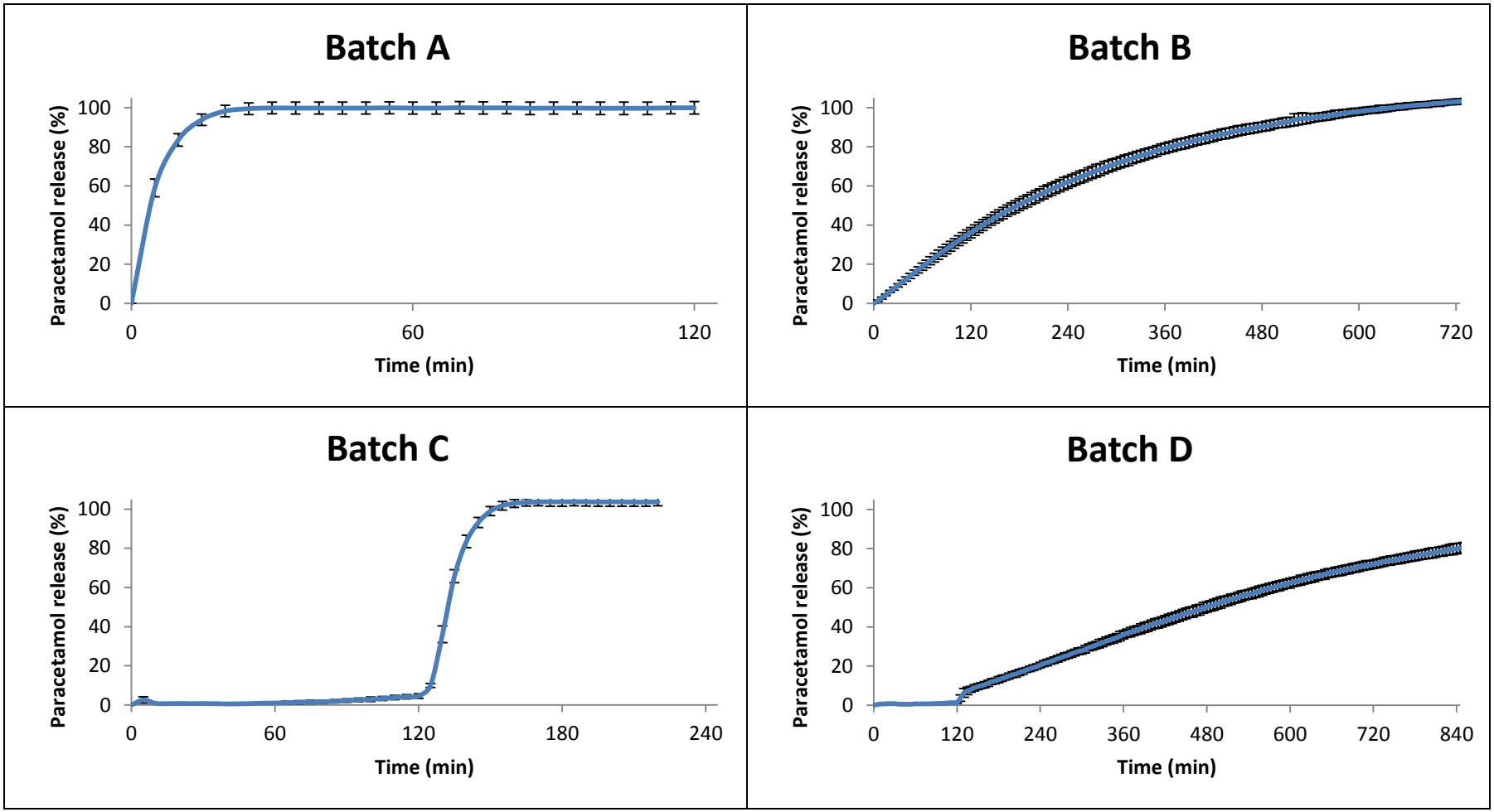


Figure 3.3. Dissolution profiles of pellets containing paracetamol (n=3; mean ±SD). Batch A: Immediate drug release formulation in distilled water. Batch B: Sustained drug release formulation in distilled water. Batch C: Time delayed pulsatile drug release formulation in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8. Batch D: Time delayed sustained drug release formulation in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8.

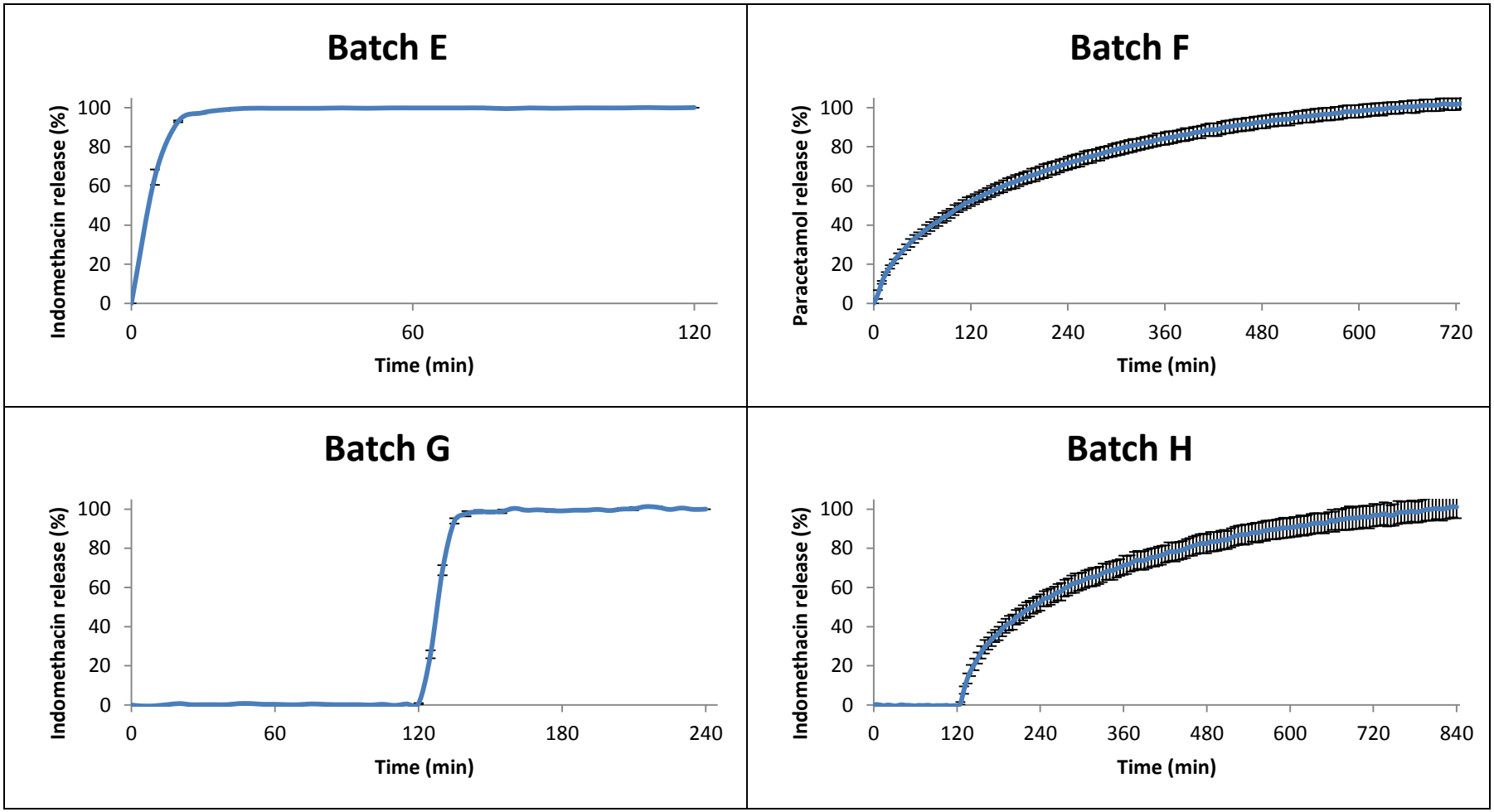


Figure 3.4. Dissolution profiles of pellets containing indomethacin (n=3; mean (±SD)). Batch E: Immediate drug release formulation in phosphate buffer pH 6.8. Batch F: Sustained drug release formulation in phosphate buffer pH 6.8. Batch G: Time delayed pulsatile drug release formulation in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8. Batch H: Time delayed sustained drug release formulation in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8.

All pellet batches in Figure 3.3 and Figure 3.4 were intact after their *in vitro* dissolution. The drug release of the extrusion-spheronised pellets was therefore mainly a diffusion-controlled process in which the drug release rate was limited by the diffusion and/or dissolution of the drug. Diffusion is the most common mechanism for controlling drug release (Arifin *et al.* 2006; Siepmann *et al.* 1999; Teng and Qiu 2011). The drug release from drug-layered pellets is due to erosion of the drug-layer rather than diffusion through pores.

3.2.4.1. Dissolution of uncoated pellet batches

The dissolution profiles of Batch A, E and F in Figure 3.3 and Figure 3.4 were achieved using uncoated pellet cores.

An immediate paracetamol release was achieved using pellet cores manufactured by the extrusion-spheronisation technique (Batch A). Due to the partially water soluble properties of paracetamol the drug quickly dissolved which caused an immediate diffusion through the pores of the pellet cores.

A sustained indomethacin release was achieved for uncoated pellet cores manufactured by the extrusion-spheronisation technique (Batch F). Due to the poorly water soluble properties of indomethacin the drug was dissolved slowly which caused a prolonged diffusion through the pores of the pellet matrix. Pinto JF *et al.* (1992) also produced extrusion-spheronised indomethacin pellets using same excipients and achieved similar drug release profiles. The size of their manufacturing scale is not stated in their published paper. They described this uncoated diffusion controlled delivery system as a hydrophilic matrix sustained release delivery system (Pinto *et al.* 1992).

An immediate release of indomethacin was achieved for uncoated pellet cores manufactured using the drug-layering technique (Batch E). The drug-layered components were similar to those used in a study carried out by Akhgari *et al.* (2005), who also achieved an immediate indomethacin release, so the results were as expected (Akhgari *et al.* 2005). The immediate release of the poorly water soluble drug was accomplished by immediate dissolution of the drug-layer rather than a prolonged diffusion through pores in a pellet matrix, which was the case for the

extrusion-spheronised pellet batches. Chapter 5 has dealt with the investigation of an immediate indomethacin release using the extrusion-spheronisation technique by the addition of a superdisintegrant or a co-solvent but without achieving the desired immediate release.

3.2.4.2. Dissolution of Surelease® coated pellet batches

A sustained paracetamol release of Batch B and D in Figure 3.3 was achieved by coating with Surelease®. Batch D was also coated with Acryl-EZE® in order to get an initial delay of the drug release.

The application of a Surelease® film coat slows the diffusion of drug from the pellet core by applying a layer of an insoluble polymer. Small pores in the polymer, created by a water soluble channelling agent, allow slow diffusion of drug. This type of diffusion-controlled delivery system achieved for Batch B and Batch D has been described as a porous reservoir system (Teng and Qiu 2011).

When comparing their dissolution profiles, after the delay of Batch D, it can be seen that the paracetamol release of Batch B was faster than Batch D. Batch B released 103.0 (±1.4)% (w/w) paracetamol and Batch D released 79.9 (±2.6)% (w/w) after 720 min of dissolution in distilled water and phosphate buffer pH 6.8 respectively. The reason for the faster paracetamol release of Batch B is presumably due to the distilled water dissolution media having low ionic strength, whereas the dissolution media for Batch D containing phosphate buffer has higher ionic strength. In contrast, decreased drug diffusion due to increased ionic strength in the dissolution media has been reported in the published literature (Asare-Addo *et al.* 2011; Colombo *et al.* 1995).

3.2.4.3. Dissolution of Acryl-EZE® coated pellet batches

The delayed drug dissolution profiles of Batch C, D, G and H in Figure 3.3 and Figure 3.4 were achieved by coating with Acryl-EZE®. All dissolution profiles of pellets coated with Acryl-EZE® resulted in less than 10% (w/w) drug release after 2 hours in hydrochloric acid pH 1.2 media. This is the requirement for delayed release dosage forms, such as gastro resistant pellets, listed in a monograph in the British Pharmacopoeia (BP online 2013, Appendix XII B. Dissolution).

3.3. Conclusion

- Eight different pellet batches were successfully manufactured in a small scale sufficient for dosing to six volunteers in a clinical study.

- The manufacturing techniques, excipients, pellet textures, drug release mechanisms and *in vitro* drug release profiles of the developed pellet batches resemble those of conventional pellet formulations.

- The pellet batches are suitable for use in scintigraphic studies involving dosing of six volunteers if a radioactive tracer can be incorporated into the pellets during the small scale manufacturing.

Chapter 4: Influence of lactose to MCC composition in extrusion-spheronised pellets

4.1. Introduction

In order to be able to manufacture a wide range of pellet formulations it is necessary to be capable of changing the excipient compositions of the pellet formulations to accommodate for different types of pellet formulation. The aim of this research was therefore to investigate whether extrusion-spheronised pellets could be manufactured in a small scale similar to that described in Chapter 3 when changing the composition of lactose to MCC, and to investigate how this has an effect on the *in vitro* drug dissolution profile.

4.2. Results and discussion

4.2.1. Influence on pellet yield

Table 4.1 illustrates the pellet yield of paracetamol (P0:90, P45:45 and P60:30) and indomethacin (I0:90, I45:45 and I60:30) containing extrusion-spheronised pellets with varying compositions of lactose to MCC.

Table 4.1. Pellet yields in the size range 1.00-1.18 mm of pellet batches prepared with various compositions of lactose:MCC.

Batch name	Yield
Batch P0:90	54% (w/w)
Batch P45:45	36% (w/w)
Batch P60:30	20% (w/w)
Batch I0:90	60% (w/w)
Batch I45:45	35% (w/w)
Batch I60:30	19% (w/w)

All the pellet batches were produced in yields higher than 15% (w/w). This was desired in order to have enough pellets to fit in the film coater, if a film coat were to be applied, or to dose six volunteers in a clinical trial. Batch P60:30 and Batch I60:30 were close to a yield of less than 15% (w/w) so these batches need standardisation in order to ensure that a yield of at least 15% (w/w) would be achieved in a reproducible manner.

It is observed in Table 4.1 that the yield of pellets within the desired size range (1.00-1.18 mm) increased as the MCC composition became higher. This was due to a more narrow size distribution of spheroids after spheronisation when increasing the MCC content. Kristensen *et al.* (2000) reported same trend for similar formulations prepared by the direct pelletisation technique, when increasing the MCC content at different levels from 10-30% (w/w). However, they did not find same trend when increasing the MCC content at different levels between 30-100% (w/w) (Kristensen *et al.* 2000). This may be due to a difference in their method compared to the method in our study.

In the study by Kristensen *et al.* (2000) pellets of reproducible size ranges were produced by altering the volume of distilled water added during wet massing of powder blends when the composition of MCC to lactose was changed. This resulted in reproducible pellet size distributions (Kristensen *et al.* 2000). In our study we altered an additional process parameter; the temperature of the spheroniser. Spheronisers are typically heated during spheronisation using the hot melt extrusion technique (Lee 2003; Rahman *et al.* 2009) but for our small scale extrusion-spheronisation method heat was necessary in order to evaporate the liquid and reduce agglomeration during spheronisation. As the addition of this extra process parameter was found to give smaller size distributions when the MCC composition increased, our developed pelletisation method, using spheronisation under heated conditions, may have advantages over conventional spheronisation carried out at room temperature.

4.2.2. Influence on drug release

Figure 4.1 shows the dissolution profiles of paracetamol pellets with various compositions of lactose to MCC (Batch P0:90, P45:45 and P60:30) and Figure 4.2 show the dissolution profiles of indomethacin pellets with various compositions of lactose to MCC (Batch I0:90, I45:45 and I60:30).

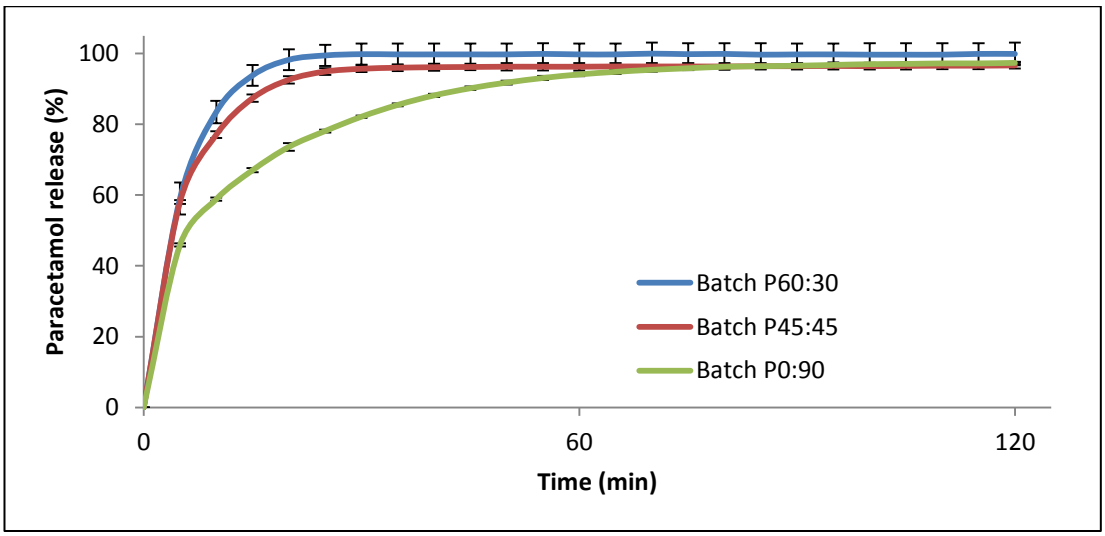


Figure 4.1. Paracetamol release in distilled water of pellet batches with different lactose to MCC compositions (n=3; mean (±SD)).

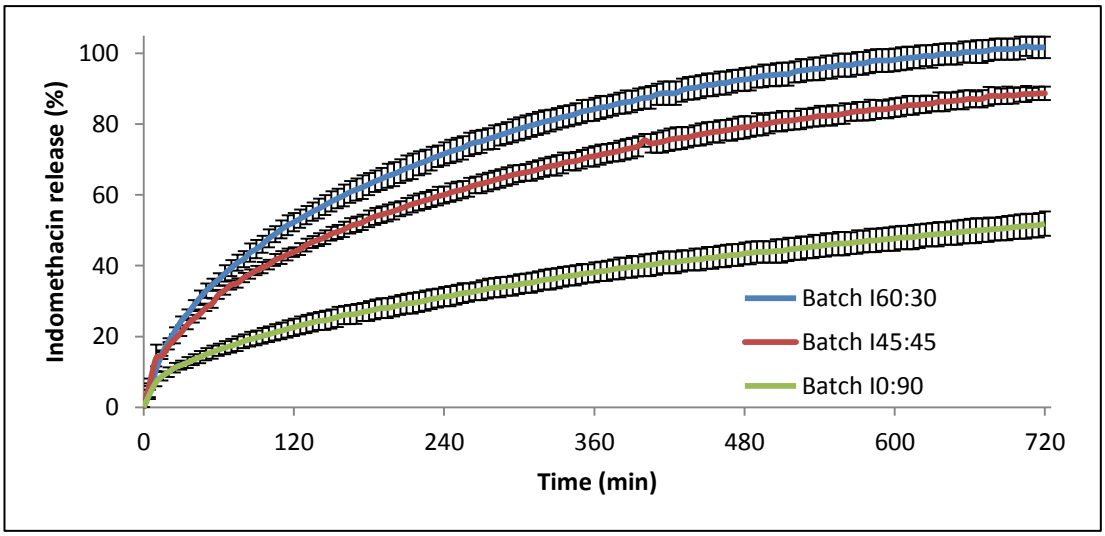


Figure 4.2. Indomethacin release in phosphate buffer pH 6.8 of pellet batches with different lactose to MCC compositions (n=3; mean (±SD)).

It is observed that an increase in the MCC content of the pellet formulations results in a decreased release rate of both paracetamol and indomethacin. In order to better compare the effect on the release rate the $T_{85\%}$ values for the paracetamol release and the $T_{80\%}$ values for indomethacin release in Figure 4.1 and Figure 4.2 respectively are listed in Table 4.2.

