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Hybrid Matrix Systems for Oral Controlled Drug Delivery

Coordinator:

Prof. Marco Mor

PhD candidate:

DAVIDE FRASCIO

Supervisor:

Prof. Ruggero Bettini

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1. Introduction

1.1 The origin of controlled release

Arthur Amos Noyes in collaboration with Willis Rodney Whitney published in 1897 the Noyes-Whitney Law, the first documented mathematical approach in describing the phenomenon of dissolution of a solid particle in a liquid medium (Noyes and Whitney, 1897). After 60 years, Takeru Higuchi published the first equation aimed at describing drug release from pharmaceutical dosage forms, deriving the Fick's Second Law of Diffusion (Higuchi, 1960). This two pioneering works represent the starting point of the process that changed the way to design pharmaceutical dosage forms, giving us the tools to understand the physical phenomenon governing drug release. Nevertheless, it would be wrong to think that the concept of controlled release was unknown before the nineteenth century. One could be surprised to know that in the Chinese Medicine, for example, the idea of "controlled release" seems to be extremely old as some manuscripts belonging to 1200 A.D. reported the practice of administering medicines in waxy excipients to release the active "slowly and gradually" (Unschuld, 1986). The practice of producing coated pills to administer bad tasting drugs is even older; Avicenna and Rhazes reported this practice in the ninth century (Thompson and Lee, 1945). It seems therefore that the idea of modifying the physical properties of drugs or even their release rate by means of technological processes is older than one could imagine. The innovation of the Noyes Whitney and Higuchi equations resides in the concept of describing with mathematical models the experimental observation. This paradigm gained popularity in the last decades with the introduction of innovative tools like the design of experiment (DoE) in the manufacturing process, to understand the relationship between process variable and product characteristics. The concept is the same and it is

quite simple: we should be able to describe a phenomenon to understand it completely.

The beginning of controlled release as a field of pharmaceutical research saw the appearance of an impressive number of pharmaceutical products, 180 in 1961 in USA, claiming to provide controlled release action, but mostly based on observation of clinical outcomes and physiological measures instead of evaluating the effective drug plasma concentration (Florence, 2011). In 1961 Lazarus and Cooper criticize the enormous enthusiasm in the growing controlled release field because it resulted in "a plethora of ideas and products, many of which should remained unborn" (Lazarus and Cooper, 1961). If we take a look at our days, there are 133 commercially available controlled drug delivery systems compared to 180 in 1961, and more than 300 controlled drug delivery systems in clinical trials (Kuick Research, 2017).

The concept of controlled release changed gradually over time, particularly with three principal factors: the discovery of new materials suitable in drug product manufacturing, the birth and the growth of new field of research such as biopharmaceutics in the sixties and the development of more accurate and precise mathematical models able to describe the release of a drugs from a pharmaceutical dosage form (Lee and Li, 2010). Alongside affording patients benefits and increased patient compliance, the need for the pharmaceutical companies to protect drugs from patents expiration has become in the latest years another important driving force in controlled release pharmaceutical forms development.

Over the years, the conception of strictly time-controlled release has evolved. Drugs are now expected to act not only *when* we want them to act but also *where* we want them to act. This is well represented by the "magic bullet" theory elaborated by Paul Ehrlich in the eighteenth century, a concept that is now one of the major aspect of clinical medicine

(Schwartz, 2004), and should be taken into consideration whenever developing a pharmaceutical dosage form.

After more than fifty years of controlled release evolution, we should admit that there are still important challenges in developing controlled release formulation and still several unmet clinical needs. Formulation of peptides for oral administration, for example, despite countless efforts is one of the most significant unreached goals. The administration of drugs to the lung appeared to be a good alternative to the oral route for peptide, as demonstrated by the FDA approval of insulin based product such as Exubera® in 2006, withdrawn from the market after only one year, and Afrezza® in 2014. The future of the inhalation insulin products will be drawn by accurate evaluation of patience compliance and risk *vs* benefit profile. Colon specific drug delivery has been also proposed in the past years (Gazzaniga *et al*., 1994), aiming to administer peptides for systemic effects, but no products are available today for this purpose and colon specific drug delivery is available only for local effects.

The challenges affecting the oral administration of drugs, along with the difficulties to reach particular district of the body such as the eye, led to the development of drug controlled delivery devices able to guarantee a constant and predictable drug release to specific areas of the body for both local and systemic effects. According to A.S. Hoffman, drug delivery devices era started in 1960 with Judah Folkman's work (Hoffman, 2008). Controlled drug delivery devices, both macroscopic and microscopic, are nowadays extremely common to deliver drugs through the skin in the form of patches or to the eye and to the vagina in the form of implants.

The introduction of clinically available biotechnological medicines changed somewhat the trends of pharmaceutical companies in small molecules and controlled release devices development, redrawing the scenario of pharmaceutical research. In 2012 the revenue for

pharmaceutical companies generated from selling biotechnological products was 71% as opposed to only 7% in 2000 (Waltz, 2014). Nevertheless looking only to the economical income can be sometime misleading. This is particularly clear if we take a look to the ten best selling drugs compared to the ten most prescribed medications in 2015 (Brown, 2015).

Top ten drugs by number of monthly prescription:

1. Synthroid (levothyroxine), 21.5 million
2. Crestor (rosuvastatin), 21.4 million
3. Ventolin HFA (albuterol), 18.2 million
4. Nexium (esomeprazole), 15.2 million
5. Advair Diskus (fluticasone), 13.7 million
6. Lantus Solostar (insulin glargine), 10.9 million
7. Vyvanse (lisdexamfetamine), 10.4 million
8. Lyrica (pregabalin), 10.0 million
9. Spiriva Handihaler (tiotropium), 9.6 million
10. Januvia (sitagliptin), 9.1 million.

Top ten drugs by sales:

1. Humira (adalimumab), $8.2 billion
2. Abilify (aripiprazole), $7.9 billion
3. Sovaldi (sofosbuvir), $6.9 billion
4. Crestor (rosuvastatin), $5.9 billion
5. Enbrel (etanercept), $5.9 billion
6. Harvoni (ledipasvir and sofosbuvir), $5.3 billion
7. Nexium (esomeprazole), $5.3 billion

8. Advair Diskus (fluticasone), $4.7 billion
9. Lantus Solostar (insulin glargine), $4.7 billion
10. Remicade (infliximab), $4.6 billion

Humira, the medicine that generated most revenues in 2015, ranked 64th by monthly prescriptions in the same year. It seems then that pharmaceutical companies are more and more focused on new therapies and more profitable investments. This happened also because of introduction of generic medicines and consequent erosion of earnings from the small molecules trading market. On the other hand health care expenses are increasing year-by-year resulting in several approaches and strategies to contain it. This is particularly true in parts of the world like Italy, where the National Health Care System provides medicines directly to patients. Biotechnological drugs, compared to the so-called "small molecules", are usually more expensive and only few of them are available for oral administration. Despite new available technologies and compelling innovative administration routes, the oral administration of drugs in the form of tablets or capsules remains, when possible, the first choice by both patients and pharmaceutical companies, considering its simplicity and convenience of administration by one side, and its easy-to-manufacture on the other. The impossibility for most biotechnological drugs to be administered in other way than with injection, with the related safety concerns and lack of patient compliance, could act therefore as a brake on the development of controlled release formulations for the administration of these drugs.

One could say that medicines should be developed and perfected to meet patient's needs as opposed to generating new profits or containing health care expenditure, but this ethical argument goes beyond the aim of this thesis. For the purpose of this thesis a consideration that could be done is

that the impact of the formulation on patient's compliance can be enormous considering the possibility to decrease the number of daily administration, reduce side effects and increase the patient adherence to therapy. These goals remain strongly desirable (Langer, 1990). The driving force leading the work of researchers in the formulation field should be therefore the desire to improve patient safety, increasing patient confidence with available treatments.

1.2 Oral Controlled Drug Delivery

It is recognized knowledge that the gastrointestinal (G.I.) tract is a complex environment where medicines face extremely variable conditions.. According to Dumez, the literature concerning the practice of producing enteric capsules by hardening gelatine capsules with formaldehyde was particularly prosperous in 1914-1915 even though the production of keratin coated enteric pills has been reported earlier (Dumez, 1893). Therefore it is not surprising that the oral controlled drug release found its roots in the field of enteric coating. Coating a pharmaceutical dosage form was indeed the first approach to try to modify the release of a drug in order to administer drugs that were irritating to the gastric mucosa, causing vomit, or to mask bad tasting medicine (Martindale and Wescott, 1924). Several authors agree on the fact that the first commercially available sustained release system was produced in 1960 by Smith Kline and French Laboratories, exploiting their patented Spansule™ technology (Helfand and Cowen 1983; Florence and Siepmann 2010). The name "spansule" was a combination of the word "span", to emphasize the fact that the release took place over a span of time, and "capsule". It is interesting to note that Spansule™ technology was based on wax-fat coated sugar beads inside a capsule and the key controlling element was the waxy layer covering the beads. More

recently, the use of lipid excipients as key-controlling elements in matrix systems has been taken into consideration to formulate poorly soluble drugs in oral controlled drug delivery (Rosiaux *et al.*, 2014). A deeper insight on this topic will be given in a separate part of this work.

In 1960 Allan Hoffman founded the Alza Corporation. Some authors consider this event and not the registration of Spansule technology in 1945 the dawn of the controlled-release era (Folkman, 1990; Hoffman 2008). Regardless, in 1960, controlled release of medicines was one of the major topics of interest in the pharmaceutical field. Polymers, able to afford a more controlled and predictable release, replaced the wax-fat coatings and were exploited for the production of matrix systems, the most diffused technology for oral controlled drug delivery nowadays.

The release of drug from controlled delivery systems can be governed by diffusion, dissolution, osmotic pressure and ion exchange (Hoffman, 2008; Florence and Siepmann, 2010). In matrix delivery systems the release can also take place thanks to the erosion of the matrix, another important phenomenon (Siepmann and Peppas, 2001). More than one mechanism can be involved simultaneously, and even thought one mechanism could prevail on the other, every contribution should be taken into consideration. Alongside matrices, other different strategies have been developed to control the release of a drug from a dosage form. These include osmotic pumps and reservoir systems in which drug release is mainly governed by diffusion. In osmotic pumps a semi-permeable membrane surrounds a by-phasic core, containing the active pharmaceutical ingredient and a swelling osmotically active excipient. The controlling release mechanism is the water penetration through the semipermeable membrane, thanks to the osmotic compartment of the device. As water come inside the matrix and swells the osmotic excipient the drug in solution or suspension is pushed outside through a laser-

drilled orifice (Malaterre *et al.*, 2009). In reservoir systems a semipermeable membrane surrounds a drug-containing core, often formulated as a pellet (Palugan *et al.*, 2015). As water penetrates, a saturated solution of the drug is formed and the release takes place through to diffusion. In all these pharmaceutical dosage forms a constant drug release rate with time can often be obtained, irrespective of the release medium conditions.

1.3 Matrix Systems in Oral Controlled Drug Delivery

In more than fifty years of drug delivery technology, several approaches have been studied to obtain a controlled and predictable drug release upon oral administration. Matrix monolithic systems, containing a discrete amount of drug dispersed in a matrix-forming polymer, are the most exploited and characterized systems (Colombo *et al.,* 2009). This is due to the fact that matrix systems are easily produced by direct compression of a homogenous mixture of drugs and excipients, making the manufacturing process extremely fast, cost-effective and reproducible (Colombo *et al.*, 1996). Another reason of the popularity of matrix systems relies in the fact that they are very versatile, allowing the formulation of both hydrophilic and hydrophobic compounds. In addition, more than one polymer is often used and it is possible, modifying the polymer composition, to obtain different release profiles. Upon contact with water, polymer chains acquire a higher degree of freedom and as a result gel formation took place on the surface of the matrix. The polymer, in other words, passes from its glassy to its rubbery state after inclusion of water molecules which act like a plasticizer decreasing the glass transition temperature (T_g) of the polymer (Colombo *et al.*, 1999). Upon polymer chains hydration the volume of the matrix can increase pronouncedly, depending on the use of swelling or

not swelling polymers. As water penetrates inside the matrix the dispersed drug passes in solution and can be released by diffusion and/or relaxation and erosion of the polymer. This event strongly depends on the solubility and viscosity of the polymer and on the solubility of the drug (Bettini *et al.,* 1994). Polymers used in manufacture of matrix systems can be classified based on the physical and chemical characteristics (hydrophilic, hydrophobic, ionizable) and/or on the production process (naturally derived, synthetic, partially synthetic). Alongside cellulose derivatives other polymers can also be exploited for matrices production also in combination with cellulose derivatives. Sodium alginate, Xanthan gum, Chitosan, Guar gum, Polyethylene oxide, Acrylic acid homo- and co-polymers are some example of available hydrophilic polymers. Polyvinyl acetate and Methacrylic acid copolymers are two water insoluble polymers widely used in controlled delivery systems formulation (Maderuelo et al., 2011). It is estimated that around 70% of sustained release drug delivery systems approved by FDA contain cellulosic derivatives (Rosiaux et al., 2014). Cellulosic polymers are the most exploited because they are easily processed with the conventional method and they are generally recognized as safe (GRAS). In addition they are available in a wide range of different derivatives showing different physicochemical properties, thus allowing to choose the best one or the optimal mixture of them to reach the desired properties. Cellulose derivatives are naturally derived polymers and they include water-soluble (Hydroxypropylmethyl cellulose, HPMC, Methylcellulose, Hydroxypropylcellulose, Hydroxyethylcellulose, Sodium carboxymethylcellulose) and water-insoluble (Ethyl Cellulose, HPMC acetate succinate, Cellulose acetate, Cellulose acetate succinate) polymers.

1.4 Hydroxypropylmethyl cellulose in matrices formulation

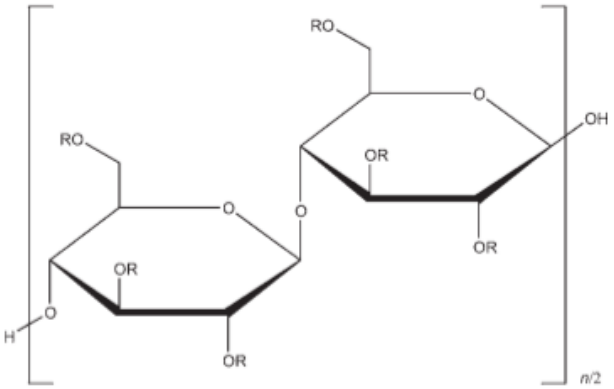


Figure 1. Chemical structure of HPMC, where R=–CH_3, –$CH_2CH(CH_3)OH$ or –H depending on the degree of substitution.

HPMC has been exploited for years in pharmaceutical product manufacturing, ranging from oral to ophthalmic, nasal and topical product formulation. HPMC can be used as binder, extended release agent, modified release agent, coating agent, bioadhesive material, dissolution enhancer, dispersing agent (Rogers, 2009).

Upon contact with water, HPMC chains hydrate and this event lower the T_g of the polymer until it reaches the experimental temperature. Once the T_g equals the experimental temperature, the polymer transition between glassy to rubbery state take place. This event is crucial because polymer chains change from a rigid and stationary state to a more relaxed and mobile state, resulting in an increase of the dimensions of the system. In a cylinder-shaped matrix, the formation of a hydrogel layer on the matrix surface is the event that controls drug release. The gel formation acts as a barrier controlling further water penetration and, thus, a boundary between the matrix core, still in a glassy state, and the rubbery gel layer surrounding the core can be observed (Colombo et al., 1995). As soon as polymer chains hydrate, they acquire enough freedom to dissolve in the dissolution medium. The erosion front separates the gel layer from the surrounding dissolution medium. Another front can be observed

between the swelling and eroding front, i.e. the diffusion front that separates dissolved from solid drug particles (Lee, 1991). This boundary is clearly visible when using a coloured drug in high (Colombo *et al.*, 1999). The relative position of these boundaries is extremely important in determining the drug release mechanism. As the release proceeds the swelling front moves inward as soon as water penetrates towards the core thus increasing the gel thickness. The movement of the erosion front is vey difficult to describe because it depends both on the core hydration and on the polymer solubility in water. The erosion front, indeed, is subjected to two different processes: the swelling of the core pushes the erosion boundary outward and the dissolution of polymer chains at the water interface works in the opposite direction. This is very important for the drug release mechanism because the polymeric swollen layer is the controlling element of drug release kinetic. Whenever we try to describe the release of drugs from HPMC matrices with a mathematical approach, the variable "thickness of the gel layer during drug release" represents a challenge in determining exact drug release rate.

In some systems, as reported by Colombo *et al.*, the swelling and eroding front can synchronize (Colombo, 2000). After an initial phase of system swelling and gel formation, the rate of water penetration into the matrix become equal to the rate of matrix dissolution, thus maintaining the thickness of the gel layer constant during a timed period of drug release. This phenomenon is known as "synchronization of the fronts". This phenomenon has been exploited to produce constant drug releasing systems (Colombo *et al.*, 1987). Nevertheless, front synchronization is more the exception then the rule and other mechanisms not related to fronts movement can be responsible of more complicate situations. Bettini *et al.* described solid drug particles translocation in HPMC matrices that turned out to be an important additive element to be

considered in mass transport through the polymeric gel layer (Bettini *et al.,* 2001). This phenomenon will be described later.

1.5 HPMC and λ-carrageenan in matrices formulation

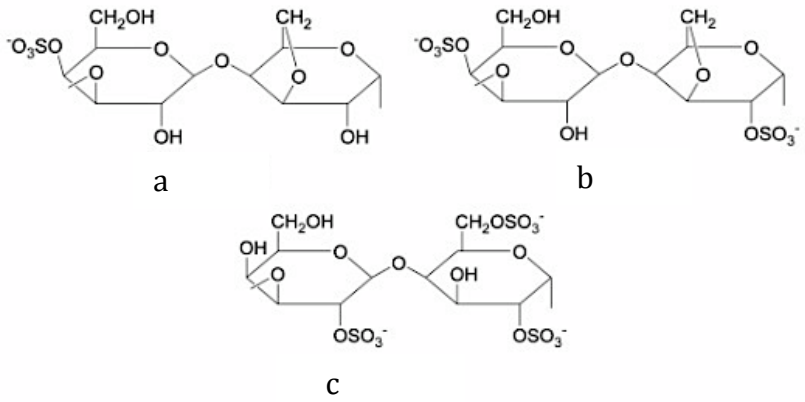


Figure 2. Molecular structure of different type of carrageenans. a = κ-carrageenan; b = ι-carrageenan; c = λ-carrageenan.

Whenever a constant drug release over time is desirable, some challenges could be encountered by using HPMC based systems. This is particularly true for freely water soluble drugs in diffusion-controlled matrices, where the initial burst effect due to the lag-time required for HPMC gel layer formation is often followed by a decrease of drug release rate due to the lower gel erosion compared with HPMC hydration (Baveja S. K., 1987). Furthermore the entity of time required for gel formation and for gel erosion is influenced by the viscosity grade of polymers used. To address this limitation, a useful solution has been found to be the formulation of matrices using a mixture of HPMC with different cellulosic and non-cellulosic pharmaceutical excipients. Mixtures of HPMC with negatively charged polymers such as sodium carboxy-methylcellulose, guar gum and carrageenans have been evaluated by Bonferoni *et al*. and have been found to afford different release profile depending on matrix composition (Bonferoni *et al.*, 1993). The possibility of introducing another element for drug release control, i.e. the ionic interaction

between positively charged drugs and anionic polymers, confers to the above-mentioned mixtures interesting additive properties.

Carrageenans are naturally occurring polymer extracted from some varieties of mosses and algae (Figure 2). These copolymers are formed by units of sulphate esters of galactose and 3,5-anhydrogalactose, alternatively linked with α-1,3 and β-1,4 bonds (Singh K. K., 2009). Carrageenans are widely used in the food industry, in cosmetics and in pharmaceutics as thickening agents listed as GRAS substance. Depending on the number of sulphate esters and the presence of 3,5-anhydrogalactose units, carrageenans are classified in three different types, i.e. λ, κ, and ι-carrageenans. λ carrageenans is a non-gelling polymer and presents the higher content of sulphate esters, three per each galactose unit, which impart a strong anionic character to the polymer, while the 3,5-anhydrogalactose residues are not present. In combination with HPMC, λ carrageenan has proven to be a suitable excipient in matrices manufacturing with the additional property of being able to afford different release rate depending on the matrix composition (Bonferoni *et al.*, 1994). The anionic strength of λ-carrageenan is responsible for the ionic interaction with basic drugs like chlorpheniramine maleate and diltiazem hydrochloride as reported by Bonferoni *et al.* and Aguzzi *et al.* (Aguzzi *et al.,* 2002; Bonferoni *et al.,* 1998). The importance of this interaction resides in the fact that by optimizing matrix composition it is possible to obtain a pH-independent drug release.

1.6 Drug release from polymeric matrix systems

Over the years, several attempts have been made to represent drug release from matrices through a mathematical approach. The importance

of obtaining a descriptive equation that consider all the processes involved in drug release, mainly drug diffusion and polymer erosion, resides in the fact that the comprehension of the underlying mechanisms helps formulators to correlate matrix composition to well defined release profiles. Unfortunately, it is not always possible to clearly identify the contribution of each physical phenomenon, and this is particularly true when more than one of them is involved.

The first equation developed to describe drug release from a matrix system was proposed by Takeru Higuchi in 1960, deriving the Fick's Second Law of Diffusion (Higuchi, 1960).

$$Q = (2A - C_s)\sqrt{\frac{Dt}{1 + \frac{2(A - C_s)}{C_s}}} \qquad (1)$$

In equation (1) Q is the amount of drug released at time t per unit of exposed area, A is the concentration of drug expressed as units/cm^3, C_s is the solubility of the drug in the external phase in units/cm^3 and D is the diffusion constant of the drug in the external phase. The Higuchi model was initially developed to describe the mass transfer in a thin planar ointment base containing a dispersed drug, and was then adapted for solid systems having different geometries (Higuchi, 1963). The Higuchi model is not always suitable to describe matrix systems, as some assumptions made to derive the Higuchi equation cannot be satisfied.

In particular:

- Drug diffusion must be one-dimensional thus allowing neglecting edge effects;

- The matrix should not swells or erodes;
- The diffusion coefficient of the drug should be constant.

The Higuchi equation is essentially useful to describe drug release when diffusion is the only mechanism involved. Considering the physical phenomena governing drug release, it is often challenging to evaluate one by one the release mechanisms individually because often more than one mechanism is involved at the same time with positive and negative effects overlapping simultaneously. This can potentially lead to cumbersome mathematical equation. For this reason, simplification had to be introduced to derive simple equations describing drug release rate. Ritger and Peppas proposed an empirical approach in 1987 to describe drug release from slabs, cylinders and spheres (Ritger and Peppas, 1987a). The Ritger and Peppas equation is reported below:

$$\frac{M_t}{M_\infty} = kt^n \qquad (2)$$

In equation (2) M_t/M_∞ is the amount of drug released at time *t*. The exponent n is the diffusional exponent, indicative of the transport mechanism involved. The equation is only valid for $M_t/M_\infty \leq 0.6$.

The importance of equation (2) resides in the fact that it can be used to fit experimental data obtaining a good understanding of the governing controlling mechanism. Nevertheless, the results should be verified and supported by strong experimental data since the Peppas and Ritger equation is quite simple but can be sometimes misleading, in particular when dealing with HPMC systems (Siepmann and Peppas, 2001). In equation (2) the value of the exponent *n* is indicative of the mechanism governing drug release. If *n* value equals 0,50, then the amount released will depend on the square root of time and the drug release will be

controlled only by Fickian diffusion, as pointed out by Higuchi (equation 1). When the value of *n* is greater than 0,50, then other mechanisms are involved and must be taken into consideration. The dependence of *n* on system geometry has been also considered. Values of *n* equal to 0,43 or 0,45 has been found to be descriptive of diffusion controlled release systems respectively from spheres and cylinders. The higher limit of the *n* exponent for non-swellable systems or swellable slabs is one and it is indicative of a Zero-Order Release (Ritger and Peppas, 1987b). In this particular case the relaxation of the polymer chains with increasing water content is the main mechanism governing drug release. This situation is known as Case II transport among polymer scientist. Values of *n* for different geometries were determined by Ritger and Peppas and are listed in Table 1.

Table 1. Values of n for systems of different geometry.

Thin Film	Cylinder	Sphere	Drug release mechanism
0,5	0,45	0,43	Fickian Diffusion
0,5<n<1,0	0,45<n<0,89	0,43<n<0,85	Anomalous transport
1,0	0,89	0,85	Case II transport

Values of *n* ranging from 0,50 and 1 are indicative of a so-called Anomalous (non-Fickian) transport. In this situation, the release is controlled both by Fickian diffusion and polymer chain relaxation and the interpretation of the relative contribution of each mechanism can be challenging. Another important mechanism involved whenever dealing with water-soluble polymers is the dissolution of the polymer in the releasing medium, a process defined as “erosion of the matrix”. Drug release in water-soluble polymers can take place also thanks to polymer erosion. It is clear then that a single term equation can result in a poor

understanding of the releasing mechanism. For this reason, Peppas and Sahlin proposed a derivation of equation (2) able to describe separately the contribution of Fickian diffusion and polymer chain relaxation to drug release (equation 3) (Peppas and Sahlin, 1989).

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \qquad (3)$$

In equation 3 the first term on the right-hand side accounts for Fickian diffusion and the second term is indicative of the Case-II relaxation of the polymer chains. Fickian diffusion is characterized by a dependence of drug released on the square root of time. From a geometrical point of view, these terms describe the shape of the release profile in the amount released vs time plot. The first term on the right-hand side accounts for a negative deviation from linearity, i.e. a decrease of amount released with time; the second term accounts for a linear contribution to the release profile, i.e. a linear dependence of the amount released with respect to time. Bettini *et al.* have successfully applied the Peppas and Sahlin equation to HPMC systems to describe the relative contribution of mechanisms governing drug release (Bettini *et al.*, 1994). Equation 3 can be considered valid, once again, only during the early stages, where $M_t/M_\infty \leq 06$.

An interesting scenario can be observed, as *n* values in equation 2 are greater than 1.00. In this case the drug release is defined as Super Case II transport and the release rate increases with time. This phenomenon is poorly characterized since it requires very complicated equations that are able to fit the experimental data and the physical laws governing this type of release can be difficult to understand. Super Case II transport has been studied in the past by Jacques *et al.* to describe the penetration of

normal hexane through polymeric films (Jacques *et al.*, 1974). A mathematical solution to describe Super Case II transport in polymeric material was not reported by Jacques *et al*. but the principle involved in determining such a non-linear dependence of penetration rate with time was proposed. It seems that the contribution of Fickian diffusion and Case II transport to Super Case II transport is essential. The diffusion of the penetrant through the glassy core of the film from the swollen region takes place thanks to Fickian diffusion. As the Fickian tail in the glassy core reaches the medium axis of the film from both surfaces, the relaxation process in the core is accelerated thus leading to an increase of the contribution of Case II transport. This process is exacerbated by the swelling stress of the outer swollen layer. It seems therefore that the assumption of a constant Fickian and non-Fickian contribution to the penetration of solvent in glassy polymer networks cannot be applied to Super Case II transport because the relative contribution changes over time. Moreover, Super Case II transport has been studied for polymers like polystyrene, which swell only to a limited extent compared to HPMC systems. An in-deepth understanding of the Super Case II transport in HPMC systems is probably difficult to reach considering the simultaneous overlapping of several different phenomena which would require cumbersome mathematical equation. Bettini *et al.* reported an interesting phenomenon, which can be seen as a Super Case II transport in HPMC matrix systems (Bettini *et al.*, 2001). This work reported the increase of drug release rate with time for poorly soluble drugs. The explanation of this event was attributed to the disappearance of the glassy core and to the increased erosion of polymeric chains while solid undissolved drug particles were still present. In particular, the increase of drug release rate can be observed only with poorly soluble drugs, where the erosion mechanism for drug release is more important.

