# UNIVERSIDADE DE LISBOA

## FACULDADE DE FARMÁCIA

### DEPARTAMENTO DE FARMÁCIA GALÉNICA E TECNOLOGIA FARMACÊUTICA



# Production of laminar extrudates containing particles of a model drug processed by supercritical fluids


**Gonçalo Emanuel Rodrigues da Cunha Correia de Oliveira**


DOUTORAMENTO EM FARMÁCIA

(Tecnologia Farmacêutica)

Tese orientada pelo Professor Doutor João F. Pinto e coorientada pelo Professor Doutor Martin A. Wahl, especialmente elaborada para a obtenção do grau de doutor no ramo de Farmácia, Especialidade de Tecnologia Farmacêutica

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*A quem muito me amou,*

*a quem muito me ama*

*e a quem eu muito amo.*

***“O caminho para se conseguir a felicidade***

***é fazer as outras pessoas felizes.”***

Baden-Powell

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**Abstract**

The aim of this work was to test the hypothesis that laminar extrudates and co-extrudates, containing particles of drugs processed by supercritical fluids, can be manufactured at room temperature and in the absence of solvents, to deliver drugs by the oral or transdermal routes of administration.

Particles of coumarin (drug) were manufactured by Rapid Expansion of Supercritical Solutions (RESS), which is a supercritical fluid micronization technique, using both a discontinuous and a continuous mode of operation. The manufactured particles of coumarin obtained from different RESS experiments, in which there were tested different experimental conditions, presented reduced sizes (between 20-40 μm, mean values) and similar properties in terms of morphology, surface area, thermal behavior, amorphous and crystalline contents, density and porosity.

It was observed that specific experimental conditions of the RESS influenced the size of the manufactured drug particles, namely the pre-expansion pressure, the initial amount of solute, and the post-expansion pressure, amongst others.

In parallell, several excipients and different formulations were developed and tested in order to manufacture extrudates with a laminar shape, at room temperature, and without including solvents in the formulations. The lipid-based materials were the excipients that better contributed for the aim of the study, as the formulations with these excipients in high proportions presented suitable physical and mechanical properties and, in consequence, a satisfactory quality.

After identifying the best formulation, the extrusion processing parameters and their influence in the properties of the manufactured extrudates, namely the extrusion rate, were properly studied. Additionally, for each extrudate, there were analyzed the dissolution profiles, the thermal behaviors of the raw materials before extruding and of the extrudates after extrusion and over the storage period, as well as the evolution of the density, of the porosity and of the mechanical properties of the

extrudates such as the bending strength, the deformation, the stiffness, and the elasticity (Young's modulus).

It was studied the release of the drug from extrudates containing particles of drugs with different sizes in order to assess the impact of the particle size of the drug on its release from the extrudates previously manufactured. It was concluded that the manufacture of extrudates at room temperature and without including solvents in the formulation is a promising technology for the manufacture of new pharmaceutical dosage forms. All the analysis performed both to the starting materials and to the obtained extrudates showed that after extrusion occurs an aging phenomenon of the extrudates, which is visible by the evident changes in their physical and mechanical properties over storage. This phenomenon is similar to the previously observed and described for the glycerides and triglycerides that mainly compose the lipid-based excipients selected for this study.

The formulations composed by different excipients originated extrudates with different properties such as the mechanical properties. Also, the bending strength, the stiffness, the deformation, the elasticity, the density and the porosity changed over time, and the observed changes were related to the composition of the formulations and of the extrusion processing conditions.

It was observed that the properties of the extrudates, namely their physical and mechanical properties, needed to be stabilized over time in order to maintain unaffected the release of the drug performance expected for certain drug and extrudate. Additionally, the extrudates including particles of drugs with smaller sizes presented higher release rates when compared with particles with higher dimensions.

The formulations and the components selected for the manufacture of extrudates at room temperature and in the absence of solvents were in the majority composed by lipid-based materials, which can sustain the release of the drug. Therefore, it was concluded that the release rate of a certain drug can be modified by

changing the composition of the formulatons and/or by changing the size of the particles of drug.