Table 4.2. $T_{85\%}$ and $T_{80\%}$ values for the release of paracetamol and indomethacin respectively from batches used for investigating different compositions of lactose to MCC.

Batch name	$T_{85\%}$ or $T_{80\%}$ values
Batch P60:30	$T_{85\%} \approx 10$ min
Batch P45:45	$T_{85\%} \approx 15$ min
Batch P0:90	$T_{85\%} \approx 35$ min
Batch I60:30	$T_{80\%} \approx 370$ min
Batch I45:45	$T_{80\%} \approx 605$ min
Batch I0:90	$T_{80\%} > 720$ min

According to an FDA Guidance for Industry, an immediate release drug formulation is considered rapidly dissolving when no less than 85% of the labelled amount of the drug substance is released within 30 minutes of *in vitro* dissolution (FDA 2000). A rapid release could thus only be achieved for paracetamol containing pellets containing lactose (Batch P60:30 and P45:45). According to another FDA Guidance for Industry, a final *in vitro* dissolution time point of sustained release formulations should be taken at 80% (w/w) drug release (FDA 1997). It was found that a $T_{80\%}$ value was not achieved during the 12 hours *in vitro* dissolution of Batch I0:90. It was therefore concluded that the addition of lactose to both paracetamol and indomethacin containing pellet formulations was necessary in order to achieve their desired drug release profiles.

A decreased indomethacin release rate caused by increasing the MCC content of extrusion-spheronised pellets was also found in a similar study by Pinto *et al.* (1992) who used the same excipients. The decreased drug release rate is therefore as expected, and has been described by Pinto *et al.* (1992) as an enhanced sustained releasing effect of the hydrophilic matrix sustained release pellet delivery system (Pinto *et al.* 1992). Kleinebudde (1994) explained that an increased MCC content in pellets reduces the drug release rate by reducing the porosity of the pellets in a shrinking process during drying (Kleinebudde 1994).

4.3. Conclusion

- Extrusion-spheronised pellets containing 30, 45 or 90% (w/w) MCC could be produced in a sufficient yield using the developed small scale extrusion-spheronisation method.

- The non-traditional use of spheronisation under heated conditions showed advantages over spheronisation at room temperature, as a narrower pellet size range could be achieved when increasing the spheronisation temperature while increasing the MCC content.

- Increasing the amount of MCC in the pellets reduced the *in vitro* drug dissolution rate. It was therefore found necessary to add lactose to the extrusion-spheronised pellet formulations in order to achieve a rapid immediate paracetamol release and a sufficient sustained indomethacin release.

Chapter 5: Investigation of PEG 400 and Explotab® as indomethacin release enhancers for extrusion-spheronised pellets

5.1. Introduction

An immediate *in vitro* release of poorly water soluble drugs in extrusion-spheronised pellets is complicated by the MCC content which restricts disintegration and causes low porosity by contraction of MCC during drying (Kleinebudde 1994; Pinto *et al.* 1992). The aim of this research was therefore to investigate whether extrusion-spheronised pellet formulations with an immediate release of a poorly water soluble drug, indomethacin can be achieved by the addition of a co-solvent, PEG 400 or a superdisintegrant, Explotab®.

5.2. Results and discussion

5.2.1. Pellet yield and drug content

Table 5.1 illustrates the yield and drug content of extrusion-spheronised pellets containing PEG 400 (I-PEG) or Explotab® (I-Exp) as indomethacin release enhancers.

Table 5.1. Yield and indomethacin content of extrusion-spheronised pellets for investigation of an immediate release of indomethacin.

Batch	Yield in size range 1.00-1.18 mm	Indomethacin content	
		Estimated content[a]	Assayed content[b]
I-PEG	13.2% (w/w)	9.5% (w/w)	12.0% (w/w)
I-Exp	15.1% (w/w)	10.0% (w/w)	16.4% (w/w)

[a]Estimated content is calculated from the amount of indomethacin initially added to the powder blend.
[b]Assayed content is measured using UV as described in the method section.

It is seen from Table 5.1 that the pellet yields in size range 1.00-1.18 mm were low for both Batch I-PEG and I-Exp. The low yield complicates further experimentation for scintigraphic studies where a reproducible yield of 15% (w/w) is required for dosing of six volunteers. An optimisation of the extrusion-spheronisation method may give higher yields but this was not carried out in the present thesis.

The assayed indomethacin contents of both batches were much higher than their estimated contents. The deviations between the estimated and assayed drug contents can be explained as follows:

- For Batch I-PEG the amount of PEG 400 added during wet massing (3.5 g) was used in the calculation of the estimated indomethacin content. As PEG 400 is a liquid it may not be well incorporated into the final pellets hindering the estimation of the indomethacin content.

- For Batch I-Exp, the addition of Explotab® caused the powder blend to become very sticky and a lot of powder was found sticking to the inside of the spheroniser. The proportion of Explotab® sticking to the spheroniser may have been higher than the proportion of indomethacin making the assayed indomethacin content of the pellets higher.

5.2.2. *In vitro* dissolution

Figure 5.1 shows the indomethacin release of extrusion-spheronised pellets containing PEG 400 (I-PEG) or Explotab® (I-Exp) as dissolution enhancers. The indomethacin release of an extrusion-spheronised pellet formulation without dissolution enhancers and identical lactose composition as Batch I-Exp (Batch I45:45), is shown as a reference.

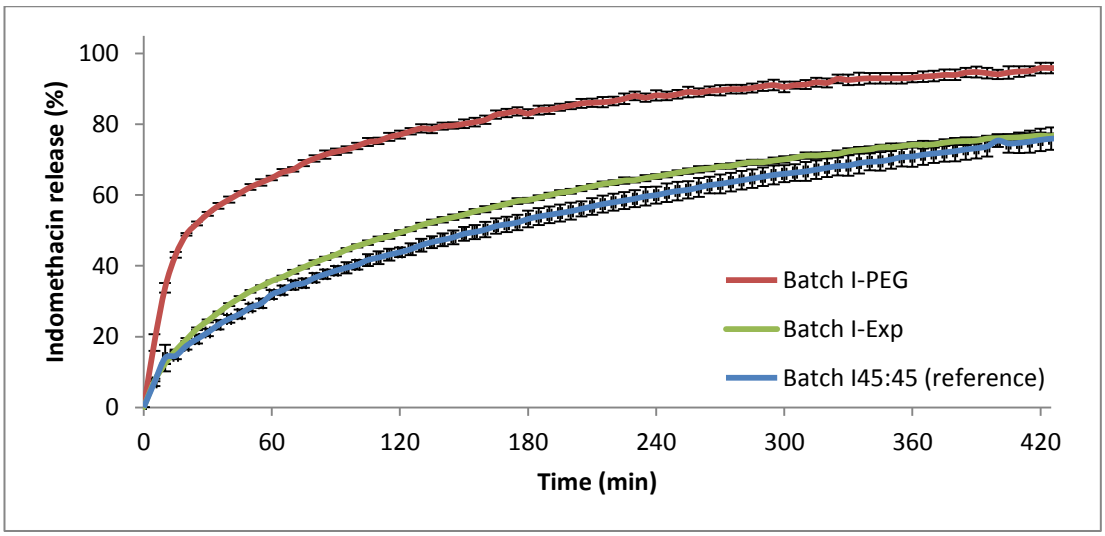


Figure 5.1. Indomethacin release of Batch I-Exp and I-PEG with Batch I45:45 shown as a reference (n=3; mean (±SD)).

No rapid immediate indomethacin release was observed for Batch I-PEG or I-Exp, as $T_{85\%}$ >30 min for both pellet batches. The incorporation of PEG 400 or Explotab® were therefore not suitable as dissolution enhancers for making an immediate indomethacin release of extrusion-spheronised pellets. Pellet drug delivery systems that do not require a drug diffusion process may therefore be the best option for producing an immediate release of a poorly water soluble drug.

Both Batch I-PEG and I-Exp were intact after the dissolution, which is in accordance to a similar study using the same superdisintegrant conducted by Souto *et al.* (2005) and a similar study using the same co-solvent as Ibrahim *et al.* (2011) (Ibrahim *et al.* 2011; Souto *et al.* 2005). However, Chamsai and Sriamornsak (2013) also carried out a similar study and found that the incorporation of 10% (w/w) PEG 400 results in pellet disintegration within 90 seconds of dissolution, and that the incorporation of a superdisintegrant together with PEG 400 further reduces the time for disintegration to five seconds. The main difference between the study by Chamsai and Sriamornsak (2013) and our study is that they used ethanol together with PEG 400 in the granulation liquid. The addition of ethanol may have caused the pellet disintegration. This is backed up by Millili and Schwarts (1990) who found that ethanol in the granulation liquid has an accelerating effect on disintegration of extrusion-spheronised pellet formulations. However, the addition of ethanol also greatly

reduces the mechanical strength of the pellets but this was not tested in their study (Millili and Schwartz 1990).

5.2.2.1. *In vitro* dissolution of pellets containing PEG 400

The purpose of adding PEG 400 in Batch I-PEG was to act as a co-solvent for indomethacin in order to facilitate the dissolution of the drug, and thereby enable a rapid release of indomethacin from the non-disintegrating pellets.

As shown in Figure 5.1 an initial rapid indomethacin release was observed for Batch I-PEG, which was followed by a slower sustained release. The rapid release of indomethacin is assumed to be due to release of indomethacin on the surface of the pellets and the sustained release due to indomethacin incorporated inside the core of the pellets. Ibrahim *et al.* (2011) found a similar initial burst of indomethacin release and also concluded that the indomethacin release is a diffusion controlled process (Ibrahim *et al.* 2011).

It was postulated that the incorporation of 60% (w/w) lactose to Batch I-PEG would cause an immediate release of indomethacin, as the lactose would increase the porosity of the pellet core. However, due to the low mechanical strength of the pellets, as described in Section 5.2.3.1, it was decided not to carry out more investigations into the use of PEG 400 as a co-solvent for rapid indomethacin release.

5.2.2.2. *In vitro* dissolution of pellets containing Explotab®

The purpose of adding Explotab® to Batch I-Exp was to investigate whether a rapid release of indomethacin by pellet disintegration through swelling of the superdisintegrant was achievable. As the pellets stayed intact after 7 hours of dissolution this purpose of adding Explotab® was not realised, and the achieved dissolution profile closely resemble the sustained indomethacin release accomplished for Batch I45:45, which was manufactured without the use of Explotab®. There is only limited evidence that a disintegrating pellet formulation can be achieved by the incorporation of a superdisintegrant, as not much literature describes this function. This may be because extrusion-spheronisation involves the use of granulation liquid to the dry blend, causing the superdisintegrant to swell during the formation of

pellets, and thereby being able to stay intact when returning to its swelled state during subsequent dissolution. However, Souto *et al.* (2005) found that the addition of superdisintegrants can have a modest effect on increasing the drug release rate through an increased porosity of the pellet formulations rather than pellet disintegration (Souto *et al.* 2005). This modest increase of indomethacin release rate was also observed in Figure 5.1.

5.2.3. Physical evaluation of the pellets

5.2.3.1. Pellets containing PEG 400

The manufactured pellets containing PEG 400 (Batch I-PEG) were yellow in colour due to solubilisation of indomethacin in PEG 400. The pellet surface was very smooth. However, due to the addition of PEG 400 the pellets had become soft and were easily broken when rolled between the fingers. The pellet texture was measured using a texture analyser in order to find out whether they would show brittleness and be able to withstand mechanical forces during potential film coating and capsule filling. A force-displacement diagram generated from the texture analyser is shown in Figure 5.2.

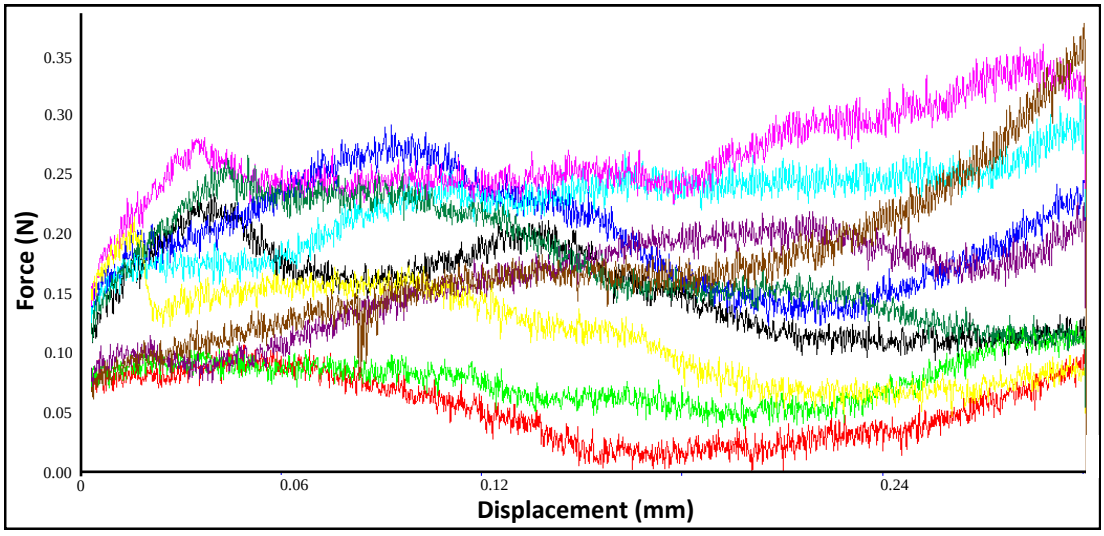


Figure 5.2. Force-displacement diagram for pellet of Batch I-PEG (n=10).

From Figure 5.2 it is observed that the pellets showed a plastic textural behaviour rather than a brittle behaviour, and were therefore considered too soft to resist the mechanical stress usually applied during film coating or capsule filling. The observed plastic textural behaviour is usually expected for pellet formulations

containing a high moisture content, which is the case in the present study where PEG 400 creates moist pellets (Bika *et al.* 2001). As plastic textural behaviour was not desired in the present study, further investigations into an immediate release of pellets containing PEG 400 was not carried out.

The plastic behaviour of the pellets, due to addition of PEG 400, was not documented by Ibrahim *et al.* (2011) who had produced similar pellet formulations with 20, 40 or 60% (w/w) PEG 400 (Ibrahim *et al.* 2011). However, Chamsai and Sriamornsak (2013) documented a decreased mechanical strength of pellets when only adding 10% (w/w) PEG 400 to extrusion-spheronised pellets (Chamsai and Sriamornsak 2013).

5.2.3.2. Pellets containing Explotab®

The manufactured pellets containing Explotab® (I-Exp) had a rough surface and looked like granules rather than pellets. This negative effect on pellet shape is opposed to the findings by Souto *et al.* (2005) and Chamsai & Sriarmornsak (2013) who both found no significant negative effect on pellet shape by the inclusion of a superdisintegrant to extrusion-spheronised pellets. The granular structure of the pellets found in our study may be explained by the use of higher spheroniser temperatures, which may have caused a more rapid evaporation of liquid of the pellets during spheronisation reducing the plasticity. Further studies into spheronisation of the pellets containing a superdisintegrant at room temperature were not performed.

5.3. Conclusion

- The additions of a co-solvent or a superdisintegrant in extrusion-spheronised pellets were tested without accomplishing a desired immediate release of a poorly water soluble drug.

- An immediate release of a poorly water soluble drug may be accomplished by adding lactose to the developed extrusion-spheronised pellets containing a co-solvent. However, these pellets show high plasticity and low mechanical resistance so further investigation into this may be irrelevant.

- The addition of a superdisintegrant to extrusion-speronised pellets only moderately increases the drug release of poorly water soluble drugs, and no disintegration of the pellet formulation is observed.

- The extrusion-spheronisation technique may not be appropriate for accomplishing an immediate release of a poorly water soluble drug. A different pelletisation technique that produces pellets with a drug release mechanism that is less dependent on drug diffusion may be required.

Chapter 6: Radiolabelling of drug loaded pellets

6.1. Introduction

There is a lack of capability in producing radiolabelled drug loaded extrusion-spheronised and drug-layered pellet formulations so that their *in vivo* performance can be scintigraphically evaluated.

Several scientific papers describe radiolabelling of preformed non-radiolabelled pellets containing an ion exchange resin by soaking them in a solution of the radioisotope. However, this method can be unsatisfactory as it may result in the release of water soluble pellet excipients or drug during the soaking procedure. In addition, soaking pellets that are already film coated may hinder the efficiency of radiolabelling, and subsequent scintigraphic imaging, and also compromise the integrity of the film coat, leading to an altered dissolution performance compared to non-radiolabelled formulation. Furthermore, radiolabelling drug-free pellet formulations using this soaking methodology can be inadequate as the size and density of the pellet remains unchanged in comparison to drug-containing formulations. Since these are potentially important factors impacting upon GI transit time such approach invalidates or severely limits resultant observations made on these drug-free pellet systems.

This chapter discusses the investigations carried out in our development of two novel radiolabeling methods, which offer advantages over the current existing radiolabelling method. We have denoted these two radiolabelling methods, which are based on the extrusion-spheronisation technique and the drug-layering technique, the "incorporating" method and the "soaking" method respectively. In this study uncoated and film coated drug loaded radiolabelled pellets are produced to validate our developed methods for a wide range of pellet formulations.

6.2. Results and discussion

6.2.1. The "incorporation" method for manufacturing of radiolabelled extrusion-spheronised pellets

The objective of this study was to develop a novel radiolabelling method, the "incorporation" method, for drug loaded extrusion-spheronised pellet formulations. A study by Mehta *et al.* (2012) described a comparable method using extrusion-spheronisation to radiolabel uncoated starch based pellets with ^{111}In (Mehta *et al.* 2012). Our developed radiolabelling method, which includes the use of more conventional extrusion-spheronisation excipients, has advantages over the method used by Mehta *et al.* (2012). We accomplished a reduced exposure to radiation by a rapid manufacturing on a small scale, containment of the radiolabel to the pellet formulation using an anion exchange resin as detailed in Section 6.2.1.1, and the incorporation of a film coat detailed in Section 6.2.1.3.

6.2.1.1. Preparation of anion exchange resin

Anion exchange resins were used for retaining the incorporated radiolabel within the extrusion-spheronised pellet formulations. The anion exchange resins were soaked in a non-radioactive solution or a solution containing the radiolabel prior to drying and incorporation during extrusion-spheronisation. The results of the prepared anion exchange resins are detailed in Table 6.1.

Table 6.1. Mass and radioactivity of incorporated anion exchange resin in extrusion-spheronised radiolabelled and non-radiolabelled dummy pellet batches.

	Batch Fd	Batch F*	Batch Dd	Batch D*
Mass of anion exchange resin prior to soaking	100 mg	100 mg	150 mg	150 mg
Mass of soaked and dried anion exchange resin	62.3 mg	58.9 mg	86.1 mg	82.6 mg
Radioactivity of anion exchange resin added[a]	N/A	730 MBq	N/A	825 MBq

[a]Radioactivity is decay corrected to 09.00 a.m. on the morning of manufacturing.

As seen from Table 6.1 the mass of anion exchange resin was found to decrease after drying. This was due to a large water content in the anion exchange resin, which is according to the product specification sheet of the manufacturer that states a water retention capacity within the anion exchange resin of 39-45%. The mass of anion exchange resin to be soaked was therefore increased in Batch Dd and Batch D* in order to achieve a larger mass of anion exchange resin in these two batches, which may ensure stronger binding of the radiolabel within the pellet cores of Batch D*.

The radioactivity of the anion exchange resin added during the extrusion-spheronisation was low compared to the radioactivity of 6.6 GBq of ^{111}In required in the comparable study by Mehta *et al.* (2012). Our developed method therefore shows advantages over the method by Mehta *et al.* (2012) as we managed to keep the radioactive exposure to the manufacturer lower, as well as incorporating the more desirable radioisotope, ^{99m}Tc during extrusion-spheronisation 24 hours prior to time of dose (TOD).

Furthermore, in the study by Mehta *et al.* (2012) no ion exchange resin was incorporated into the pellet formulations so the radiolabel could easily diffuse from their pellet formulation and thereby give a false indication of the site of their formulation. Also, since their pellets were of a disintegrating formulation type an ion exchange resin would easily be released from the pellets after disintegrating (Mehta *et al.* 2012).