Several other mathematical approaches have been proposed over the years to describe drug release from matrices. The Hixson and Crowell model, the Weibull model, the Hopfenberg model, the Baker-Lonsdale model, the Brazel and Peppas model are just some of the methods proposed to describe drug release depending on the shape and geometry of the releasing system (Hixson and Crowell, 1931; Katzhendler *et al.*, 1997; Baker and Lonsdale, 1974; Brazel and Peppas, 2000; Siepmann and Peppas; 2008; Grassi and Grassi, 2005).

Considering the choice of a suitable model to describe drug release from controlled delivery systems, one should take into consideration the limitation of each model, the characteristics of the system to be described and the information expected from the application of the model. Finally, it seems useful to underline that the model chosen must be able to fit the experimental data with a strong correlation supported by a statistical analysis.

1.7 Lipid excipients in oral controlled drug delivery

Despite the fact that lipid excipients have been used for years in pharmaceutical dosage forms like ointments, suppositories, creams and emulsion, their importance and suitability in controlled release dosage forms manufacturing increased quite recently. This can be attributed to:

- Increased availability of chemically modified lipids: The esterification of fatty acids with several different compounds like glycerol and polyols in general, allows to obtain lipid excipients characterized by different HLB (Hydrophobic-Hydrophylic Balance) and different melting point (Weiner, 2001);
- Reformulation of already registered drugs: The interest of pharmaceutical companies in finding new strategies to preserve revenue from drug whose patent expired and the challenging formulation of poorly soluble drugs, have been the driving forces for the development of different strategies and technologies based on innovative excipients (Rosiaux *et al.*, 2014).
- Increasing interest in new manufacturing technologies: Increasing attention has been devoted to cost-effective processes to produce innovative drug products. The strong versatility of lipid excipients allows formulators to choose between several processing technologies without the restrictions imposed by other excipients (Ozyazici *et al.*, 2006).
- Regulatory requirements: An increasing number of efforts have been made in the research of inert excipients, which can be employed in pharmaceutical dosage form production without safety concerns. The safety profile of natural lipid excipients is well characterized considering their employment in food production, and several naturally derived lipids are GRAS listed excipients (Sandeep *et al.*, 2013).

Lipids are a heterogeneous class of molecules, widely diffused in eukaryotic and prokaryotic cells, characterized by different chemical structure and physical properties. According to Fahy *et al.*, which proposed an exhaustive classification of lipids, this class of molecules can be synthesized in part or completely by condensation of thioesters and/or isoprene units (Fahy *et al.*, 2005). The classification of Fahy *et al*. divided lipids in different classes, i.e., fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides with a total of eight primary classes. The interesting aspect of this classification is that it allows the characterization also of synthetically derived lipids.

Among lipids exploited in pharmaceutical technology, for the topic treated in this thesis, those defined as "solid lipid excipients" are the most interesting. Solid lipid excipients have been used for years in pharmaceutical field primarily as lubricants in manufacturing processes where a good flowability of the powder and a reduced adherence to surfaces is required (i.e. tablets production). Their popularity in matrices production as controlling release elements is on the other hand quite recent. The most interesting feature of this class of excipients is the high process flexibility when compared to polymeric materials. Solid lipid excipients can be easily processed with "cold" techniques like direct compression and with "hot" techniques like hot melt extrusion thus conferring to the final product, depending on the process employed, specific features. The use of solid lipid excipients in tablet production is particularly convenient because they can act as binders, lubricants and controlling release elements at the same time (Rosiaux *et al.*, 2014). The rationale beyond the use of lipids in oral controlled drug delivery is not only limited to technological processing.

The evidence that lipids quantity as low as 2 grams can positively influence the absorption and disposition of poorly soluble drugs opened new perspective (Kossena *et al.*, 2007). The latter evidence, along with the possibility of obtaining floating systems retained longer in the stomach, can be useful to prolong and increase drug release (Shimpi *et al.*, 2004).

Another interesting application of lipid excipients in oral drug delivery is the achievements of higher bioavailability. The evidence that some drugs are likely to be absorbed by the lymphatic system following the chylomicrons route when administered with food is universally accepted (Porter et al., 2007). The absorption through the lymphatic system entails the bypassing of the liver metabolism thus increasing oral bioavailability. Unfortunately this mechanism of absorption is not only poorly characterized but it is also limited to drugs having precise physic-chemical properties. According to Charman *et al*. a drug should have a $LogD_{7,4}$ value of 5 or more, and a solubility in long chain triglycerides of 50 mg per g (Charman *et al.*, 1986). An interesting application of this concept is the esterification of methylnortestosterone with undecanoic acid forming a pro-drug that is absorbed through the lymphatic system when taken with food, thus increasing the bioavailability of methylnortestosterone by 50 times (White et al., 2009). DDT (Dichlorodiphenyltrichloroethane) is proven to follow the lymphatic route when ingested as well (Sieber *et al.*, 1974).

Among the materials used in the production of the hybrid matrices described in this thesis, Compritol®888 ATO is a solid lipid excipient. Glycerides include a large number of esters of glycerol widely employed in formulation. Compritol® is a mixture of mono- di- and tri-glycerides of behenic acid, a fatty acid consisting of a chain of 22 atoms of carbon. The

percentage composition and the main properties of Compritol® are listed in Table 2.

Table 2. Percentage composition and main properties of Compritol®888 ATO as reported by Mona and Badr-Eldin. (Mona and Badr-Eldin, 2014)

Monoglycerides	12-18% w/w
Diglycerides	45-54% w/w
Triglycerides	28-32% w/w
Melting point	69-74°C
HLB	≈2

Glycerilbehenate esters are GRAS-listed substances by regulatory authorities (FDA 21CFR §184.1328 GRAS; USP/NF, EP, and JSFA compliance; DMF n. 4663) and are widely employed in foods, pharmaceuticals and cosmetics manufacturing as emulsifier; in tablets and capsules manufacturing they are used as formulation adjuvants for their lubricant, stiffening, brightening and binding properties (Ash M., Ash I., 2008). The use of glyceride bases in extended release dosage form production gained importance in the last 30 years (Sutananta et al., 1995; Barthelemy, 1999). The suitability of Compritol® for direct compression processes and hot melt extrusion techniques contributed significantly in spreading its popularity among pharmaceutical companies and researchers. Compritol® has ben successfully employed for the production of different controlled release formulations such as nano- and microparticles (Nastruzzi, 2005), matrix tablet systems, (Gu *et al.*, 2004; Li *et al.*, 2006) and hot melt extruded matrices and pellets (Liu *et al.*, 2001).

1.8 Statins and statins properties

Several epidemiological and clinical studies underlined the pivotal role of high lipids levels in the blood for the pathogenesis of potentially lethal cardiovascular diseases. Cardiovascular diseases (CVDs) are a group of clinical conditions including, among others, coronary heart disease, peripheral arterial disease and cerebrovascular disease. According to the World Health Organization, coronary heart disease and stroke are estimated to be responsible respectively of 7,4 and 6,7 million of deaths in 2015, being the deaths reconducible to CVDs the 31% of all global deaths (http://www.who.int/mediacentre/factsheets/fs317/en/).

Cholesterol is a fundamental structural lipid of the cells membrane but its function is not limited to this important role. From the same biosynthetic pathway used to synthesize cholesterol, i.e. the mevalonate pathway, several important biomolecules like vitamin D, hormones and bile salts are synthesized in specific cells (Goldstein and Brown, 1990). Cholesterol is also involved in some severe pathological conditions. High cholesterol and triglycerides levels in the blood can have a genetic aetiology like in the familial hypercholesterolemia and hypertriglyceridemia but can also be the results of high lipids containing diet, hypertension and diabetes. Other related risk factors for developing hypercholesterolemia are physical inactivity, tobacco use, obesity and alcohol abuse. As a result, the first approach to treat non-genetic hyperlipidaemia often is the modification of dangerous life habits. When the latter did not result in decreasing cholesterol levels then a pharmacological intervention is required.

Statins have been used for decades as first line treatment to successfully lower cholesterol levels in the blood and eventually the risk of CVDs (Gelissen and McLachlan, 2014). In 1970 Akira Endo hypothesized that cholesterol levels in the blood could be reduced by inhibiting its

biosynthesis in the liver and observed that some bacteria were able to synthesize compounds to self-defend against other microbes, which require mevalonate pathway related products for their growth (Endo, 2004). This successful observation culminated in the discovery of ML-236B, today known with the name mevastatin, a powerful inhibitor of cholesterol biosynthesis in human cells (Endo, 1976). Endo *et al.* elucidated the mechanism of action of mevastatin in 1976, attributing the powerful hypocholesterolemic effect to the competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), a key enzyme in cholesterol biosynthesis in cells (Endo *et al.*, 1976). After the discovery of mevastatin, which was never commercialized, several other statins were developed for the patients at high risk of cardiovascular diseases, i.e. lovastatin, simvastatin, fluvastatin, atorvastatin, rosuvastatin, pravastatin and the latest one pitavastatin. Cerivastatin was withdrawn from the market in 2001 because of severe side effects like kidney failure and rhabdomyolisis (Furberg and Pitt, 2001). Despite differences in physico-chemical properties of the drugs belonging to this class, they are all characterized by the presence of a β-hydroxy carboxylic acid that mimics the natural substrate of HMG-CoA reductase. The conversion of the β-hydroxyl carboxylic acid in a closed lactone structure is also a difference among statins. The closed lactone is converted to the acid form *in vivo*, and, according to Hoffmann and Nowosielski these two forms are coexisting in equilibrium *in vivo* (Hoffmann and Nowosielski, 2008). The acid form of statins is very similar to 3-hydroxy-3-methylglutaryl coenzyme A, the natural substrate of the enzyme, but it cannot be converted into mevalonate and hence the inhibition occurs. Once mevalonate synthesis, a key step in cholesterol biosynthesis, is inhibited, the expression of receptors for LDL cholesterol in the liver increases and LDL clearance in the liver becomes more

important. This is a key event in hypercholesterolemia treatment since high blood levels of LDL cholesterol, also known as “bad cholesterol”, are universally accepted to be directly correlated to the risk of developing CVDs, while the opposite is true for HDL cholesterol, also known as “good cholesterol”. Cholesterol and other lipids cannot be transported freely in the blood due to their low solubility in water. They have to be included in higher structure to mask their hydrophobicity. Lipoproteins are a class of carriers responsible for lipids transportation in the blood. Lipoproteins are classified in reason of their density, composition and structural proteins (apoproteins) in chylomicrons, chylomicrons remnants, intermediate density lipoproteins (IDL), high-density lipoproteins (HDLs), low-density lipoproteins (LDLs) and lipoprotein A (Lp(a)) (Feingold and Grunfeld, 2015). High LDL cholesterol in the blood is involved in the genesis of atherosclerosis, a modification of the arteries walls that implies the formation of rich lipids and macrophages plaques that narrows the arteries diameter. The rupture of plaques, resulting from mechanical stresses, can lead to stroke and heart failure. It is accepted that the oxidation of circulating LDLs makes this lipoproteins particularly likely to be captured by macrophages in the artery plaques (Steinberg *et al.*, 1989). Therefore it is of great utility to decrease the levels of circulating LDLs to reduce CVDs. Also triglycerides have been proved to be involved in the incidence of CVDs (Hokanson and Austin, 1996). The ability of statins in reducing circulating LDLs is not the only effect reducing the risk of CDVs. Statins proved to be also able to reduce triglycerides level and increase HDL cholesterol. Furthermore it seems that other effects, aside of those cited above, could contribute to the overall benefits of statins treatment (Davignon, 2004). These effects are less characterized and seem to be not related to the inhibition of HMG-

CoA. The improvement of endothelial dysfunction is recognized to be an important positive effect of statins treatment. This is the result of:

- Reduced inflammation and oxidation of endothelial cells;
- Increased bioavailability of nitric oxide, an important messenger in the vasodilatation of arteries;
- Plaque-stabilizing effects;

All these benefits can be observed also at low statins dose and seem to be class-related and not compound-related effects.

Another interesting property of statins seems to be the anti-carcinogenic effect, even thought not well characterized. It seems that only for prostate aggressive cancer there is evidence enough for the preventive effect of statins (Boudreau *et al.*, 2010).

Statins are generally well-tolerated drugs and the risk *vs* benefit is generally considered favourable (Stone *et al.*, 2014).

1.9 Statins side effects and patient compliance

The treatment of hypercholesterolemia with a statin drug generally allows good outcomes even thought the deficiency of patient compliance is one of the main obstacle to therapy success. The poor patients compliance can be attributed in several cases to the side effects typically associated with statins treatment, mainly myotoxicity. Myotoxicity includes different pathological conditions characterized by muscle pain with or without creatin-kinase (CK) blood level elevation. Myalgia, according to Baer and Wortmann, is defined as muscle ache without CK increase, myositis is defined as muscle inflammation with elevation of CK blood level and rabhdomyolisis is characterized by myalgia and myositis associated with renal insufficiency (Baer and Wortmann, 2007). Rabhdomyolisis is a rare adverse effect attributed to the interference of statins with the muscle metabolism. It leads to muscle cells massive

disintegration. Rabhdomyolisis can be potentially fatal. Death may occur because of kidney failure induced by huge amounts of myoglobin reaching the kidney after the muscle cells membrane rupture. The incidence of rabhdomyolisis in two cohort studies has been determined to be 3.4 (1.6 to 6.5) per 100,000 patients/year, with an associated fatality of 10% (Law and Rudnicka, 2006). The underlying mechanism of muscle toxicity has not been clearly understood yet, but it seems that more than one factor is involved. Membrane excitability, mitochondrial dysfunction, ubiquinone depletion, calcium homeostasis alteration, apoptosis induction and genetic determinants are all mechanisms that participate in statin-induced muscle toxicity (Tomaszewski *et al.*, 2011). The event that triggers muscle cells apoptosis is the activation of caspases due to the intracellular Ca^{2+} increase and ATP depletion. Caspases are intracellular proteins responsible of activating the pro-apoptotic pathway. Other side effects include the increase of hepatic transaminase, cognitive disorders and increased incidence of type II diabetes mellitus. These side effects were rare in clinical trials and can be observed often in patients receiving more than one medicine (Thompson et al., 2016). Multi-therapy is a commonly occurring scenario, considering the success of combination of more than one drug, a choice that needs to be considered with caution whenever two or more drugs share the same enzymatic system or the same target. The withdrawal of Baycol, the association of Cerivastatin and Gemfibrozil, responsible of 52 deaths in USA for rhabdomyolisis-related kidney failure, strongly remarked the importance of evaluating additive and cumulative effect of combined therapies (Furberg and Pitt, 2001). This is particularly true for the CVDs prevention and treatment, since more than one pharmacological approach is often required (Gradman et al., 2010).

1.10 Controlled release of statins

Considering the high potency of statins in reducing cholesterol and triglycerides levels in the blood and the lack of good patient compliance for the above-mentioned reasons, different strategies should be considered to improve patient's adherence to therapy. One the main target of controlled release formulations is to reduce side effects of therapy to increase patient compliance. As reported by Petyaev, the active targeting to the liver, where statins are expected to act, could be a promising strategy to decrease systemic exposure and eventually side effects in the muscle cells (Marzo, 2007; Petyaev, 2015). Considering that there is evidence showing that statin-related side effects are dose-dependent, that these effects are directly related to systemic exposure and that statins undergo a massive first pass effect metabolism, a controlled release dosage form for statins, able to release the active pharmaceutical ingredient in a controlled manner without saturating the enzymatic capacity of the liver, could be an ideal strategy to achieve the active targeting suggested by Petyaev. Unfortunately, there are few controlled release formulations of statins currently available. Lescol®XL and Altoprev® are controlled drug delivery systems available in U.S.A. for the administration of fluvastatin and lovastatin respectively. Only Lescol®, a prolonged release formulation of fluvastatin is available in Italy. As underlined by the Global Dyslipidemia Drugs Market 2017-2021 report (March 2017, TechNavio) the future trends in the therapy of lipids disorder will be devoted to the development of lipid-lowering agents characterized by a safer profile.

2. Aim of the project

Taking into consideration the problems of patients compliance related to the statins side effects, the starting point of the present thesis work has been the assumption that a suitable controlled release formulation for the oral delivery of statins would be of great value to improve the efficacy of the clinical therapy. In fact, the myopathy associated with the statins treatment has been attributed to both patient characteristics (age, gender, renal and hepatic functionality, diet) and statins properties (lipophilicity, high absorption, limited protein binding). The combination of these two aspects eventually may lead to patient drop out from therapy.

Given these premises, an ideal dosage form for statins administration would be characterized by a drug delivery rate and subsequent intestinal absorption rate perfectly synchronized with the drug hepatic uptake and metabolism. In this way, the systemic exposure to the statin would be minimized or even made equal to zero. Thus, side effects related to statin plasma concentration would be avoided and patient compliance increased.

Therefore, the aim of the present doctorate project was to design and develop innovative formulations for controlled oral delivery of high doses simvastatin taken as a model compound of this class of drugs and characterized by high lipophilicity and good permeability.

The project was carried by investigating ternary hybrid matrices constituted of hydrophilic polymers and a solid lipid excipient.

An additional goal was the investigation of the mechanism controlling drug release from such ternary systems, which has obtained little attention in the available literature.

A further aim has been the study of the suitability of hot melt extrusion as a technique for large-scale production of the matrices here developed.

This final part of the project has been carried out at the University of Toronto (Canada) under the supervision of Prof. Ping I. Lee.

3. Materials and methods

3.1 **Materials**

Simvastatin, batch c*20070435, (Polichimica srl, Italy);

Compritol® 888 ATO, batch n° 3884, was kindly donated as sample by Gattefossè SAS, France;

Methocel® K15 (HPMC) batch n° MM91012912K, was kindly donated by Colorcon Limited (United Kingdom);

Viscarin® PH 209 (λ-carrageenan) batch n°30610141, was kindly donated by FMC biopolymers, (United Kingdom);

Vegetable magnesium stearate, batch n°C1402005, was purchased from ACEF spa, Italy.

Sodium phosphate monosodic, batch n°F1450002, ACEF spa, Italia;

Sodium dodecyl sulphate, batch n° BCBM0747V, Sigma Aldrich, Germany;

Sodium hydroxide pellets batch n° 15480604 (Scharlau, Spain).

Sinvacor 40 mg tablets, MSD ITALIA Srl, batch n° E000421.

Novum SLE Tubes 3cc cartridges for liquid exctraction, sample volume (before dilution) 200 µl, recommended elution volume 1.8 ml., batch: S15-002716 (Phenomenex, USA);

Purified water (0.055 µS/cm, TOC 1ppb) was obtained with a Purelab pulse + Flex ultra-pure water (Elga Veolia, Milan, Italy);

Ammonium Acetate, batch n° 0405214 (VWR International, France);

Metanol HPLC grade, batch n° 15K060512, (VWR Chemicals, France);

Acetonitrile HPLC grade, batch n° STBG0319V, (Sigma- Aldrich, Poland);

Acetone analytical grade, batch n° 15F250939, (VWR Chemicals, Belgium);

Ethyl acetate HPLC grade, batch n° 12L140508, (VWR Chemicals, France);

Hydrochloric acid 37%, batch n° 11K180511 (VWR International, France);

Formic acid 85%, batch n° 21570, (Sigma-Aldrich, Germany);
Chloroform analytical grade, batch n° SZI3D3310V, (Sigma Aldrich, USA);
High purity standards (>98%) of Simvastatin and Lovastatin were purchased from Vinci Biochem, Italy.

3.2 Methods

DSC measurements on simvastatin:Compritol® binary mixture

DSC measurements were performed using an Indium calibrated DSC 821e instrument (Mettler Toledo, USA) driven by STARe software. DSC traces were recorded by placing accurately weighed quantities (5-10 mg) of powder sample in a 40 µL aluminium pan that was then sealed and pierced twice. Scans were performed between 25 °C and 200 °C at a scanning rate of 10 °C min^{-1} under a purging nitrogen atmosphere (100 mL min^{-1}).

DSC measurements on simvastatin:Compritol® hot melt extruded powders

DSC measurements were performed between 25 °C and 200 °C at a scanning rate of 10 °C min^{-1} under a purging nitrogen atmosphere (50 mL min^{-1}) using a TA Instrument Q200 DSC (USA) driven by a Universal V3.9A software (TA instruments). Samples were prepared placing accurately weighed quantities (5-10 mg) of powder sample in a 40 µL sealed aluminium pan.

Simvastatin *in vitro* dissolution tests

Simvastatin UV detection

The concentration of unknown simvastatin samples was determined by measuring the absorbance at 231 nm with a UV spectrophotomer (Lambda 25, Perkin Elmer Milan, Italy). The optimal wavelength was

determined by measuring the UV absorbance of a simvastatin solution in the range 200-400 nm. The calibration curve for the quantification of simvastatin in solution was prepared by measuring the absorbance of a series of samples of known concentration. Samples were prepared by dissolving an accurate amount of simvastatin in acetonitrile and diluting the solution thus obtained with phosphate buffer at pH 6.8 in order to obtain solutions of simvastatin concentration ranging from 0.25 and 20 μg mL^{-1}. The buffer used for the preparation of the diluted solutions contained 0.25% w/v of sodium dodecyl phosphate (SDS). The calibration curve is reported in Figure 3. It can be observed a good correlation between the simvastatin concentration and the relevant solution absorbance (R^2 = 0.9998).

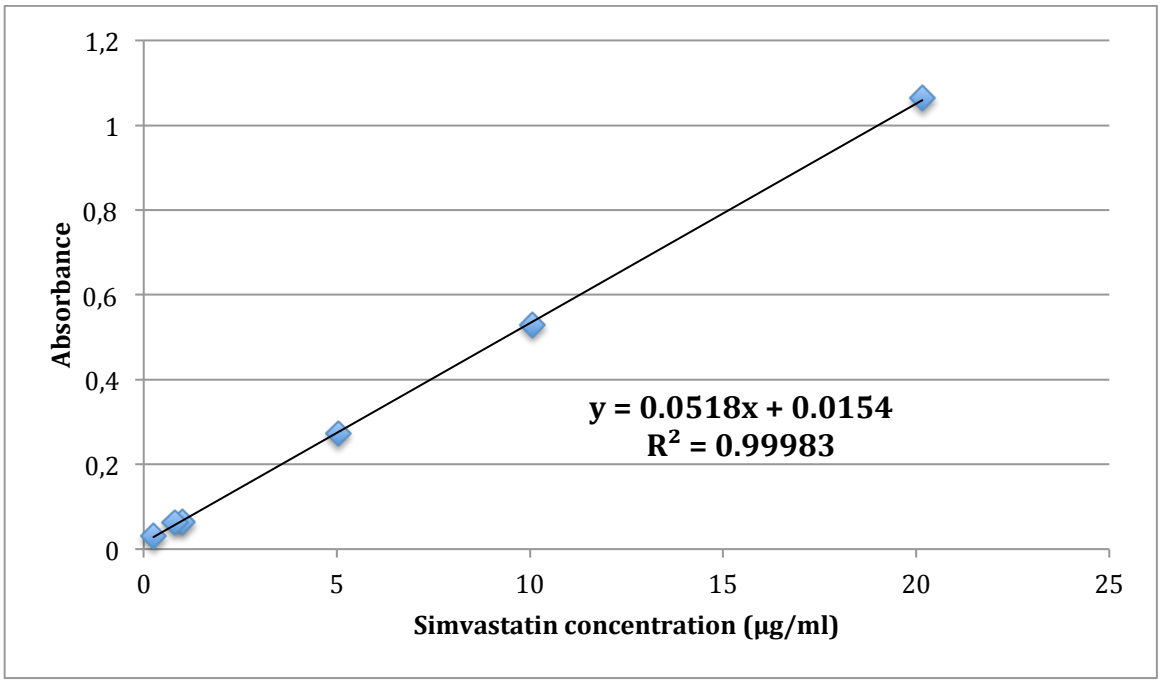


Figure 3. Simvastatin calibration curve in phosphate buffer pH 6.8 containing 0.25% v/v of SDS. The absorbance values reported are the medium of ten consecutive readings, obtained automatically by the Lambda 25 software.

LOD (Limit of Detection):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not quantified with an acceptable accuracy.

According to ICH Harmonised Tripartite Guideline on Validation of Analytical Procedures LOD can be determined as:

$$LOD = \frac{3.3 * \sigma}{b} \qquad (4)$$

where

σ is the residual standard deviation of a regression line, obtained from analysis of variance of the calibration curve;

b the slope of calibration curve.

LOQ (Limit of Quantification):

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

LOQ was determined from the equation:

$$LOQ = \frac{10 * \sigma}{b} \qquad (5)$$

where

σ is the residual standard deviation of the regression line, obtained from the analysis of variance of the calibration curve;

b the slope of calibration curve.

The computed LOD and LOQ were 0.043 and 0.144 μg mL^{-1} respectively.

Simvastatin solubility in phosphate buffer pH 6.8 was calculated by placing an excess amount of simvastatin in 10 ml of phosphate buffer pH 6.8, prepared according to the Second Supplement to USP 35-NF 30. The

samples (n=3) were left equilibrating overnight in a water bath maintained at 37°C by a heater M900-TI Basic (MPM Instruments, Italy). Thereafter the samples were centrifuged at 3000 rpm (Medifuge centrifuge, Heraeus Sepatech, Germany) for 15minutes at ambient temperature, then left equilibrating again in the water bath at 37°C for two hours. The supernatant was then withdrawn from each vial, diluted with phosphate buffer and its concentration determined with the UV method described above. The solubility of simvastatin in phosphate buffer pH 6.8 was 0.03 ± 0.002 μg mL^{-1}. Aiming to test 40mg simvastatin tablets in 500 mL^{-1} of buffer, resulting in a concentration of 80 μg mL^{-1}, the solubility of simvastatin should be 10 times the experimental concentration in the vessel, *i.e.* 0.8 mg mL^{-1}, to assure sink condition. This value is very far from the value observed and because of that SDS was added to the buffer to increase simvastatin solubility. The amount of SDS necessary to assure sink condition was determined experimentally by measuring in triplicate the solubility of the active ingredient in buffer containing 0.25 and 0.5 SDS w/v, considering the amount of SDS usually used in dissolution testing which is 0.1 to 3 % w/v (Zhao et al., 2004). The solubility values were plotted with respect to SDS percentage (fig. 4), to determine the exact amount of SDS to be added.