The entire approach for the formulation development and for the study of the extrusion process and of the properties of the extrudates was replicated for the development of the laminar co-extrudates (with two or three layers). The properties and results obtained were similar to those observed for the extrudates with a single layer.

The particles of coumarin micronized by RESS were included in laminar extrudates manufactured without changing the temperature during processing and avoiding the inclusion of solvents in the formulation. The release of coumarin from these extrudates was assessed and it was concluded that this pharmaceutical dosage form could successfully transport and release coumarin.

Those laminar extrudates were also tested for its ability in delivering coumarin obtained by RESS and levothyroxine by both the oral and the transdermal routes of administration. The particles of both drugs, in separate, were included in formulations of extrudates and its *in-vitro* release, *ex-vivo* permeation and *in-vivo* absorption / permeation were properly assessed.

Several portions of extrudates containing coumarin or levothyroxine were placed in the donor compartments of diffusion cells (Franz cells) containing a barrier composed by human skin (*ex-vivo* experiment) and it was observed that both drugs were successfully released and permeated through the skin. Also, several portions of extrudates containing levothyroxine were delivered to Wistar rats by both routes of administration (oral and transdermal) and it was observed that levothyroxine was respectively absorbed and permeated, being detected in the blood mainstream of the mice.

All the findings described in this work allowed to conclude that the hypothesis was valid and that it is possible to manufacture extrudates and co-extrudates at room

temperature, without including solvents containing particles of a drug manufactured by supercritical fluids.

**Keywords**

Co-extrusion

Laminar extrusion

Solvent-free extrusion

Particle design

Supercritical fluids

**Resumo**

O trabalho desenvolvido no âmbito desta Tese pretendeu testar a hipótese de que extrudidos e co-extrudidos laminares contendo partículas de fármacos micronizadas por uma técnica de fluidos supercríticos, podem ser produzidos à temperatura ambiente e sem a inclusão de solventes, permitindo a veiculação de fármacos pelas vias de administração oral e transdérmica.

A produção de partículas de cumarina (fármaco) por intermédio de uma técnica de micronização por fluidos supercríticos – *Rapid Expansion of Supercritical Solutions* (RESS) – foi testada segundo duas modalidades de operação distintas: operação descontínua e operação contínua.

Nas experiências de RESS em modo descontínuo, uma porção de cumarina (soluto) foi colocada na câmara de extração e o dióxido de carbono (solvente) foi injetado até obtidas determinadas pressão (15-42 MPa) e temperatura (40-60 ºC) atingindo o estado supercrítico. Todo o soluto foi dissolvido no dióxido de carbono supercrítico, formando-se uma solução supercrítica de cumarina. De seguida, a solução supercrítica foi transferida para a câmara de expansão que se encontrava a pressão e temperatura atmosféricas. Esta mudança abrupta das condições de pressão e temperatura levou à transformação do dióxido de carbono supercrítico em gás e provocou a super-saturação da solução supercrítica com a consequente precipitação da cumarina em partículas. Toda esta mudança brusca de condições (temperatura e pressão) levou ao crescimento extremamente rápido de núcleos de cristalização, dando origem a partículas muito finas e com diâmetros com cerca de 30 a 50 µm (valores médios).

De forma a extrair a maior quantidade possível de cumarina, o decréscimo de pressão verificado na câmara de extração aquando da transferência da solução supercrítica para a câmara de expansão, foi compensado pela injeção de novas quantidades de dióxido de carbono na câmara de extração, reestabelecendo-se

assim as condições iniciais de pressão e completando-se um ciclo de expansão. Vários ciclos foram realizados de forma a melhorar o rendimento do processo.

No caso das experiências RESS em modo contínuo, foi estabelecida a circulação contínua da solução supercrítica de cumarina entre as câmaras de extração e de expansão, através da injeção contínua e constante de dióxido de carbono na câmara de extração. Desta forma, toda a cumarina foi extraída e transferida para a câmara de expansão sem qualquer paragem do processo e sem a necessidade de injeção de porções de dióxido de carbono para restabelecimento da pressão interna na câmara de extração. O processo decorreu de forma contínua.