6.2.1.2. Pellet yield of extrusion-spheronised pellets

Table 6.2 illustrates the pellet yields for the radiolabelled batches and the non-radiolabelled dummy batches prepared by extrusion-spheronisation.

Table 6.2. Pellet yields in size range 1.00-1.18 mm of extrusion-spheronised radiolabelled and non-radiolabelled dummy pellet batches.

	Batch Fd	Batch F*	Batch Dd[a]	Batch D*
Pellet yield	4.4% (w/w)	17.7% (w/w)	9.6% (w/w) and 9.0% (w/w)	30.3% (w/w)

[a]**Pellet cores of Batch Dd were produced in two lots.**

A pellet yield ≥15% (w/w) within the desired size range of 1.00-1.18 mm was necessary in order to have enough pellets for further film coating and *in vitro* radiolabel release studies. However, it was challenging to achieve this pellet yield:

- Pellet cores of Batch Fd were produced in a yield smaller than 15% (w/w). This yield was sufficient for *in vitro* drug release studies, and the pellet cores did not require subsequent film coating or *in vitro* radiolabel release studies, so the extrusion-spheronisation was not repeated.

- Radiolabelled pellet cores of Batch F* were initially produced in a yield that was less than 15% (w/w). The pellet cores were therefore re-granulated immediately after spheronisation, with sufficient granulation liquid to produce a sufficient pellet yield within the desired size range.

- Pellet cores of Batch Dd were produced in two lots in order to have sufficient pellet cores for subsequent film coating.

- Radiolabelled pellet cores of Batch D* were produced in a sufficient yield in first attempt of extrusion-spheronisation as an optimised extrusion-spheronisation method was taken in use.

The results of Batch F* indicate that radiolabelled extrusion-spheronised pellet cores can be produced by re-granulation of the powder blend if the yield is too low. However, as the re-granulation procedure requires more time the exposure to radioactivity is increased. It is therefore more desirable to obtain a sufficient

reproducible yield of extrusion-spheronised pellets within the desired size range after the first attempted extrusion-spheronisation. The results of the pellet yields indicate that an optimisation and standardisation of the extrusion-spheronisation method is required so that a reproducible pellet yield within the desired size range can be obtained. This is achievable as evidenced by the results of the pellet yield of Batch D*. The optimised extrusion-spheronisation method is discussed further in Chapter 7.

6.2.1.3. Film coating of radiolabelled extrusion-spheronised pellets

Table 6.3 illustrates that a radiolabelled extrusion-spheronised pellet batch and a non-radiolabelled dummy extrusion-spheronised pellet batch prepared by the "incorporation" method were successfully film coated with the desired weight gain.

Table 6.3. Weight gain of film coat applied to one batch of extrusion-spheronised radiolabelled pellets and one batch of non-radiolabelled dummy pellets.

	Batch Dd	Batch D*
Weight gain of Surelease® film coat	10.2% (w/w)	10.3% (w/w)
Weight gain of Acryl-EZE® film coat	40.1% (w/w)	39.7% (w/w)

Due to the small batch size of Batch D* we managed to film coat the radiolabelled pellets in a short time period using a laboratory scale fluidised bed film coater, thereby reducing the radioactive exposure to the manufacturer.

In the comparable study by Mehta *et al.* (2012) no film coat was applied (Mehta *et al.* 2012). It is uncertain whether the crushing strength of the radiolabelled pellets produced by Mehta *et al.* (2012) would have been strong enough to withstand the mechanical force exerted during film coating. The successful film coating of the radiolabelled pellet formulations in our study therefore shows further advantages over the radiolabelling method used by Mehta *et al.* (2012).

6.2.2. The "soaking" method for manufacturing of radiolabelled drug-layered pellets

The objective of this study was to develop a novel radiolabelling method, the "soaking" method, for drug-layered pellet formulations. Various published papers

describe how pellet formulations can be radiolabelled through a soaking procedure. Our described method is unique in its method of radiolabelling beads prior to drug-layering and possible film coating. Drug-layering and film coating were accomplished by fluidising a mix of commercially available non-radiolabelled beads, Cellets 700® with radiolabelled manufactured beads containing an anion exchange resin during the application.

6.2.2.1. Manufacturing of inert beads containing an anion exchange resin

In order to manufacture radiolabelled drug-layered pellets, beads containing an anion exchange resin to bind with the radiolabel are required. These beads are not readily commercially available, and therefore need to be manufactured. Manufactured beads were thus prepared in this study and mixed with the commercially available non-radiolabelled beads, Cellets 700®. In order to establish that the manufactured beads and Cellets 700® are interchangeable, and will have same behaviour during drug-layering, film coating and GI transit, tests including size distribution (Section 6.2.2.1.2), density (Section 6.2.2.1.3) and crushing strengths (Section 6.2.2.1.4) were carried out.

6.2.2.1.1. Yield

One batch of manufactured beads was produced in a yield of 78.8% (w/w) within the size range 710-1000 µm. This high yield meant that sufficient manufactured beads were produced for manufacturing of Batch E*, Ed, G* and Gd using this batch of manufactured beads. The relatively high yield in the desired size range was probably due to the relatively large desired size range (710-1000 µm) compared to the desired size range of the drug loaded extrusion-spheronised pellets described in the present study (1.00-1.18 mm). Furthermore, the high content of MCC (99%) in the manufactured beads also enabled a higher yield by making beads of a narrow size distribution. This effect of increasing the MCC content was discussed in more details in Chapter 4, Section 4.2.1.

6.2.2.1.2. Size distributions

Figure 6.1 shows the size distribution of the manufactured beads and Cellets 700®. A normal distribution is observed.

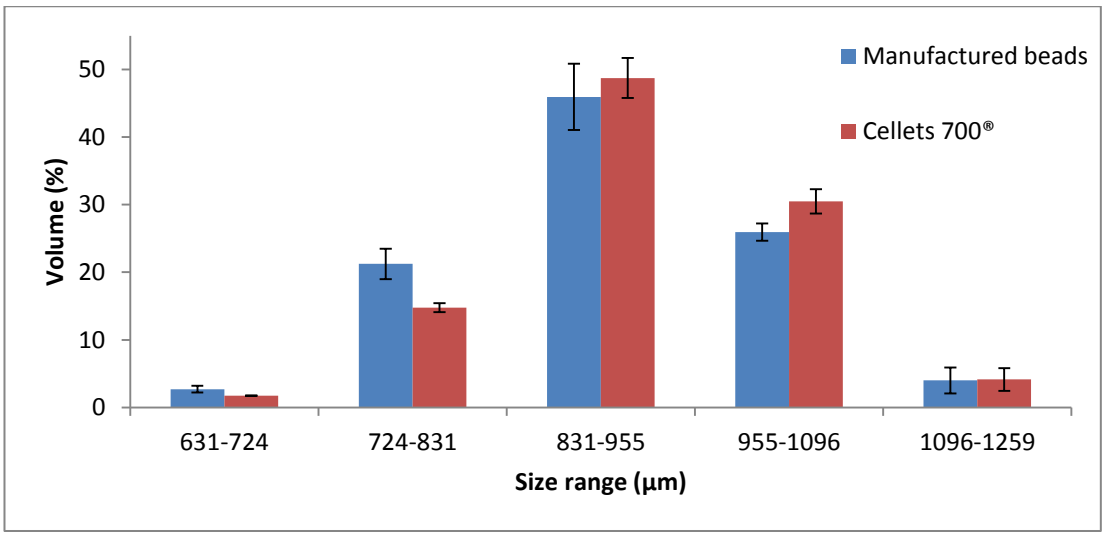


Figure 6.1. Size distribution of manufactured beads with comparison to the commercially available inert pellet cores, Cellets 700® (n=3; mean (±SD)).

Most of the inert pellet cores were within the size range 831-955 µm in which the amount of manufactured beads and Cellets 700® was the same ($p<0.05$). The size distribution of the manufactured beads and Cellets 700® was therefore considered identical.

It is noted that the measured size distributions in Figure 6.1 may only be used for comparison between the two types of inert pellet cores and not an indication of their actual size. This is because MCC is known to swell when exposed to water and water was used as a dispersant in the laser diffraction carried out for the measurement of the size distribution. The measurement time for laser diffraction was 10 seconds so the expansion was limited by a short time period of exposure to water. In order to get a precise measurement of the size distribution of the manufactured beads the analysis should be carried out using an organic solution as the dispersant instead of water. However, it is worth to mention that the increased pellet diameter on contact with water for uncoated pellets presumably mirrors reality should they be taken orally.

The importance of the manufactured beads and Cellets 700® having same size distribution may be negligible when comparing their effect on GI transit (Clarke *et al.* 1993; Davis *et al.* 1984). However, the size may be of importance when comparing the flow during fluidisation for drug-layering or film coating. If the two types of beads do not have same flow the applied drug layer or film coat may differ when the beads are mixed and fluidised together.

6.2.2.1.3. Density

The densities of the manufactured beads and Cellets 700® are shown in Table 6.4.

Table 6.4. Size, mass and density of manufactured beads and Cellets 700®. Median size and mass/pellet were used for calculations of densities (n=3; mean (±SD)).

Density of manufactured beads			Density of Cellets 700®		
Median size (mm)	**Mass/pellet (mg)**	**Density (g/cm^3)**	**Median size (mm)**	**Mass (mg)**	**Density (g/cm^3)**
0.855	0.426 (±0.018)	1.301 (±0.054)	0.850	0.472 (±0.010)	1.468 (±0.032)

The density of the manufactured beads was the same as the density of Cellets 700® ($p<0.01$). Similar to the explanation in Section 6.2.2.1.2 about size distribution, the importance of density in GI transit may be negligible but may have importance in fluidising for drug-layering and film coating (Clarke *et al.* 1995; Clarke *et al.* 1993; Davis *et al.* 1984).

6.2.2.1.4. Crushing strength

The crushing strengths of the manufactured beads and Cellets 700® are shown in Table 6.5.

Table 6.5. Crushing strengths of manufactured beads and Cellets 700® (n=10; mean (±SD)).

	Manufactured beads	**Cellets 700®**
Crushing strength	9.51 (±2.20) N	12.87 (±1.96) N

The two types of beads have relatively high crushing strengths compared to the crushing strengths of approximately 5.60 N and 5.21 N of manufactured pellet cores in Chapter 3, Table 3.2. The high crushing strengths is due to the high content of MCC in the beads which reduces the porosity and thereby the mechanical strength of the pellets (Kleinebudde 1994).

The manufactured beads had lower crushing strength than Cellets 700®. Philippe *et al.* (2010) found that MCC beads of pure MCC had lower abrasion (0.4 % (w/w)) than MCC beads containing 5 % (w/w) anion exchange resin (3.52% (w/w)) (Philippe *et al.* 2010). The crushing strength may therefore have been reduced by the addition of an anion exchange resin. However, as only 1% (w/w) anion exchange

resin was added to the manufactured beads it may be more likely that the reduced crushing strength is caused by other differences in the manufacturing methods, such as volume of granulation liquid, spheronisation speed and time, and drying method, which can all influence the mechanical strength of extrusion-spheronised pellets (Baert and Remon 1993; Lee 2003; Pinto *et al.* 1992; Vertommen and Kinget 1997). Even though the crushing strengths of the two inert pellet cores differed they were still expected to have similar behaviour during drug layering, coating and GI transit as they were not expected to break during any of these processes. Force-displacement diagrams are shown in Figure 6.2 and Figure 6.3 for the manufactured beads and Cellets 700® respectively.

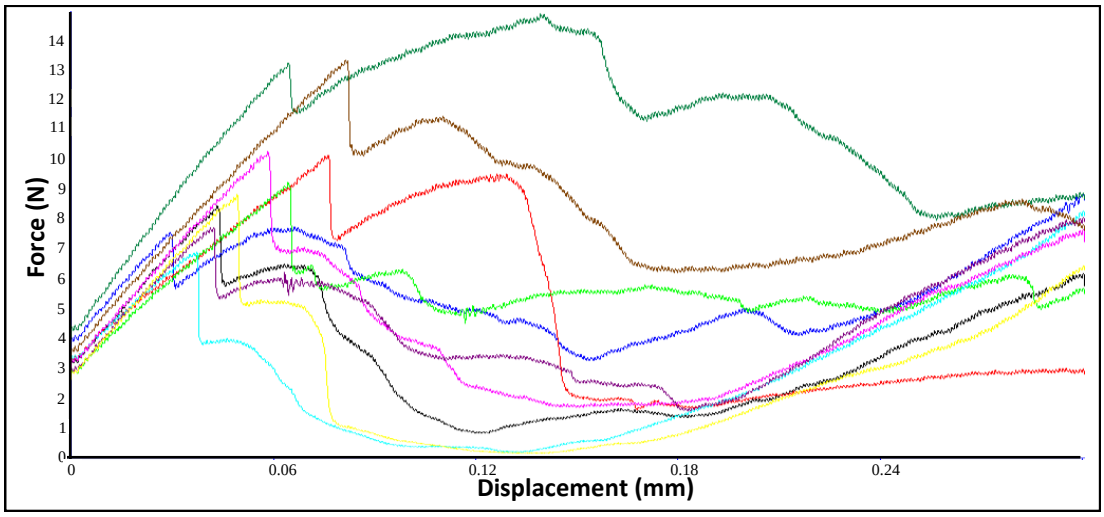


Figure 6.2. Force-displacement diagram for manufactured beads (n=10).

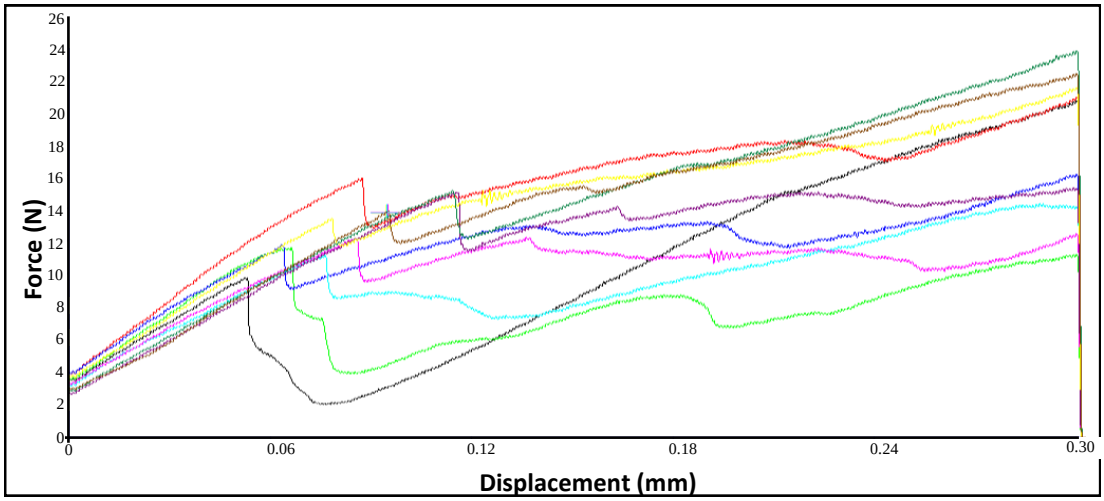


Figure 6.3. Force-displacement diagram for Cellets 700® (n=10).

It is observed that both types of inert pellet cores show brittle behaviour by the initial peak on the force-displacement diagrams in Figure 6.2 and Figure 6.3, which shows a further similarity of the two types of beads.

6.2.2.2. Radiolabelling of inert beads containing an anion exchange resin

Table 6.6 shows the radiolabel uptake of the inert beads containing an anion exchange resin.

Table 6.6. Radioactivity of manufactured beads in Batch E* and G*.

	Radioactivity of soaking solution[a]	Radioactive uptake of manufactured beads[a]	Percentage radioactive uptake of manufactured beads
Batch E*	580 MBq	358 MBq	61.7%
Batch G*	618MBq	81.10 MBq	13.1%

[a]Radioactivity is decay corrected to 09.00 a.m. on the morning of manufacturing.

The radioactive uptake of manufactured beads belonging to Batch E* was relatively high compared to Batch G*. This may be due to differences in the radiolabelling procedures. In the radiolabelling procedure used for Batch E* smaller volumes of liquid were used for soaking and washing. This gave more satisfactory results than the procedure used for Batch G*, involving larger volumes of liquids. The procedure used for Batch E* was therefore preferred from that of Batch G*. However, an optimised method for radiolabelling inert pellet cores in future studies is described in Chapter 9.

Various published literature describe a similar radiolabelling procedure for pellet containing at least 5% (w/w) anion exchange resin, and which may contain a film coat prior to soaking. Only 1 % (w/w) anion exchange resin was added to our manufactured beads in order to resemble Cellets 700® as much as possible. In a study by Philippe *et al.* (2010) it was found that increasing the amount of anion exchange resin from 1% (w/w) to 5% (w/w) results in the same uptake of radiolabel but a stronger binding of radiolabel to the anion exchange resin (Philippe *et al.* 2010). A high uptake of radiolabel was therefore expected in the present study. The binding of radiolabel to the anion exchange resin is discussed in Section 6.2.5 where it was

concluded that 1% (w/w) anion exchange resin is sufficient for radiolabelling of pellet cores using the "soaking" method.

6.2.2.3. Drug-layering of radiolabelled beads

Table 6.7 illustrates that batches of radiolabelled beads prepared by the "soaking" method and batches of non-radiolabelled beads containing an anion exchange resin were successfully drug-layered.

Table 6.7. Estimated and assayed drug content of drug-layered batches prepared using the "soaking" method.

	Estimated drug content[a]	Assayed drug content[b] ((n=3; mean (±SD))
Batch E*	12.7% (w/w)	11.4 (±0.4)% (w/w)
Batch Ed	12.3% (w/w)	12.7 (±0.3)% (w/w)
Batch G*	11.8% (w/w)	11.1 (±1.0)% (w/w)
Batch Gd	14.3% (w/w)	15.0 (±0.5)% (w/w)

[a]Estimated drug content for drug layered pellets is calculated based on their weight gain as described in the method Section 2.2.3.2 and Appendix I.
[b]Assayed drug content of drug layered pellets is measured on the actual batch as described in the method Section 2.9.4.2.

It is seen from Table 6.7 that the estimated drug content of the drug layered pellets varied from the assayed drug content. This finding was also observed for the non-radiolabelled drug-layered pellets, Batch E and G, which consisted solely of Cellets 700® beads. This difficulty in estimating the drug content using weight gain was discussed in more detail in Chapter 3, Section 3.2.2. The incorporation of 1% (w/w) anion exchange resin to the beads was therefore not found to have negative influence on the drug-layering process.

A similar technique of drug-layering radiolabelled beads has not been found in the literature. However, some published papers describe a comparable method of soaking drug loaded pellets (Blok *et al.* 1991; Davis *et al.* 1987; Subhabrota *et al.* 2011). Soaking of drug loaded pellets cannot be accomplished in the present study as it will cause a removal of the drug layer from the pellet formulations, which are designed for the purpose of immediate drug release. We thus accomplished to drug layer our manufactured beads post radiolabelling by reducing the exposure to radioactivity by manufacturing in a small scale.

6.2.2.4. Film coating of radiolabelled drug-layered pellets

Table 6.8 illustrates that a radiolabelled drug-layered pellet batch and a non-radiolabelled dummy drug-layered pellet batch prepared by the "soaking" method were successfully film coated with the desired weight gain.

Table 6.8. Weight gain of film coat applied to one batch of drug-layered radiolabelled pellets and one batch of non-radiolabelled dummy pellet.

	Batch G*	**Batch Gd**
Acryl-EZE® weight gain	40.5% (w/w)	40.7% (w/w)

Blok *et al.* (1991) and Subhabrota *et al.* (2011) described a similar method whereby radiolabelled drug loaded pellet formulations are film coated post radiolabelling. However, the amount of radioactivity that the manufacturer was exposed to during film coating was not described (Blok *et al.* 1991; Subhabrota *et al.* 2011). Subhabrota *et al.* (2011) managed to reduce radioactive exposure during film coating by using a small batch of 600 mg radiolabelled drug-layered pellets. However, their exposure to radioactivity could have been greatly reduced by using our method whereby radiolabelled and non-radiolabelled pellets were mixed during film coating.