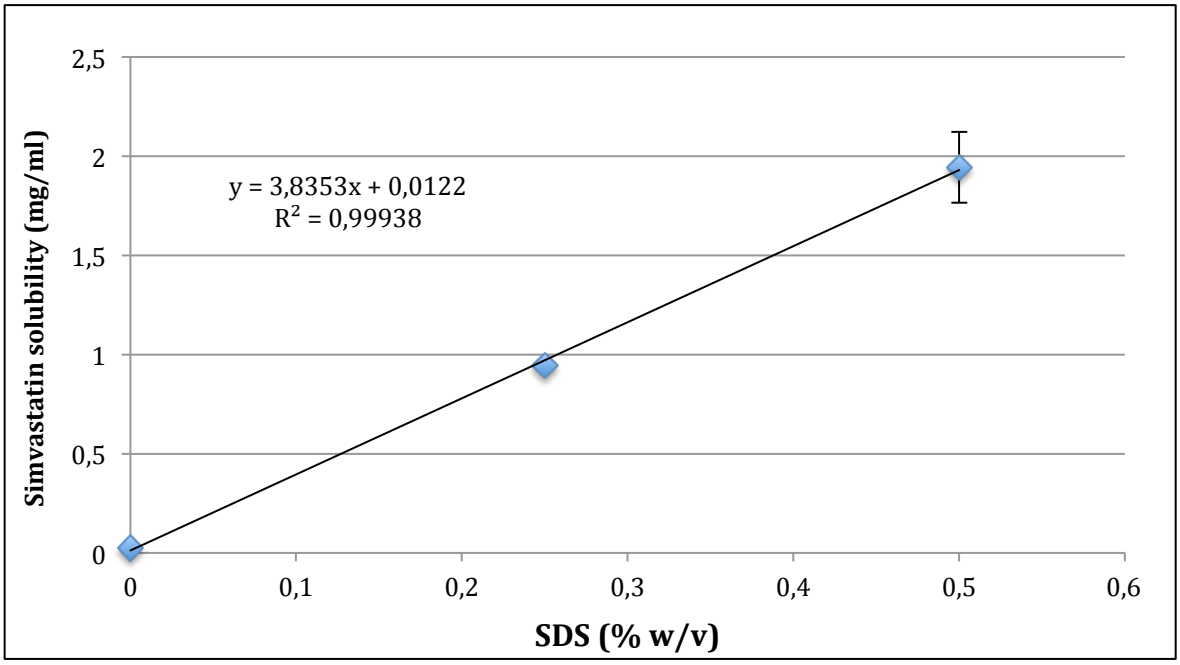


Figure 4. Simvastatin solubility values in presence of increasing SDS amounts.

The solubility of simvastatin in phosphate buffer pH 6.8 containing 0.25 and 0.5% w/v SDS was calculated to be 0.94 ± 0.02 and 1.94 ± 0.18 mg mL^{-1} respectively, and a good linearity can be appreciated in Figure 4 (R^2=0.9994). The amount of SDS required to obtain sink condition in the dissolution vessel was therefore calculated according to the buffer volume, *i.e.* 0.15 % w/v for 750 ml and 0.25 w/v for 500 ml.

Simvastatin tablets production

Simvastatin tablets containing the physical mixture of the active ingredient and excipients or the powder obtained by the fusion method were produced with direct compression. To obtain the powder by the fusion method, 2 g of Compritol® were placed in a 100 mL beaker in a heating bath (BM4, Falc Instruments, Italy) set at 85 °C. After Compritol® fusion was completed, 2 g of simvastatin were added under gentle stirring. The suspension thus obtained was cooled gradually to assure the formation of the stable glyceride beta form (Pattarino *et al.*, 2014) and once ambient temperature was reached the mass was grinded in a

mortar and sieved to select a particle size between 125 and 300 μm. Simvastatin content in this powder was measured and resulted to be 42.9 ± 2.5 % w/w.

The lower than expected (50%) value of simvastatin content in the mixture was explained by taking into account the loss of active ingredient as a consequence of its partial sticking on the wall of the container.

To produce simvastatin tablets (12 tablet batch size), simvastatin and excipients were weighed separately and placed in a plastic container then loaded in a Turbula® mixer (WAB, Basilea, CH). Tablets containing fusion method powder were produced mixing with the other excipients the amount of fusion method powder required to ensure 40 mg simvastatin dose in each tablet. Magnesium stearate 1% w/w was added to the mixture as lubricant. The powder was mixed for 30 minutes to obtain a homogenous mixture. The mixture was then compressed by applying a force of about 15 kN, using an EKO single punch tableting machine (Korsh AG, Germany), equipped with 7 mm diameter, cylindrical, flat faced punches.

The composition of the tablets produced is reported in Table 3.

The active content of the produced tablets was determined by grinding three tablets in a mortar, transferring three aliquots of about 80 mg of the grinded powder in a 100 mL volumetric flask with acetonitrile and sonicating for 5 minutes each specimen prior to add acetonitrile to made the solution to volume.

Table 3. Composition of hybrid matrices produced with direct compression

	F1	F2	F3	F4	F5
Simvastatin (mg)	40	40	40	40	40
Compritol® (mg)	40	40	40	40	40
HPMC (mg)	80	60	40	20	
λ-carrageenan (mg)		20	40	60	80
Magnesium Stearate (mg)	1.6	1.6	1.6	1.6	1.6

	F6	F7	F8	F9	F10
Simvastatin- Compritol® (Fusion Method) (mg)	80	80	80	80	80
HPMC (mg)	80	60	40	20	
λ-carrageenan (mg)		20	40	60	80
Magnesium Stearate (mg)	1.6	1.6	1.6	1.6	1.6

The dissolution rate of simvastatin from simvastatin raw material, simvastatin-Compritol® powder obtained by fusion method and simvastatin-Compritol® physical mixture was determined *in vitro* with an USP Apparatus II (Varian 750 DS, Varian Inc., USA) equipped with paddles rotating at 100 rpm in 750 ml of phosphate buffer pH 6.8 containing 0.15% w/v SDS. At predetermined time points an aliquot of the dissolution medium was withdrawn, filtered (35μm) and sent to the spectrophotometer through an auto sampling peristaltic pump (Ismatec IPC High precision multichannel dispenser, Ismatec, Germany) in order to quantify the dissolved active ingredient.

Similar *in vitro* dissolution tests were performed on the 10 types of prepared tablets using a basket apparatus (Apparatus I, Varian 750 DS, Varian Inc., USA) set at 100 rpm. The volume of vessels was adjusted to 500 ml of phosphate buffer pH 6.8 containing 0.25% w/v SDS for tablets testing to reduce chemicals and water waste in the screening phase.

Simvastatin tablets *in vivo* testing

Simvastatin HPLC-MS method development

Simvastatin concentration in rat plasma samples was determined using a liquid chromatographic system interfaced with a triple quadrupole mass spectrometer (LC-MS-MS). This technique was chosen because of its sensibility to very low concentration of the analytes. The method for the quantification of simvastatin in plasma samples with LC-MS/MS system was developed by modifying the to methods reported by Barrett and Apostolou (Barret *et al.*, 2006; Apostolou *et al.*, 2008).

Chromatographic conditions

The chromatographic separation was performed with a reverse phase partitioning method in a Synergi 4u Fusion C18 100 Å (50 x 2 mm, 4 μm) column, operated on an Agilent 1200 series HPLC system (Agilent, CA, USA) equipped with binary pump, degasser and refrigerated (8 °C) auto sampler. The mobile phase was a mixture of 5mM ammonium acetate aqueous solution adjusted to pH 4.5 (eluent A) and acetonitrile (eluent B), added with 0.1% v/v formic acid to obtain a good ionization of the analyte, as reported by Carlucci *et al.* and Nováková *et al.* (Carlucci *et al.*, 1992; Nováková *et al.*, 2008). The mobile phase was pumped at 200μLmin^{-1} constant flow, according to the a gradient elution reported in Table 4.

Table 4. Mobile phase v/v percentage composition for the detection of simvastatin

Step	Time (min)	A (%)	B (%)
0	0.00	70.0	30.0
1	1.00	70.0	30.0
2	1.50	10.0	90.0
3	6.00	10.0	90.0
4	6.50	70.0	30.0
5	10.00	70.0	30.0

Mass Spectrometry

Analyte revelation and quantification was performed on a triple quadrupole API 150EX mass spectrometer equipped with a TurboIonSpray source (ABSCIEX, MA, USA); the instrument was controlled by Analyst software 4.1 (ABSCIEX, MA, USA).

The electrospray interface parameters were set as follows:

Source temperature, 350°C;

Capillary voltage, 5.5 kV;

Curtain gas: Nitrogen: 10 a.u.;

Collision Gas (CAD): Medium;

Ion Source Gas 1 (GS1): 20.0 a.u.;

Ion Source Gas 2 (GS2): 20.0 a.u.;

Selective reaction monitoring was exploited to monitor the mass transition of simvastatin m/z 419.200 (Q1) –> 199.100 (Q3), time = 50ms.

Simvastatin extraction from plasma samples

Solid phase extraction (SPE) and liquid-liquid extraction (LLE) were evaluated as suitable methods for simvastatin extraction from plasma.

The efficiency of the extraction was evaluated in order to choose the most effective method.

Solid phase extraction

SPE was performed using Phenomenex Novum SLE extraction cartridges. The cartridge was conditioned with 1.5 mL of methanol and 1.5 mL of aqueous 100mM ammonium acetate solution pH 4.5 prior to sample loading. Samples were prepared by diluting 100 µL of blank plasma spiked with a dimethyl sulphoxide solution of the analyte, with different volumes of 100mM ammonium acetate pH 4.5 or formic acid 0.1% aqueous solution. 400 µL of sample thus obtained, were loaded on the cartridge and washed with 1ml of water and methanol 95:5 v/v. The sample was eluted in a glass vial with 1.8 mL of acetone, dried under a nitrogen flow and reconstituted with a variable volume of a 50:50 v/v mixture of acetonitrile and 100mM ammonium acetate solution pH 4.5.

Liquid-liquid extraction

LLE was performed on samples prepared in Eppendorf® safe lock tubes, spiking blank plasma with dimethyl sulphoxide solutions of the analyte, and diluting the samples with different solvents, namely:

- acetonitrile;
- methanol;
- 0.1M HCl;
- 5:1 v/v acetonitrile:0.1M HCl;
- 5:1 v/v methanol:0.1M HCl;
- 5:1:0.08 v/v/v methanol:0.1 M HCl:formic acid;
- 100mM ammonium acetate aqueous solution pH 4.5.

The diluted samples were mixed with the extraction solvent (chloroform or ethyl acetate) with a Vortex mixer and centrifuged at 15000 rpm for 7

minutes in a micro-centrifuge (Scilogex D3024). The supernatant was withdrawn and transferred in another Eppendorf® and the extraction procedure was repeated twice on the precipitate. The supernatants were dried under nitrogen flow and reconstituted with 100 µL of methanol:formic acid 100:0.1 v/v.

LLE using chloroform as extraction solvent was selected as the most efficient method for the extraction of simvastatin from plasma, considering the good recovering value even at low analyte concentrations (112 ± 18% and 65 ± 6%, at concentrations of 50 ng/ml and 10 ng/ml respectively)

LC-MS/MS system suitability

To assess the suitability of the method developed a calibration curve was constructed. Briefly, five plasma blank aliquots were treated with the LLE method described above and simvastatin aliquots where added after reconstitution in order to obtain simvastatin solution of concentration ranging from 5 to 100 ng mL^{-1}). Final samples volume was 100 µL. The calibration curve is reported in Figure 5.

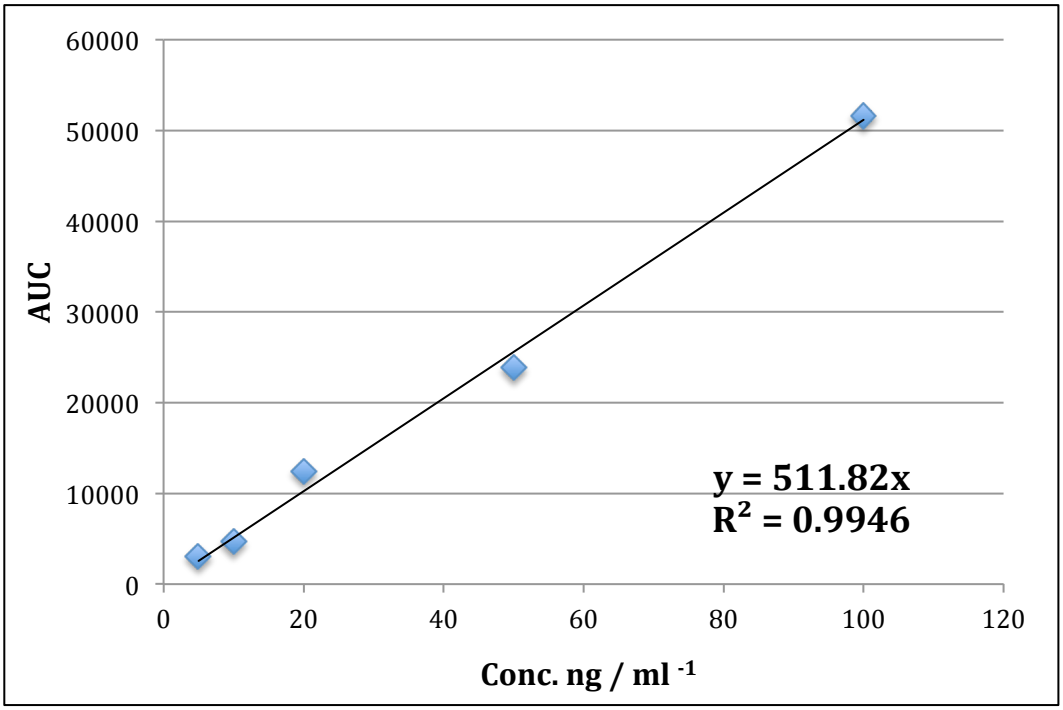


Figure 5. Calibration curve for the quantification of simvastatin in plasma samples. Each AUC value is the medium of two injections of the same sample.

The limit of detection and the limit of quantification, were calculated in accordance to equations 4 and 5 using the standard deviation of the area under the curve of the signal obtained with 10 blank plasma samples as σ.

LOD and LOQ resulted to be 4.8 ng/mL and 14.7ng/mL respectively.

Precision was calculated evaluating the relative standard deviation (RSD) as follows:

$$RSD = \frac{100 * \sigma}{\bar{X}} \qquad (6)$$

Where:

σ= Sample standard deviation;

$\bar{X}$= Sample mean.

Accuracy was calculated as follows:

$$Accuracy = \frac{X_e * 100}{X_t} \qquad (7)$$

Where:

X_e= Experimentally determined value;

X_t= Theoretical expected value.

Accuracy and precision are summarized in Table 5.

Table 5. Precision and accuracy of the HPLC-MS/MS method developed for simvastatin quantification in plasma samples.

Concentration (ng/ml)	Precision (%)	Accuracy (%)
5	6.90%	119%
10	9.50%	92%
20	0.17%	122%
50	1.30%	93%
100	0.98%	100%

Simvastatin pharmacokinetics

In vivo testing was performed using rats following the protocols approved by the Italian Ministry of Health, in agreement with the CEE directive n°609/86 concerning the protection of animals exploited for experimental and other scientific purposes (DL 116/92).

Simvastatin formulation F8 was selected for *in vivo* testing. Sinvacor®, the immediate release formulation of simvastatin available on the market, was also tested for comparative purpose.

Tablets were reformulated to adapt the human dosage to rats. Considering the higher dose of simvastatin administered to patients, i.e. 80 mg, and an average human weight of 70 kg, the dose of active/kg was

calculated to be 1.143 mg/kg. Considering a medium rat weight of 0.3 kg, the corresponding dosage was determined to be 0.343mg. However as underlined by Nair and Jacob (Nair and Jacob, 2016 Mar) a dose scaling on a gravimetric basis is not the correct approach since bigger animals shows often a slower metabolism. The dose was therefore increased nine folds. The composition of the tablets produced was:

Simvastatin-Compritol® (obtained by fusion method powder) 6 mg (corresponding to 3 mg of simvastatin);

HPMC 3 mg;

λ-carrageenan 3 mg;

Magnesium stearate 0.12 mg.

The tablets were produced by direct compression with an alternative EKO tableting machine (Korsh, Germany) equipped with 2 mm concave faced punches. 2 mm tablets resulted to be suitable for an easy administration to rats.

Scaling down of Sinvacor 40 mg tablet was performed by weighing and grinding the tablet and determining the amount of powder required for a 3 mg simvastatin tablet. The amount of powder required was 30.6 mg, hardly compactable with 2mm punches. Sinvacor was then formulated in 15.3 mg tablets produced by direct compression with an alternative EKO tableting machine (Korsh, Germany) equipped with 2mm concave faced punches. Two tablets were administered to each rat. The influence of the number of tablets was assumed to be irrelevant not being Sinvacor a controlled release formulation.

Simvastatin *in vivo* testing

Eight Wistar strain male rats of about 300 g weight, were assigned randomly to two groups, one receiving formulation F8 and one receiving Sinvacor-like tablet. Rats were kept fasted for 12 hours before tablets

administration while water was given *ad libitum*. Tablets were administered with oral gavage technique. Blood samples (100-200 µl) were withdrawn from the sublingual artery after mild sedation. Blood samples were collected after 0, 30, 60, 120, 240, 360 and 1440 minutes and refrigerated at -80 °C until the extraction of simvastatin and analysis were performed with the methods described above.

3.3 Simvastatin-Compritol Hot Melt Extruded Powder Production and Testing

3.3.1 Materials

Haake MiniCTW Twin Screws Extruder, Thermo Fisher Scientific, MS, USA;

Carver IR laboratory press (Carver Inc., IN, USA);

Sodium dihydrogen phosphate monohydrate, batch 3F29969, Bioshop Canada Inc., Canada;

Sodium Hydroxide, batch 020M00071V, Sigma-Aldrich, MO, USA;

Sodium acetate anhydrous, batch 7C48364, Bioshop Canada Inc., Canada;

Acetic acid, batch 45200, EMD chemicals, Germany;

HPMC K15M, batch SE10012N12, Dow Chemical Company, MI, USA;

Compritol® 888 ATO, batch 144062, Gattefossé, France;

3.3.2 Methods

Simvastatin-Compritol Hot Melt Extruded Powder Production

The Simvastatin-Compritol® hot melt extruded powder was produced to evaluate the suitability of the extrusion process for simvastatin-compritol micromatrices production as alternative to fusion method. One of the major challenges in fusion method processes is indeed the lack of

suitable and cost-effective technique to obtain large batches of product. For this reason hot melt extrusion was selected as promising alternative technique. Moreover, hot melt extrusion technique allows working in milder conditions inducing the fusion of Compritol® at lower temperature thanks to high shear force developed by the screws (Breitenbach J., 2002). This is always desirable to avoid the use of excessively high temperature that can result in the degradation of the active ingredient.

Some preliminary studies, aimed at defining the parameters for simvastatin-compritol 50% w/w extrusion, indicated that 65 °C was the lower temperature suitable to obtain a good extruded product in milder conditions. In these studies only the physical shape and texture of the product extruded were evaluated. The extruder temperature was then fixed at 65 °C.

To extrude simvastatin-Compritol® 50:50 w/w mixture, the powders were weighed and mixed in a mortar to ensure a high degree of homogeneity. Fractions of the powder were then loaded manually into the feeder with a plastic spatula, waiting for about 30 seconds between two consecutive loadings to allow the powder to proceed into the barrel. 5 grams (the maximum capacity of the extruder, 7 cm^3) of mixture were loaded into the extruder with the screws turning at 50 run per minute and the temperature kept at 65 °C. Powder loading was completed in 5 minutes. Thereafter the mixture was left flushing for 10 minutes to achieve a good mixing and to complete the fusion of Compritol®. Then the screws speed was set at 10 rpm in the extrusion phase.

After 30 min the extrusion was stopped as nothing exited the extruder.

Simvastatin quantification

Simvastatin quantification in dissolution media and solution prepared to determine the extruded products actual drug content was performed with an HPLC (Hitachi Elite Lachrom, Hitachi, Japan) equipped with UV detector (Hitachi L2400), binary pump (Hitachi L210-213) and autosampler (Hitachi L2200). The UV detector was set at 231nm. The mobile phase was a mixture of acetonitrile:acetate buffer pH 4, 70:30 v/v. Acetate buffer pH 4 was produced according to the Sigma database for buffer solutions. 100 mL of acetate buffer solution pH 4 was produced by mixing 18 mL of a 0.2 M sodium acetate solution with 82 mL of a 0.2 M acetic acid. The pH was adjusted to 4.00 ± 0.05 with 2 M sodium hydroxide or 2 M acetic acid solutions. Buffer thus obtained was filtered (0.45 μm) and degassed applying high vacuum for 30 minutes. The buffer was kept refrigerated when not in use. Mobile phase was pumped at 0.5 mL min^{-1}, with a runtime of 15 min. Simvastatin retention time was 11 minutes.

To determine simvastatin content in extruded products, samples were firstly grinded in a mortar, then 10 mg aliquots were weighed and placed in a 25 mL volumetric flask with 20 mL of acetonitrile, sonicated for 15 seconds before adding acetonitrile to volume. The samples were then filtered, diluted with phosphate buffer, placed into glass vials and analysed by HPLC. The linearity of the response of the HPLC system used to determine simvastatin concentration in the samples was assessed by analysing simvastatin standard solutions in the range 7-60 μg mL^{-1}. All solutions were injected six times and the medium AUC value was used to plot the data. The resulting calibration curve is shown in Figure 6.

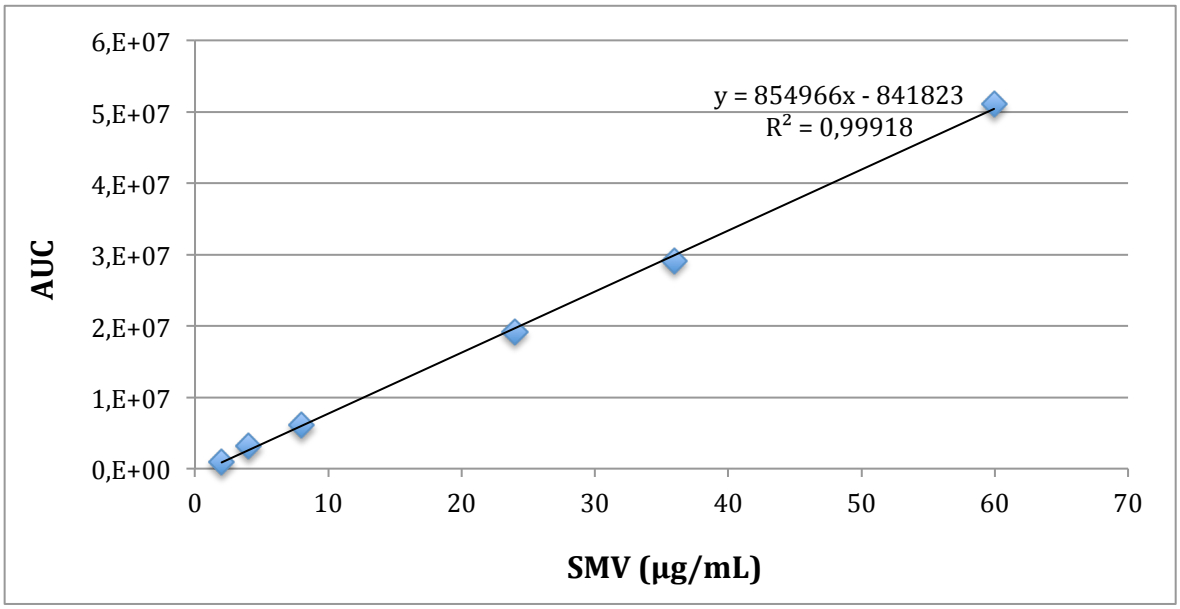


Figure 6. Simvastatin calibration curve in the HPLC system described above. Each point represents the medium value of three injections.

As it can be appreciated to method showed a good linearity (R^2=0.9991) in the range of concentration tested.

Dissolution testing

The dissolution apparatus used was a paddle (Type II) apparatus (VK7000, Vankel, CA, USA) set at 100 rpm for powders and a basket apparatus (Type I) set at 100 rpm for matrices. Phosphate buffer pH 6.8 was prepared according to the USP (Second Supplement to USP 35 –NF 30). Temperature was maintained at 37 °C for the duration of the experiment. 500 mL of phosphate buffer and 1.25 g of sodium dodecyl sulphate were poured in each vessel with a graduated cylinder. 5 mL of dissolution buffer were withdrawn manually with a syringe at each set point and the dissolution volumes were corrected accordingly for the calculation of the dissolved amount of simvastatin.

4. Results and Discussion

4.1 Pre-formulative study on simvastatin/Compritol® miscibility

A pre-formulative study was carried out to assess the solubility of simvastatin in Compritol®. In order to do that, DSC were performed in order to achieve accurate and quick informations. Compritol® melts around 73 °C, while simvastatin shows a pronounced endothermic event, i.e. the fusion of crystals at 139° (data not reported).

Physical mixtures of Compritol® and simvastatin at different ratios were then produced and analysed in DSC.

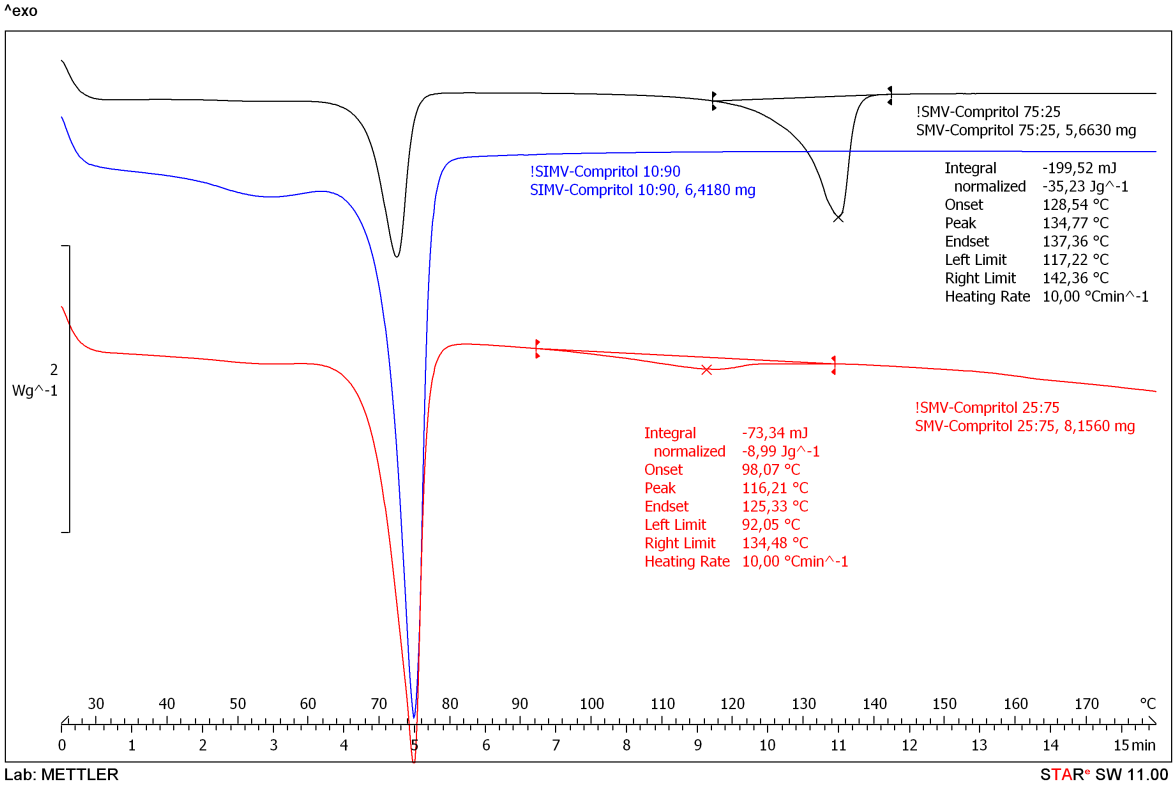


Figure 7. DSC traces of physical mixture of Compritol®-simvastatin at three different ratios.