Em ambas as modalidades de operação da tecnologia RESS, verificou-se a formação de partículas de cumarina com dimensões reduzidas (20-40 µm, valores médios). No entanto, também se verificou que as várias condições de processo selecionadas podem influenciar as propriedades das partículas obtidas por RESS, nomeadamente: o aumento da temperatura de pré-expansão e da pressão de pré-expansão, dão origem ao aumento do tamanho médio das partículas obtidas. Ao contrário, verificou-se que a diminuição da temperatura de expansão e da pressão de expansão, levam à diminuição do tamanho médio das partículas obtidas, pelo que o controlo das condições de processamento é bastante crítico para a obtenção de partículas com propriedades específicas.

Para além do estudo do tamanho da partícula e da distribuição dos tamanhos de partícula, também foram estudadas outras propriedades das partículas de cumarina – como a área de superfície, a densidade, o conteúdo amorfo / cristalino e o comportamento térmico – e a influência das condições de processo da tecnologia RESS nessas propriedades, concluindo-se que ambas as modalidades de operação (descontínua e contínua) e que as várias alterações testadas às condições de processo, dão origem a partículas com propriedades equivalentes.

Em paralelo, e de forma produzir extrudidos laminares sem a inclusão de solventes nas formulações e sem alteração das condições de temperatura ambiente durante o processo, foram desenvolvidas e testadas várias formulações. Foram experimentados igualmente vários excipientes e verificou-se que os excipientes lipídicos sólidos são os materiais mais promissores, na medida em que as formulações que os continham em maior proporção produziram os extrudidos com melhor qualidade. De igual forma, várias combinações e proporções de excipientes foram ensaiadas até serem definidas as formulações mais adequadas.

Numa primeira fase, e depois de selecionada uma formulação base, foram estudados os parâmetros de processo e a sua influência nas propriedades dos extrudidos produzidos, nomeadamente a velocidade de extrusão utilizada. Para cada extrudido foram analisados os perfis de extrusão, o comportamento térmico dos excipientes antes da extrusão e o comportamento térmico dos extrudidos após a extrusão ao longo do tempo (período de armazenamento), assim como a evolução da densidade, da porosidade e das suas propriedades mecânicas, nomeadamente a resistência à flexão, a deformação, a rigidez e a elasticidade (módulo de *Young*), após a extrusão e durante o armazenamento.

Foi igualmente estudada a libertação do fármaco a partir de extrudidos contendo partículas de fármaco com diferentes tamanhos e diferentes distribuições de tamanho de partícula, de forma a avaliar o impacto do tamanho da partícula do fármaco na sua libertação a partir dos extrudidos anteriormente produzidos.

Concluiu-se que a produção de extrudidos laminares à temperatura ambiente e sem a inclusão de solventes na formulação é uma tecnologia promissora para o fabrico de novas formas farmacêuticas. Toda a análise efetuada aos materiais e aos extrudidos obtidos permitiu verificar que após extrusão ocorre um processo de envelhecimento dos extrudidos que se manifestou pela alteração das suas propriedades físicas e mecânicas ao longo do tempo. Este fenómeno foi semelhante

ao previamente observado e descrito para os glicerídeos e para os triglicerídeos, que compõem grande parte dos excipientes lipídicos utilizados nas formulações. Observou-se igualmente que formulações compostas por diferentes excipientes ou por diferentes proporções dos mesmos excipientes originaram extrudidos com propriedades diferentes, nomeadamente as propriedades mecânicas. A resistência à flexão, a rigidez, a deformação, a elasticidade, a densidade e a porosidade dos extrudidos alterou-se ao longo do tempo, sendo que esta alteração é dependente da composição e das condições de processo durante a extrusão.

Concluiu-se que as propriedades dos extrudidos, nomeadamente as propriedades físicas e mecânicas, necessitam de ser estabilizadas ao longo do tempo de forma a que estas alterações não afetem a performance de libertação do fármaco esperada para determinados extrudidos.