6.2.3. *In vitro* drug dissolution

Dissolution experiments were carried out to compare the drug release of radiolabelled batches and the dummy experiment batches to a reference of non-radiolabelled batches described in Chapter 3. The drug release profiles of the radiolabelled pellet batches (Batch F*, D*, E* and G*) together with their dummy experiment batches (Batch Fd, Dd, Ed and Gd) and non-radiolabelled reference batches (Batch F, D, E and G) are shown in Figure 6.4.

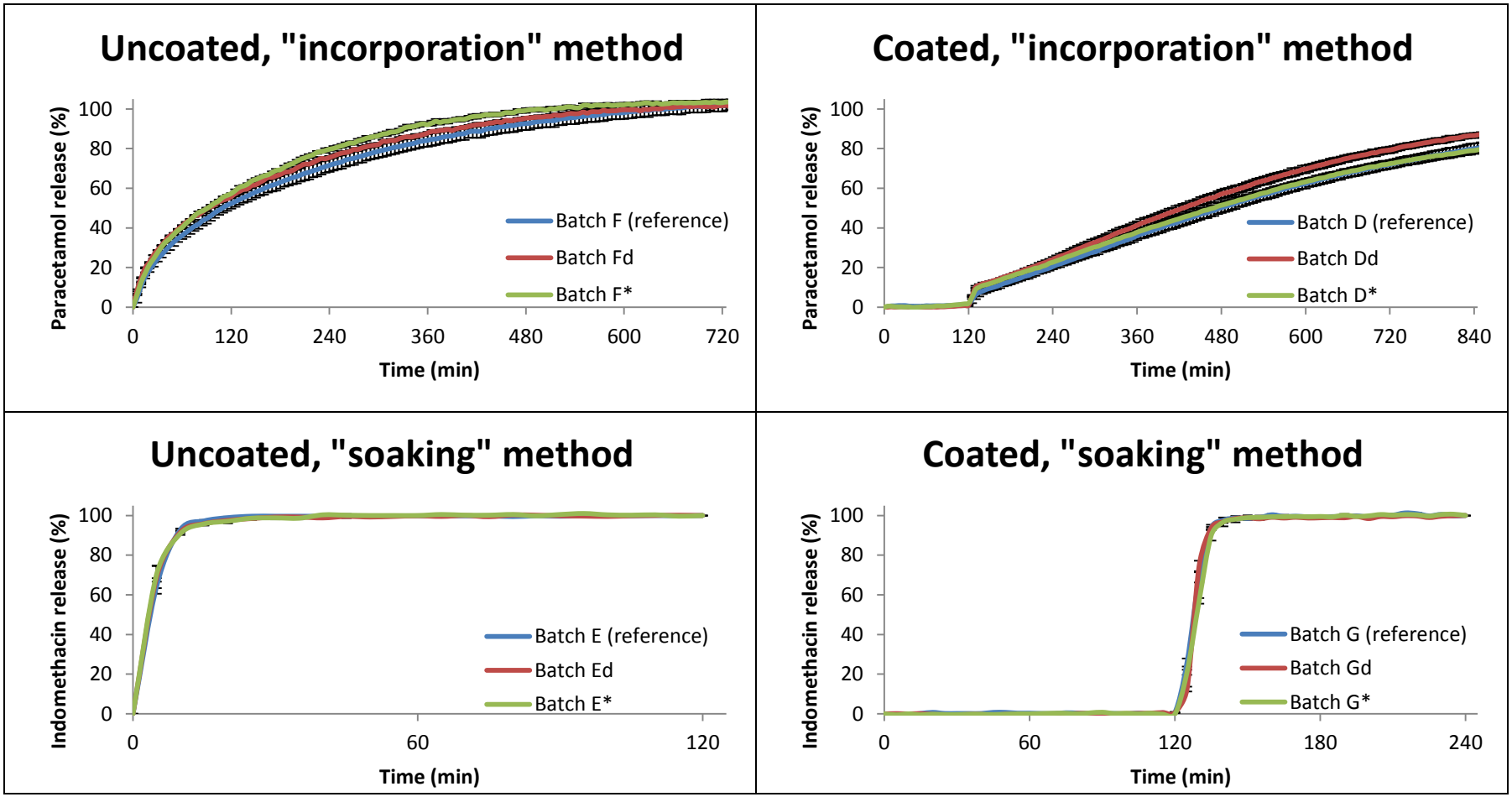


Figure 6.4. Dissolution profiles of radiolabelled pellets with the dummy experiment and reference (n=3; mean (±SD)). Uncoated, "incorporation" method: Indomethacin release in phosphate buffer pH 6.8. Coated, "incorporation" method: Paracetamol release in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8. Uncoated, "soaking" method: Indomethacin release in phosphate buffer pH 6.8. Coated, "soaking" method: Indomethacin release in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8.

It was found that the radiolabelled batches, the dummy batches and the non-radiolabelled reference batches had similar drug release profiles. It was therefore concluded that the anion exchange resin and the radioisotope did not interfere with the drug release. Radiolabelled uncoated and coated pellet formulations can therefore become radiolabelled without changing the drug release properties of the pellet formulation when using the "incorporation" method or the "soaking" method. The sections below discuss the comparisons of the drug release profiles in detail (Sections 6.2.3.1 to Section 6.2.3.3).

6.2.3.1. Immediate and delayed pulsatile drug release

The immediate drug release profiles (Batch E*, Ed and E) had more than 85% (w/w) of the labelled amount of drug released within 15 min of start of dissolution. Their drug release profiles can on this basis be considered similar without the use of an f1 and f2 test. The delayed pulsatile drug release profiles (Batch G*, Gd and G) had more than 85% (w/w) of the labelled amount of drug released within 15 min of start of dissolution in phosphate buffer pH 6.8. Their drug release profiles can therefore also be considered similar.

6.2.3.2. Sustained and delayed sustained drug release

The sustained drug release profiles (Batch F*, Fd and F) were compared from start of dissolution until 80 % of drug was released using f_1 and f_2 tests. The delayed sustained drug release profiles (Batch D*, Dd and D) were compared after two hours of dissolution until 80% of the drug was released also using f_1 and f_2 tests. The f_1 and f_2 values are shown in Table 6.9.

Table 6.9. f1- and f2-values for comparisons of the sustained drug release profiles of Batch F and D with their radiolabelled batch and their dummy experiment batch.

Batches compared	f_1-value	f_2-value
F vs. F*	11.1	59.5
F vs. Fd	7.6	68.2
D vs. D*	2.7	87.1
D vs. Dd	12.4	60.4

All the f_1 values were below 15 and all the f_2 values are above 50 (Table 6.9). It was therefore concluded that all the sustained and delayed sustained release profiles were identical.

6.2.3.3. Delayed drug release

Batch D*, Dd, D, G*, Gd and G were all pellet formulations of delayed drug release. All the delayed drug release formulations had released less than 10% (w/w) drug after 2 hours of dissolution in hydrochloric acid pH 1.2 and thereby fulfilled the requirements listed in a monograph in the British Pharmacopoeia (BP online 2013, Appendix XII B. Dissolution).

6.2.4. Dose preparation

Each of the four radiolabelled drug loaded pellet batches (Batch D*, E*, F* and G*) were prepared into doses that were used for *in vitro* radiolabel release studies in Section 6.2.5. The prepared doses represent film coated and uncoated extrusion-spheronised and drug-layered pellet formulations. The result of the dose preparation is illustrated in Table 6.10 and discussed in the sections below.

Table 6.10. Radioactivity, drug content, mass, and corresponding capsule size of prepared doses from each of the radiolabelled drug loaded pellet batches (n=3; mean (±SD)).

	Radioactive dose[a]	Drug dose	Mass of dose	Corresponding capsule size
Batch D*	1.86 (±0.01) MBq	36.8 mg	617.3 (±0.7) mg	00
Batch E*	3.01 (±0.35) MBq	22.8 mg	199.9 (±0.0) mg	3
Batch F*	1.14 (±0.01) MBq	45.6 mg	401.1 (±0.7) mg	0
Batch G*	0.56 (±0.25) MBq	42.7 mg	401.4 (±0.9) mg	0

[a]All radioactivity is decay corrected to 09.00 a.m. on the morning after manufacturing when subjected to *in vitro* dissolution.

6.2.4.1. Radioactive dose

As seen from Table 6.10 the radioactive dose of Batch D*, F* and G* is below the range of 2-4 MBq per dose desired in the present study. This desired radioactive dose range was based on the radioactive dose commonly used in oral dosages administered for scintigraphic purposes (Ghimire *et al.* 2011; Ghimire *et al.* 2010; McConville *et al.* 2009; Wilson *et al.* 2011). Even though the radioactive doses were below the desired doses they were found to be sufficient to carry out the *in vitro*

radiolabel release study detailed in Section 6.2.5 for validation of the radiolabel technique.

The radioactive dose of pellet formulations used for dosing to humans found in the published literature is higher than the radioactive dose commonly used in scintigraphic studies and used for the *in vitro* studies carried out in the present study. Clarke *et al.* (1995) and Basit *et al.* (2001) prepared pellet doses of 3.7 MBq and 7.4 MBq respectively, which were filled into size 0 capsules (Basit *et al.* 2001; Clarke *et al.* 1995). These high radioactive doses may have been required as the pellets were dosed to humans. This is assumed due to the fact that pellets disperse in the GI tract after ingestion, which complicates scintigraphic monitoring of an individual dispersed pellet that will only contain a small fraction of the total radioactive dose. It is uncertain why Basit *et al.* (2001) used twice as high a radioactive dose as Clarke *et al.* (1995) but it may have been to ease scintigraphic monitoring of pellets dispersed in the GI tract.

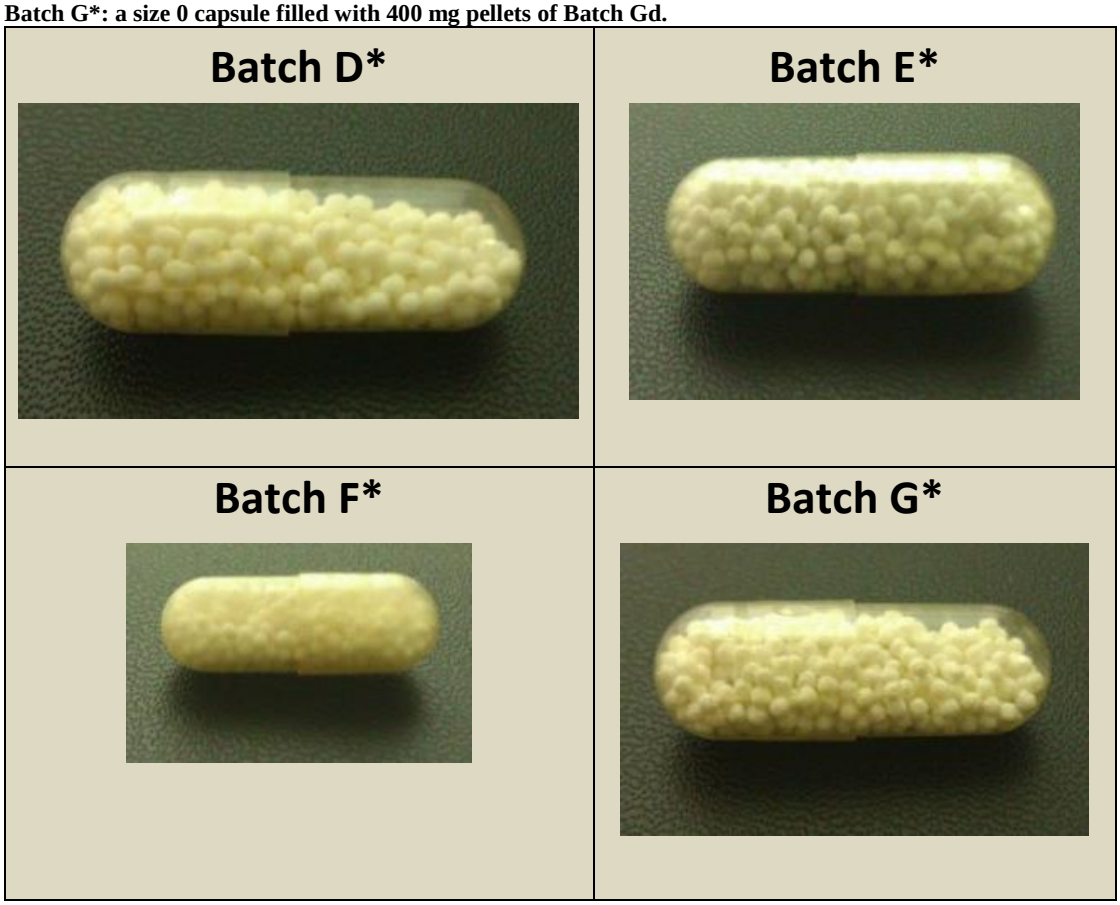
In order for the pellet formulations in the present study to have sufficient radioactivity for scintigraphic GI transit monitoring, their radioactive dose may need to be increased. The radioactive dose of the extrusion-spheronised pellet formulations (Batch D* and F*) can be increased by adding more radiolabel to the anion exchange resin prior to extrusion-spheronisation. Furthermore, it may be necessary to radiolabel these pellet formulations a few hours prior to dosing, rather than 24 hours prior to dosing, in order to reduce the radioactive exposure during manufacturing. The radioactive dose of the drug-layered pellet formulations (Batch E* and G*) can easily be increased by changing the radiolabelling method and adding more radioactivity during radiolabelling by soaking. The low uptake of radiolabel by Batch G* compared to Batch E* was discussed in Section 6.2.2.2 where it was recommended to use the "wet and dry" method that will be discussed in Chapter 9. The two drug-layered pellet batches consisted of a mix of radiolabelled and non-radiolabelled pellets so the radioactivity of these batches can also be increased by increasing the proportion of radiolabelled pellets in the batch.

6.2.4.2. Mass, drug content and capsule size

Commercially available paracetamol tablets typically contain 500 mg drug and commercially available capsules of indomethacin typically contain 25 mg or 50 mg drug (The Electronic Medicines Compendium 2013). The comparably low paracetamol content in the paracetamol containing pellet formulation (Batch D*) was not considered an issue in the present study. This is due to the requirement of the drug component to act as a model drug rather than dosing to humans for a therapeutic effect. Both model drugs used in the present study, indomethacin and paracetamol, can easily be replaced by a different drug of similar solubility. It is also possible to increase the drug content of extrusion-spheronised pellets by replacing some of the lactose, incorporated in the pellets during manufacturing, with more of the drug component. However, the results still indicate that it is complicated to make a single dose of extrusion-spheronised pellets containing sufficient paracetamol for a therapeutic effect as the content of 500 mg required exceeds the total mass of the pellets that fit into a size 0 capsule. This is perhaps the reason why extrusion-spheronised paracetamol pellet formulations are not commercially available (The Electronic Medicines Compendium 2013).

Figure 6.5 illustrates how each pellet dose fits into their corresponding capsule size described in Table 6.10.

Figure 6.5. Photos illustrating capsules containing an amount of pellets corresponding to the prepared doses of radiolabelled drug loaded pellet formulations. The corresponding dummy batches were used for capsule filling. Batch D*: size 00 capsule filled with 617 mg pellets of Batch Dd. Batch E*: size 0 capsule filled with 200 mg pellets of Batch Ed. Batch F*: size 3 capsule filled with 400 mg pellets of Batch Fd. Batch G*: a size 0 capsule filled with 400 mg pellets of Batch Gd.



It is observed that the sizes of the radiolabelled drug loaded pellet batches differed in their mass and corresponding capsule size. Dosing of pellet formulations for scintigraphic purposes has been found in the published literature to be ranging from a size 4 to a size 000 capsule (Hunter *et al.* 1982; Yuen *et al.* 1993). It was decided not to prepare doses of corresponding capsule sizes larger than a size 0 capsule in order to keep the capsules within an acceptable size for ease of potential ingestion. However, it was found necessary to dose Batch D* into a larger size 00 capsule as the pellet size was greatly increased due to the application of two film coats. The radioactivity of Batch F* was sufficient for dosing in a smaller size 3 capsule.

6.2.5. *In vitro* radiolabel release study of pellets

Attachment of radiolabel to the anion exchange resin of pellets has been validated in several of the published papers. These published papers found that pellets containing

an ion exchange resin that are radiolabelled by using a soaking method could retain the radiolabel during submersion in hydrochloric acid or phosphate buffer (Basit *et al.* 2001; Blok *et al.* 1991; Davis *et al.* 1987; Yuen *et al.* 1993). Similar results were thus expected in the present study where a radiolabel release was investigated by carrying out *in vitro* dissolution of the prepared doses described in Section 6.2.4. The *in vitro* dissolution conditions corresponded to those for investigation of *in vitro* drug release. The results of these *in vitro* dissolution studies are illustrated in Figure 6.6, and will be discussed further in the sections below.

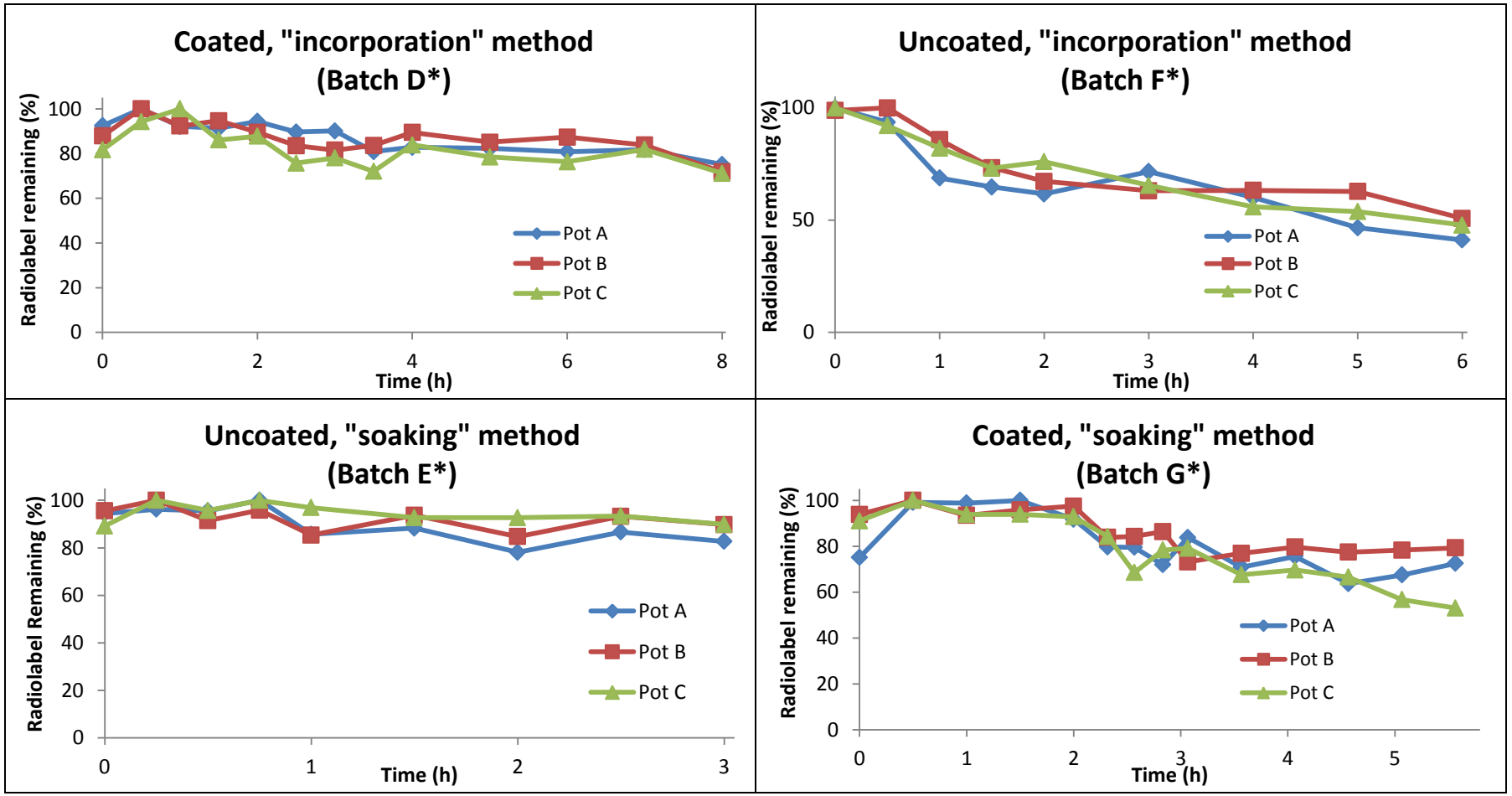


Figure 6.6. Graphs of change in radioactive counts in the ROI of three dissolution pots (Pot A-C) containing radiolabelled drug loaded pellet doses. Coated, "incorporation" method (Batch D*): Paracetamol release in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8. Uncoated, "incorporation" method (Batch F*): Indomethacin release in phosphate buffer pH 6.8. Uncoated, "soaking" method (Batch E*): Indomethacin release in phosphate buffer pH 6.8. Coated, "soaking" method (Batch G*): Indomethacin release in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8.