As an example, Figure 7 reports the DSC traces recorded for binary mixtures containing simvastatin at 10, 25 and 75 % w/w respectively.

It can be observed that the enthalpy of fusion of simvastatin (area under the melting peak) decreased as the Compritol percentage in the mixture

was higher. Moreover, the enthalpy of fusion of simvastatin was not exactly proportional to the drug concentration in the binary mixture, meaning that a partial dissolution of the drug occurred in the molten Compritol® during the DSC run.

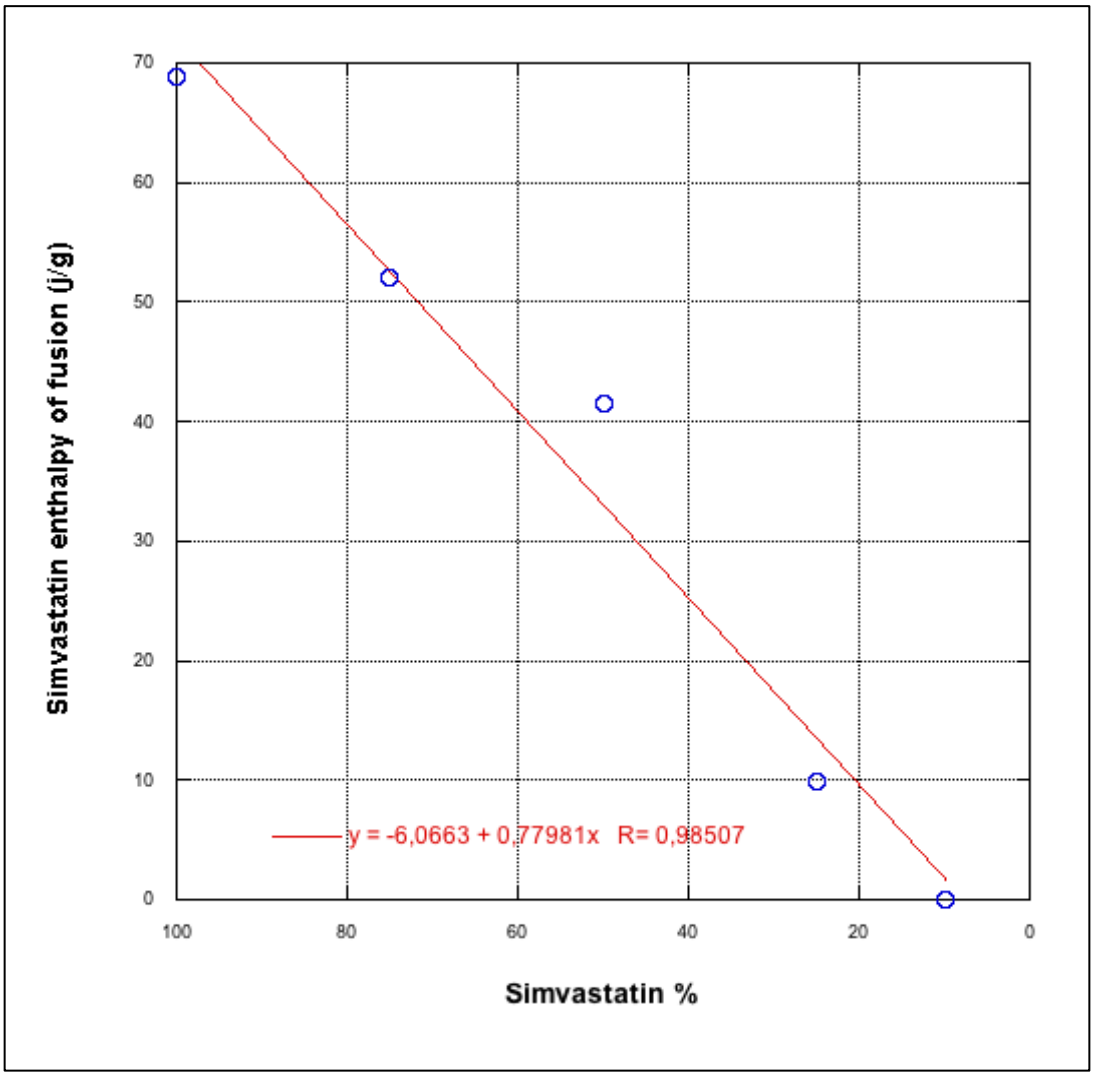


Figure 8. Simvastatin melting enthalpy values plotted *versus* simvastatin w/w percentage in the mixture.

The maximum value of simvastatin dissolved in Compritol in these conditions represents the solubility of the drug in the excipient.

To calculate this value the enthalpy of fusion of simvastatin for each binary mixture was plotted against the simvastatin concentration in the binary to build a phase diagram whose intercept with the x-axis

represents the maximum drug concentration that can dissolve into the lipid (Figure 8). The solubility of simvastatin in the lipid resulted to be 7.8 % w/w.

The data suggest that in a typical 50:50 mixture Compritol-simvastatin the drug would be present largely in crystalline form.

4.2 Dissolution profile of simvastatin fusion method powder

The dissolution profiles of simvastatin raw material, simvastatin-Compritol® physical mixture and simvastatin-Compritol® obtained with fusion method are reported in Figure 9.

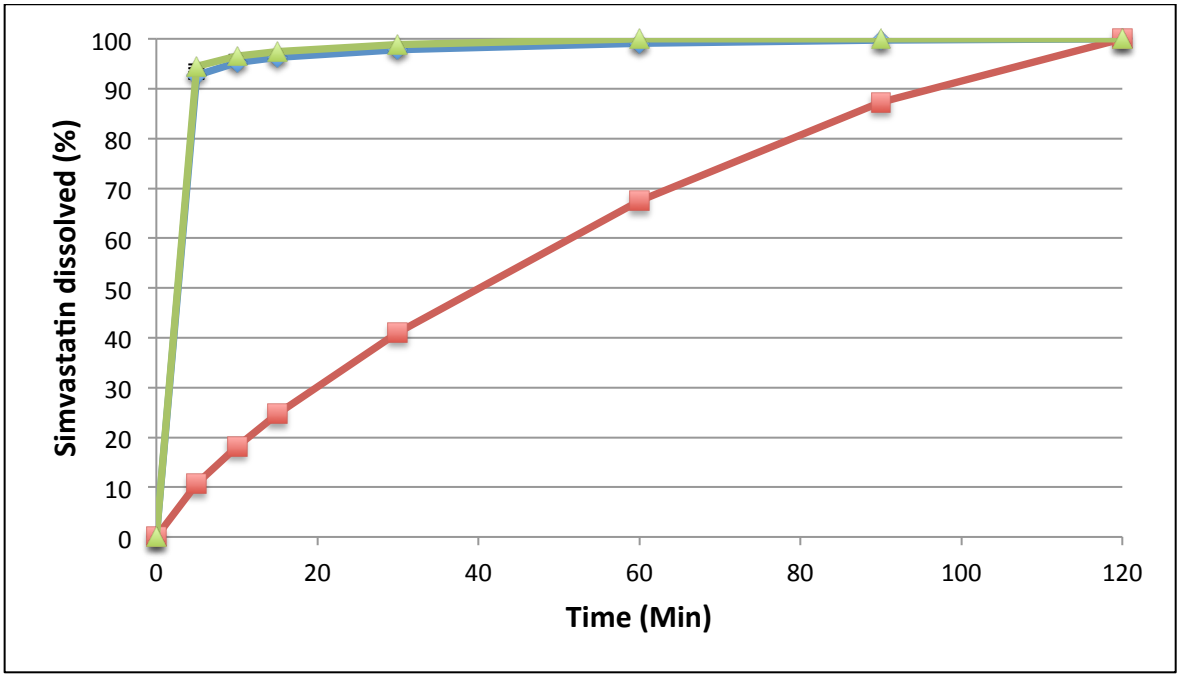


Figure 9. Dissolution profile of simvastatin raw material (green triangles), simvastatin- Compritol® physical mixture (blue diamonds) and simvastatin- Compritol® fusion method (red squares) powders in 750ml phosphate buffer pH 6.8, SDS 0.15%. The bars represent the standard deviation (n=3).

As it can be appreciated, simvastatin raw material and simvastatin-Compritol® physical mixture shows the same steep dissolution profile, with 95% of simvastatin dissolved in five minutes. On the other hand, simvastatin-Compritol® powder obtained with the fusion method shows a slow increase of the amount of simvastatin in solution. This can be attributed to the different physical state of simvastatin in the fusion method produced powder. In fusion method produced powder indeed, simvastatin and Compritol® are in more intimate contact and Compritol® acts as a matrix surrounding simvastatin particles eventually controlling the dissolution rate of the active. As a matter of fact the dissolution rate

of simvastatin resulted to be well controlled for up to two hours. The ability of Compritol® 888 ATO to act as a matrix forming agent was reported by Fini *et al.*, which evaluate the release of theophylline from Compritol® 888 ATO, HD5 ATO and E ATO microspheres (Fini *et al.*, 2010).

A deeper insight on the mechanism governing simvastatin release from Compritol® micro-matrices produced with the melting method can be achieved applying equation (2) (Ritger and Peppas, 1987). The curve fitting of the release profile is reported in Figure 10.

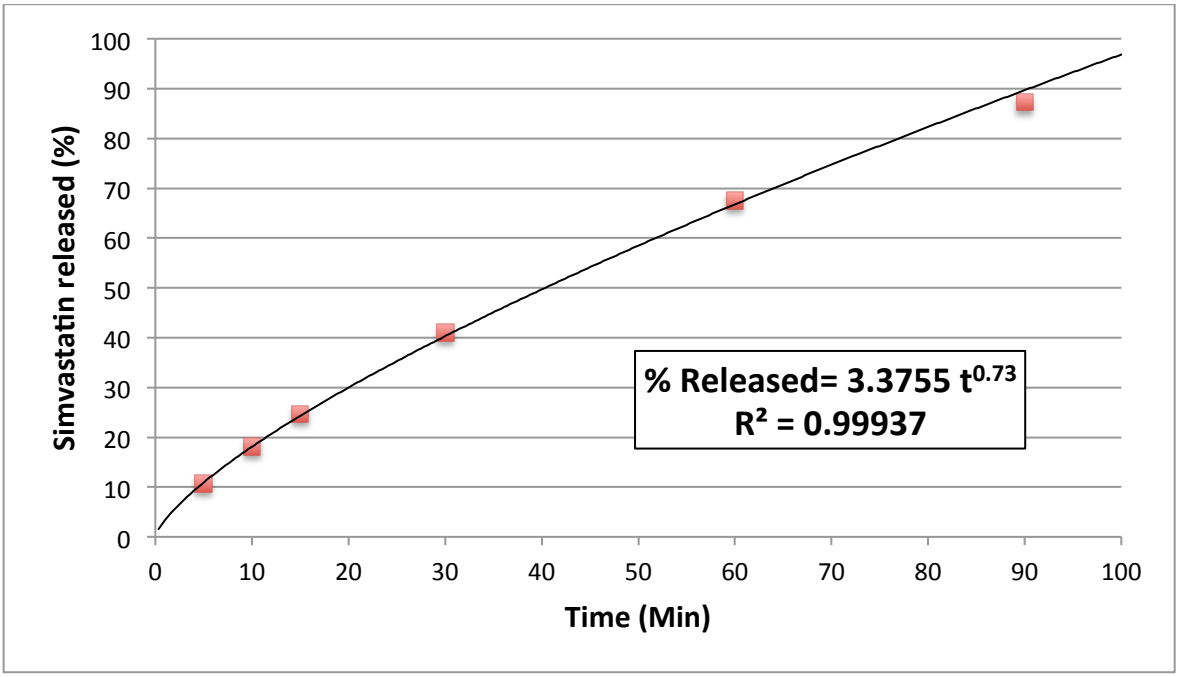


Figure 10. Experimental data of the amount of simvastatin released from melting method powder with respect to time (red squares) and curve fitting with the Peppas power law (solid black line).

The very good fitting of the power equation to experimental data is indicated by the R^2 value > than 0.99. According to Ritger and Peppas the n exponent of t is indicative of the mechanism governing drug release; it resulted to be 0.73. It seemed therefore, that this situation falls between a diffusion-controlled and an erosion/relaxation-controlled release, defined as Anomalous mass transport. If we consider the fact that Compritol® is not a polymer, polymer chain relaxation cannot be

rationally considered to explain the deviation observed from a merely diffusion-controlled drug release which would imply a dependence on the square root of time. Nevertheless, Fini *et al.* studied the mechanism governing the release of theophylline from three types of Compritol® concluding that the drug was released by simple diffusion (Fini *et al.*, 2010). The results of these authors are supported by the work of Sutananta *et al.* who studied the release of the same active, i.e. theophylline, from glycerides bases. It was concluded that the release was determined by a diffusion mechanism and no swelling or erosion were involved (Sutananta *et al.*, 1995). It has to be underline that in both these works theophylline, a slightly water soluble compound, was used. A slightly soluble active ingredient can better dissolve and diffuse into the water-filled pores of the matrix and would have a relatively low tendency to partitioning into the matrix itself. Simvastatin on the other hand is almost insoluble in phosphate buffer (0.03 mg/mL) and the migration of the active in solution toward the dissolution medium through the pores of the matrix, can be excluded while has been considered for slightly soluble compounds. This can explain, at least partially, the difference with respect to the data reported in the cited literature. Another reason for the Anomalous release observed can be found considering the behaviour of Compritol®. As already said, swelling and relaxation of glycerides can be excluded *a priori*, but we should consider the contribution of SDS to the dissolution of lipid excipients. SDS indeed, acting as a surfactant, increases water solubility of hydrophobic compounds and can justify some erosional contribution to drug release that can explain the Anomalous drug transport observed.

4.3 Simvastatin release from hybrid matrices

To make the reading easier the composition of the formulations produced is summarized in Table 6 along with the actual (experimentally determined) active content.

Table 6. Hybrid matrices composition with determined active content.

	F1	F2	F3	F4	F5
Simvastatin (mg)	40	40	40	40	40
Compritol® (mg)	40	40	40	40	40
HPMC (mg)	80	60	40	20	
λ-carrageenan (mg)		20	40	60	80
Magnesium Stearate (mg)	1.6	1.6	1.6	1.6	1.6
Active content %	103 ± 11.25	108 ± 10.93	106 ± 5.33	115 ± 6.80	102 ± 3,79

	F6	F7	F8	F9	F10
Simvastatin-Compritol® (Fusion Method) (mg)	80	80	80	80	80
HPMC (mg)	80	60	40	20	
λ-carrageenan (mg)		20	40	60	80
Magnesium Stearate (mg)	1.6	1.6	1.6	1.6	1.6
Active content %	100 ± 6.79	101 ± 9.52	103 ± 4.56	109 ± 3.38	100 ± 0.54

Considering a nominal active content of 40 mg, a good agreement between the nominal values and the determined values can be observed. In general, the actual values are within the 10% of the nominal dose, i.e. 40 mg.

The release profiles of hybrid matrices obtained through direct compression of physical mixture are reported in Figure 11. As it can be appreciated, different release kinetics were obtained by varying the matrix composition. Matrix containing HPMC in larger amount (formulations F1 and F2) showed an almost linear drug release profile with slower release rate during the 24 hours compared to those containing larger amounts of λ-carrageenan (formulations F4 and F5). This can be explained considering the behaviour of these polymers upon contact with water. HPMC is a gel-forming agent in water and the thickness of the gel formed strongly depends on HPMC viscosity grade (Siepmann and Peppas 2001; Bettini *et al.,* 1994). λ-carrageenan is a non-gelling excipient instead, it forms viscous solutions but the presence of sulphate ester in position 2 hinders the formation of an ordinate structure in contrast to other carrageenans (Li *et al.*, 2014), and therefore the gel cannot be formed.

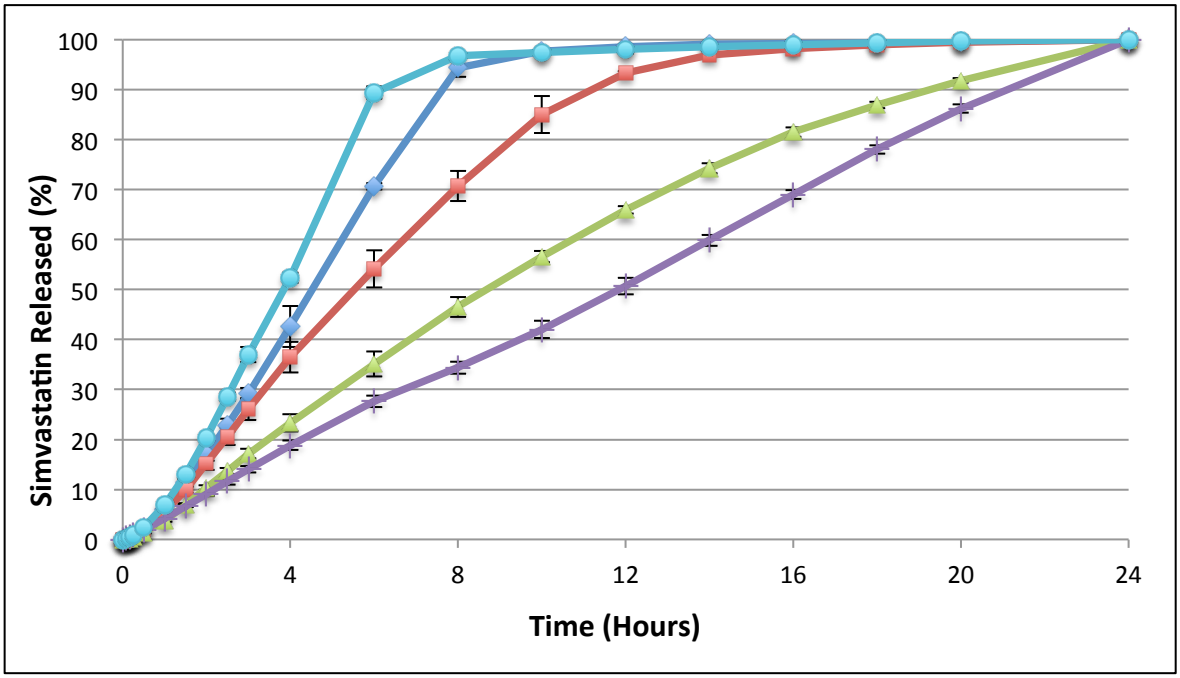


Figure 11. Simvastatin release profiles from matrices produced by direct compression of physical mixture. F1=purple crosses, F2=green triangles, F3=red squares, F4=blue diamonds, F5=aquamarine circles. The bars represent the standard deviation (n=3).

As a matter of fact, the viscosity for 2% w/v aqueous HPMC K15M solution is reported to be 15000 mPas (Rogers T. L., 2009), compared to 450 to 750 mPas of a 1.5 w/v aqueous solution of λ-carrageenan (Technical data sheet provided from the producer). Moreover, HPMC and λ-carrageenan are characterized by different solubility in water being HPMC freely soluble in cold water and practically insoluble in hot water while the opposite occurs for carrageenan (Singh K. K., 2009).

In matrices containing larger amounts of λ-carrageenan the erosion rate will be then more important compared to matrices containing larger amounts of HPMC, a behaviour already observed by Bonferoni *et al.* (Bonferoni *et al.*, 1993; Bonferoni *et al.*, 1994).

At early stages (within 1 hour from the beginning of the release experiment), the matrices containing larger amounts of λ-carrageenan showed a release rate comparable to systems containing larger amounts of HPMC.

Once λ-carrageenan hydrates, the lower viscosity of the gel formed and the higher solubility of the polymer allow water to penetrate faster in the system whereas, HPMC forms a very thick gel thus slowing down water penetration in the system. In physical mixture systems therefore, the characteristics of the gel formed and the solubility of polymers are strongly affecting drug release rate. The formulation containing equal amounts of HPMC and λ-carrageenan (formulation F3) showed an intermediate situation with an initial slower phase in the first hours followed by an almost linear drug release until 10 hours and then slowing down, completing drug release in 16 hours.

The release profiles of matrices produced through direct compression of simvastatin-Compritol powders obtained by the fusion method are reported in Figure 12. The same trend observed in matrices produced from physical mixture can be appreciated, even though the difference between formulations containing

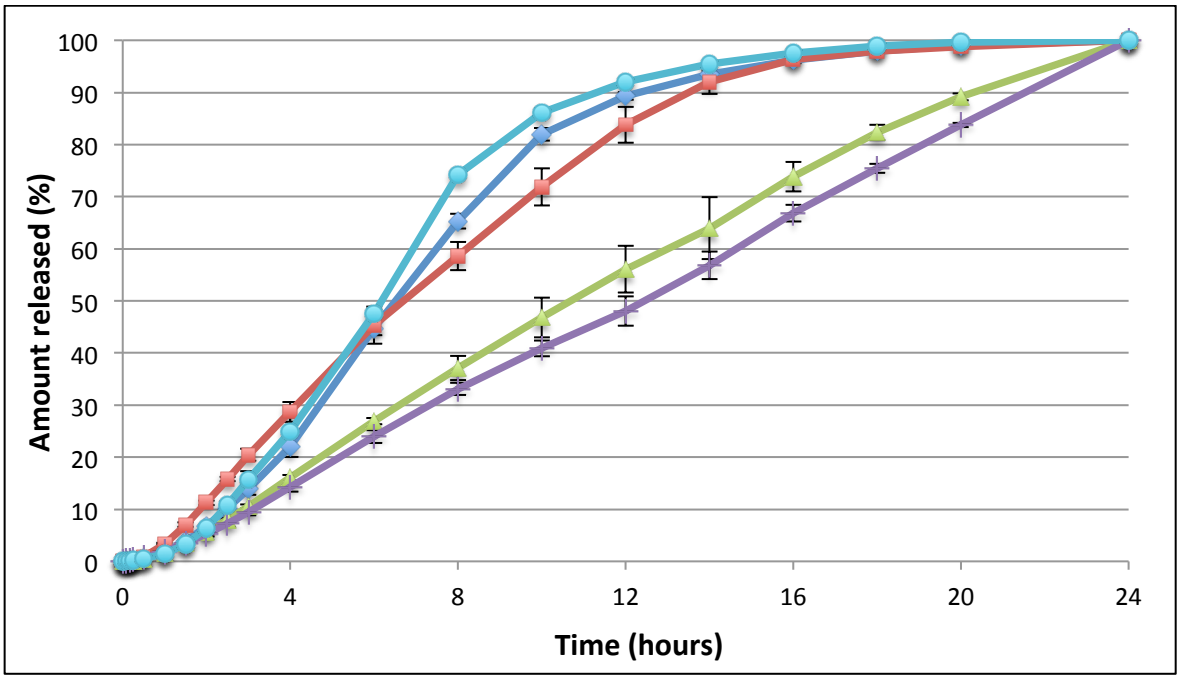
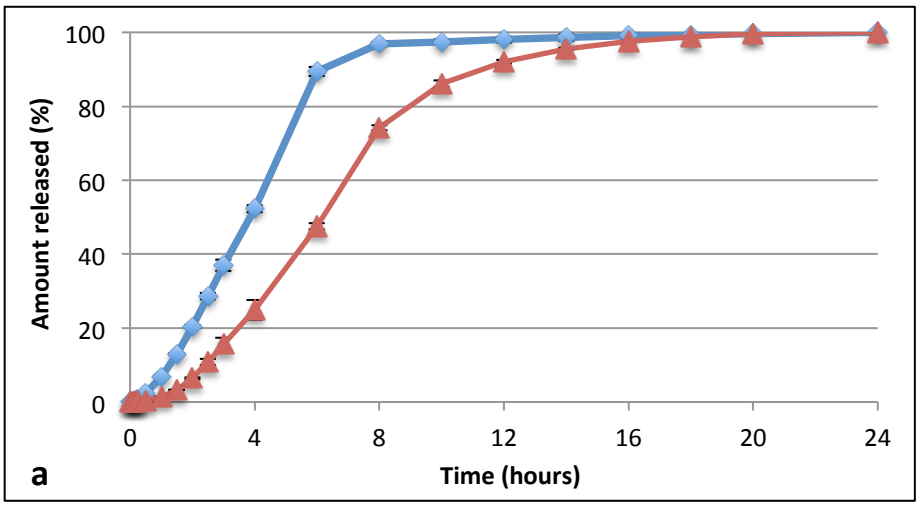


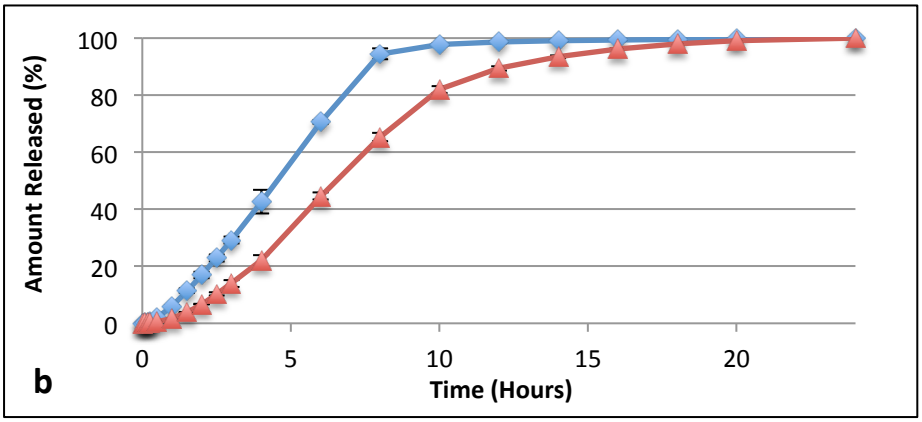
Figure 12. Simvastatin release profiles of matrices produced by direct compression of fusion method powder. F6=purple crosses, F7=green triangles, F8=red squares, F9=blue diamonds, F10=aquamarine circles. The bars represent the standard deviation (n=3).