Verificou-se ainda que os extrudidos incluindo partículas de fármaco com diferentes tamanhos apresentaram perfis de libertação diferentes. Os extrudidos com as partículas de cumarina mais pequenas apresentaram taxas de libertação do fármaco mais elevadas quando comparadas com partículas de maiores dimensões.

Estes extrudidos laminares, ao serem compostos maioritariamente por excipientes lipídicos, apresentaram uma libertação retardada do fármaco. Dessa forma, pode-se concluir que as taxas de libertação do fármaco podem ser modificadas através da alteração da composição da formulação e/ou alterando o tamanho das partículas de fármaco incluídas.

Toda a abordagem de desenvolvimento das formulações, do estudo do processo de extrusão e do estudo das propriedades dos extrudidos foi replicada para o desenvolvimento de co-extrudidos laminares, contendo duas ou três camadas justapostas. Os resultados obtidos foram similares aos verificados para os extrudidos de uma única camada.

As partículas de cumarina obtidas pela tecnologia de RESS foram posteriormente incluídas em extrudidos laminares produzidos sem a inclusão de qualquer solvente e sem aquecimento do extrusor durante o processo. A libertação da cumarina a partir destes extrudidos foi avaliada e concluiu-se que esta forma farmacêutica permitiu o transporte e libertação destas partículas de cumarina.

Os extrudidos laminares foram testados quanto à sua capacidade de veiculação e administração pelas vias de administração oral e transdérmica de partículas de cumarina obtidas pelo método de RESS e de partículas de levotiroxina.

As partículas de ambos os fármacos, em separado, foram incluídas em formulações de extrudidos semelhantes às descritas anteriormente e os extrudidos laminares obtidos e a sua libertação *in-vitro*, permeação *ex-vivo* e absorção / permeação *in-vivo* foram avaliadas e estudadas.

Várias porções de extrudidos contendo cumarina e de extrudidos contendo levotiroxina foram colocados em compartimentos dadores de células de difusão (células de Franz) de forma a confirmar a capacidade destes extrudidos em libertar os fármacos e de estes permearem uma barreira constituída por pele humana (estudo *ex-vivo*). Verificou-se que ambos os fármacos foram libertados pelos extrudidos e atravessaram a pele com sucesso.

De igual modo, as várias porções de extrudidos contendo levotiroxina administradas a ratos Wistar (macho) pelas vias de administração oral e transdérmica, libertaram o fármaco e este foi permeado e absorvido com sucesso, sendo detectado no sangue dos ratinhos.

**Palavras-chave**

Co-extrusão

Design de partículas

Extrusão laminar

Extrusão sem solventes

Fluidos supercríticos

## Abbreviations

| | |
|---|---|
| **ANOVA** | Analysis of variance |
| **BET** | Brunauer, Emmett and Teller |
| **$CO_2$** | Carbon dioxide |
| **$F_{ss}$** | Extrusion force at steady state |
| **HSD** | Honest significant difference |
| ***n*** | Release exponent (according to Korsmeyer-Peppas model) |
| **PEG** | Polyethylene glycol |
| **PGSS** | Particles from gas saturated solutions |
| **$R^2$** | Coefficient of determination |
| **RESS** | Rapid expansion of supercritical solutions method |
| **RH** | Relative humidity |
| **SAS** | Supercritical anti-solvent method |
| **SC** | *Stratum corneum* |
| **SCF** | Supercritical fluid |
| **$ScCO_2$** | Supercritical carbon dioxide |
| **SEM** | Scanning electron microscopy |
| **T4** | Levothyroxine (free acid) |
| **UV** | Ultraviolet |

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### Research articles

***Characterization of laminar extrudates manufactured at room temperature in the absence of solvents for the delivery of drugs***, G. Oliveira, M. A. Wahl, J. F. Pinto, International Journal of Pharmaceutics 454 (2013) 90– 98.