6.2.5.1. Coated pellets prepared using the "incorporation" method

As seen from Figure 6.6 the pellets of Batch D* were not found to lose any radiolabel during the radioactive *in vitro* dissolution study. This is also illustrated in Figure 6.7, which shows the scintigraphic images at 0 and 7 hours of dissolution, where no radiolabel was observed to release into the dissolution pots.

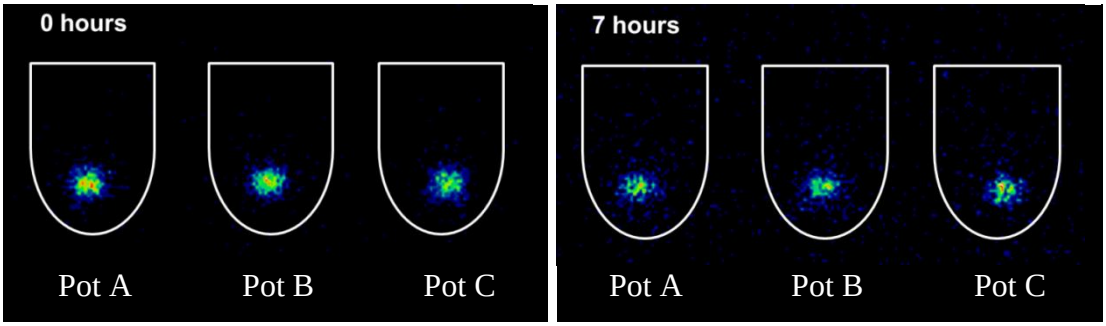


Figure 6.7. Scintigraphic images of Batch D* at 0 hours and 7 hours of a radioactive *in vitro* dissolution study. The dissolution pots (Pot A-C) are drawn on the images.

The results were as expected as the published literature also found that radiolabel remains attached to anion exchange resins during dissolution in hydrochloric acid and phosphate buffer (Basit *et al.* 2001; Blok *et al.* 1991; Davis *et al.* 1987; Yuen *et al.* 1993). However, this published literature incorporated 5% (w/w) anion exchange resin into the pellet formulations instead of 1% (w/w). This higher concentration of anion exchange has been reported to bind tighter to the radiolabel (Philippe *et al.* 2010). However, securing of the radiolabel has been further improved in our study through the incorporation of the radiolabelled anion exchange resin into the pellet matrix, rather than radiolabel sitting on the outer pellet surface, and film coating of the pellet formulations post radiolabelling rather than prior to radiolabelling. Coated extrusion-spheronised pellet formulations can therefore be traced *in vivo* using scintigraphic detection by radiolabelling using the "incorporation" method.

6.2.5.2. Uncoated pellets prepared using the "incorporation" method

As seen from Figure 6.6 pellets of Batch F* were losing their radiolabel during the radioactive *in vitro* dissolution study. Approximately 50% of the radiolabel came off the pellets and dispersed in the dissolution vessel after 6 hours of dissolution. The loss of radiolabel is also illustrated in Figure 6.8, which shows the scintigraphic

images at 0 and 5 hours of dissolution. It is seen that radiolabel has leaked into the dissolution media on the scintigraphic image acquired at 5 hours of dissolution.

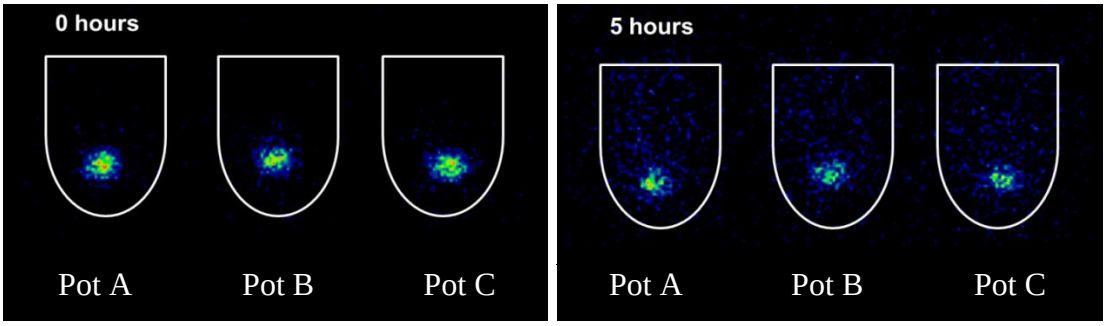


Figure 6.8. Scintigraphic images of Batch F* at 0 hours and 5 hours of a radioactive *in vitro* dissolution study. The dissolution pots (Pot A-C) are drawn on the images.

This result was unexpected as the literature suggests that the radiolabel stays attached to the anion exchange resin after exposure to phosphate buffer. The reason that radiolabel is coming off the pellets can be explained by the lack of a film coat. The anion exchange resin containing the radiolabel may erode during diffusion of lactose and indomethacin from the pellet core. As there is no film coat to create a diffusion barrier between eroded components and the dissolution media, the radiolabelled anion exchange resin can flow freely into the dissolution pots. This correlates with the findings in Section 6.2.5.1 where no radiolabel was released from the extrusion-spheronised pellets after the application of a film coat.

The radiolabel release from Batch F* may not be observed in a scintigraphic image after digestion by a human because it is released in small quantities which will disperse throughout the GI tract. The radiolabel released will thus be very low and probably be recognised as eroded pellet components rather than pellets.

No similar validation was carried out by Mehta *et al.* (2011), who used a similar method for radiolabelling uncoated pellet formulations (Mehta *et al.* 2012). It is therefore uncertain whether their disintegrating pellet formulations were being successfully traced *in vivo* or if it was radiolabelled anion exchange resin that had been separated from the pellet formulation that was being traced.

6.2.5.2.1. Radiolabel bound to anion exchange resin

It was investigated whether the observed radiolabel released from Batch F* remained bound to the eroded anion exchange resin. This was investigated as unbound radiolabel can cause health and safety issues due to systemic absorbance of radiolabel, whereas radiolabel bound to an anion exchange resin cannot be absorbed systemically.

Figure 6.9 shows a scintigraphic image taken after radiolabelled anion exchange resin had settled in a dissolution pot for 20 hours.

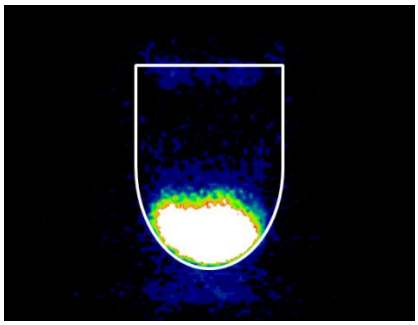


Figure 6.9. Scintigraphic image after 20 hours of radiolabelled anion exchange resin settling in a dissolution pot containing phosphate buffer pH 6.8. The radioactivity at time of the image was 41.9 MBq and the dissolution pot is drawn on the image.

It is observed that the radiolabel remains bound to the anion exchange resin, which has sunk to the bottom of the dissolution pot, and that some radiolabelled anion exchange resin is floating on top of the dissolution pot. No free radiolabel is observed in the middle of the pot which indicates that all radiolabel is bound to anion exchange resin. There is observed a large flare on the scintigraphic image which is caused by the high amount of radioactivity inside the dissolution pot (41.9 MBq). This makes it seem as though radiolabel has escaped the dissolution pot and makes it difficult to confirm that no radiolabel is unbound.

Measurements of the radioactivity in the mother liquor of the filtered dissolution media are shown in

Table 6.11. The data confirms that observed radiolabel was bound to the anion exchange resin.

Table 6.11. Measurement of the mother liquor (100 mL) of the filtered dissolution media using a dose calibrator and a Geiger counter with calculated values for the total radioactivity and percentage of unbound radiolabel.

	Measured radioactivity	Total radioactivity in dissolution media (900 mL)	Percentage of unbound radiolabel[b]
Dose calibrator	0.0 MBq	0.0 MBq	0.0%
Geiger counter[a]	500 Bq	0.0045 MBq	0.01%

[a]**Data is approximate as the readings of the Geiger counter had small variations.**
[b]**Calculated on the basis of a decay correction to a total radioactivity of 41.9 MBq.**

Approximately 0.01% of the radiolabel was measured as unbound to the anion exchange resin. This could only be measured using a Geiger counter due to the low radioactivity of the mother liquor. This is not a recognised analytical method for the quantification of radioactivity so the data may not be totally precise. However, the result shows that some insignificant unbound radiolabel was detected in the dissolution media, which had radioactivity above background readings.

This finding indicates that the radiolabel has higher affinity to the anion exchange resin than the phosphate buffer used as dissolution media. It is thereby confirmed that the radiolabel release from Batch F* during *in vitro* dissolution in phosphate buffer is due to erosion of the pellet matrix and subsequent escape of anion exchange resin into bulk water rather than displacement of radiolabel from the anion exchange resin by the ions in the phosphate buffer. Even though the phosphate ions have strong affinity to anion exchange resins (Alchin 2013), which is due to their high electronegativity, no radiolabel was being displaced. It is therefore also postulated that the radiolabel will not be displaced from the anion exchange resin due to ions encountered during GI transit after ingestion by a human. Furthermore, Davis *et al.* also found no release of radiolabel from pure anion exchange resin during *in vitro* dissolution with conditions simulating GI pH and ionic strength (Davis *et al.* 1984). Our results combined with those by Davis *et al.* (1984) thus indicate that the radiolabel remains attached to the anion exchange resin during their entire GI transit. Pellets prepared using the "incorporation" method can therefore be detected *in vivo* without causing health and safety issues as no systemic absorption of radiolabel is expected due to binding to the anion exchange resin.

6.2.5.2.2. Radiolabel release vs. indomethacin release

The release of radiolabel was found to be due to erosion during dissolution of indomethacin and lactose. If the release of the poorly water soluble indomethacin was also entirely due to erosion of the drug it can be hypothesised that the indomethacin release profile would be similar to a release profile of the radiolabel. These release profiles are compared and shown in Figure 6.10.

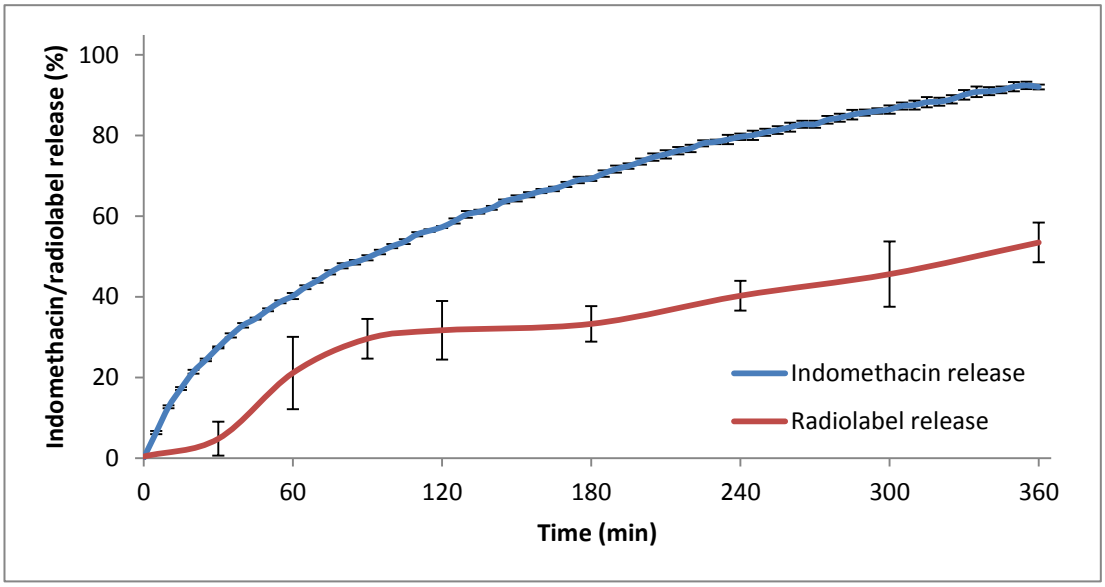


Figure 6.10. Radioactive release and indomethacin release of Batch F* (n=3; mean (±SD)).

The indomethacin release and the release of radiolabel from Batch F* were found to be different (f_1=86 and f_2=26). The faster release of indomethacin can be explained by a release due to a combination of diffusion and erosion whereas that of the radiolabel is only due to erosion.

The presence of a diffusional indomethacin release mechanism in uncoated extrusion-spheronised pellet formulations was described in Chapter 3 and mentioned by Pinto *et al.* (1992). This was assumed due to the formulations remaining intact post drug release (Pinto *et al.* 1992). The finding in the present study, that an erosion mechanism is also taking place within the uncoated extrusion-spheronised pellets formulations, was not reported earlier.

6.2.5.3. Uncoated pellets prepared using the "soaking" method

As seen from Figure 6.6 the pellets of Batch E* were not found to lose any radiolabel during the radioactive *in vitro* dissolution study. It was thus proved that the incorporation of 1% (w/w) anion exchange resin is sufficient for radiolabelling of pellet formulations using the "soaking" method, as opposed to at least 5 % (w/w) anion exchange used in the published literature (Basit *et al.* 2001; Blok *et al.* 1991; Davis *et al.* 1987; Yuen *et al.* 1993). This was also found for the "incorporating" method discussed in Section 6.2.5.1 and Section 6.2.5.2 where it was assumed that the radiolabel was retained due to incorporation to the matrix of the radiolabelled anion exchange resin.

Even though no significant release of radiolabel was found from the radiolabelled drug-layered pellets some release could be observed on a scintigraphic image. This is illustrated in Figure 6.11, which compares the scintigraphic images at 0 and 3 hours of dissolution. This leaked radiolabel was observed on all scintigraphic images preceding the initial image acquired at 0 hours of dissolution.

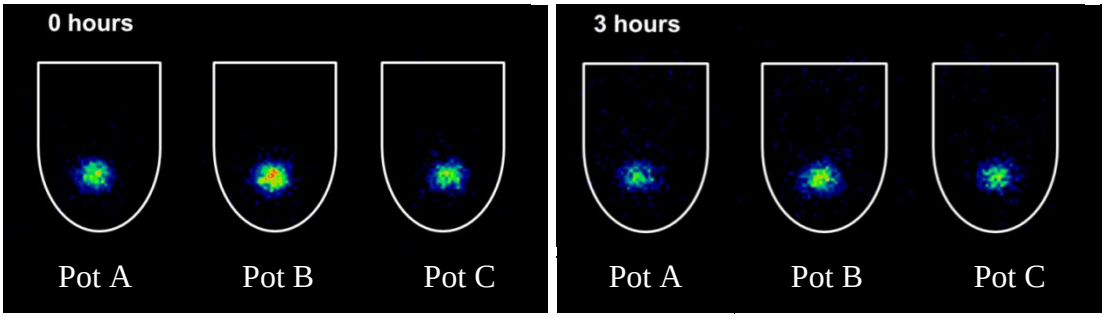


Figure 6.11. Scintigraphic images of Batch E* at 0 hours and 3 hours of a radioactive *in vitro* dissolution study. The dissolution pots (Pot A-C) are drawn on the images.

The observed radiolabel release is minimal and is not expected to interfere with the high radioactivity of the pellet formulations. The released radiolabel is assumed to have been loosely attached to the pellet core and unbound to the anion exchange resin. This is assumed because the anion exchange resin was incorporated into the matrix of the nonporous pellet core and therefore unable to erode from the pellets. Even though the unbound radiolabel will be absorbed systemically it is not expected to have any health and safety issues due to its small quantity. In the published literature a radiolabel release of 3.7 ±1.5% from a dose of 7.5 MBq ^{99m}Tc has been

reported and considered an acceptable release (Yuen *et al.* 1993). The release of radioactivity from Batch E* is considered lower than reported in the literature.

6.2.5.4. Coated pellets prepared using the "soaking" method

Due to the findings of no significant radiolabel being released from uncoated drug-layered pellets in Section 6.2.5.3, no release of radiolabel was expected to be observed from the coated drug-layered pellets in the present section. As seen from Figure 6.6 a drop in radiolabel attached to the pellets is only observed at 2 hours of dissolution. This drop occurred during dissolution media change where plastic pots were changed to glass pots. Glass attenuates radioactivity so the drop is possibly caused by attenuation of the dissolution pots rather than a release of radiolabel. Furthermore, after dissolution media change the enteric film coat of the drug-layered pellets dissolves and the radiolabel is exposed to the dissolution media. This may have caused a small leak of radiolabel as described for the uncoated drug-layered pellets in Section 6.2.5.3.

When taking the background readings into considerations it is also seen from Figure 6.12 that no release of radiolabel is observed after 5 hours of dissolution.

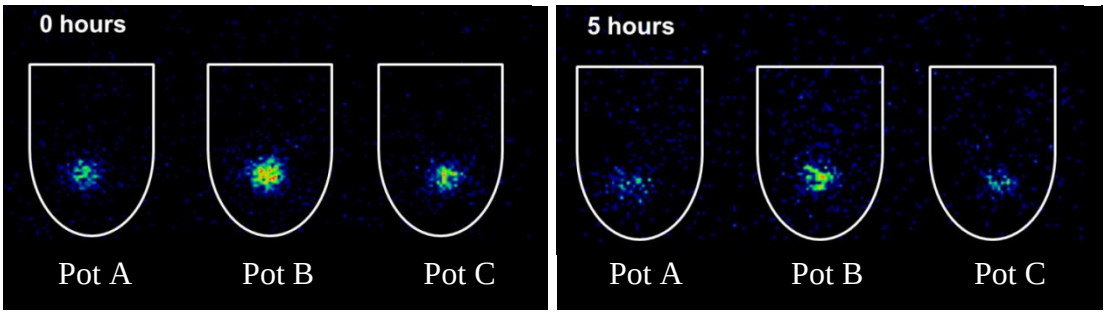


Figure 6.12. Scintigraphic images of Batch F* at 0 hours and 5 hours of a radioactive *in vitro* dissolution study. The dissolution pots (Pot A-C) are drawn on the images.

As concluded in the discussion for uncoated drug-layered pellets in Section 6.2.5.3 only a diminished amount of radiolabel is released during the *in vitro* dissolution verifying that there are no health and safety issues due to systemic absorption of radioactivity.

In Section 6.2.5.1 it was shown that coating of radiolabelled pellet formulations has a significant effect of retaining the radiolabelled anion exchange resin within the porous extrusion-spheronised pellets formulations. For non-porous beads made for

drug-layering the same effect of retaining the radiolabel may be observed in the case that the GI fluid contains components are able to displace the radiolabel from the anion exchange resin.

A commonly used method for radiolabelling of pellet formulations prior to film coating has been described in the literature. It is described that film coating prior to radiolabelling requires penetration of radiolabel through the film coat prior to attachment to the pellet core (Basit *et al.* 2001; Basit *et al.* 2004; Clarke *et al.* 1995; Davis *et al.* 1987; McConnell *et al.* 2008; Yuen *et al.* 1993). Using this method compromises the film coat as it must be able to facilitate penetration of radiolabel. This type of film coat will therefore not retain the radiolabel during GI transit so our method of film coating post radiolabelling shows advantages over the commonly used method.

6.3. Conclusion

- Two novel methods of radiolabelling pellet formulations, the "soaking" method and the "incorporating" method can be used for tracing pellet formulations *in vivo* using gamma scintigraphy.