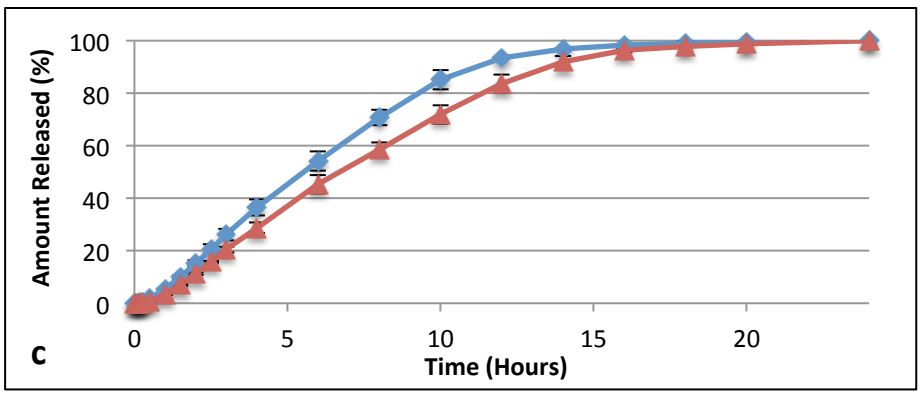
different amounts of HPMC and λ-carrageenan are less pronounced whereas some differences could be detected in the first 8 hours among the formulations containing the lower amount of HPMC. Formulation F9 and F10, containing respectively 37.5 % and 50 % λ-carrageenan, showed an initial lag phase which is more pronounced in comparison with formulation F4 and F5, *i.e.* the same formulations containing the physical mixture of simvastatin and Compritol®. Furthermore, compared to formulation F8, the drug release from formulations F9 and F10 was slower in the early phase and faster in the late time; the crossover point was around 5 hours. This behaviour was not observed in the physical mixture tablets. Therefore, the fusion method used for the production of simvastatin/Compritol® mixture, contributes in changing simvastatin release.

As previously stated, the fusion method gave rise to micromatrices where the drug was intimately embedded within the lipid excipient. In the polymeric matrices containing such micromatrices slower release rates, compared to the same formulations produced from physical mixture, were observed. This aspect is better evidenced in Figure 13 where the release profiles of matrices prepared starting from the powder obtained by physical mixture (blue diamonds) or by fusion method (red triangles) are directly compared. It is visually clear that the higher the HPMC amount in the matrix, the less pronounced the difference between release profiles. In other words, in matrices containing a larger amount of λ-carrageenan, the contribution of the lipid micromatrices became more evident in slowing down the release rate of the drug. This can be explained once again with the different swelling and erosion rate of the polymers upon contact with water. In F9 and F10 formulations, the control of the λ-carrageenan on drug release rate was negligible due to its fast dissolution rate, thus, the matrix effect of Compritol® became more

important in controlling the release of simvastatin. In F1 and F6 matrices, (containing larger amounts of HPMC), the erosion of Compritol® combined with the HPMC matrix effect and became less important. In fact simvastatin release profiles from F1 and F6 formulations were practically superimposable (Figure13, Panel e).







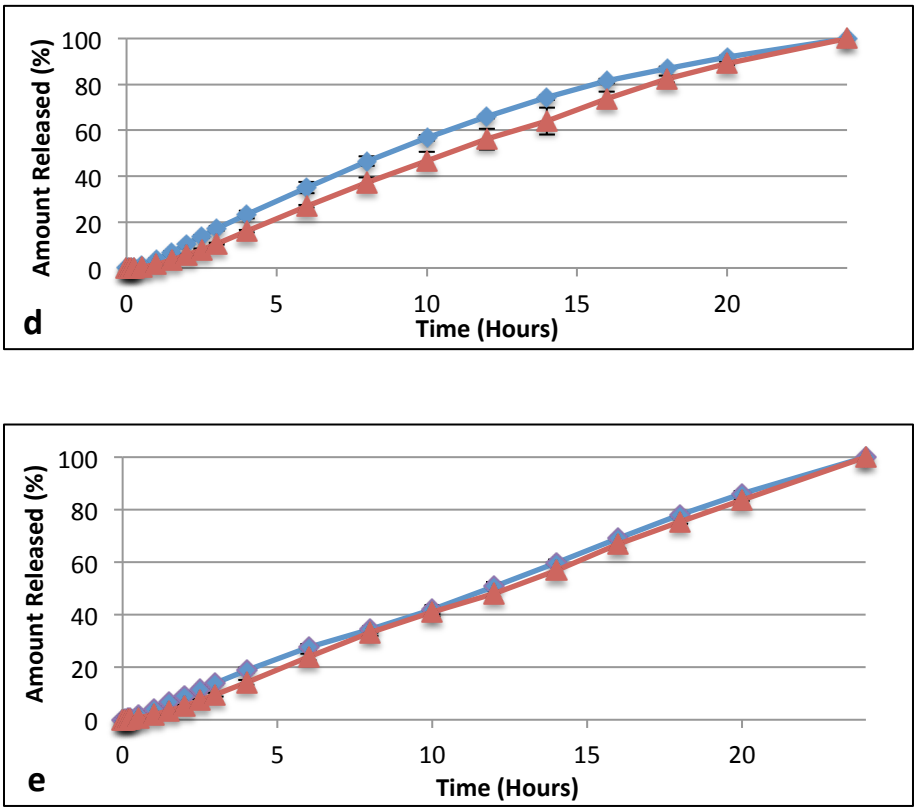


Figure 13. Comparison of simvastatin release profiles of matrices produced from physical mixture (blue diamonds) and fusion method (red triangles) powders. a= F5, F10; b= F4, F9; c= F3, F8, d= F2, F7; e= F1, F6. The bars represent the standard deviation (n=3).

The contribution of the HPMC : λ-carrageenan ratio to the control of release rate can be better evaluated by directly plotting the time required for the release of 80% of the dose against the relative polymer composition of the matrices. The results are shown in Figure 14. It can be appreciated that the time required to release 80% of the dose was always higher in matrices obtained starting from the fusion method powders. Furthermore, the $t_{80\%}$ in formulation F1 and F6 was 18.5 and 19.1 hours respectively, while for formulation F5 and F10 was 5.5 and 9.0 hours respectively.

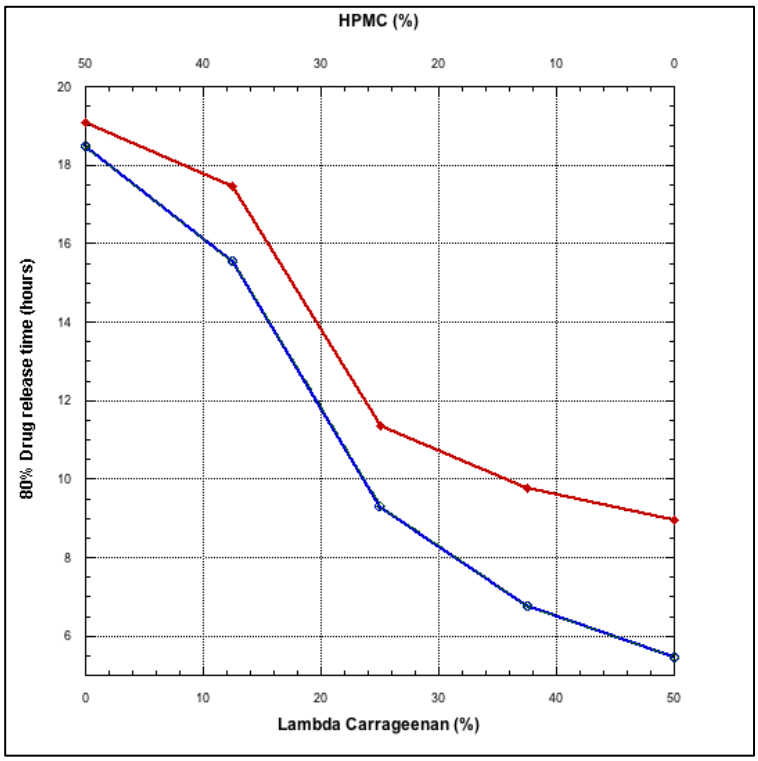


Figure 14. Time required to release 80% of drug with respect to matrices polymers composition. Blue line= tablets from simvastatin : Compritol® physical mixture powder, Red line= tablets from simvastatin : Compritol® fusion method powder.

4.4 Mathematical description of drug release

The understanding of the mechanisms governing drug release from hybrid matrices is not an easy task as the whole drug release process is complex and a simple description of the system would not provide a reliable picture (Colombo et al. 2000). The simultaneous cooperation of several mechanisms with different relative kinetics and relative contribution makes the scenario complicated to describe. Nevertheless, some mechanistic interpretation will be proposed hereafter.

Hybrid matrix systems studied in the present work are characterized by a large degree of complexity in consideration of their composition. The coexistence in the same system of two polymeric excipients, namely HPMC and λ-carrageenan, with different solubility in water and different physical behaviour upon contact with water is the first reason of complexity. The introduction of large amounts of Compritol®, a hydrophobic excipient, accounts for a second level of complexity. Moreover, for systems produced with the fusion method, the incorporation of the poorly water-soluble simvastatin in hydrophobic micromatrices, in turn embedded in a hydrophilic matrix imparts a further element of complication.

In general terms the release controlling mechanisms in such matrices can be identified as:

- Fickian diffusion of the drug through the polymeric network (Bettini *et al.,* 1994)
- Fickian diffusion of the drug in the hydrophobic matrix (Ozyazici *et al.*, 2006). This aspect would be more important for matrices produced with the fusion method due to the more intimate contact between drug and excipient.

- Relaxation/Dissolution of polymeric chains which depends upon the polymer physico-chemical characteristics (Bonferoni *et al.*, 1993);
- Dissolution of the hydrophobic excipient (Sutananta *et al.*, 1995);
- Translocation of solid drug or drug/hydrophobic micromatrix particles suspended in the swollen polymer due to swelling of polymeric chains (Bettini *et al.*, 2001);
- Osmotically driven water penetration in the partially glassy core (Catellani *et al.*, 1998);
- Drug partitioning between a hydrophobic and a hydrophilic phase.

Considering all these mechanisms involved at the same time it is not surprising that the models commonly applied to describe drug release from hydrophilic matrices could be reductive and could lead to an extreme simplification of the reality.

The first attempts to use the power law (eq. 2) resulted in passable fitting of experimental data as indicated by R^2 values reported in Table 7 and graphically in Figures 15 and 16 (as an example only F3 and F8 Formulation profiles are reported).

Table 7. R^2 and n values obtained applying power law equation to the experimental data.

	Fusion Method		Physical Mixture	
	R^2	*n*	R^2	*n*
λ carrageenan 50%	0.99891	1.6298	0.99745	1.4566
λ carrageenan 37.5% HPMC 12,5%	0.99915	1.6209	0.99204	1.5213
λ carrageenan 25% HPMC 25%	0.96258	1.5596	0.97293	1.4352
λ carrageenan 12.5% HPMC 37,5%	0.97131	1.5798	0.97997	1.3119
HPMC 50%	0.99382	1.2639	0.99711	1.0442

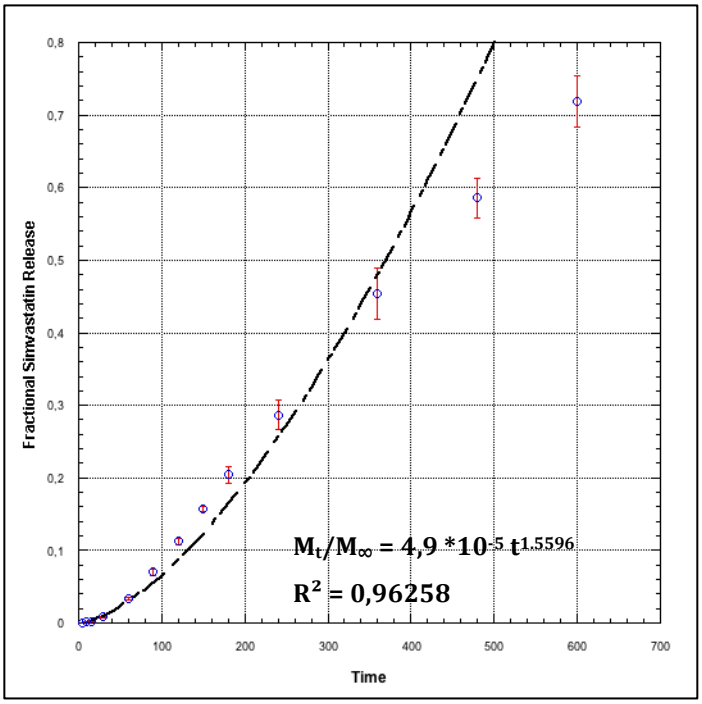


Figure 15. Drug release from matrices produced by compression of powder produced with fusion method. Matrix composition: λ Carrageenan: 25%; HPMC: 25%; Compritol: 25%; Simvastatin: 25%. Blue circles: Experimental data; Black broken line: Curve fit using the power law.

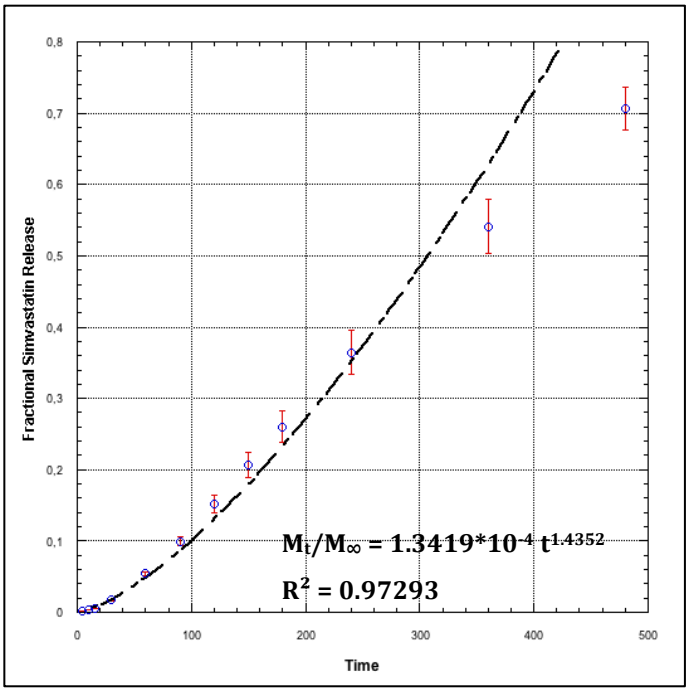


Figure 16. Drug release from matrices produced by compression of physical mixture. Matrix composition: λ Carrageenan: 25%; HPMC: 25%; Compritol: 25%; Simvastatin: 25%. Blue circles: Experimental data; Black broken line: Curve fit using the power law.

As it can be appreciated, the fitting of the results is suggested by the R^2 values so one could think that this model fits satisfactorily experimental data. If we take a look to the values of the *n* exponent in the power equation, we immediately realize that, as already told in the Introduction part (paragraph 1.6), the model would suggest that the mechanism involved in the drug release is a Super Case II transport. Considering the fact that it is hard to describe systems governed by this kind of mass transport (Alfrey *et al*., 1966), we should consider that a simple number, i.e. the value of exponent *n*, can be reductive to describe the relative contribution of each physical phenomenon in an exhaustive manner. As a matter of fact, the n exponent values for all the matrices produced, reported in Table 7, are all > 1 although this observation is not sufficient to hypothesize a specific transport mechanism.

Another approach is therefore required. In an attempt to better understand the contribution of Fickian and non-Fickian transport, the Peppas-Sahlin equation (eq. 3) was applied. The exponent of time was set at 0.45, which is reported to describe better the release from cylinder-shaped matrix. The application of Peppas-Sahlin equation resulted in pretty good fitting of the experimental data (Table 8). The two terms on the right end side of the equation accounts for diffusion and linear contribution (likely stemming from erosion) to drug release respectively.

Table 8. k_1, k_2, and R^2 values obtained applying the Peppas-Sahlin equation to experimental data.

	Fusion Method			Physical Mixture		
	k_1	k_2	R^2	k_1	k_2	R^2
λ carrageenan 50%	-0,0552	0,0058	0,9959	-0,0458	0,0071	0,9989
λ carrageenan 37,5% HPMC 12,5%	-0,0339	0,0044	0,9879	-0,0282	0,0053	0,9963
λ carrageenan 25% HPMC 25%	-0,0120	0,0030	0,9979	-0,0137	0,0036	0,9983
λ carrageenan 12,5% HPMC 37,5%	-0,0111	0,0020	0,9985	-0,0042	0,0020	0,9988
HPMC 50%	-0,0096	0,0018	0,9990	-0,0018	0,0015	0,9992

Nevertheless, as discussed previously, in matrices obtained starting from powders prepared with the fusion method the erosion of both lipid excipient and polymers took place at the same time and a relative contribution of each phenomenon can be hardly quantified with the Peppas and Sahlin equation.

We thought therefore that, from a merely mathematical point of view, the interpolation of the curves with a third degree equation, could have been more suitable for a an accurate representation of the obtained drug release profiles.

$$\frac{M_t}{M_\infty} = M_1 + M_2 t^b + M_3 t^{2b} + M_4 t^{3b} \qquad (8)$$

Here M_t/M_∞ is the fraction of drug released at time *t*. M_2, M_3 and M_4 are the coefficient having dimensions of t^{-b}, t^{-2b} and t^{-3b} respectively. The value of the b exponent was set at 0.45 according to Peppas and Sahlin (Peppas and Sahlin, 1989) and Ritger and Peppas (Ritger and Peppas, 1987) who indicated this value for the diffusional exponent in case of cylindrical-shaped matrices.

The fitting curves for all the releasing profile are reported in Figures 17-26.

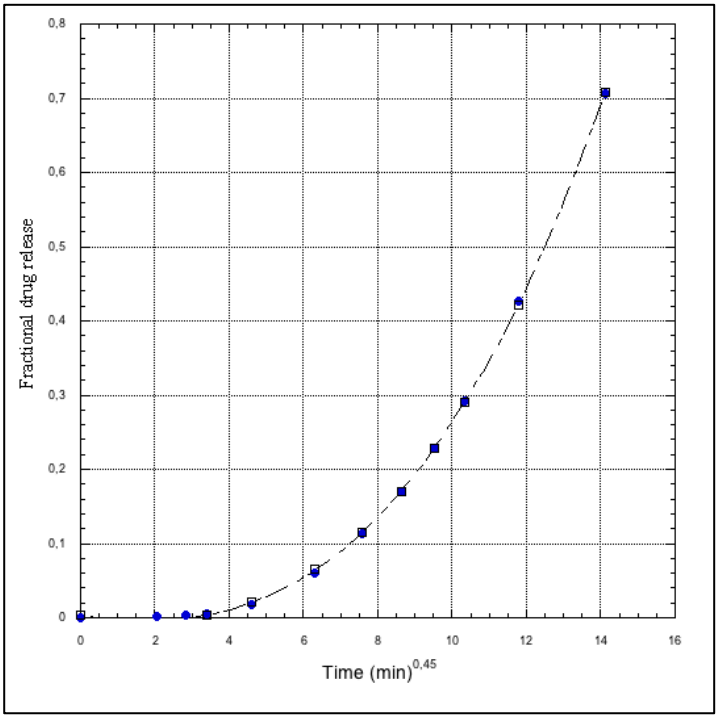


Figure 17. Drug release from matrices produced by compression of powder produced with fusion method. Matrix composition: λ Carrageenan: 50%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=3.23*10^{-3}-0.68*10^{-6}\ t^b-1.32*10^{-3}\ t^{2b}+0.26*10^{-3}\ t^{3b}$; $R^2=0.9996$.

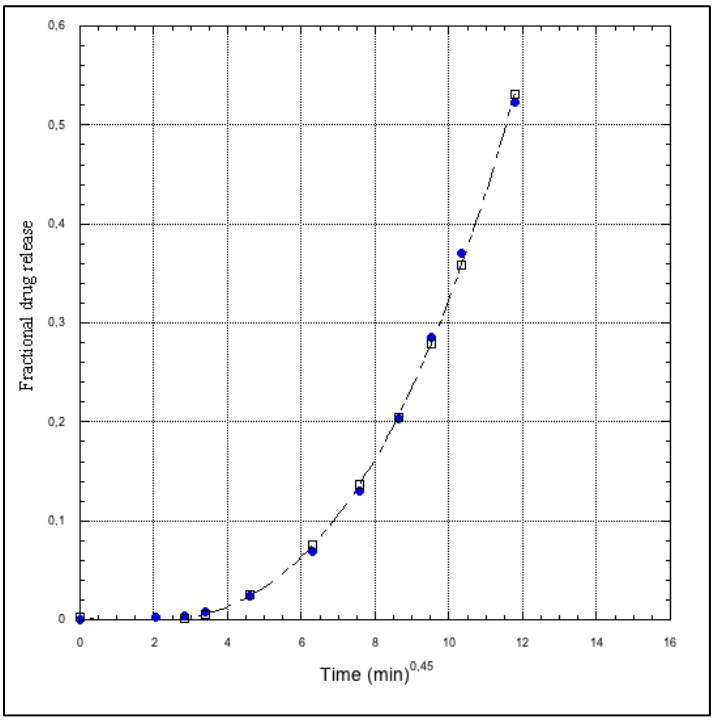


Figure 18. Drug release from matrices produced by compression of physical mixture. Matrix composition: λ Carrageenan: 50%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line $M_t/M_\infty=3.32*10^{-3}-5.33*10^{-3}\ t^b+0.81*10^{-3}\ t^{2b}+0.29*10^{-3}\ t^{3b}$; $R^2=0.9988$.

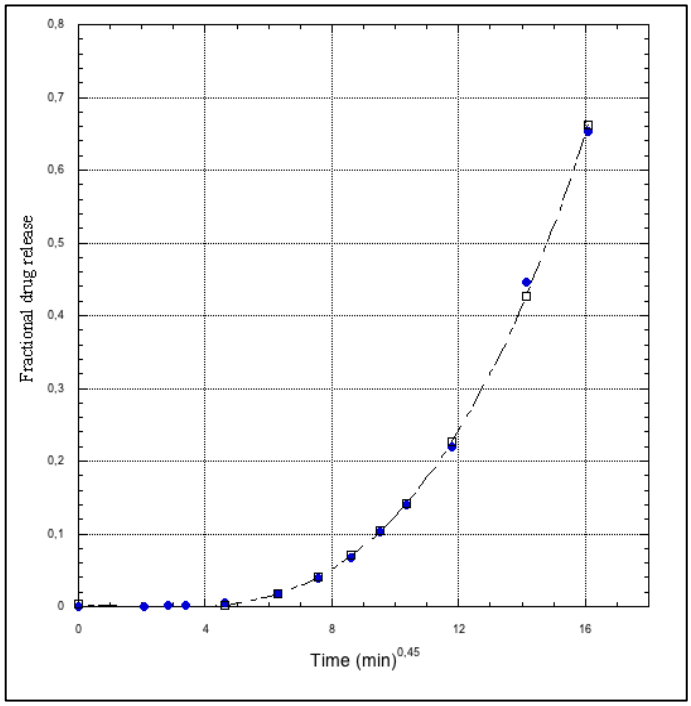


Figure 19. Drug release from matrices produced by compression of powder produced with fusion method. Matrix composition: λ Carrageenan: 37,5%; HPMC: 12,5%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=3.37*10^{-3}-1.07*10^{-3}\ t^b\ -0.80*10^{-3}\ t^{2b}\ +0.21*10^{-3}\ t^{3b}$; $R^2=0.9988$.

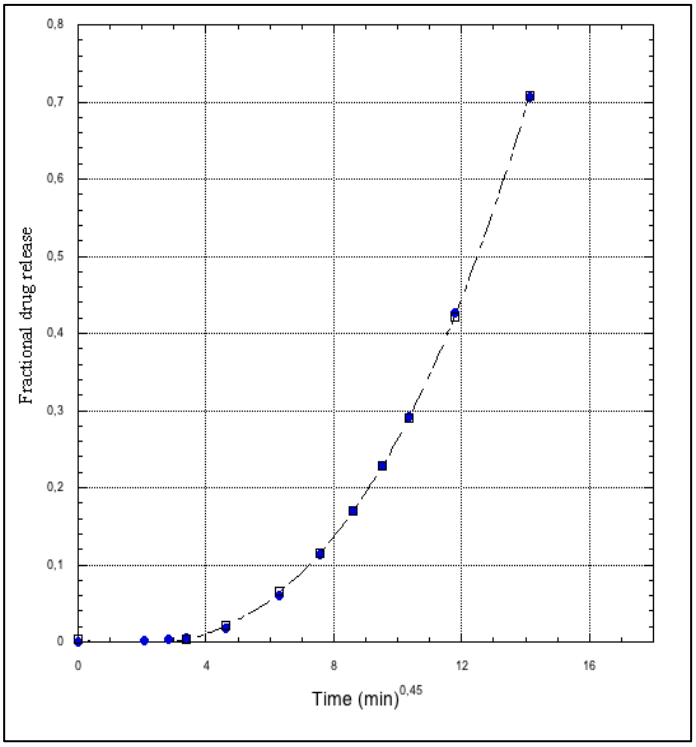


Figure 20. Drug release from matrices produced by compression of physical mixture. Matrix composition: λ Carrageenan: 37,5%; HPMC: 12,5%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=3.25*10^{-3}-6.96*10^{-3}\ t^b\ +1.53*10^{-3}\ t^{2b}\ +0.18*10^{-3}\ t^{3b}$; $R^2=0.9995$.

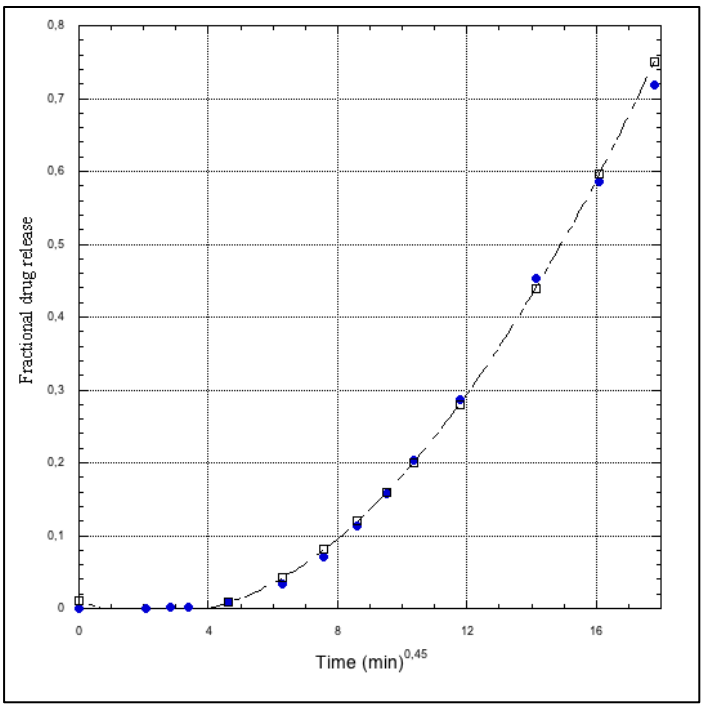


Figure 21. Drug release from matrices produced by compression of powder produced with fusion method. Matrix composition: λ Carrageenan: 25%; HPMC: 25%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=1.08*10^{-2}-1.64*10^{-2}\ t^b+3.49*10^{-3}\ t^{2b}-1.29*10^{-6}\ t^{3b}$; $R^2=0.9978$.