***Delivery of Drugs from Laminar Co-Extrudates Manufactured by a Solvent-Free Process at Room Temperature***, G. Oliveira, M. A. Wahl, J. F. Pinto, Journal of Pharmaceutical Sciences (2014) 103(11):3501-10.

***Influence of process variables on the properties of coumarin particles micronized by supercritical fluids (RESS) to be delivered in a laminar extrudate***, G. Oliveira, J. F. Pinto, AAPS PharmSciTech (2015) [submitted].

***In-vivo evaluation of levothyroxine (free acid) administered through laminar extrudates by the oral and transdermal routes of administration in mice***, G. Oliveira, M. A. Wahl, J. F. Pinto, Journal of Controlled Release [in preparation].

# THESIS

## 1. Challenges

The pharmaceutical development and the manufacture of medicinal products comprise important challenges that must be appropriately addressed. In the majority of the cases, these challenges represent severe obstacles to the delivery of drugs and, in consequence, limit the therapeutic options available. Therefore, it is crucial to find strategies for overcoming these limitations, widening the pharmaceutical alternatives for delivering drugs and contribute to the lifecycle management of medicines.

The processing of thermo-sensitive and/or solvent-sensitive drugs is an example of the referred limitations. Also, the poor bioavailability of drugs, resulting of a non-complete absorption or permeation, can impair the desired therapeutic goals.

Additionally, the need for delivering more than one drug in the same dosage form, the benefit from delivering a drug with two different release profiles within the same dosage form, and the need to provide adequate and specific doses to special groups of patients that do not have an appropriate dosage available in the market (e.g. pediatrics), represent strong limitations to the delivery of drugs and require mitigations by pharmaceutical scientists.

From a different perspective, the existing pressure for reducing manufacturing costs and to meet ecological demands represents daily challenges for pharmaceutical scientists.

## 2. Motivation and aim

The motivation for this study was to explore the potential of two specific technologies – supercritical fluids and extrusion – for addressing some of the referred drug delivery obstacles and overcoming the identified barriers, providing new

proposals for the problems and representing a step forward in the development of new pharmaceutical dosage forms.

The aim of this thesis is to challenge both technologies and to identify the best strategies for using and testing up most of the advantages of the selected technologies and to develop the best methodologies for obtaining pharmaceutical dosage forms that will deliver drugs to patients in ideal conditions. More specifically, to explore new experimental conditions for manufacturing drug particles with reduced sizes by a supercritical fluids technology, and to develop new formulations for extrusion allowing the preservation of the properties of the drug particles previously manufactured, together with new ways of performing the extrusion process.

## 3. Hypothesis

***Laminar extrudates and co-extrudates, containing particles of drugs processed by supercritical fluids, can be manufactured at room temperature and in the absence of solvents, to deliver drugs by the oral or transdermal routes of administration.***

## 4. Research objectives and outcomes

The main purpose of this work was to use two existing technologies – supercritical fluids and extrusion – to build up alternatives for overcoming the pharmaceutical challenges described previously.

The research objectives pursued were:

- to manufacture and characterize particles of model drugs with reduced sizes by a supercritical fluid technology – Rapid Expansion of Supercritical Solutions (RESS);

- to manufacture and characterize extrudates and co-extrudates with a laminar shape in the absence of solvents minimizing changes of temperature during processing (green technologies);
- to deliver the manufactured drug particles with reduced sizes within the laminar extrudates and co-extrudates prepared.

The expected research outputs were:

- particles of a model drug with reduced size (micro and nanometer scale) engineered by RESS;
- laminar extrudates and co-extrudates containing at least a layer with particles of the model drug manufactured by RESS.