- Manufactured beads containing an anion exchange resin resemble the size and density of Cellets 700® and they all have relatively high crushing strengths. Both types of beads are interchangeable for use as inert beads for drug-layering.

- The drug release profile of pellet formulations is unaffected by the incorporation of 1% (w/w) anion exchange resin which can be bound to ^{99m}Tc-pertechnetate ions.

- Size 0 capsules can be dosed with pellets of sufficient radiolabel for scintigraphic monitoring during dispersion in the GI tract. The radioactive dose can be adjusted through the following:
    - Adjustment of proportion between radiolabelled and non-radiolabelled pellets in a dosed capsule
    - Adjustment of radioactivity during the "incorporating" method
    - Adjustment of time from manufacturing to dosing

- Anion exchange resins tightly bind to the radiolabel during *in vitro* dissolutions, and a film coat applied post radiolabelling assists containing the radiolabelled anion exchange resin to the pellet core.

Chapter 7: Optimisation and reproducibility of the small scale extrusion-spheronisation technique

7.1. Introduction

Radiolabelling using the "incorporation" method requires a reproducible pellet yield of ≥ 15% (w/w) within the desired pellet size range of 1.00-1.18 mm. As discussed in previous chapters, this pellet yield is required in order to have sufficient pellets for dosing during clinical scintigraphic studies and possible film coating.

The extrusion-spheronised non-radiolabelled pellet batches described in Chapter 6 were easily repeated until a pellet yield of ≥ 15% (w/w) within the desired pellet size range of 1.00-1.18 mm was achieved. However, when using the "incorporating" method the pelletisation cannot easily be repeated due to limitations by the use of radioactivity. The importance of manufacturing the desired yield in a reproducible manner when using the "incorporation" method was highlighted in Chapter 6, Section 6.2.1.2.

Wan *et al.* (1993) concluded from their investigations that spheroniser speed and time, and the amount of granulation liquid are variables that must be taken into account for successful production of spheroids within a certain size range. Furthermore, it has been reported that the volume of granulation liquid also can affect the drug release profile (Baert and Remon 1993; Lee 2003; Pinto *et al.* 1992; Vertommen and Kinget 1997). This chapter describes the results after optimisation and standardisation of these parameters, as well as the spheroniser temperature, in order to reproducibly manufacture the highest possible yield of pellets in size range of 1.00-1.18 mm.

7.2. Results and discussion

7.2.1. Yield

Table 7.1 shows the pellet yield in the desired size range of 1.00-1.18 mm for the optimised pellet batches (Batch P1-P5, I1-I5 and Ires).

Table 7.1. Pellet yield in the desired size range of 1.00-1.18 mm for the 11 optimised pellet batches which include a pellet batch without resin.

Batch name	P1	P2	P3	P4	P5	I1	I2	I3	I4	I5	Ires
Pellet yields (% (w/w))	29.9	28.4	27.4	21.5	27.6	26.2	25.9	28.8	29.2	27.5	28.5
Average pellet yields (±SD)	27.0 (±3.2)% (w/w)					27.5 (±1.5)% (w/w)					

All pellet batches were produced with yields above 15% (w/w) without having to discard a batch or re-granulate. The optimised extrusion-spheronisation technique could therefore be used in order to produce pellets with yields above 15% (w/w) without having to re-granulate pellet batches that were outside the size range of 1.00-1.18 mm.

The manufacturing of Batch I-res also proved that the addition of 1 % (w/w) anion exchange to the pellet powder blend of pellets containing indomethacin did not cause a significant change to the yield of the extrusion-spheronised pellets ($p<0.05$).

An important extrusion-spheronisation parameter optimised in the present study, in order to produce the highest yield possible, was the volume of distilled water for granulation. It has been reported that the volume of granulation liquid influences the pellet size without a direct influence on the pellet size distribution (Pinto *et al.* 1992; Vertommen and Kinget 1997; Wan *et al.* 1993). It was therefore important to optimise the amount of granulation liquid in order for the pellets to distribute evenly within the desired size range. Furthermore, it may be suggested that an increased spheroniser temperature has a similar effect on pellet yield as a reduction in granulation liquid volume due to evaporation of water at higher spheronisation temperatures. However, the effect on pellet morphology is assumed to remain unchanged. The optimised granulation liquid volume and spheroniser temperature were therefore considered of major contribution to the high pellet yields achieved.

It has been reported that an increased spheronisation time and speed results in pellets that are more round and large due to more opportunity for agglomeration (Wan *et al.* 1993). However, Lee (2003) found that an increased spheronisation time of wax based pellets gives larger size distributions and Wan *et al.* (1993) found that a further increased spheronisation speed causes high destructive forces generating smaller and less rounded pellets. In the present study the spheronisation speed and time were optimised in order to produce desirable spheres.

7.2.2. Dose variation

Changes in the extrusion-spheronisation variables, in order to optimise the pellet batches, have not been reported in the published literature to influence the resultant drug content of the pellets. The drug content was therefore only assayed for the purpose of investigating the dose variation in the extrusion-spheronised pellet batches. The results of the assayed drug contents are shown in Table 7.2.

Table 7.2. Assayed drug contents of optimised pellet batches. Assayed content is measured using UV as described in the method section.

Batch name	P1	P2	P3	P4	I1	I2	I3	Ires
Assayed drug content (% (w/w))	9.3	9.1	9.1	9.3	10.6	11.3	12.0	11.8
Average assayed drug contents (labelled content) (±SD)	9.2 (±0.1)% (w/w)				11.4 (±0.6)% (w/w)			

It is observed that the assayed drug contents vary from the drug content added in the dry blend [10 % (w/w)]. Paracetamol added to the dry blend was 8 (±1)% (w/w) lower than the assayed content and the indomethacin added to the dry blend was 14 (±6)% (w/w) higher than the assayed content. These deviations could be due to the hygroscopic properties of the drug, the loss of water during drying of pellets or inaccurate UV absorption readings.

Pellets were produced with reproducible drug contents, shown by their relatively small standard deviations. The average assayed drug contents were used as the

labelled content in the present study for all extrusion-spheronised pellet formulations composed of indomethacin or paracetamol with lactose and/or MCC as excipients.

7.2.3. Indomethacin release

It has been reported that an increased amount of granulation liquid sustains the drug release from extrusion-spheronised pellet formulations by contraction of the matrix, which reduces the porosity of the pellets (Baert and Remon 1993). Furthermore, an increased spheroniser temperature is assumed to have similar effect as a reduced granulation liquid volume due to larger water evaporation during spheronisation at higher temperature. No literature has been found to have investigated the effect on the drug dissolution profiles when changing the spheroniser time or speed, so these variables are presumed to have no effect. The reproducibility of the drug dissolution profiles of the optimised pellet batches was therefore investigated using the indomethacin containing pellet batches (Batch I1-I3 and Ires), and the dissolution profiles were compared to a batch prepared prior to the optimisation (Batch F*). These drug dissolution profiles are shown in Figure 7.1.

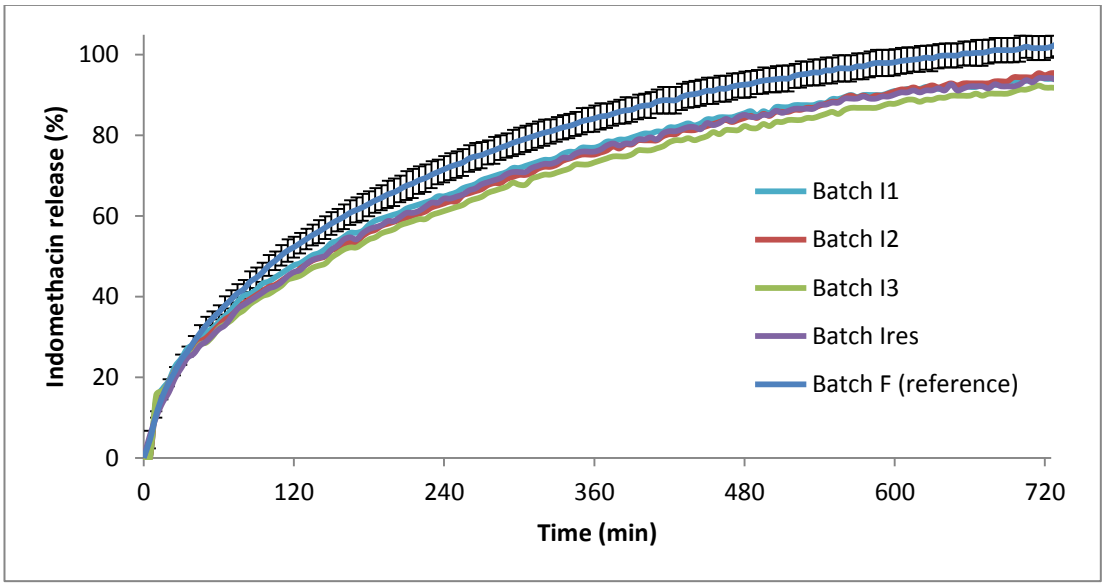


Figure 7.1. Indomethacin release of Batch I1-I3 and Ires together with the indomethacin release profile of Batch F as a reference (n=3; mean (±SD)).

As seen in Figure 7.1 the indomethacin release of Batch I1-I3 and Ires look similar. This means that the drug release of the pellets prepared by the optimised extrusion-

spheronisation method is produced in a reproducible manner and that the incorporation of an anion exchange resin has no effect on the dissolution profile.

The indomethacin release of the batches prepared using the optimised settings look slightly faster than the indomethacin release of the reference prepared prior to optimisation. However, a statistical comparison between the indomethacin release profiles of the optimised batch, Batch I3 and the reference batch prior to optimisation, Batch F found that this difference was insignificant (f_1=13.7 and f_2=60.3). The changes in granulation liquid volumes and spheroniser temperature between the optimised and previous pellet batches therefore had no significant effect on the dissolution profiles. This may be because the magnitude of the changes was considerably small. Pinto *et al.* (1992) also found no difference between the *in vitro* indomethacin dissolution profiles when decreasing the volume of distilled water for granulation by 12 % (v/v).

7.3. Conclusion

- Optimised small scale pellet batches were produced in a desirable yield of 1.00-1.18 mm without the necessity of re-granulating.

- Reproducible pellet yields, drug contents and *in vitro* drug dissolution profiles were achieved for the manufactured small scale pellet batches.

- The optimised extrusion-spheronisation variables had no significant effect on the *in vitro* drug dissolution profiles of the small scale pellet batches.

Chapter 8: Radiolabelled GI transit markers for drug loaded pellets

8.1. Introduction

Radiolabelling using the "incorporation" method and the "soaking" method described in Chapter 6 is limited to the use of conventional extrusion-spheronised pellets or drug-layered pellets using MCC beads. In order to scintigraphically monitor drug loaded pellet formulations, which cannot become radiolabelled using the "incorporation" or the "soaking" methods as described in Chapter 6, the use of radiolabelled inert pellets as GI transit markers for drug loaded pellets may be considered. This method has been described widely in the published literature but there are limitations to its use due to the necessity of mimicking the size and density of the pellet formulations being traced during GI transit.

Two different pellet formulations may transit the GI tract at different rates due to differences in size and/or density. However, Clarke *et al.* (1995) found that there is no difference in GI transit for pellets of size 1.2-1.4 mm and density below 2.4 g/cm^3 (Clarke *et al.* 1995).

When using inert pellets as markers for GI transit of an investigated pellet formulation, it is important that the inert pellets mimic the size and density of the investigated pellet formulation or that both pellets are of size 1.2-1.4 mm and density below 2.4 g/cm^3. This study validates the use in gamma scintigraphic studies of radiolabelled placebo pellets as GI transit markers for drug loaded pellets of similar size containing an insoluble film coat in.

8.2. Results and discussion

8.2.1. Manufacturing of GI transit markers

8.2.1.1. Extrusion and spheronisation

GI transit marker pellet cores, Batch B*, consisting of MCC with 1% (w/w) anion exchange resin, were produced using the small scale extrusion-spheronisation method with a yield of 54.7% within the desired size range (1.00-1.18 mm).

The high yield in the desired size range was caused by a narrow pellet size distribution, which was contributed by the combination of a high content of MCC (99%) in the beads together with spheronisation under heated conditions. The contribution to a high yield from these factors was discussed in more detail in Chapter 4.

8.2.1.2. Radiolabelling

The radioactive uptake of pellet cores of Batch B* is shown in Table 8.1.

Table 8.1. Radioactive uptake of Batch B*.

Radioactive uptake of Batch B*[a]	Percentage radioactive uptake of Batch B*
33.4 MBq	37.6%

[a]Radioactivity is decay corrected to 09.00 a.m. on the morning of manufacturing.

The radioactive uptake of the pellet cores was relatively low but still high enough to be detected scintigraphically during the scintigraphic dissolution experiment in Section 8.2.2 that was performed on the same day as the radiolabelling. It was found in Chapter 6, Section 6.2.2.2 that a higher radioactive uptake could be achieved by reducing the liquid volumes during radiolabelling. However, the relatively large volumes were necessary for radiolabelling of the pellet cores because the 1500 mg of pellet cores being radiolabelled could not fit in a smaller container. An optimised and standardised radiolabelling technique is described in Chapter 8.

8.2.1.3. Film coating

As shown in Table 8.2 the pellet cores of Batch B* were successfully film coated with 10 % (w/w) Surelease® and the film coat therefore mimicked that of the drug loaded pellets of Batch B described in Chapter 3.

Table 8.2. Surelease® weight gain for Batch B* and the drug loaded pellets being mimicked, Batch B

Batch B* Surelease® weight gain	Batch B Surelease® weight gain
10.1 % (w/w)	9.9 % (w/w)

In the published literature, inert pellet cores have typically been radiolabelled post the addition of a film coat. This required film coats of different types of formulae; the drug loaded pellets require a film coat to control the drug release whereas that of

the inert pellets require a film coat with sufficient channels for the radioisotope to penetrate and attach to the ion exchange resin in the pellet core (Yuen *et al.* 1993). Our method of radiolabelling prior to film coating shows advantages over the commonly published methods as we can mimic the pellet surface of the drug loaded pellets by applying same types of film coat.

The importance of adding a film coat to radiolabelled pellets was discussed in Chapter 6, where Section 6.2.5 showed that the film coat assists in retaining the radiolabel within the formulation. Further to the importance of retaining the radiolabel, Yuen *et al.* (1993) also suggested that a film coat should be applied to the GI transit markers in order to mimic the hydrophilicity of the surface of the drug loaded pellet, as it may influence the ability of the pellets dispersing throughout the GI tract. This is especially important when the surface of the GI transit markers has high concentrations of ion exchange resin (Yuen *et al.* 1993).

In a study by Basit *et al.* (2004) radiolabelled GI transit markers also contained a Surelease® film coat with a weight gain which is not stated. The GI transit markers were prepared by radiolabelling post film coating. The drug loaded pellets being mimicked contained a different type of film coat applied for the purpose of enteric protection. A Surelease® film coat was applied to the GI transit markers in order to facilitate penetration of radiolabel, as enteric protective film coats do not contain pores for penetration and are designed to dissolve at pH near neutral. In a preliminary study we found that the radiolabel could not penetrate the Surelease® film coat when applied at a 10 % (w/w) weight gain so it is assumed that Basit *et al.* (2004) applied less film coat (Basit *et al.* 2004).

8.2.2. *In vitro* radiolabel dissolution of GI transit markers

The doses prepared for the *in vitro* radiolabel dissolution experiment is illustrated in Table 8.3.

Table 8.3. Mass and radioactivity at time of dissolution (03.00 p.m. on the day of manufacturing) Batch B* in dissolution Pot 1, 2 and 3.

	Pot 1	**Pot 2**	**Pot 3**
Mass	184.3 mg	200.8 mg	198.7 mg
Radioactivity	1.8 MBq	2.4 MBq	1.7 MBq

As seen in Table 8.3 approximately 200 mg of inert pellets radiolabelled with approximately 2 MBq at time of dissolution were prepared. This dose was sufficient to be monitored with the gamma camera during 18 hours of the *in vitro* dissolution.

As seen from Figure 8.1 the change in radioactive counts within Batch B* after 18 h of dissolution was minimal and the radiolabelled inert pellets were not observed to lose any radiolabel during the radioactive *in vitro* dissolution study.

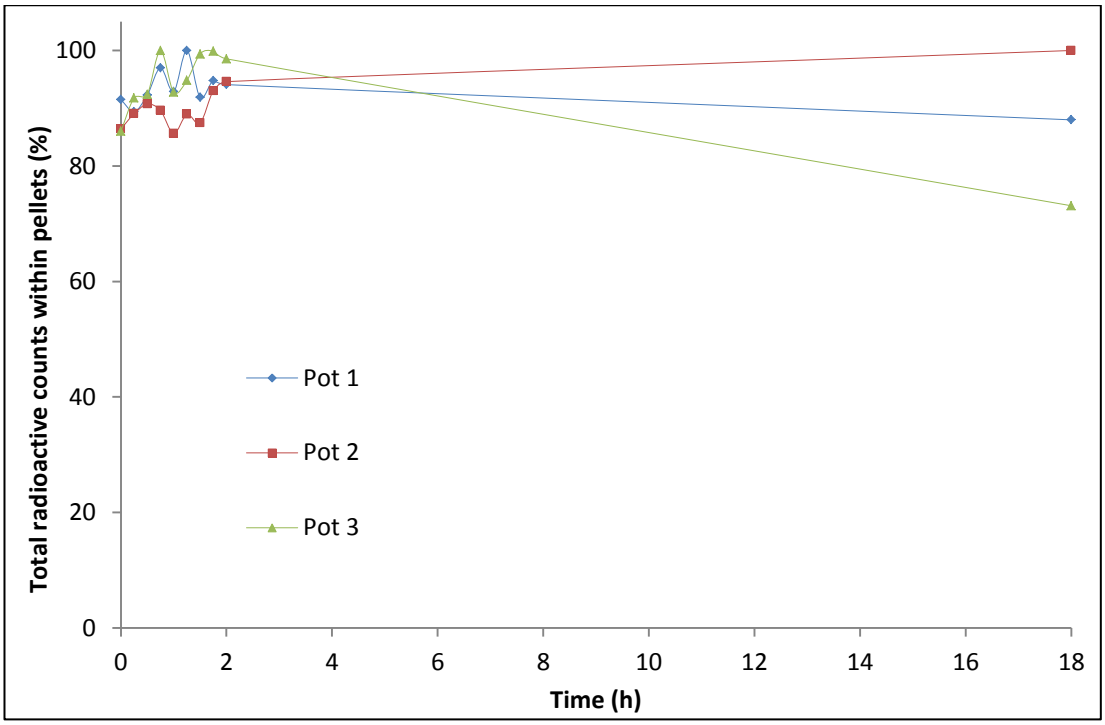


Figure 8.1. Graphs of change in radioactive counts in the ROI of Batch B* in the three dissolution pots (Pot 1-3) containing distilled water

In vitro dissolution of inert pellet cores similar to the GI transit markers in the present study was also carried out and discussed in more detail in Chapter 6, Sections 6.2.5.3 and 6.2.5.4. As no release of radiolabel was found in these studies, a similar result was expected in the present study. This is especially assumed due to Batch B* containing a non-dissolving film coat applied post radiolabelling that could retain the radiolabel.

8.2.3. Comparison of size and density during *in vitro* dissolution of drug loaded pellets and GI transit markers

Data for size and density of Batch B* were compared to those of the drug loaded pellets being mimicked, Batch B, prior to and post 18 hours of *in vitro* dissolution.

These measurements were carried out in order to investigate whether the two pellet formulations would be expected to have similar GI transit. The results are shown in Table 8.4.

Table 8.4. Size and densities of Batch B and B* with the effect of dissolution (n=3; mean (±SD)).

Time post-dissolution start (h)	Batch B size (mm)	Batch B* size (mm)	Batch B density (g/cm^3)	Batch B* density (g/cm^3)
0	1.25 (±0.01)	1.30 (±0.01)	1.04 (±0.02)	0.84 (±0.02)
2	1.26 (±0.01)		0.81 (±0.02)	
4	1.21 (±0.04)		0.71 (±0.01)	
12	1.26 (±0.04)		0.52 (±0.01)	
18	1.23 (±0.03)	1.29 (±0.03)	0.56 (±0.01)	0.85 (±0.02)

Prior to dissolution the diameter of Batch B and B* was similar ($p < 0.01$) but the density of Batch B* was lower than Batch B. Batch B* had no size and density changes after 18 hours of dissolution ($p < 0.05$). The size of Batch B also did not change during dissolution ($p < 0.01$) but the density decreased over time until stabilising.