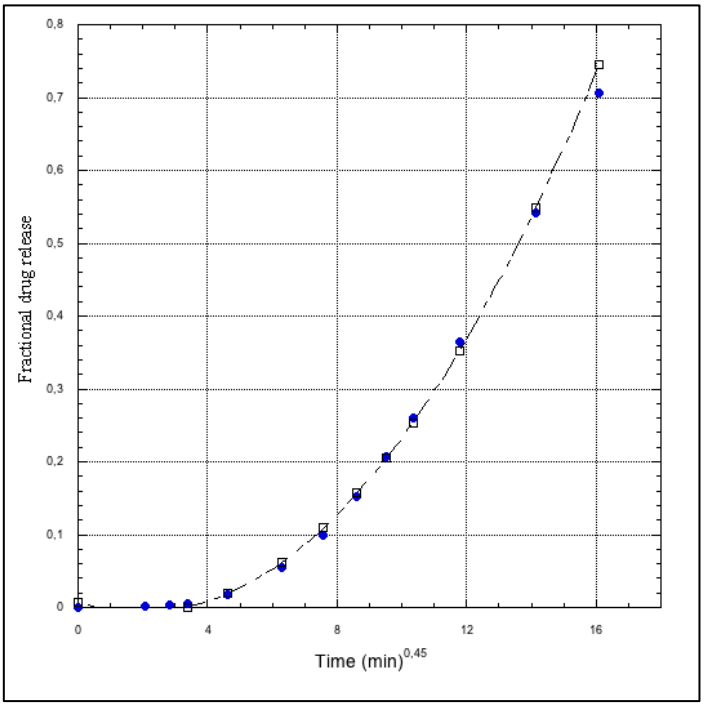


Figure 22. Drug release from matrices produced by compression of physical mixture. Matrix composition: λ Carrageenan: 25%; HPMC: 25%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=7.34*10^{-3}-1.41*10^{-2}\ t^b+3.56*10^{-3}\ t^{2b}-1.01*10^{-6}\ t^{3b}$; $R^2=0.9981$.

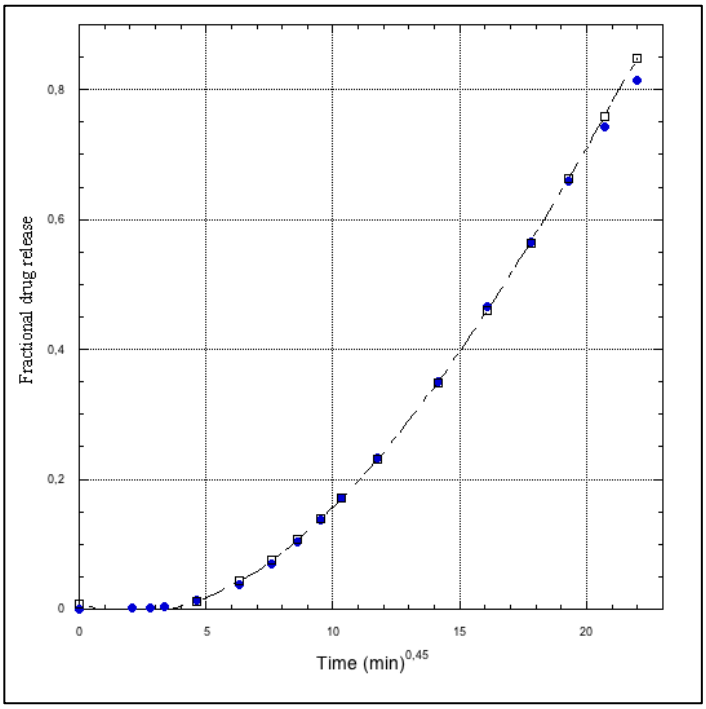


Figure 23. Drug release from matrices produced by compression of powder produced with fusion method. Matrix composition: λ Carrageenan: 12,5%; HPMC: 37,5%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=1.05*10^{-2}-1.18*10^{-2}\ t^b+2.04*10^{-3}\ t^{2b}+3.25*10^{-6}\ t^{3b}$; $R^2=0.9996$.

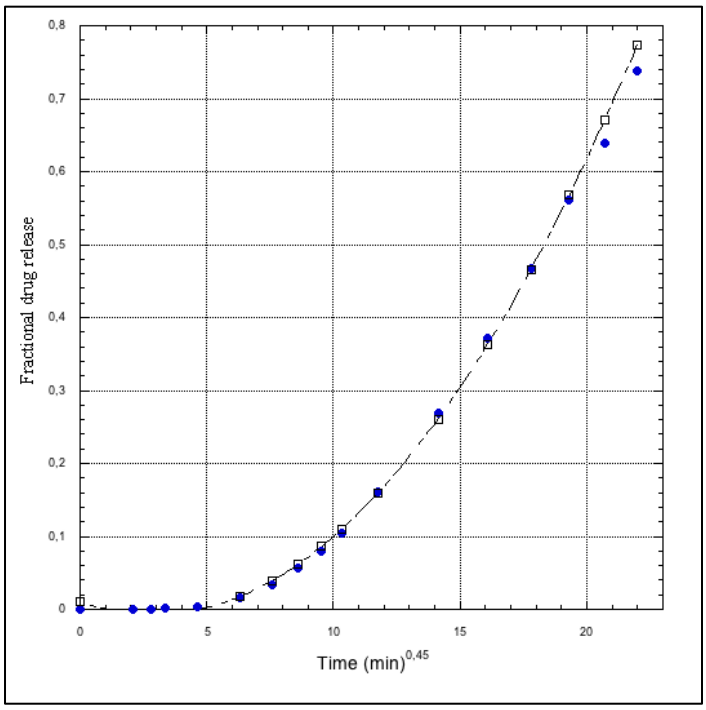


Figure 24. Drug release from matrices produced by compression of physical mixture. Matrix composition λ Carrageenan: 12,5%; HPMC: 37,5%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=8.30*10^{-3}-1.28*10^{-2}\ t^b+3.14*10^{-3}\ t^{2b}-3.70*10^{-5}\ t^{3b}$; $R^2=0.9988$.

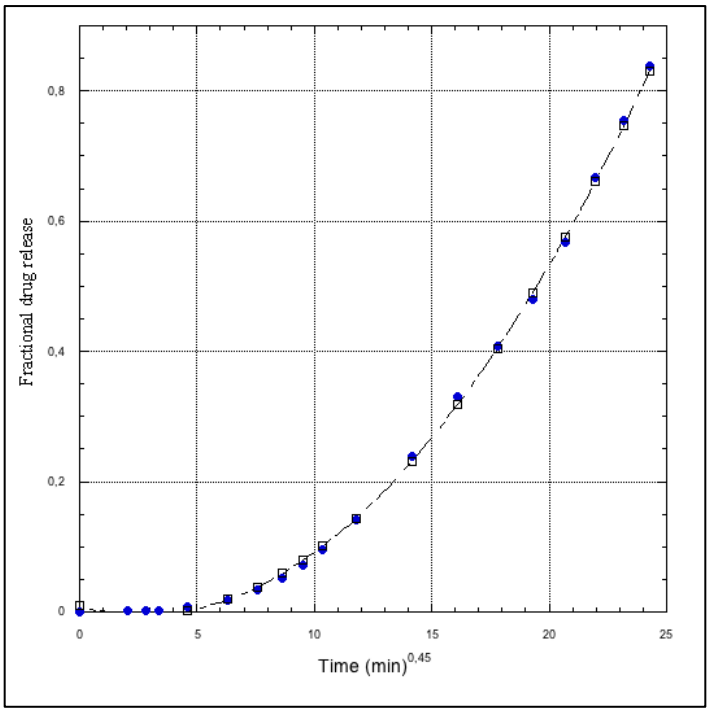


Figure 25. Drug release from matrices produced by compression of powder produced with fusion method. Matrix composition: HPMC: 50%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $Mt/M\infty=9.32*10^{-3}-9.92*10^{-3}\ t^{b}+1.83*10^{-3}\ t^{2b}-1.16*10^{-6}\ t^{3b}$; $R^2=0.9999$.

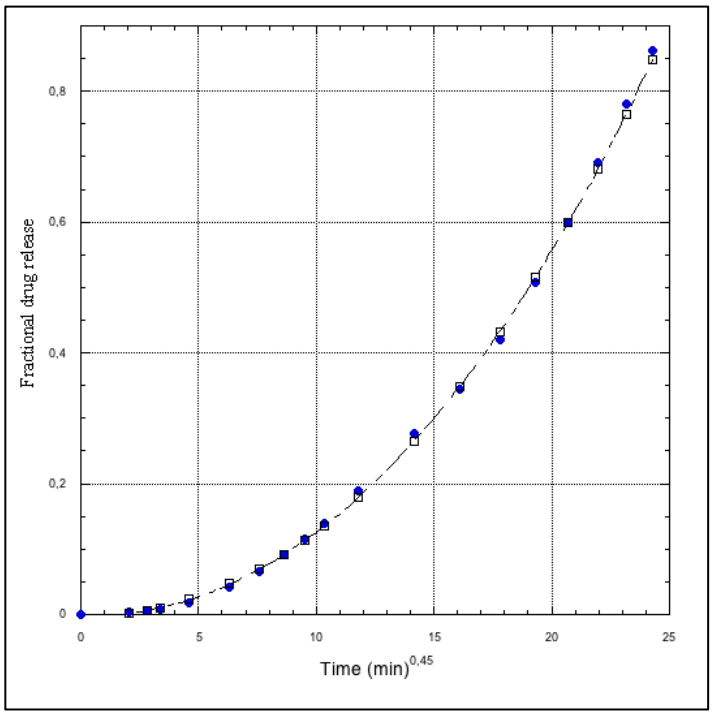


Figure 26. Drug release from matrices produced by compression of physical mixture. Matrix composition: HPMC: 50%; Compritol: 25%; Simvastatin: 25%. Blue line: Experimental data; Black Broken line: $M_t/M_\infty=-3.60*10^{-3}+1.50*10^{-4}\ t^{b}+1.18*10^{-3}\ t^{2b}-1.07*10^{-5}\ t^{3b}$; $R^2=0.9992$.

As expected, a very good fitting was obtained for all the matrices produced. Considering the shape of the curves from a geometrical standpoint, in equation 8, the coefficient M_2 contributes to a negative deviation from linearity of the curve; the coefficient M_3 accounts for the linear relationship between the variables while coefficient M_4 introduces a positive deviation from linearity of the curve.

On the other side by taking into account a possible mechanistic approach, the coefficient M_2 can be considered as indicative of Fickian diffusion release mechanism, while it is hard to assign the others coefficients to a single defined physical mechanism considering the complexity of the system. Some interesting considerations can be done by plotting the value of the three coefficients as a function of the matrix composition in terms of relative polymers content Figures 27 and 28.

Coefficients M_2 and M_3 showed specular trends, the former decreasing from 0 until 25 % HPMC (from 50 to 25 % λ Carrageenan) and then increasing from 25 to 50% HPMC (from 25 to 0 % λ Carrageenan). The opposite behaviour could be observed for coefficient M_3. This behaviour was noticed for both matrices prepared from physical mixtures and fusion method powders, being 1:1 polymers ratio the upper or lower limit for the change between positive and negative trend.

The coefficient M_4 showed a specular behaviour compared to M_3 and changed in the same direction of M_2. A slight different behaviour was observed for coefficient M_4 between the matrices obtained starting from the fusion method relative to those obtained from the physical mixture. For coefficient M_4 the change between negative and positive trend was observed at 25 % and 37.5 % HPMC ratio in tablets produced from fusion method and physical mixture powder respectively. It is worthy to underline that for both matrix types the value of M_4 became almost zero above 25 % HPMC. As stated above, M_4 accounts for the positive

deviation from linearity of the release profile. In fact, for both matrices above 25% HPMC the curve resulted practically linear.

These observations, along with those relevant to Figures 13 and 14, allow hypothesizing that M_4 may be representative of the contribution of the lipid micromatrices erosion while M_3 would account for the polymer erosion.

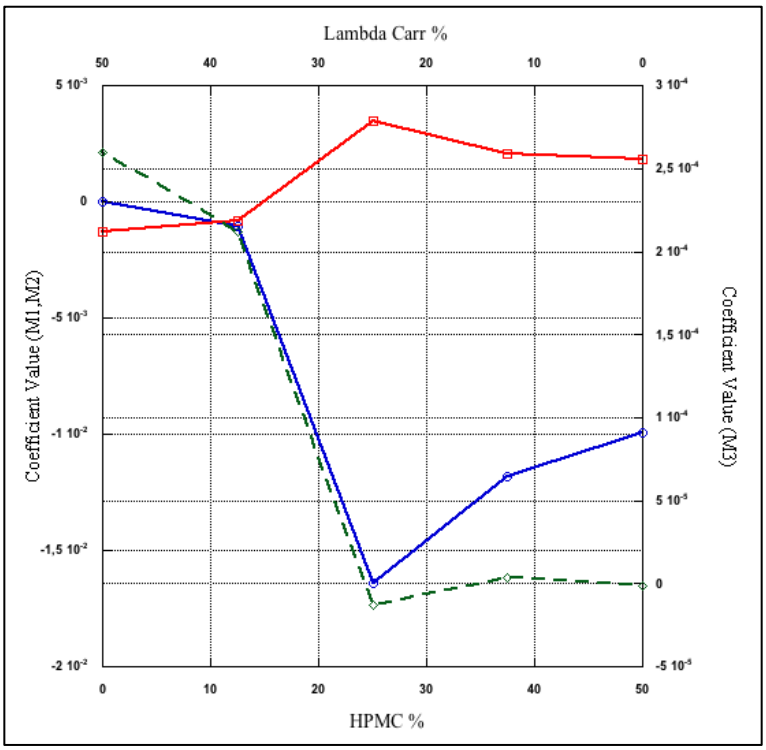


Figure 27. M_2, M_3 and M_4 coefficient values extrapolated from equation 8 obtained from matrices produced by compression of powder produced with fusion method. M_2=blue line, M_3=red line, M_4=green broken line.

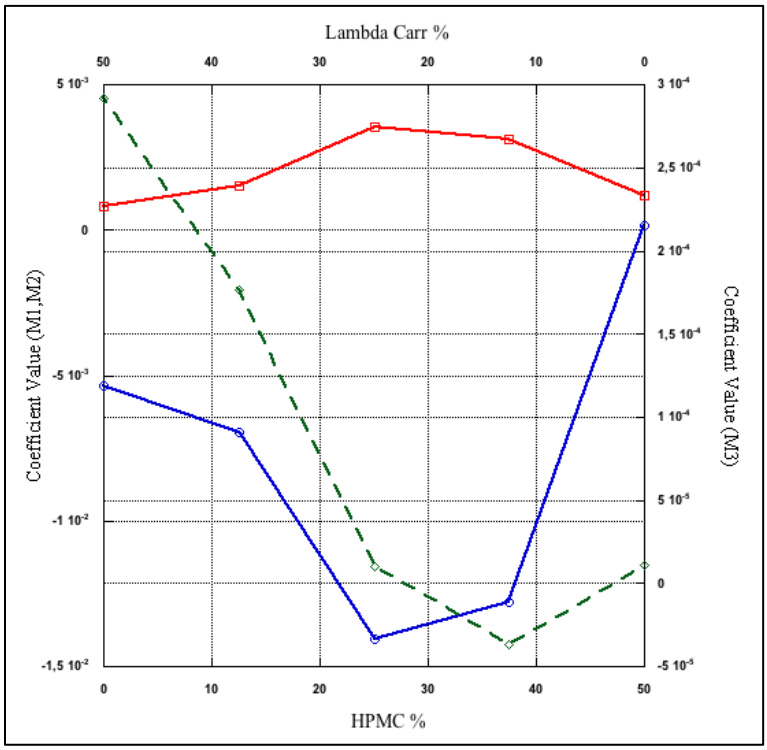


Figure 28. M_2, M_3 and M_4 coefficient values extrapolated from equation 8 obtained from matrices produced by compression of physical mixture. M_2=blue line, M_3=red line, M_4=green broken line.

4.5 Dissolution testing in hydroalcoholic media

Among the studied formulations F8 showed an intermediate drug release behaviour. For this reason it was selected to study the likelihood of the hybrid matrix to alcohol-induced dose dumping (ADD) and was tested in dissolution media containing different ethanol concentrations.

Dose dumping is defined by FDA as "unintended, rapid release in a short period of time of the entire amount or a significant fraction of the drug contained in a modified release dosage form" (Meyer *et al*., 2005). ADD can happen when patients, intentionally or not, take medicines together with alcoholic beverages, and can potentially result in severe adverse reactions. The ADD underlying mechanism resides in the fact that ethanol-water mixtures have different properties compared to aqueous dissolution media. Ethanol has a lower hydrogen bond capacity and it is less polar than water. This can modify the solubility of the active pharmaceutical ingredient and/or excipients compromising the performances of a controlled release formulation. The interaction of the dosage form with ethanol may result either in negative ethanol effect with decreased bioavailability thus compromising efficacy, or positive effect with abnormal increased bioavailability thus compromising safety. ADD should be therefore considered whenever developing a controlled release formulation (Fadda et al. 2008). This can be particularly recommended for drugs that have to be administered chronically and could led to serous side effects, i.e. simvastatin.

To perform dissolutions in alcoholic media, phosphate buffer pH 6.8 (USP) containing 5, 10, 20% v/v ethanol was used, as recommended by the European Medicine Agency (EMA, 2009). The obtained results are reported in Figure 29.

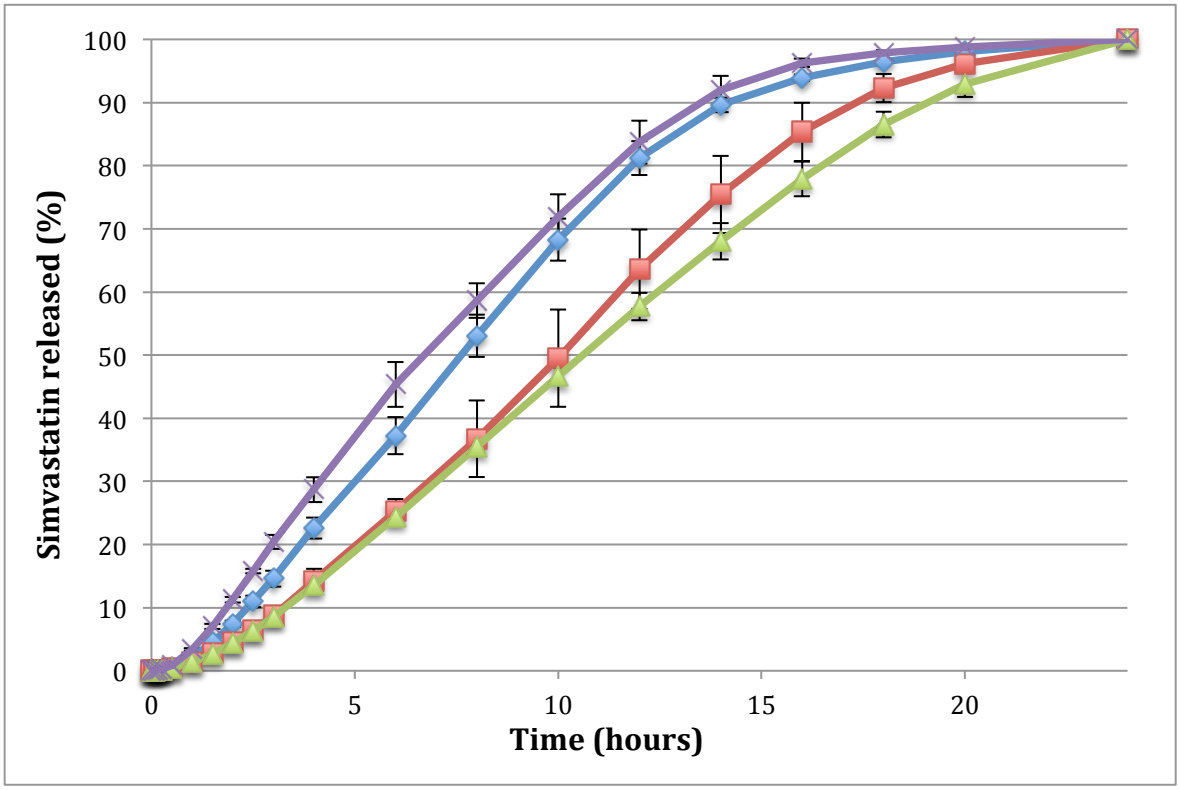


Figure 29. Simvastatin release from hybrid matrix formulation F8 in presence of 5% (blue diamonds), 10% (red squares) and 20% (green triangles) v/v ethanol concentration. The behaviour of formulation F8 in phosphate buffer pH 6.8 is also reported (purple crosses) for comparison purpose.

Simvastatin is a hydrophobic compound, and for this reason it was expected to show increased dissolution rate in presence of ethanol. However, the results obtained suggested a different scenario. As it can be seen a significant negative effect of ethanol was observed at 10 and 20% v/v concentration while at 5% ethanol effect was less pronounced.

This phenomenon can explained if we consider the dosage form tested with particular focus on the excipients employed. In fact, whenever the dissolution rate of an active ingredient is decreased by ethanol, despite its increased solubility, the explanation has been found considering the interaction of ethanol with the formulation excipients (Roberts *et al.*, 2007). HPMC solubility, as well as degree of swelling are lower in ethanol relative to pure water (Rogers T. L., 2009, Roberts *et al.*, 2007). Therefore in water-ethanol mixtures the release rate of a hydrophilic

compound would be increased because, as a consequence of the lower solvent-polymer interaction, the controlling release element, *i.e.* the HPMC gel layer, would be less structured and therefore, less efficient in controlling drug release. This is not what we observed in formulation F8, and the reason of this behaviour can be ascribed to the micromatrices of Compritol®. Compritol® is indeed insoluble in alcohol and for this reason it has been widely employed in formulation of ADD-resistant pharmaceutical dosage forms (Jedinger *et al.*, 2015). Formulation F8 seems to be therefore vulnerable in hydro-alcoholic media containing 10 and 20% of ethanol, where a decrease in the releasing rate of drug can be observed. Rubbens *et al.* recently reported that a maximum concentration of 8.5% ethanol was found in the stomach of healthy volunteers upon intake of two glasses of super- alcoholic (Rubbens *et al.*, 2017). In these conditions, dangerous increase of the release rate of simvastatin from formulation F8 resulting in increased bioavailability is not likely to occur, thus allowing us to consider F8 formulation relatively safe from this point of view.

4.6 In vivo absorption of simvastatin

The developed formulations were administered to rats in comparison to the marketed product Sinvacor® in order to investigate its pharmacokinetics.

4.6.1 Sinvacor® *in vitro* dissolution behaviour

Firstly, the in vitro dissolution behaviour of Sinvacor®, the branded simvastatin formulation available on the market, was studied for comparison purpose. Sinvacor® is an immediate release formulation produced by MSD Italia s.r.l. and it is available in three different strength i.e. 10, 20 and 40 mg. Sinvacor® 40mg is the higher dosage available and it is frequently prescribed for a twice-a-day administration in the therapy of severe cases of hypercholesterolemia. Considering the strong dependence of side effects on the dosage administered and recalling that the aim of this work was to develop new controlled release formulations with reduced side effects the choice of the strength automatically felt on the 40mg dose. The dissolution profile of Sinvacor® 40 mg tablets is reported in Figure 30.

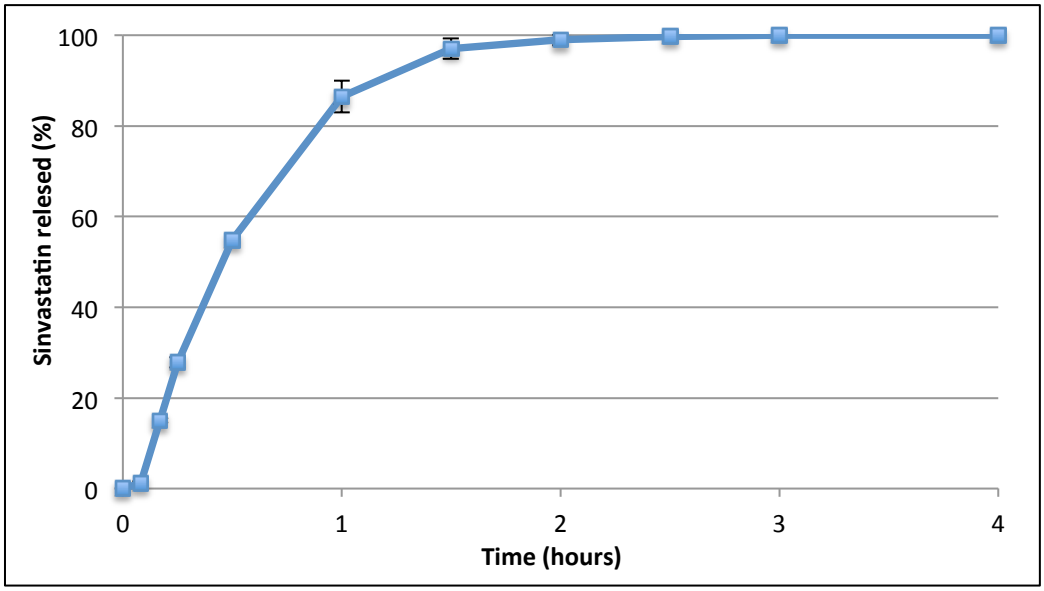


Figure 30. Sinvacor release profile in phosphate buffer pH 6.8 0.25% w/v SDS. The bar represent the standard deviation (n=3).

Sinvacor® is characterized by a relatively fast drug release with the 80% of the dose released in less than 1.5 hours. An initial lag phase lasting 5 minutes can be observed and it can be attributed to the coating of the tablet that requires a certain time to dissolve in phosphate buffer.

Among the formulation produced and characterized with *in vitro* dissolution studies, formulation F8 was chosen for *in vivo* testing because of its release profile which is faster compared with F6 and F7 formulations but slower compared with F9 and F10 formulations. Matrices produced by compression of the powder obtained by the fusion method were preferred due to their slower drug release, thus allowing covering a larger period of release time relative to the matrices produced from the physical mixture. Moreover, simvastatin/compritol® mixtures, produced with the fusion method, appeared more flowable compared to the physical mixture; this is an aspect that can strongly contribute to easier processing and dosage uniformity.

Formulation F8 was selected as the more suitable candidate for synchronizing the drug release rate/absorption with its liver uptake/metabolism in order to decrease systemic exposure to simvastatin thus resulting in lower tendency in giving statin-related myopathy (Marzo 2007, Petyaev, 2015).

4.6.2 Simvastatin pharmacokinetics

Aiming to study the behaviour of simvastatin F8 formulation in a more representative environment, *i.e.* a living organism, the *in vivo* behaviour of simvastatin tablets formulation F8 was evaluated with a pharmacokinetic study in rats upon oral administration. Sinvacor® tablets were scaled down to 2 mm diameter and tested as well for comparative purpose.

Figure 31 reports the plasma concentration of simvastatin after oral administration of F8 2 mm matrices and Sinvacor®-like 2 mm tablets.

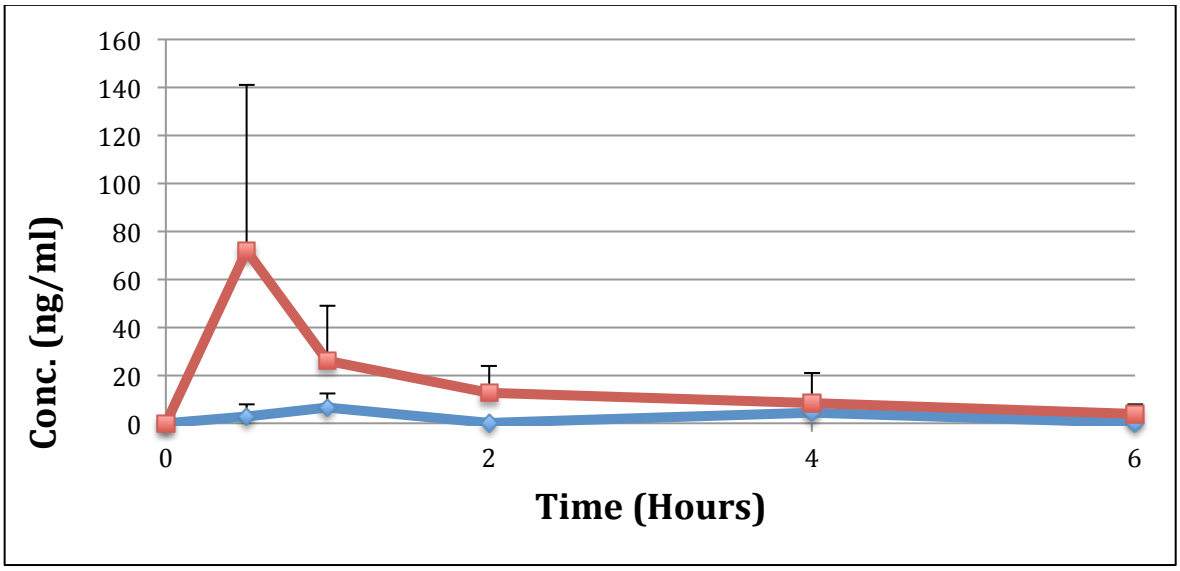


Figure 31. Simvastatin concentration in blood samples of rats receiving F8 (blue diamonds) and Sinvacor® (red squares) formulation. n= 4

The plasma profiles of the two dosage forms reflected the difference already observed from the relevant *in vitro* profiles. Rats treated with

Sinvacor® showed a higher peak in simvastatin plasma concentration after 30 minutes, with a C_{max} of 70 ng/mL. The AUC, obtained integrating the concentration *versus* time curve with KaleidaGraph® software (Version 4.5 Synergy Software, USA), resulted to be 9159,6 ng/mL/min. Rats treated with formulation F8 showed, on the other hand, a peak after 60 minutes with a C_{max} of 7ng/ml. The relevant AUC was 1508,1 ng/mL/min. Interestingly, formulation F8 showed a second lower peak after 240 minutes from administration. This phenomenon has been already reported in simvastatin *in vivo* testing and has been attributed to particular pharmacokinetic characteristics of simvastatin (Jin *et al.*, 2014). Simvastatin is well absorbed once released from the pharmaceutical dosage form and undergoes massive metabolism in the liver (Sirtori, 2014). Intestinal metabolism of simvastatin by CYP450 3A was reported to be important in determining simvastatin bioavailability by Ogasawara *et al.*, and the presence of inhibitors of this isoform can increase markedly simvastatin bioavailability (Ogasawara *et al.*, 2008). Once metabolized in the liver simvastatin is eliminated through the bile and it is subjected to enterohepatic re-circulation; this phenomenon can account for the second peak observed.

Interestingly no double peaks were observed in rats treated with the Sinvacor-like tablets, suggesting that release mode can also be involved. The calculated AUC ratio upon administration of F8 formulation resulted to be 16.5% compared with Sinvacor-like formulation, confirming that the blood exposition of simvastatin can be effectively reduced by hybrid matrix F8.

In order to assess whether the very low simvastatin plasma level measured upon administration of F8 matrix can be attributed to effective synchronization between drug release/absorption and liver metabolism or rather to the incomplete release of the drug from the dosage form, the

faeces of rats receiving formulation F8 were collected for 24 hours after dosing and kept in refrigerator at -80 °C until further analysis. The extensive enterohepatic circulation of simvastatin makes its quantification in faecal samples of limited value in the evaluation of the undissolved/unreleased amount. The faecal matter was therefore inspected only visually. Faeces were gently scrambled in order to make it in granular form. The obtained granules were then observed under the lens of a stereoscope (Citoval2 Jena, Germany) and carefully examined to seek possible residues of the matrix. The faecal granules were heterogeneous in nature showing the presence of different components of rat litter, however any matrix residue could be observed.

4.8 Simvastatin Extrusion

4.8.1 Compritol Hot Melt Extruded Powder Production

Figure 32 reports the torque force variation and the rotation of the screw during the extrusion process. As to the latter, it was set at 50 and 10 rpm during the flushing/mixing and the extrusion phase respectively. The torque force increased with the introduction of the mixture in the feeder then it slowly decreased due to the material softening. The peaks between 3 and 7 minutes accounts for each powder loading. Extrusion occurred between 10 and 40 minutes, in this time interval the torque force progressively decreased as a consequence of the delivery of the material from the extruder.

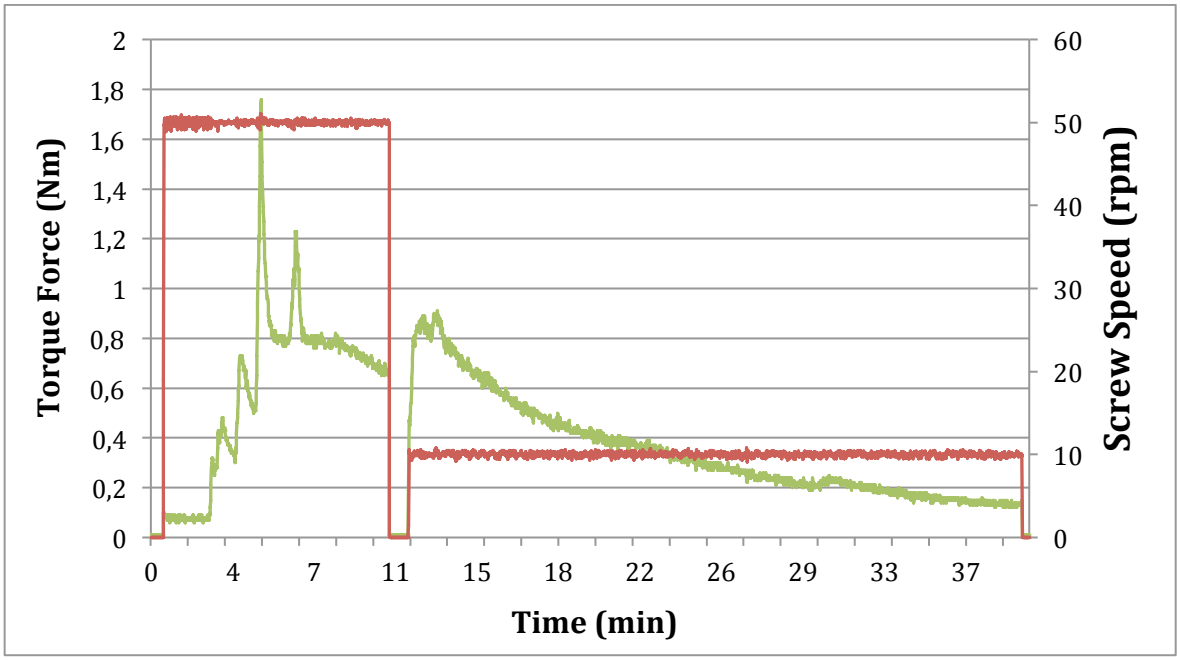


Figure 32. Torque force (green) and the screws speed (red) as a function of time during the production of Simvastatin/Compritol 50% w/w hot melt extrudate.

The mixture of Compritol®-simvastatin obtained by fusion method was produced with the procedure previously described (pp 43). Dissolution tests were made on Simvastatin-Compritol® extruded powder, Simvastatin-Compritol® physical mixture and Simvastatin-Compritol® powder obtained by fusion method.

Samples were prepared by grinding in a mortar hot melt extruded and fusion method produced materials in order to obtain powdered samples that were subsequently calibratied by passing them through a 300 μm sieve. The particle size thus selected was between 106 μm and 300 μm.

The product exiting the extrusor upon loading the binary mixture, was collected after 10, 20 and 30 minutes in three separate fractions, to evaluate to effect of the residence time on drug stability. Extruded product was slightly grey suggesting possible partial drug degradation. On the other hand a contamination stemming from the material release from the apparatus could not be excluded *a priori*. To verify these hypotheses simvastatin content in these samples was quantified by HPLC (Table 10).

The computed active content in the fractions extruded suggested a decrease in simvastatin content with time thus confirming that the extrusion process slightly affected the drug stability.

Simvastatin oxidation in the dosage form is a phenomenon that should be considered in the development of formulations, as confirmed by fact that antioxidants such as butylated hydroxyanisole (E320) and ascorbic acid (E300) are present in the commercially available Sinvacor® formulation (Sinvacor® technical data sheet).

However, the complete formulation development was beyond the scope of the present work, whose main goal was to pave the way for matrix design and release mechanism understanding.

Table 10. Simvastatin/compritol hot melt extruded powder active content.

Sample	% SMV Content, w/w	Standard Dev.
SMV/Compritol 50% w/w 1rst fraction	52.38	1.79
SMV/Compritol 50% w/w 2nd fraction	47.18	0.27
SMV/Compritol 50% w/w 3rd fraction	45.89	0.65

Simvastatin-Compritol powder produced by hot melt extrusion was tested for dissolution behaviour in phosphate buffer pH 6,8 with 0,25% w/v SDS. For comparison purpose Compritol®/Simvastatin 50% w/w physical mixture and Compritol/Simvastatin 50% w/w powders obtained by fusion method were tested as well (Figure 35). It can be appreciated that hot melt extruded and fusion method powders showed a slow release of simvastatin due to the matrix effect of Compritol already observed. Interestingly, simvastatin release from extruded micromatrices was slower in comparison with that of the powder obtained by fusion method. This can be attributed to the high shear force developed inside the extruder that would create a more intimate contact between the simvastatin particles and the surrounding lipid excipient.

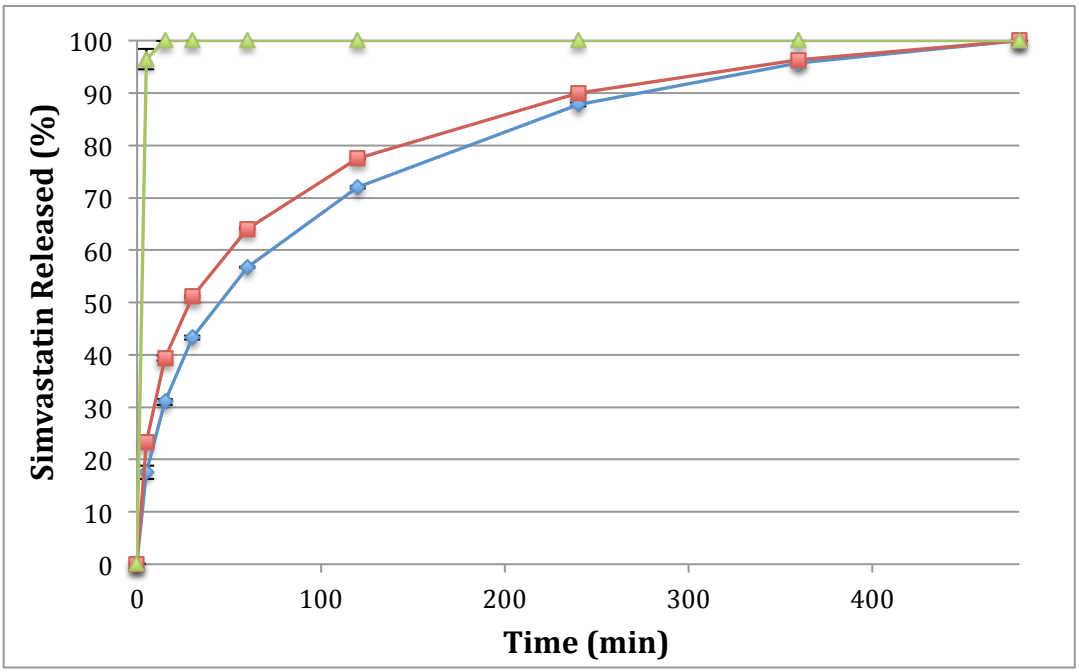


Figure 35. Simvastatin release profiles from simvastatin/Compritol® 50%w/w physical mixture (green triangles) and hot melt extruded (blue diamonds) and fusion method (red squares) powders.

DSC measurements were performed to assess the solid-state properties of the powder produced. DSC traces (Figure 36) evidenced melting point of both compritol and simvastatin indicating that simvastatin crystal structure was maintained, at least partially, during the hot melting process. The same thermal event can be appreciated also in the DSC trace of powder produced with the fusion method (Figure 37). We can conclude that in both powders simvastatin was present in solid, crystalline form.

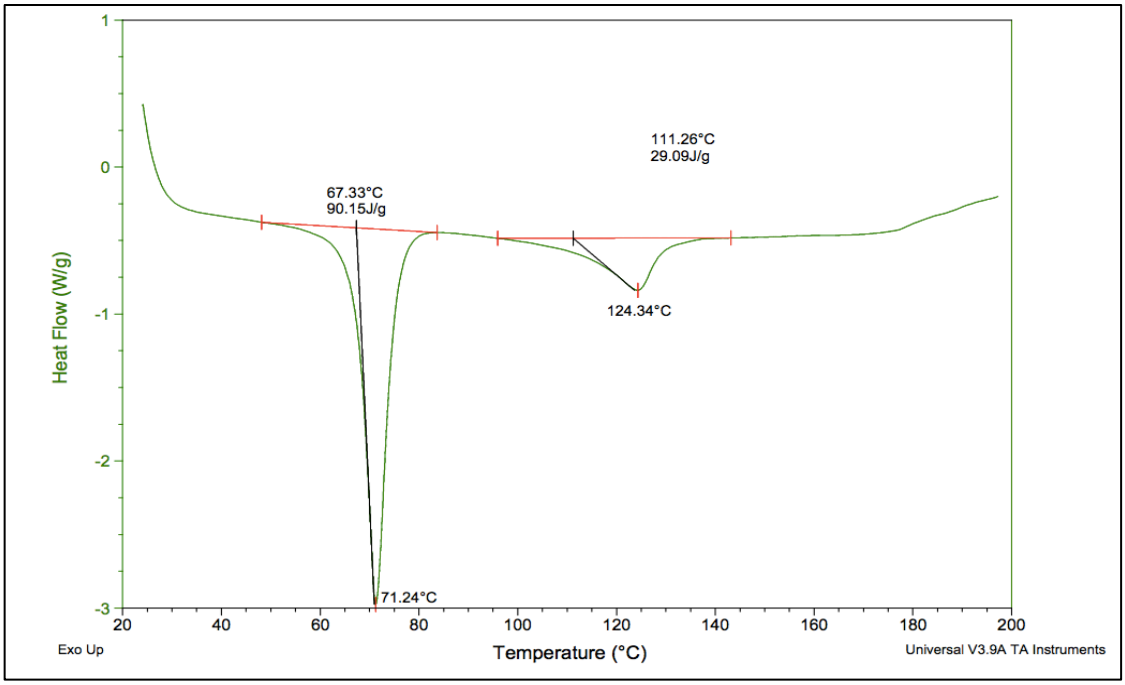


Figure 36. DSC trace of simvastatin hot melt extruded powder.

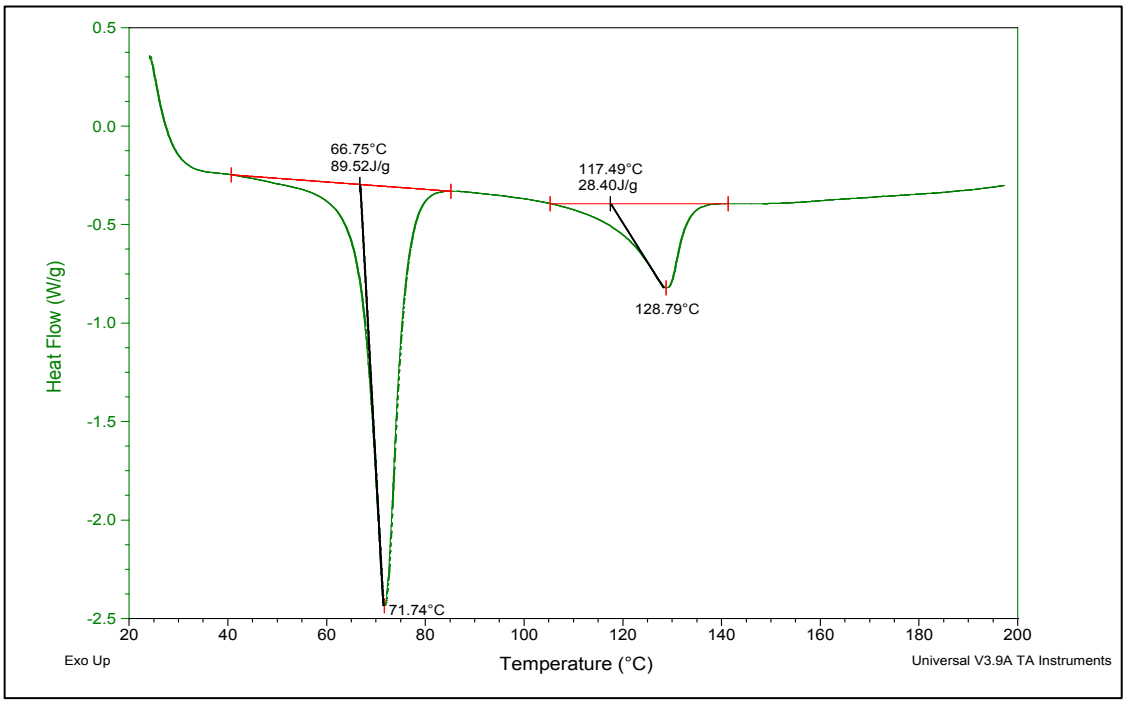


Figure 37. DSC trace of simvastatin fusion method powder.

4.8.2 Simvastatin hybrid matrix extrusion and testing

After the extrusion of simvastatin:Compritol 50 : 50 w/w, an attempt to obtain slower releasing matrices was performed introducing HPMC in the system. Formulation F1 HME containing a large amount of HPMC, was chosen in consideration of the literature available on this topic on one side (Baert *et al.*, 1997, Ghebremeskel *et al.*, 2006, Javeer et al., 2013) and of the lack of characterization of HPMC-Compritol extruded systems on the other. Extrusion temperature was set at 80°C to obtain the fusion of Compritol® that was expected to act as binder and controlling release excipient at the same time. Demineralized water was used to obtain the plasticization of HPMC at this temperature. The amount of water needed for plasticization of HPMC in the ternary mixture was determined empirically, performing hot melt extrusion on mixtures of powders with increasing water amount and recording the torque force of the screws. Water was dispersed over a HPMC bed with a nebulizer to facilitate the uniform hydration of the polymer, thus reducing the formation of agglomerates. Wetted HPMC was then added to other components, mixed gently in a mortar with a plastic spatula and introduced manually in the feeder of the extruder. The most critical parameter during the extrusion resulted to be the torque force of the screws. The screws should be able to turn inside the extruder to mix and extrude the material but some attrition is on the other hand needed to move the powder inside the extruder. A good compromise between these two situations was found. The plasticization of HPMC was obtained by introducing 0.32g of water for 1g of HPMC.

It should be underlined that this amount of water was not enough to extrude HPMC:simvastatin 50:50 w/w binary mixtures since the torque force was extremely high. This put into evidence the pivotal contribution

of Compritol® that acted not only as binder and controlling release excipient, but also as lubricant allowing extrusion of the ternary mixture.
The extrusion of Simvastatin:Compritol:HPMC 25:25:50 was successful even thought it was necessary to remove the die to allow to the material to exit the extruder. At the exit of the extruded indeed, water evaporated and the temperature was high due to the high shear force. The molten mixture therefore dried and stiffened in the die, hindering extrusion.
After collection the extruded material was placed in a vacuum oven at 30 °C for 12 hours before proceeding with the determination of the active content. Figure 33 reports the extrusion profile of this mixture.

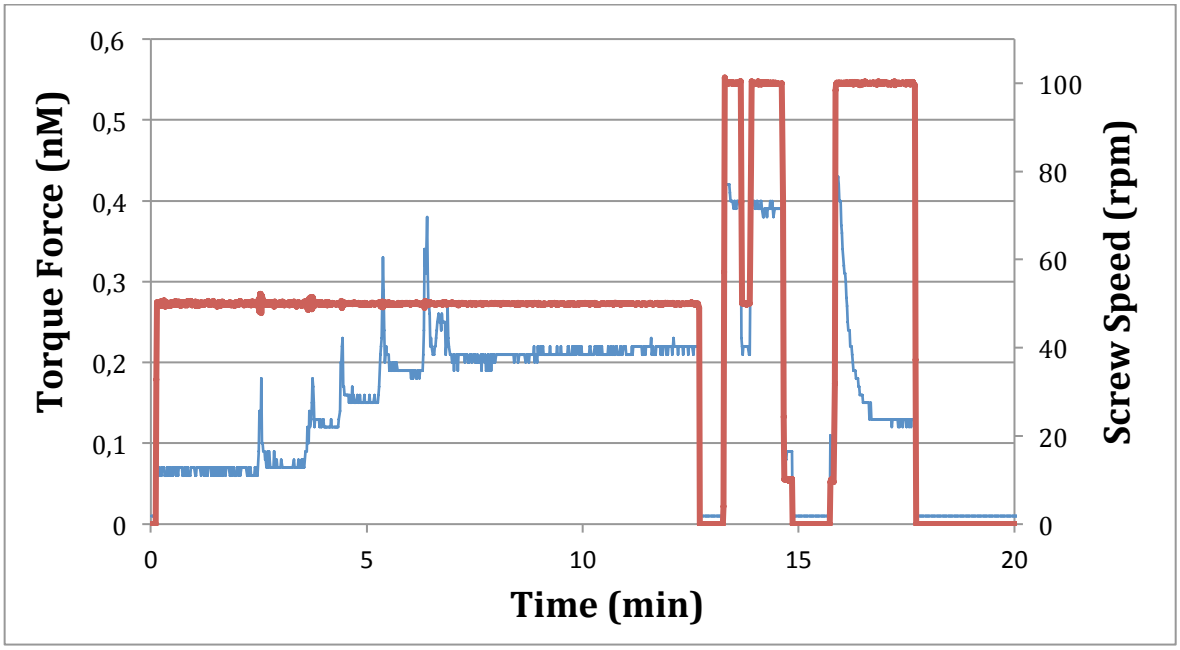


Figure 33. Torque force (blue line) and the screws speed (red line) are reported as a function of time during the production of hybrid matrix with hot melt extrusion

It can be observed that, contrary to the extrusion process of the binary mixture (Figure 32) the screws speed was set at 50rpm for the loading/mixing step and at 100rpm during the extrusion.
The loading step lasted for 12 minutes and was performed through 6 consecutive powder loadings. The extrusion phase was carried out

between 14 and 18 min and implied the realization of three subsequent steps due to the difficulty in material delivery.

The extruded bars were cut in matrices rectangular having the dimensions 2.9x1.97x5 mm Figure 34. Each rectangular matrix weighted 24.8 ±1.8 mg.

The dissolution testing was performed placing a weighed quantity of rectangular matrices in each basket (about 6-7 matrices), in order to obtain the equivalent amount of a 40 mg simvastatin dose in each vessel.

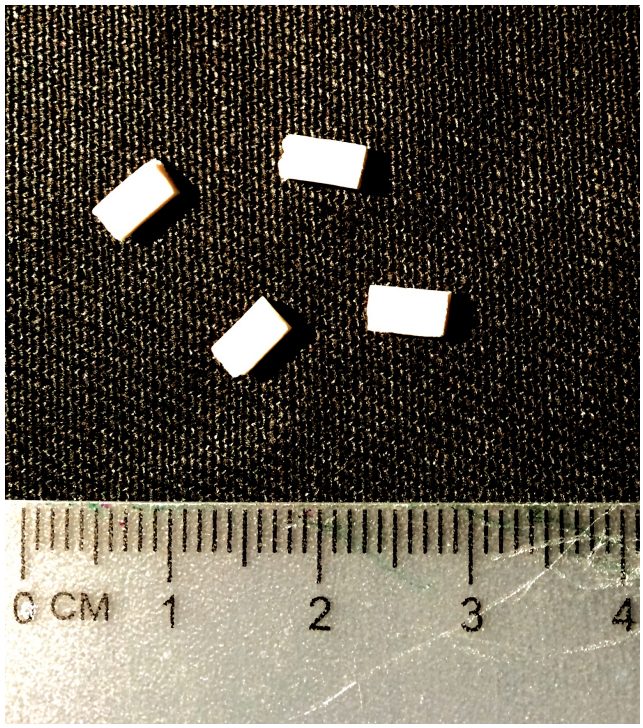


Figure 34. Matrices cut from the hot melt extruded barrel.

Simvastatin matrix tablets, obtained by direct compression of the physical mixture, were produced for comparison purpose. Simvastatin 40 mg tablets were produced using a hydraulic press operated at about 15kN for two seconds to obtain a disk-shaped matrix having a diameter of 5mm and a thickness of 1.97mm. The composition of these matrices is reported in Table 9.

Table 9 Composition of simvastatin tablets produced by direct compression of the physical mixture.

HPMC	80 mg
Compritol	40 mg
Simvastatin	40 mg
Magnesium stearate	1% w/w

The active content of the extruded mixture was evaluated with HPLC and resulted 22.56 ± 0.22 %, a little less than expected (theoretical value= 25%). This slight reduction was one again attributed to a partial degradation of the active. However, it is worthy underling that no additional peaks, ascribable to simvastatin degradation product were detected in the HPLC chromatogram.

As previously stated, the dissolution testing on the matrices produced was performed by placing the equivalent amount of a 40 mg simvastatin dose in each vessel. Unfortunately, it was not possible to cut perfectly shaped matrices since the material was extremely crumbly. For this reason it was hard to obtain the exact equivalent amount of a 40mg dose of simvastatin, considering an active content of 22,6% (Table number 10).

Table 10. Number of matrices, cumulative weight, and calculated active content of the hot melt extruded matrices tested.

	Number of rectangular matrices	Total Weight (mg)	SMV Content (mg)
Vessel 1	7	161.22	36.38
Vessel 2	7	173,08	39.05
Vessel 3	6	159.88	36.08

The dissolution profiles of simvastatin hot melt extruded (F1 HME) and direct compression disk-shaped matrices (F1 DCD) are reported together for comparison in Figure number 38.

Table 11. Diameter-thickness ratio (2a/l) and surface area initially exposed to the dissolution medium in formulations F1 produced with different methods and having different shapes. F1 HME: Hot melt extruded F1; F1 DCD: direct compression disk-shaped F1; F1: direct compression cylindrical shaped F1; a= radius, l=thickness.