The large decrease in density of Batch B is due to drug and excipient loss as a consequence of dissolution. This is illustrated in Figure 8.2 where it can be observed that the density change comes to a halt as the pellets approach 100% drug release.

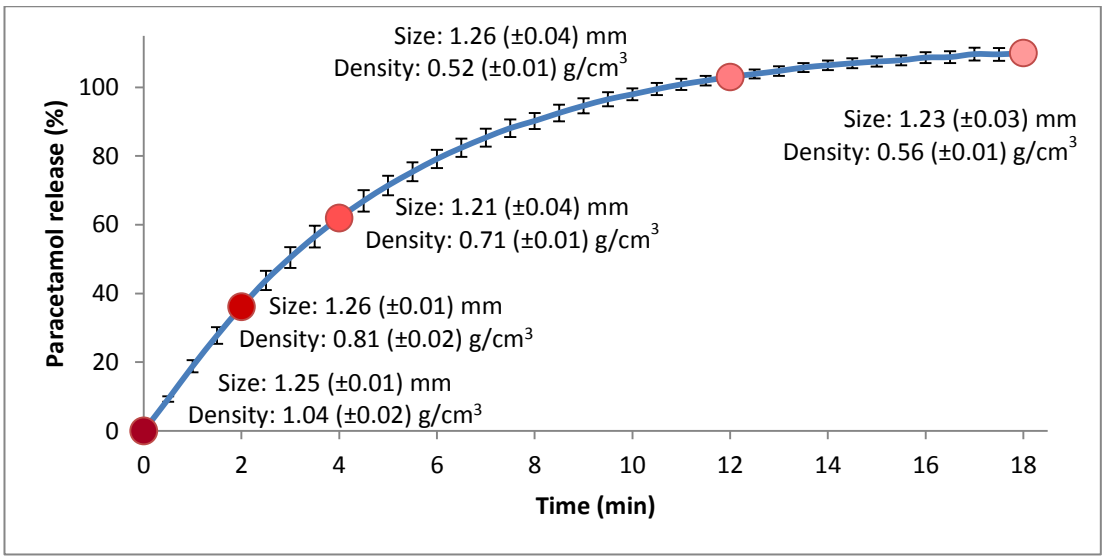


Figure 8.2. Size and densities during dissolution of Batch B at dissolution points represented by dots (n=3; mean (±SD)).

Further to replicating the size and film coat of Batch B it has been shown that the density prior to dissolution can be replicated by adding barium sulphate, a highly dense compound, to the GI transit marker pellets (Basit *et al.* 2001; Basit *et al.* 2004; Clarke *et al.* 1995; McConnell *et al.* 2008; Yuen *et al.* 1993). However, this will not replicate the change in density that occurs during dissolution. The addition of barium sulphate to further replicate the density will thus be mostly suitable for scintigraphic monitoring of time and site of drug release from delayed drug release pellet formulations that remain unchanged until time of drug release.

In the present study the size of the pellets in Batch B and Batch B* remained similar and within 1.2-1.4 mm during the *in vitro* dissolution, and the density remained below 2.4 g/cm^3, so, as reported by Clarke *et al.* (1995), it was not required to mimic the density of the drug loaded pellets (Clarke *et al.* 1995). This confirms that during any *in vivo* scintigraphic study any image captured should accurately reflect the GI position of the drug loaded pellets. Using the findings by Clarke *et al.* (1995) it can be concluded that this method of using radiolabelled GI transit markers is potentially suitable for scintigraphic monitoring of GI transit of non-disintegrating drug loaded pellets.

Further to the discussed results it should be noted that the size of the pellet formulation has no effect on the rate of gastric emptying (Clarke *et al.* 1993; Davis *et*

al. 1986). Replicating the size of drug loaded pellets is therefore not of importance in scintigraphic investigations where the gastric compartment is the only region of interest.

8.2.4. Imaging and loading of GI transit markers

Figure 8.3 shows a scintigraphic image of ten GI transit markers, from a batch prepared on a later occasion (Batch W*), which were subjected to a bag of simulated chyme.

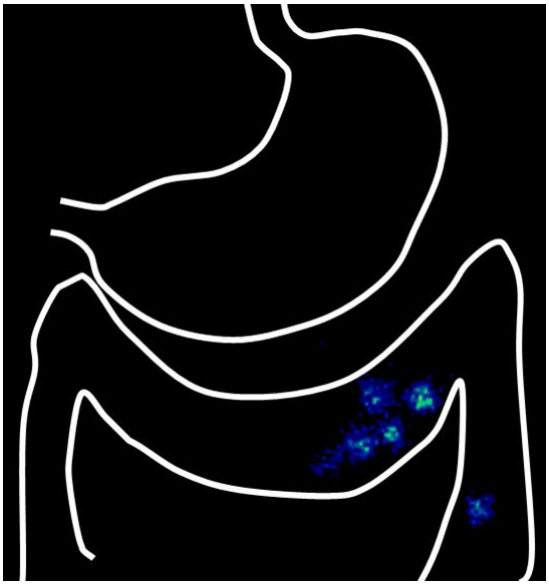


Figure 8.3. Ten GI transit markers (Batch W*) of total radioactivity 0.5 MBq in simulated chyme. The outlines of a colon and a gastric compartment are drawn for comparisons of the size and position of the GI transit markers in relation to the GI tract.

The scintigraphic image in Figure 8.3 proves that the distribution of ten GI transit markers of Batch W*, with a total activity of 0.50 MBq, can potentially be observed scintigraphically after ingestion by a human. All ten individual GI transit markers are not visualised due to overlapping. The total activity of the ten GI transit markers is low compared to the radioactive dose of 2-4 MBq commonly used in scintigraphic studies (Ghimire *et al.* 2011; Ghimire *et al.* 2010; McConville *et al.* 2009; Wilson *et al.* 2011). The low radioactive dose was used due to the small number of GI transit markers subjected to simulated chyme in this investigation. A larger number of GI transit markers will therefore still be within the radioactive dose limit of 2-4 MBq and results in a better overall representation of the potential distribution of drug loaded pellets.

As no drug dose is being mimicked by the GI transit markers, a small number of GI transit markers may be used without causing an effect on the total drug dose. In this case the radioactive strength of individual GI transit markers must be increased to achieve a total activity of 2-4 MBq. Ahmed & Ayers (2011) and Ebel *et al.* (1993) used five radiolabelled pellets in their investigations where pellets contained $^{152}Sm_2O_3$ as a radioisotope (Ahmed and Ayres 2011; Ebel *et al.* 1993). It is uncertain why they used a reduced number of GI transit markers in their study but it may be of advantage in cases where e.g. a single pellet GI movement is desired to be investigated.

In case the number of GI transit markers is increased further, the radioactive strength of each marker may have to be reduced in order to reduce the radioactive exposure and remain within a radioactive dose of 2-4 MBq. This may complicate visualisation of the individual pellets but as the number of pellets is also increased it can be expected that areas with groups of pellets can be visualised instead. In this case the transit of radiolabelled GI transit markers can been measured as a percentage of the total radioactivity within the different ROIs of the GI tract (Yuen *et al.* 1993).

8.3. Conclusion

- Radiolabelled film coated GI transit marker pellets were successfully manufactured.

- No radiolabel was released from the GI transit markers during an *in vitro* radiolabel release study and it was thus confirmed that the GI transit markers can be followed *in vivo* using scintigraphic detection.

- GI transit marker pellets and the drug loaded pellets being mimicked remained within the size and density limits indicated by Clarke *et al.* (1995), of which GI transit is the same. Therefore during any *in vivo* scintigraphic study any image captured will accurately reflect the GI position of the drug loaded placebo pellets.

- There is evidence that the dispersion of a small number of GI transit markers can be imaged scintigraphically after ingestion.

Chapter 9: The “wet and dry” method

9.1. Introduction

The “wet and dry” method is an optimised and standardised radiolabelling method investigated as an alternative to the “soaking” method. The need for an optimised and standardised “soaking” method was identified after results in Chapter 6 and Chapter 8, and also after finding large variations in the similar methods employed for radiolabelling of pellets in the published literature. In the “wet and dry” method a procedure for achieving the largest uptake of radiolabel in the shortest time period possible was investigated. The “wet and dry” method was belatedly developed in the present thesis, and has therefore not been used for radiolabelling the inert pellet cores described in previous chapters.

9.2. Results and discussion

9.2.1. Manufacturing of inert pellet cores

The manufactured beads were the same as those used for the manufacturing of radiolabelled pellet cores for drug layering discussed in Section 6.2.2.1.

9.2.2. Radiolabelling of inert pellet cores

The radioactive uptake by the manufactured beads of Batch W* is shown in Table 9.1.

Table 9.1. Radioactive uptake of Batch W*.

Radioactive uptake of Batch W*[a]	Percentage radioactive uptake of Batch W*
515 MBq	99.0%

[a]Radioactivity is decay corrected to 09.00 a.m. on the morning of radiolabelling.

Batch W* was successfully radiolabelled with a high uptake of radiolabel compared to the results of Batch B* (37.6%), Batch E* (61.7% uptake) and Batch G* (13.1% uptake).

The high radiolabel uptake proved that the “wet and dry” method was more efficient than the “soaking” method. However, no washing step was incorporated into the

"wet and dry" method so the actual uptake through binding to the anion exchange resin may be lower.

The short radiolabelling time of approximately 10 min using the "wet and dry" method proved that this method was faster than the "soaking" method where pellets were radiolabelled by soaking for 1 hour. It has been reported in the published literature that pellets radiolabelled by a soaking method have greater uptake of radiolabel the longer they are being soaked for. It has been recommended to soak uncoated inert pellets in a solution with radiolabel for at least 40 or 60 min to get a high uptake of radiolabel (Philippe *et al.* 2010). Film coated pellet formulations may require a longer soaking time due to extra time required for the radiolabel to penetrate through pores in the film coat. Clarke *et al.* (1995) reported a 2 hours soaking time in their study of radiolabelling film coated pellets by a soaking method.

9.2.3. *In vitro* radiolabel dissolution

The *in vitro* radiolabel dissolution of Batch W* was carried out in three dissolution pots (Pot A-C) containing beads with mass and radioactivity described in Table 9.2. There was only a small variation in the radioactivity of Batch W* transferred to the three pots.

Table 9.2. Mass and radioactivity of Batch W* transferred to Pot A, B and C for a radioactive dissolution study.

	Pot A	Pot B	Pot C
Mass	32.9 mg	33.1 mg	33.1 mg
Radioactivity[a]	4.75 MBq	5.06 MBq	4.99 MBq

[a]**All radioactivity is decay corrected to 09.00 a.m. on the morning after manufacturing when subjected to *in vitro* dissolution.**

The radioactivity in each pot is relatively high and the amount of pellets low. This is due to the high efficiency of the "wet and dry" method.

As seen from Figure 9.1 the pellets of Batch W* were observed to lose up to 20% radiolabel during the first 15 min of the *in vitro* dissolution study.

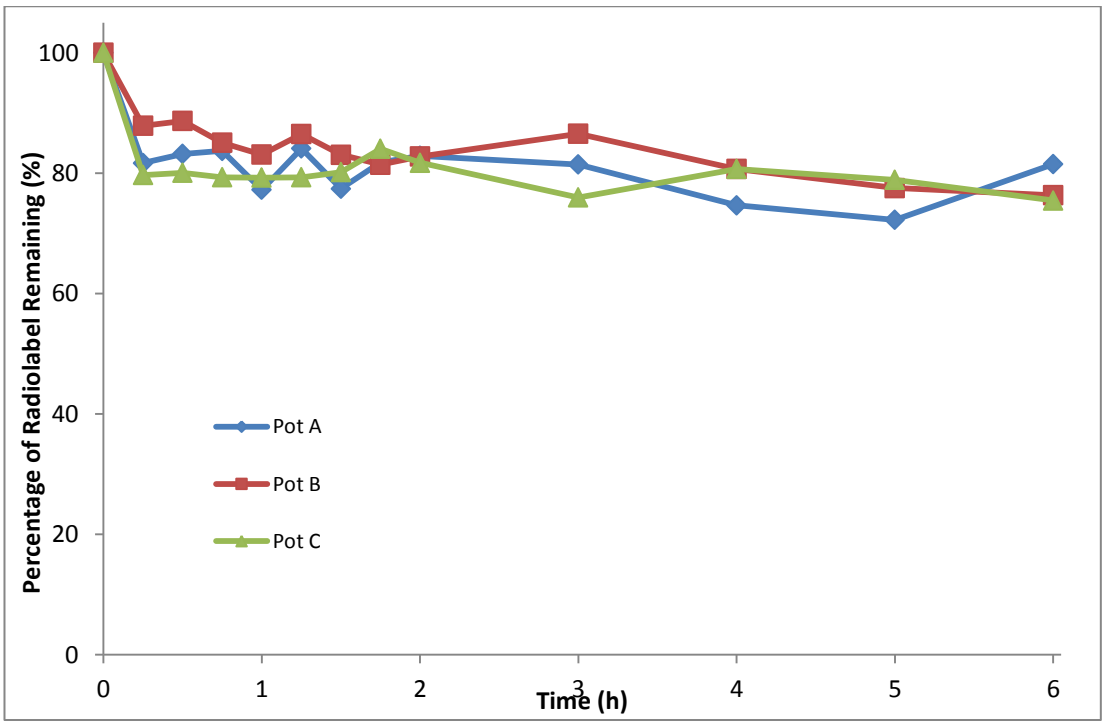


Figure 9.1. Graphs of change in radioactive counts in the ROI of Batch W* in the three dissolution pots (Pot A-C) containing distilled water.

After the first imaging at 15 min of dissolution no more radiolabel was lost. This is also illustrated in Figure 9.2 which shows the scintigraphic images at 0 min, 15 min and 3 hours of dissolution where no extra radiolabel is observed to be released between 15 min and 3 hours of dissolution.

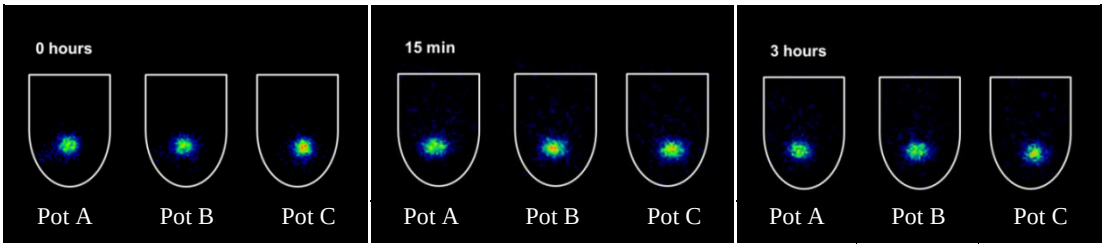


Figure 9.2. Scintigraphic images of Batch W* at 0 hours, 15 min and 3 hours of a radioactive *in vitro* dissolution study. The dissolution pots (Pot A-C) are drawn on the images.

Our "wet and dry" method was quick and easy but did not include a washing step and it is therefore expected that a high amount of radiolabel will be released during the *in vitro* radiolabel release study. The results of the *in vitro* radiolabel release study suggest that by incorporating a washing step no radiolabel is expected to be released from the pellets during GI transit. Yuen *et al.* (1993) carried out a washing step by soaking film coated radiolabelled pellets in saline for 1 hour, so the same amount of time was used in the "soaking" method previously used in Chapter 6 and

Chapter 8 (Yuen *et al.* 1993). However, Philippe *et al.* (2010) washed uncoated radiolabelled pellets by shaking for 1 min in distilled water (Philippe *et al.* 2010). Our *in vitro* radiolabel dissolution results together with the method used by Philippe *et al.* (2010) therefore suggest that 1 min of washing may be sufficient for radiolabelling of inert uncoated pellets radiolabelled using the "wet and dry" method.

9.3. Conclusion

- The new "wet and dry" method is a faster and more efficient alternative to the "soaking" method.

- A 99% radiolabel uptake is achieved after drying radiolabel onto the inert beads and approximately 20 % radiolabel is expected to be washed off after adding a washing step to the radiolabelling procedure.

- After washing the uncoated radiolabelled inert pellets no radiolabel is expected to be released during GI transit.

Chapter 10: General discussion and possible future work

10.1. Final discussion

Eight desired drug release profiles for pellet formulations containing paracetamol or indomethacin were achieved using small scale manufacturing methods. Small scale extrusion-spheronisation was accomplished using sieve extrusion and spheronisation under heated conditions. Small scale drug-layering and film coating was accomplished by fluidising on a small scale under increased dynamic conditions.

The addition of lactose as excipient for the extrusion-spheronised pellet formulations was shown to cause a larger size distribution and a more rapid drug release of the final pellet cores. The addition of lactose was found necessary in order to achieve a sufficiently rapid release of paracetamol or indomethacin.

The addition of Explotab® or PEG 400 as excipients for extrusion-spheronised pellet formulations containing indomethacin, did not give an immediate indomethacin release and the pellets produced were not of the desired physical properties. An immediate release of indomethacin was only achieved using the drug-layering pelletisation technique.

An optimised extrusion-spheronisation procedure for pellets containing paracetamol and indomethacin was shown to give yields >15% (w/w) within the desired size range of 1.00-1.18 mm and had a reproducible sustained indomethacin release.

Radiolabelled pellet formulations were successfully produced using the "incorporation" method for extrusion-spheronised pellet formulations and the "soaking" method for drug-layered pellet formulations. These radiolabelling methods could be applied to both uncoated and film coated pellet formulations. Radiolabelled pellet formulations had the same drug release profiles as the non-radiolabelled pellet formulation they were mimicking and were potentially suitable for *in vivo* gamma scintigraphic studies of GI transit.

A method of using radiolabelled placebo pellets for *in vivo* gamma scintigraphic studies of GI transit of non-radiolabelled drug loaded pellets was found suitable for

film coated pellet formulations with a size range of 1.2-1.4 mm during drug release and density below 2.4 g/cm^3. This method can be used as an alternative scintigraphic detection for pellets or mini-tablets that cannot be radiolabelled using the developed "incorporation" method or "soaking" method. There is evidence that the dispersion of a small number of GI transit markers can be imaged scintigraphically after ingestion.

A "wet and dry" method for radiolabelling of inert pellet cores was found to be faster, easier and more efficient than the "soaking" method used in this study.

10.2. Final conclusion

Two small scale pelletisation techniques were developed for extrusion-spheronised and drug-layered pellet formulations, and two radiolabelling methods were developed in order to incorporate a radioisotope into the formulations for *in vivo* scintigraphic detection. For the *in vivo* scintigraphic detection of mini-tablets or pellet formulations that cannot be replicated using the developed small scale pelletisation technique, a validated method of using GI transit markers for drug loaded pellets may be used.

10.3. Future perspectives

The following work is suggested for future studies related to the manufacturing of non-radiolabelled and radiolabelled pellet batches of different drug release profiles:

- The small scale manufacturing methods should be optimised using higher drug doses. The lactose content in extrusion-spheronised pellet formulations should be reduced and replaced by drug.

- The fixed rotation speed of the spheroniser should be measured using a tachometer in order to get the correct rotation speed if it has been changed.

- Approximately 25% (w/w) weight gain of Acryl-EZE® instead of 40% (w/w) weigh gain should be investigated. Previous experiments showed that >10 % (w/w) drug was being released after two hours of dissolution in HCl pH 1.2 when coating with 10 % (w/w) weight gain Acryl-EZE®. However, the observed drug

release may have been due to UV interference of Acryl-EZE® and not due to a drug release.

- Investigate the reproducibility of the sustained paracetamol release from pellets coated with Surelease® as it was later found difficult to reproduce these results. It may be necessary to investigate further optimisations of the coating procedure in order to make it reproducible.

- All pellets radiolabelled using the "soaking" method should be radiolabelled using the "wet and dry" method instead. The "wet and dry" method needs a washing procedure and the method should be standardised so the amount of radiolabel that is washed off is known.