	2a/l	Surface Area (mm^2)
F1 HME		319.4
F1 DCD	12.90	171.21
F1	1.98	82.06

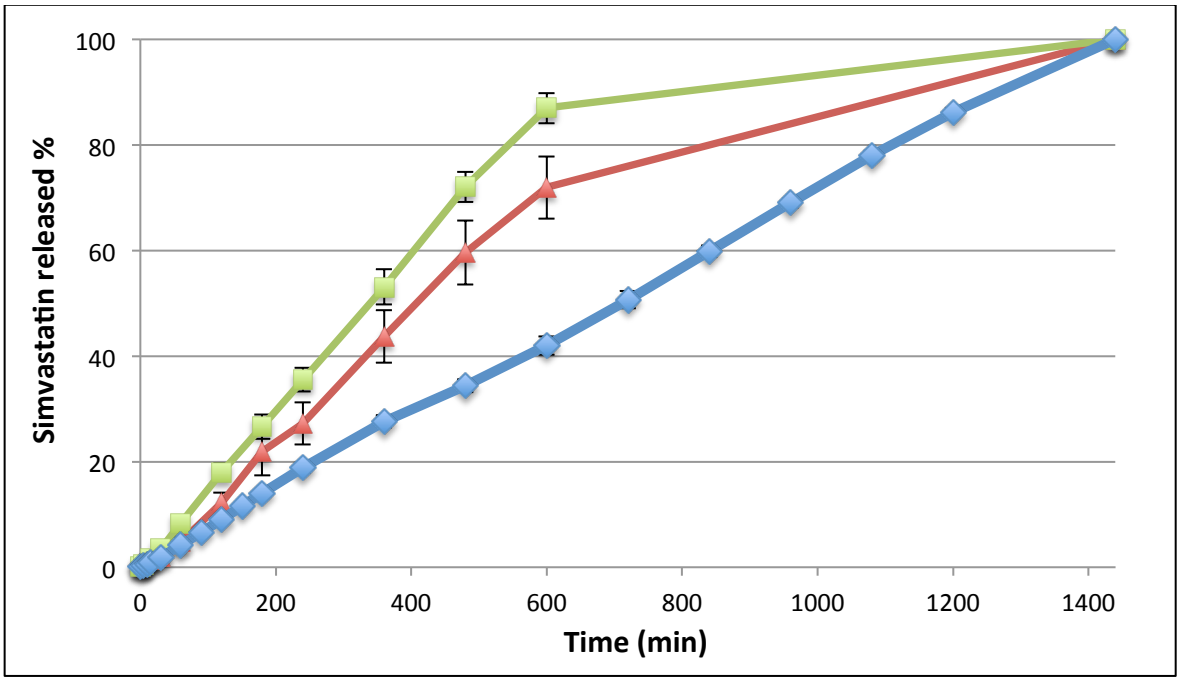


Figure38. Dissolution profiles of F1 HME (green squares), F1 DCD (red trianlges) and F1 (blue diamonds). The bars represent the standard deviation (n=3).

Both matrices afforded linear drug release. Simvastatin release rate from hot melt extruded matrices was faster compared with direct compression disk-shaped matrices. This could be due to both the differences in term of density and geometry of the systems. Hot melt extruded matrices indeed presented a higher surface area available for drug release and water interaction compared to tablets (319.4 mm^2 versus 171,21 mm^2 respectively, Table 11). Furthermore, hot melt extruded tablets were expected to present an increased porosity due to the evaporation of water during the extruding process (Vo *et al.,* 2017). An experimental observation supporting this assumption is that hot melt extruded

matrices floated on the dissolution medium for 10 hours from the beginning of the experiment. This can be attributed to the low density of the systems and appears to be of particular interest as this phenomenon may impart floating properties to the systems that may be retained in the stomach upon oral administration, thus prolonging the time available for simvastatin controlled release and absorption.

It is interesting to note that formulation F8 released the drug in with significantly lower rate, although with the same linear kinetics.

Once again the slower drug release was ascribed to the significantly lower initial release surface and diameter/thickness ratio (82.06 mm^2 and 1.98 respectively, Table 11).

5. Conclusions

In the present thesis work we have addressed the problem of the availability of a controlled-release dosage form for the simvastatin oral administration, aiming at designing a matrix capable to slowly release the drug, in order to allow complete or almost complete uptake by the liver after intestinal absorption. The rational is the dramatic reduction of simvastatin systemic exposure in order to avoid the associated side effects.

The adopted approach is based on the construction of binary or ternary matrices, in which the drug is firstly dispersed in crystalline form into solid lipid micromatrices that, in turn, are dispersed into a hydrophilic matrix based on HPMC or a mixture of HPMC and λ-carrageenan.

In systems containing 25% w/w simvastatin, by simply modifying the relative content of HPMC and λ-carrageenan while keeping fixed at 25% w/w the amount of solid lipid excipient, it is possible to get a fine tuning of drug release rate.

The kinetics obtained by these systems can be described as linear or Super case II (drug release rate increasing with time).
The occurrence of the first, rather than the second, is related to the relative importance of the contribution of solid lipid erosion, λ-carrageenan dissolution and HPMC swelling.

Pharmacokinetic experiment in rats evidenced the capability of one of the developed formulation to dramatically reduce the plasma concentration of simvastatin, in comparison with the formulation available on the market.

This reduction is the consequence of the slow drug absorption associated with the slow drug release, rather then of the incomplete release of the active ingredient from the dosage form.

Finally, during the traineeship carried out at the University of Toronto, a hot melt extrusion method has been developed for the production of binary matrices containing equal amount of solid lipid excipient and simvastatin. Hot melt extrusion is a continuous production method that would be very promising for the scaling up of the production.

6. Bibliography

Aguzzi C., Bonferoni M. C., Fortich M. R. O., Rossi S., Ferrari F., Caramella C**.,** ***Influence of complex solubility on formulations based on lambda carrageenan and basic drugs***, AAPS PharmSciTech., 3, 2002, pp 83-89.

Alfrey T., Gurnee E. F. and Lloyd, W. G., ***Diffusion in glassy polymers***, J. polym. sci., C Polym. symp., 12, 1966, 249–261.

Apostolou C., Kousoulos C, Dotsikas Y., Soumelas G. S., Kolocouri F., Ziaka A., Loukas T. L., ***An improved and fully validated LC–MS/MS method for the simultaneous quantification of simvastatin and simvastatin acid in human plasma***, Journal of Pharmaceutical and Biomedical Analysis, 46, 2008, pp 771-779.

Ash M., Ash I., ***Handbook of fillers, extenders, and diluents***, Endicott, NY, 2008, Synapse Information Resources.

Baer A. N., Wortmann R. L., ***Myotoxicity associated with lipid-lowering drugs***, Curr. Opin. Rheumatol., 19, 2007, pp 67–73.

Baert L., Elvire C., Verreck G., ***Antiretroviral compositions with improved bioavailability***, Eur. Patent, 0,872,233, 1997.

Baker R. W., Lonsdale H. S., ***Controlled release: mechanisms and rates*****,** In A. C. Taquary, & R. E. Lacey (Eds.), Controlled release of biologically active agents, 1974, New York: Plenum, pp 15– 71.

Barrett B., Huclová J., Bořek-Dohalský V., Němec B., Jelínek I., ***Validated HPLC-MS/MS method for simultaneou sdetermination of simvastatin and simvastatin hydroxy acid in human plasma,*** Journal of Pharmaceutical and Biomedical Analysis, 41, 2006, pp 517-526.

Barthelemy P., Laforet J. P., Farah N., Joachim J., ***Compritol® 888 ATO: an innovative hot-melt coating agent for prolonged-release drug formations*****,** Eur. J. Pharm. Biopharm., 47, 1999, pp 87–90.

Baveja S. K., Ranga Rao K. V., Padmalatha Devi K., ***Zero-order release hydrophilic matrix tablets of β-adrenergic blockers***, International Journal of Pharmaceutics, 39, 1987, pp 39-45.

Bettini R., Colombo P., Massimo G., Catellani P. L., Vitali T., ***Swelling and drug release in hydrogel matrices: polymer viscosity and matrix porosity effects***, European Journal of Pharmaceutical Sciences, 2, 1994, pp 213-219.

Bonferoni M. C., Rossi S., Ferrari F., Bertoni M., Bolhuis G. K., Caramella C., ***On the employment of λ carrageenan in a matrix system. III. Optimization of a λ carrageenan–HPMC hydrophilic matrix***, Journal of Controlled Release, 51, 1998, pp 231-239.

Bonferoni M. C., Rossi S., Tamayo M., Pedraz J. L., Dominguez-Gil A., Caramella C., ***On the employment of λ-carrageenan in a matrix system. I. Sensitivity to dissolution medium and comparison with Na carboxymethylcellulose and xanthan gum*****,** Journal of Controlled Release, 26, 1993, pp 119-127.

Bonferoni M. C., Rossi S., Tamayo M., Pedraz J. L., Dominguez-Gil A., Caramella C., ***On the employment of λ-carrageenan in a matrix system. II. λ-Carrageenan and hydroxypropylmethylcellulose mixtures***, Journal of Controlled Release, 30, 1994, pp 175-182.

Boudreau D. M., Yu O., Johnson J., ***Statin use and cancer risk: a comprehensive review***, Expert Opin. Drug Saf., 9, 2010, pp 603-621.

Brazel C. S., Peppas N. A., ***Modeling of drug release from swellable polymers***, Eur J Pharm Biopharm., 49, 2000, pp 47-58.

Breitenbach J., ***Melt extrusion: from process to drug delivery technology,*** Eur J Pharm Biopharm., 54, 2002, 107-117.

Carlucci G., Mazzeo P., Biordi L., Bologna M., ***Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high-performance liquid chromatography with UV detection***, Journal of Pharmaceutical and Biomedical Analysis, 10, 1992, pp 693-697.

Catellani P. L., Colombo P., Peppas N. A., Santi P., Bettini R., ***Partial permselective coating adds an osmotic contribution to drug release from swellable matrixes***, J. Pharm. Sci., 87, 1998, pp 726–731.

Charman W. N., Stella V. J., ***Estimating the maximum potential for intestinal lymphatic transport of lipophilic drug molecules***, Int. J. Pharm., 34, 1986, pp 175–178.

Colombo P., Bettini R., Massimo G., Catellani P. L., Santi P., Peppas N.A., ***Drug diffusion front movement is important in drug release control from swellable matrix tablets***. J. Pharm. Sci., 84, 1995, pp 991-997.

Colombo P., Bettini R., Peppas N.A., ***Observation of swelling process and diffusion front position during swelling in hydroxypropyl methyl cellulose (HPMC) matrices containing a soluble drug***, J. Control. Release, 61, 1999, pp 83-91.

Colombo P., Bettini R., Santi P., De Ascentiis A., Peppas, N. A., ***Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport***, Journal of Controlled Release, 39, 1996, pp 231-237.

Colombo P., Bettini R., Santi P., Peppas N. A., ***Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance***, Pharmaceutical Science & Technology Today, 3, 2000, pp 198-204.

Colombo P., Gazzaniga, A., Caramella C., ***In vitro programmable zero-order release drug delivery system***, Acta Pharmaceutica Technologica, 33, 1987, pp 15-20.

Colombo P., Sonvico F., Colombo G., Bettini R., ***Novel platforms for oral drug delivery***, Pharm Res., 26, 2009, pp 601-611.

Davignon J., ***Beneficial cardiovascular pleiotropic effects of statins***, Circulation, 109, 2004, pp III39- III43.

Dumez A. G., ***A contribution to the history of the development of the enteric capsule***, J. Pharm. Sci., 10, 1921, pp 372-376.

EMA, Quality of medicines questions and answers: Part 2, Subchapter: Specific types of product—***Need for in-vitro dissolution studies with alcohol for modified release oral products including opioid drug products***, 2009, http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/q_and_a/q_and_a_detail_000072.jsp#section10.

Endo A., Kuroda M., Tanzawa K., ***Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme a reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity***, FEBS Letters, 72, 1976, pp 323-326.

Endo A., Kuroda M., Tsujita Y., ***ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by Penicillium citrinium***, J. Antibiot., 29, 1976, pp 1346-1348.

Endo A., ***The origin of the statins***, Atheroscler. Suppl., 5, 2004, pp 125-130.

Fadda H. M., Mohamed M. A. M., Basit A. W., ***Impairment of the in vitro drug release behaviour of oral modified release preparations in the presence of alcohol***, Int. J. Pharm., 360, 2008, 171-176.

Fahy E., Subramaniam S., Brown H. A., Glass C. K., Merrill Jr. A. H., Murphy R.C., Raetz C.R., Russell D. W., Seyama Y., Shaw W., Shimizu T., F. Spener, Van Meer G., VanNieuwenhze M.S., White S. H., Witztum J. L., Dennis E. A., ***A comprehensive classification system for lipids***, J. Lipid Res. 46, 2005, pp 839–861.

Feingold K. R., Grunfeld C., ***Introduction to Lipids and Lipoproteins***, In: De Groot L. J., Chrousos G., Dungan K., *et al*., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK305896/.

Fini A., Cavallari C., Ospitali F., Gonzalez-Rodriguez M. L., ***Theophylline-Loaded Compritol Microspheres Prepared by Ultrasound-Assisted Atomization***, Journal of Pharmaceutical Sciences, 100, 2010, pp 743–757.

Florence A. T., ***A short history of controlled drug release and an introduction***, In: Wilson C., Crowley P., editors. Controlled Release in Oral Drug Delivery, New York: Springer Science and Business Media; 2011, pp 1-21.

Florence A. T., Siepmann J., ***Modern Pharmaceutics Volume 1, Basic Principles and Systems***, Fifth Edition, CRC Press , 2010, pp 2-3.

Folkman J., ***How the field of controlled-release technology began, and its central role in the development of angiogenesis research***, Biomaterials, 11, 1990, pp 615–618.

Furberg C. D., Pitt B., ***Withdrawal of cerivastatin from the world market***, Current Controlled Trials in Cardiovascular Medicine, 5, 2001, pp 205-207.

Gazzaniga A., Iamartino P., Maffione G., Sangalli M. E., ***Oral delayed-release system for colonic specific delivery***, International Journal of Pharmaceutics, 108, 1994, pp 77-83.

Gelissen I. C., McLachlan A. J., ***The pharmacogenomics of statins***, Pharmacological Research, 88, 2014, pp 99-106.

Ghebremeskel N. A., Vemavarapu C., Lodaya M., ***Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble api: Stability testing of selected solid dispersions***, Pharm. Res., 23, 2006, pp 1928–1936.

Goldstein J. L., Brown M. S., ***Regulation of the mevalonate pathway***, Nature, 343, 1990, pp 425-430.

Gradman A. H., Basile J. N., Carter B. L., Bakris G. L., ***Combination therapy in hypertension***, Journal of the American Society of Hypertension, 4, 2010, pp 42 – 50.

Grassi M., Grassi G., ***Mathematical modelling and controlled drug delivery: matrix systems***, Curr Drug Deliv., 2, 2005, pp 97-116.

Gu X., Fediuk D. J., Simons F. E., Simons K. J., ***Evaluation and comparison of five matrix excipients for the controlled release of acrivastine and pseudoephedrine***, Drug Dev. Ind. Pharm., 30, 2004, pp 1009-1017.

Helfand W. H., Cowen D. L., ***Evolution of pharmaceutical dosage forms,*** Pharm. Hist. 25, 1983, pp 3–18.

Higuchi T., ***Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices,*** J. Pharm. Sci., 52, 1963, pp 1145-1149.

Higuchi T., ***Physical chemical analysis of percutaneous absorption process from creams and ointments***, Soc. Cosmetic Chemists., 11, 1960, pp 85-97.

Hixson A. W., Crowell J. H., ***Dependence of Reaction Velocity upon surface and Agitation***, J. Industrial & Engineering Chemistry, 23, 1931, pp 923-931.

Hoffman A. S., ***The origins and evolution of "controlled" drug delivery systems*****,** Journal of Controlled Release, 132, 2008, pp 153-163.

Hoffmann M., Nowosielski M., ***DFT study on hydroxy acid-lactone interconversion of statins: the case of atorvastatin***, Org. Biomol. Chem., 6, 2008, pp 3527-3531.

Hokanson J. E., Austin M. A., ***Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies***, J. Cardiovasc. Risk, 3, 1996 pp 213–219.

Imtaiyaz H. M., Khan M., ***Mathematical modeling as a tool for improving the action of drugs***, Journal of Basic and Applied Sciences., 4 , 2008, pp 81-88.

Jacques C. H. M., Hopfenberg H. B., Stannett V., ***Super Case II Transport of Organic Vapors in Glassy Polymers,*** In: Hopfenberg H.B. (eds) Permeability of Plastic Films and Coatings. Polymer Science and Technology, vol 6., 1974, Springer, Boston, MA, pp 73-86.

Javeer S. D., Patole R., Amin P., ***Enhanced solubility and dissolution of simvastatin by HPMC-based solid dispersions prepared by hot melt extrusion and spray-drying method***, Journal of Pharmaceutical Investigation, 43, 2013, pp 471-480.

Jedinger N., Schrank S., Mohr S., Feichtinger A., Khinast J, Roblegg E., ***Alcohol dose dumping: The influence of ethanol on hot-melt extruded pellets comprising solid lipids***, European Journal of Pharmaceutics and Biopharmaceutics, 92, 2015, pp 83–95.

Jin S.J., Bae K.S., Cho S.H., Jung J.A., Kim U., Choe S., Ghim J.L., Noh Y.H., Park H.J., Kim H.S., Lim H.S., ***Population pharmacokinetic analysis of simvastatin and its active metabolite with the characterization of atypical complex absorption kinetics***, Pharm. Res., 31, 2014, pp 1801-1812.

Katzhendler I., Hofman A., Goldberger A., Friedman M., ***Modeling of drug release from erodible tablets,*** Journal of Pharmaceutical Sciences, 1997, 86, pp 110–115.

Kossena G. A., Charman W. N., Wilson C. G., O'Mahony B., Lindsay B., Hempenstall J. M., Davison C. L., Crowley P. J., Porter C. J., ***Low dose lipid formulations: effects on gastric emptying and biliary secretion***, Pharm Res., 24, 2007, pp 2084-2096.

Kuick Research, ***Global Controlled Drug Delivery Market & Clinical Trial Outlook 2022***, Global Report, May 2017, ID: 4279005.

Langer R., ***New Methods of Drug Delivery***, Science, 249, 1990, pp 1527–1533.

Law M., Rudnicka A.R., ***Statin safety: a systematic review***, Am. J. Cardiol., 97, 2006, pp 52C-60C.

Lazarus J., Cooper J., ***Absorption, testing and clinical evaluation of oral prolonged- action drugs***, J Pharm Sci, 50, 1961, 715-732.

Lee P. I., Li J. X., ***Evolution of Oral Controlled Release Dosage Forms*****, in** ***Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice,*** (eds H. Wen and K. Park), John Wiley & Sons, 2010, pp 21-31.

Lee P.I., Kim C. J., ***Probing the mechanisms of drug release from hydrogels***, J. Control. Release 16, 1991, pp 229–236.

Li F. Q., Hu J. H., Deng J. X., Su H., Xu S., Liu J. Y., ***In vitro controlled release of sodium ferulate from Compritol 888 ATO-based matrix tablets***, Int. J. Pharm., 324, 2006, 152-157.

Li L., Ni R., Shao Y, Mao S., ***Carrageenan and its applications in drug delivery***, Carbohydrate Polymers, 103, 2014, pp 1-11.

Liu J., Zhang F., McGinity J.W., ***Properties of lipophilic matrix tablets containing phenylpropanolamine hydrochloride prepared by hot-melt extrusion***, Eur. J. Pharm. Biopharm., 52, 2001, pp 181-190.

Maderuelo C., Zarzuelo A., Lanao J. M., ***Critical factors in the release of drugs from sustained release hydrophilic matrices*****,** Journal of Controlled Release, 154, 2011, pp 2-19.

Malaterre V., Ogorka J., Loggia N., Gurny R., ***Approach to design push–pull osmotic pumps*****,** International Journal of Pharmaceutics, 376, 2009, pp 56-62.

Martindale W. H., Wescott W. W., ***The extra pharmacopoeia***, vol 1, 18th edn. H. K. Lewis, London, 1924, pp 682–683.

Marzo A., ***Open questions on bioequivalence: an updated reappraisal***, Curr. Clin. Pharmacol.., 2, 2007, pp 179-189.

Mona H Aburahma M. H. and Badr-Eldin S. M., ***Compritol 888 ATO: a multifunctional lipid excipient in drug delivery systems and nanopharmaceuticals,*** Expert Opin. Drug Deliv., 2014.

Nair A. B., Jacob S., ***A simple practice guide for dose conversion between animals and human***, J. Basic Clin. Pharm., 7, 2016, pp 27-31.

Nastruzzi C., ***Lipospheres in drug targets and delivery: Approaches, methods and applications***, CRC Press; New York: 2004.

Nováková L., Šatínský D., Solich P., ***HPLC methods for the determination of simvastatin and atorvastatin***, Trends in Analytical Chemistry, 27, 2008, pp 352-367.

Noyes A. A., Whitney W. R., ***The rate of solution of solid substances in their own solutions***, Journal of the American Chemical Society, 19, 1897, pp 930–934.

Ogasawara A., Utoh M., Nii K., Ueda A., Yoshikawa T., Kume T., Fukuzaki K., ***Effect of oral ketoconazole on oral and intravenous pharmacokinetics of simvastatin and its acid in cynomolgus monkeys***, Drug Metab. Dispos., 37, 2009, pp 122-128.

Ozyazici M., Gokce E.H., Ertan G., ***Release and diffusional modeling of metronidazole lipid matrices***, Eur. J. Pharm. Biopharm., 63, 2006, pp 331–339.

Palugan L., Cerea M., Zema L., Gazzaniga A., Maroni A., ***Coated pellets for oral colon delivery***, Journal of Drug Delivery Science and Technology, 25, 2015, pp 1-15.

Pattarino F., Bettini R., Foglio Bonda A., Della Bella A., Giovannelli L., ***Polymorphism and kinetic behaviour of binary mixtures of triglycerides***, International Journal of Pharmaceutics, 473, 2014, pp 87-94.

Peppas N. A., Sahlin J. J., ***A simple equation for the description of solute release. III. Coupling of diffusion and relaxation***, International Journal of Pharmaceutics, 57, 1989, pp 169-172.

Petyaev I. M., ***Improvement of hepatic bioavailability as a new step for the future of statin***, Arch. Med. Sci., 11, 2015, pp 406-410.

Porter C. J., Trevaskis N. L., Charman W. N., ***Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs,*** Nat. Rev. Drug Discov., 6, 2007, pp 231-248.

R. Bettini, P.L. Catellani, P. Santi, G. Massimo, N.A. Peppas, P. Colombo, ***Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate***, Journal of Controlled Release, 70, 2001, pp 383-391.

Ritchie J., ***Brief notes of several cases of acute diarrhœa treated with keratin-coated carbolic acid pills***, The Lancet, 142, 1893, pp 1305 – 1306.

Ritger P. L., Peppas N. A., ***A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs,*** Journal of Controlled Release, 5, 1987a, pp 23-36.

Ritger P. L., Peppas N. A., ***A simple equation for description of solute release II. Fickian and anomalous release from swellable devices***, Journal of Controlled Release, 5, 1987b, pp 37-42.

Robert J. Meyer, M.D. and Ajaz S. Hussain, ***Mitigating the Risks of Ethanol Induced Dose Dumping from Oral Sustained/Controlled Release Dosage Forms***, FDA's ACPS Meeting, October 2005.

Roberts M., Cespi M., Ford J. L., Dyas A. M., Downing J., Martini L. G., Crowley P.J., ***Influence of ethanol on aspirin release from hypromellose matrices***, Int. J. Pharm, 332, 2007, pp 31–37.

Rogers T. L., ***Hypromellose monography***, in Rowe R. C., Sheskey P. J., Weller P. J., 2009, ***Handbook of pharmaceutical excipients,*** London, Pharmaceutical Press.

Rosiaux Y., Jannin V., Hughes S., Marchaud D., ***Solid lipid excipients — Matrix agents for sustained drug delivery*****,** Journal of Controlled Release, 188, 2014, pp 18-30.

Rubbens J., Brouwers J., Wolfs K., Adams E., Tack J., Augustijns P., ***Ethanol concentrations in the human gastrointestinal tract after intake of alcoholic beverages,*** Eur J Pharm Sci. 2016 Apr 30;86:91-5.

Sandeep K., Mohanvarma M., Veerabhadhraswamy P., ***Oral lipid-based drug delivery systems – an overview***, Acta Pharmaceutica Sinica B, 3, 2013, pp 361-372.

Schwartz R. S., ***Paul Ehrlich' s magic bullets***, N. Engl. J. Med., 350, 2004, pp 1079–1080.

Shimpi S., Chauhan B., Mahadik K.R., Paradkar A., ***Preparation and evaluation of diltiazem hydrochloride-gelucire 43/01 floating granules prepared by melt granulation***, AAPS PharmSciTech, 5, 2004, pp 51-56.

Sieber S. M., Cohn V. H., Wynn W. T., ***The entry of foreign compounds into the thoracic duct lymph of the rat***, Xenobiotica 4, 1974, pp 265–284.

Siepmann J., Peppas N. A., ***Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC)***, Adv Drug Deliv Rev., 48, 2001, pp 139-147.

Singh K. K., ***Carrageenan monography***, in Rowe R. C., Sheskey P. J., Weller, P. J. (2009). ***Handbook of pharmaceutical excipients.*** London: Pharmaceutical Press.

Sirtori C. R., ***The pharmacology of statins***, Pharmacological Research, 88, 2014, pp 3-11.

Steinberg D., Parthasarathy S., Carew T. E., Khoo J. C., Witztum J, L., ***Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity***, N. Engl. J. Med., 320, 1989, pp 915-924.

Stone N., Robinson J., Lichtenstein A. (2014). Erratum: 2013 ACC/AHA ***Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines***, J Am. Coll. Cardiol., 63, 2013, pp 3024-3025.

Sutananta W., Craig D. Q. M., Newton J. M., ***An evaluation of the mechanisms of drug release from glyceride bases*****,** J. Pharm. Pharmacol. 47, 1995, pp 182–187.

Thompson H. O., Lee C. O., ***History, literature, and theory of enteric coatings***, J. Am. Pharm. Assoc. 34, 1945, pp 135–138.

Thompson P. D., Panza G., Zaleski A., Taylor B., ***Statin-Associated Side Effects***, Journal of the American College of Cardiology, 67, 2016, pp 2395-2410.

Tomaszewski M., Stępień K. M., Tomaszewska J., Czuczwar S. J., ***Statin-induced myopathies***, Pharmacol. Rep., 63, 2011, pp 859-866.

Unschuld P. U., ***Medicine in China: A History of Pharmaceutics***, University of California Press, 1986, pp. 104–117.

Waltz E., ***It's official: biologics are pharma's darlings***, Nature Biotechnology, 32, 2014,117.

Weiner A. L., ***Lipids as excipients in pharmaceutical dosage forms***, Encyclopedia of Pharmaceutical Technology, New York, 2001. 1659–1673.

White K. L., Nguyen G., Charman W. N., Edwards G. A., Faassen W. A., Porter C. J. H., ***Lymphatic transport of methylnortestosterone undecanoate (MU) and the bioavailability of methylnortestosterone are highly sensitive to the mass of coadministered lipid after oral administration of MU***, J. Pharmacol. Exp. Ther., 331, 2009, pp 700–709.

Vo A. Q., Feng X., Morott J. T., Pimparade M. B., Tiwari R. V., Feng Zhang F., Repka M. A., ***A Novel Floating Controlled Release Drug Delivery System Prepared by Hot-Melt Extrusion***, European journal of Pharmaceutics and Biopharmaceutics, 98, 2016, pp 108–121.

Zhao F., Malayev V., Rao V., Hussain M., ***Effect of sodium lauryl sulfate in dissolution media on dissolution of hard gelatin capsule shells***, Pharm. Res., 21, 2004, pp 144-148..