- The drug content of drug-layered pellets coated with Acryl-EZE® (Batch G and G*) should be assayed prior to film coating. For the radiolabelled batch (Batch G*) a small sample (300 mg) should be kept as a quality control to measure the drug content after decaying of radioactivity.

- All radiolabelled and non-radiolabelled pellet batches produced using the extrusion-spheronisation technique (Batch A-D, F, D* and F*) should be made using the optimised extrusion-spheronisation technique.

- It would be interesting to investigate the displacement of ^{99m}Tc-pertechnetate ions from anion exchange resins using other dissolution media than a phosphate buffer. The dissolution media should contain bile acids, fatty acids or amino acids to resemble the environment in the human gut, as these components also have the potential of displacing the ^{99m}Tc-pertechnetate ions. An example of a dissolution media that contains bile acids and fatty acids is FeSSIF.

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List of Appendices

Appendix I: Calculation of drug layering weight gain in order to obtain pellets containing 10% (w/w) indomethacin

- Mass of inert pellet cores/Cellets 700®:

1,500 mg

- Mass of inert pellet cores/Cellets 700® after water has evaporated:

$$1{,}500 \text{ mg} \times \frac{97\%}{100\%} = 1{,}455 \text{ mg}$$

- Desired indomethacin strength in pellets:

10 % (w/w)

- Solid content of indomethacin in an indomethacin:PVP K30 (30:2 w/w) aqueous suspension:

$$\frac{30 \text{ g}}{30 \text{ g} + 2 \text{ g}} \times 100\% \approx 93.75\% \text{ (w/w)}$$

- Weight gain of 1,455 mg pellets using an indomethacin:PVP K30 (60:4 w/w) aqueous suspension (x):

$$\frac{x \times 93.75 \text{ \% (w/w)}}{1{,}455 \text{ mg} + x} = 10 \text{ \% (w/w)}$$

$$\Leftrightarrow x \times 93.75\% \text{ (w/w)} = 14{,}550 \text{ mg} \times \% \text{ (w/w)} + x \times 10\% \text{ (w/w)}$$

$$\Leftrightarrow x \times 83.75\% \text{ (w/w)} = 14{,}550 \text{ mg} \times \% \text{ (w/w)}$$

$$\Leftrightarrow x \approx 173.7 \text{ mg}$$

Appendix II: Calibration curve for quantification of paracetamol

Paracetamol stock solution

A paracetamol stock solution was prepared by dissolving paracetamol (2.0003 g) in distilled water (250 mL) using a volumetric flask which was sonicated for 10 min

Paracetamol row of standards

Paracetamol row of standards (n=2) were prepared by transferring the required amounts of stock solution to 100 mL volumetric flasks diluted with distilled water.

UV readings of paracetamol

The UV absorbance of the row of standards were analysed by UV at 257 nm. The results are shown in Table AII-i and the calibration curve is shown in Figure AII-i.

Table AII-i. Data for calibration curve for paracetamol.

Paracetamol concentration (mg/900 mL)	Average absorption (A)
0	0 (±0)
9	0. 45075 (±0.010394)
9.45	0.472 (±0.006788)
9.9	0.4942 (±0.007778)
10.35	0.5196 (±0.00792)
10.8	0.5384 (±0.009475)

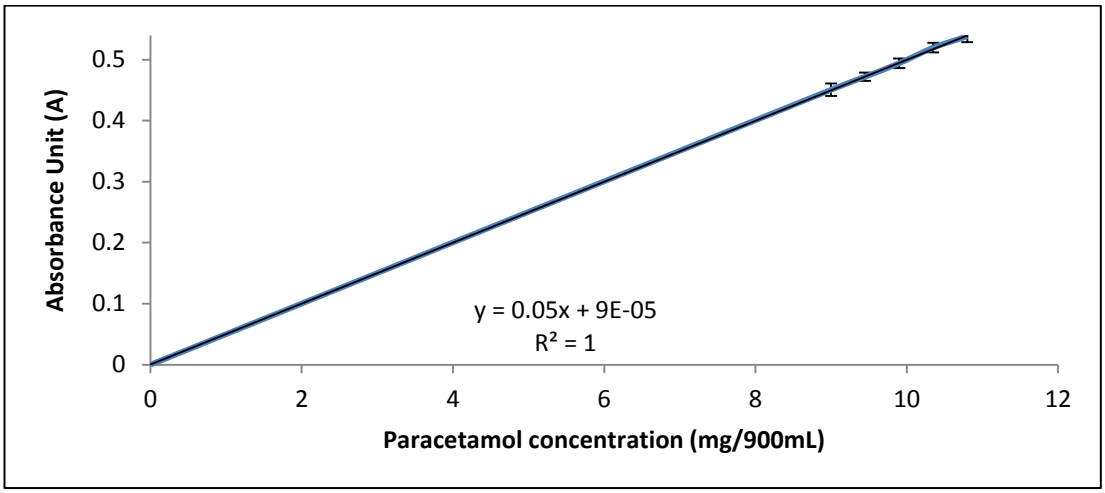

Figure AII-i. Calibration curve for paracetamol.

Appendix III: Calibration curve for quantification of indomethacin

Indomethacin stock solution

An indomethacin stock solution was prepared by dissolving indomethacin (1.0139 g) in ethanol (250 mL) using a volumetric flask which was sonicated for 10 min

Indomethacin row of standards

Indomethacin row of standards (n=2) were prepared by transferring the required amounts of stock solution to 200 mL volumetric flasks diluted with distilled water.

UV readings of indomethacin

The UV absorbance of the row of standards were analysed by UV at 320 nm. The results are shown in Table AIII-i and the calibration curve is shown in Figure AIII-i.

Table AIII-i. Data for calibration curve for indomethacin.

Indomethacin concentration (mg/900 mL)	Average absorption (A)
0	0 (±0)
7.2	0.12555 (±0.00502)
8.1	0.13900 (±0.003818)
9.0	0.15755 (±0.001909)
9.9	0.16955 (±0.002899)
10.8	0.18725 (±0.002899)

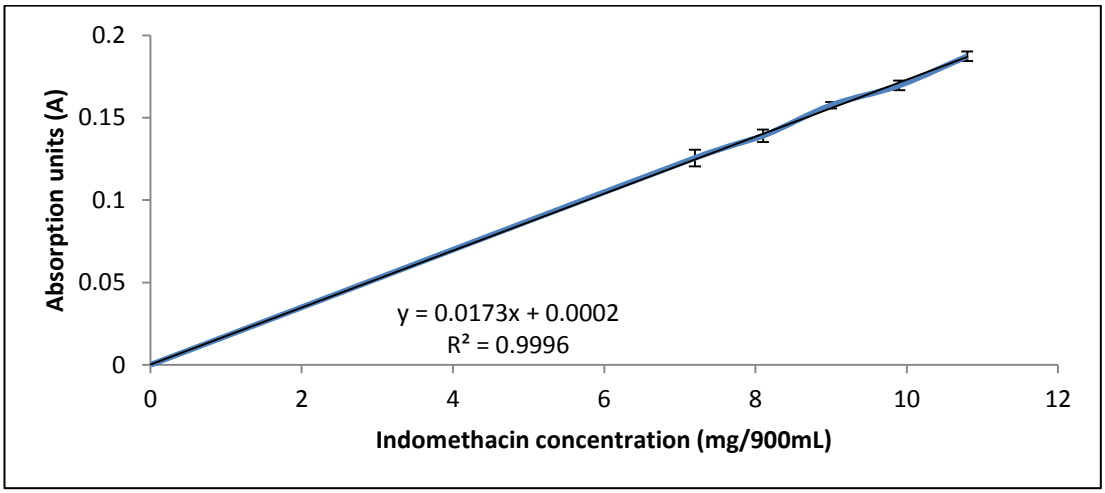

Figure AIII-i. Calibration curve for indomethacin.

Appendix IV: Acryl-EZE® interference with UV absorption

Inert pellet cores coated with Acryl-EZE®

Insoluble inert pellet cores (1.00-1.18 mm) composed of MCC were produced by extrusion-spheronisation on a previous occasion. The inert pellet cores were film coated with Acryl-EZE®.

UV scan of Acryl-EZE®

Inert pellet cores coated with Acryl-EZE (15.6 mg) were transferred to a 250 mL volumetric flask filled with phosphate buffer. The volumetric flask was sonicated for 20 min and shaken prior to transfer into cuvettes used for a UV scan at 200-380 nm, illustrated in Figure AIV-i.

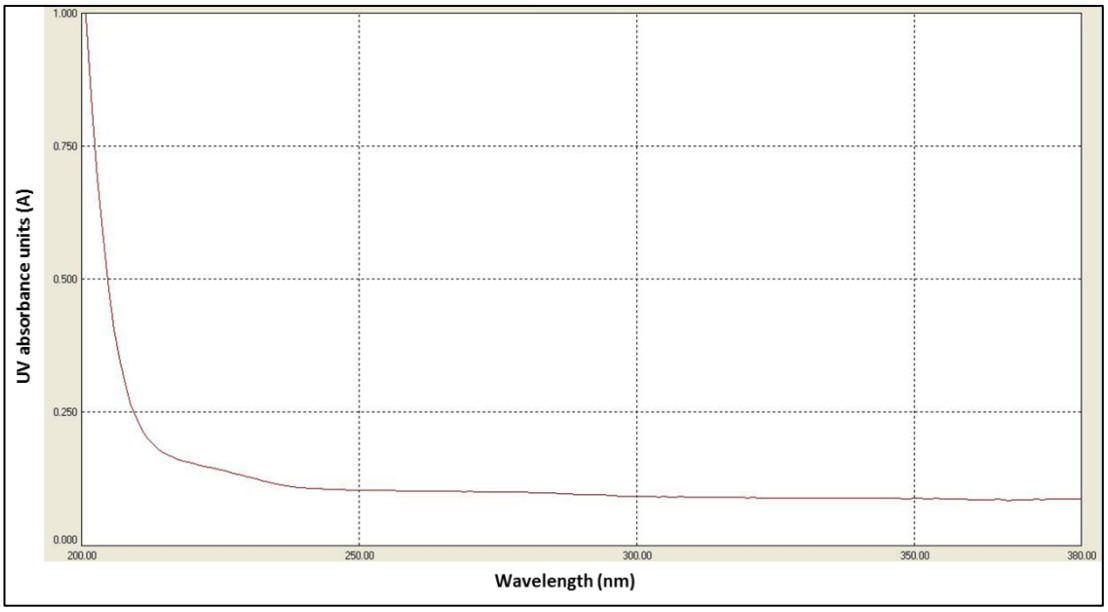

Figure AIV-i. UV scan of Acryl-EZE® dissolved in phosphate buffer pH 6.8.

The UV absorbance shown in Figure AIV-I indicates that there is an absorbance of Acryl-EZE® within the entire wavelength investigated. It was assumed that the observed absorbance was due to the presence of titanium dioxide in the Acryl-EZE® film coat causing reflections rather than absorbance of the incoming UV beam. The UV interference could therefore not be avoided by analysing the drugs at different

wavelengths. The UV absorbance therefore had to be analysed by subtracting the absorbance occurring during dissolution of blank pellets coated with Acryl-EZE®.

Subtraction of Acryl-EZE® UV absorption during paracetamol and indomethacin *in vitro* dissolution

In vitro dissolutions of inert pellet cores coated with Acryl-EZE® were carried out at the absorbance unit of paracetamol (257 nm) and indomethacin (320 nm)

The *in vitro* dissolution profiles of Acryl-EZE® coated pellets containing paracetamol or indomethacin were drawn by subtracting the UV absorption of the inert Acryl-EZE® coated pellets as illustrated in Figure AIV-ii and Figure AIV-iii.

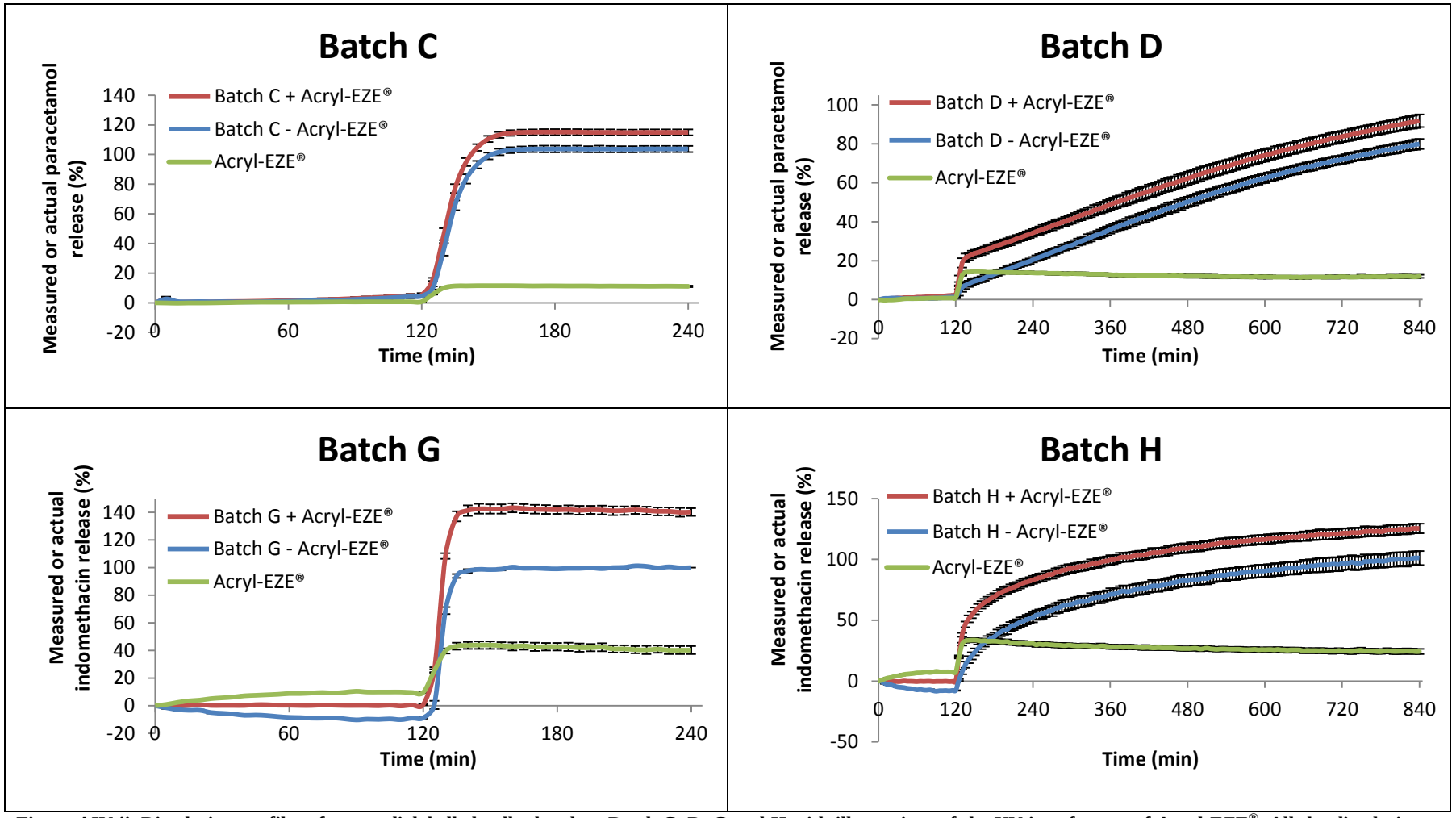

Figure AIV-ii. Dissolution profiles of non-radiolabelled pellet batches, Batch C, D, G and H with illustrations of the UV interference of Acryl-EZE®. All the dissolutions are carried out in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8 (n=3; mean (±SD)). Dissolution profiles of the blank formulation (Acryl-EZE®), the drug-loaded formulation (Batch + Acryl-EZE®) and the drug loaded formulation minus the blank dissolution profile (Batch - Acryl-EZE®) are shown.

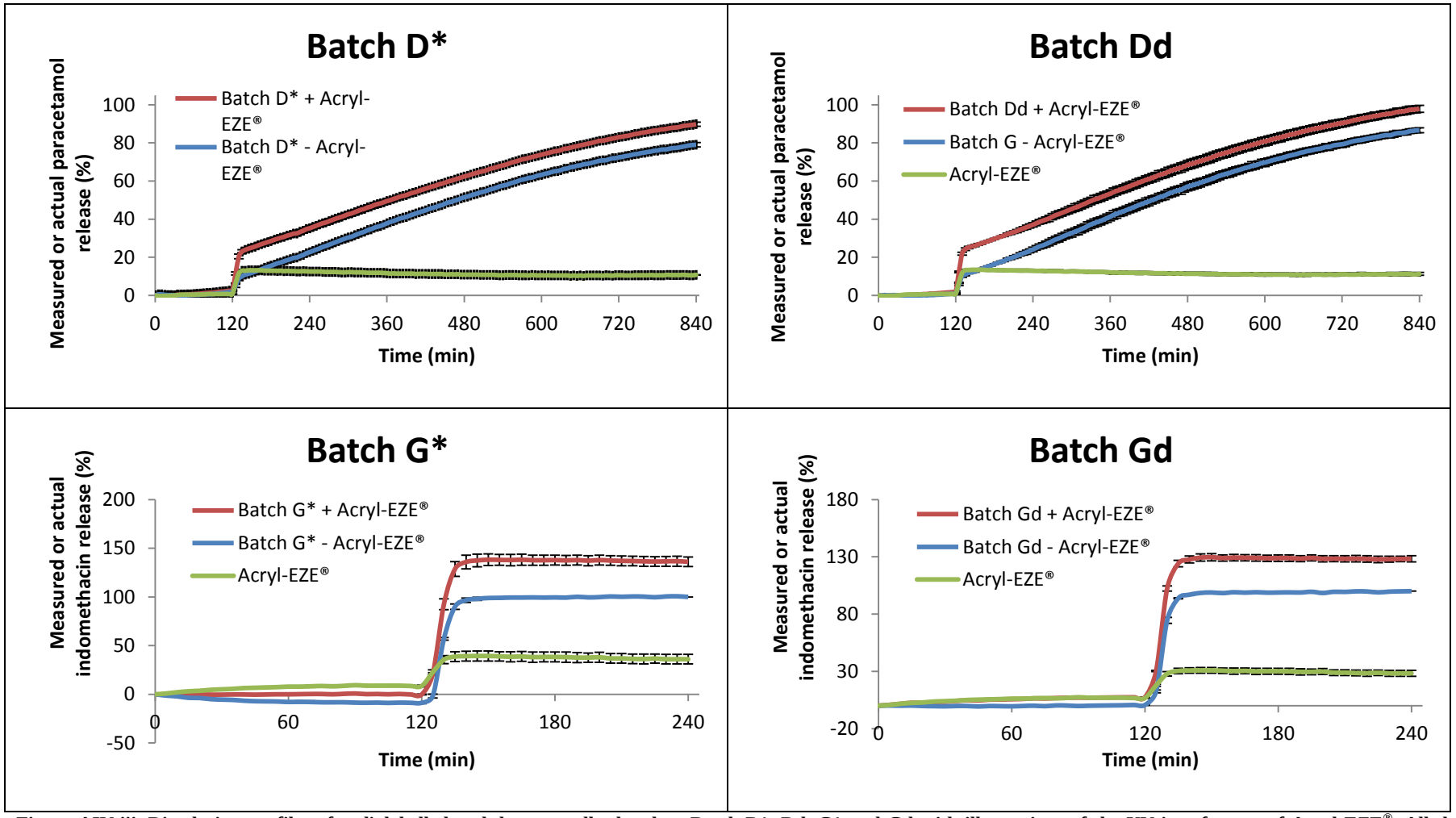

Figure AIV-iii. Dissolution profiles of radiolabelled and dummy pellet batches, Batch D*, Dd, G* and Gd with illustrations of the UV interference of Acryl-EZE®. All the dissolutions are carried out in HCl pH 1.2 for 2 h followed by phosphate buffer pH 6.8 (n=3; mean (±SD)). Dissolution profiles of the blank formulation (Acryl-EZE®), the drug loaded formulation (Batch + Acryl-EZE®) and the drug loaded formulation minus the blank dissolution profile (Batch - Acryl-EZE®) are show