## 5. Organization of the thesis

The thesis comprises several chapters that describe the technologies selected and products available for the study (Chapter 1) and summarize all the laboratorial work and research findings (Chapters 2 – 6), more specifically:

- the manufacture and characterization of particles with reduced size by the Rapid Expansion of Supercritical Solutions (RESS) technology (bottom-up approach) by comparison with the conventional milling (top-down approach) (chapter 2):
- the manufacture and characterization of laminar extrudates at room temperature and in the absence of solvents, focusing on the description and assessment of the innovative extrusion process (chapter 3);
- the manufacture and characterization of innovative laminar co-extrudates at room temperature and in the absence of solvents (chapter 4);

- the *in-vitro* assessment of the release of drugs from laminar extrudates and co-extrudates containing particles of drugs manufactured by supercritical fluids (chapter 5);
- the study of the *in-vivo* delivery of model drugs by oral and transdermal routes of administration from laminar extrudates (proof-of-concept) (chapter 6);

Chapter 7 summarizes the findings together with the overall conclusions drawn, demonstrating the hypothesis raised in the beginning of this work.

The last section, 'future perspectives' raises some questions, which were not answered in this work, and new questions beyond the scope of this work enabling the reader to get a better view of this research area.

It must be referred that some chapters were adapted from articles published in peer-reviewed journals and, therefore, follow the original structure of those journals.

# CHAPTER 1

## Introduction

## 1.1. Reduction of the size of particles

The size of drug particles can be reduced by decreasing the size of the original materials (top-down approach) or by increasing the size from solutions (bottom-up approach). Classically, the reduction of the particle size achieved by mechanical comminution considering techniques such as milling exemplify the top-down approach, whereas spray-drying, freeze-drying, precipitation or crystallization from solutions are alternative techniques representing the bottom-up approach.

However, these micronizing technologies present disadvantages that limit their use, such as the production of coarse particles or broad particle size distributions (1), the contamination of the particles with solvent residues or other impurities, the use of large amounts of solvents (that create environmental problems related to the solvent disposal), and/or the degradation of the substances due to thermal, chemical or mechanical stresses (2). To minimize these limitations, alternative technologies have been developed in recent years, namely the application of supercritical fluid (SCF) technologies in designing particles of drugs (3).

### 1.1.1. Top-down approaches

In the micronization of solid drug particles by mechanical comminution (e.g. milling, crushing, grinding) to obtain particles with smaller sizes, the reduction of size occurs by at least one of the following mechanisms: pressure, friction, attrition, impact, or shearing. Generally, more than one of these mechanisms is observed simultaneously. There is no nucleation for creating a new particle but only the alteration of the existing particles. It is important to refer that all other properties than size of the obtained particles also result directly from these mechanical phenomena, namely the amorphous / crystalline content, the density and porosity, amongst others (4).

### 1.1.2. Bottom-up approaches

In all bottom-up approaches for designing particles of drugs with reduced sizes, there is a complete solubilization of the solute / drug being processed in the solvent(s). Afterwards, taking into consideration specific methodologies related to each technology, the solution is transformed into solid particles with reduced sizes by eliminating the solvent(s) and further precipitating the solute, which acquires its solid-state properties. In general, the speed of both the elimination of the solvent(s) and the change of the solute to the solid-state, influences the size of the obtained particles (5).

Techniques such as the spray-drying (6), the freeze-drying (7) or the supercritical fluids (8), are examples of technologies applied in the reduction of the size of the particles of drugs with bottom-up approaches.

#### 1.1.2.1. Supercritical fluids techniques

Several technologies based on the use of supercritical fluids for obtaining particles with reduced sizes have been developed in recent years. The supercritical anti-solvent (SAS), the supercritical-assisted atomization (SAA), the gas anti-solvent (GAS), the aerosol solvent extraction system (ASES), particles from gas-saturated solutions (PGSS), and the rapid expansion of supercritical solutions (RESS), amongst others, are examples of those technologies (9).

The RESS technique has been more attractive than the others as the obtained particles are free of residual solvents, the size of the particles can be controlled and adjusted, and the operating conditions in terms of temperature and pressure are considered mild. Therefore, the RESS method has been widely applied in the micronization of particles of drugs (10).

## 1.2. Rapid expansion of supercritical solutions (RESS)

The rapid expansion of a solution in the supercritical state has been used for designing small, uniform and solvent-free particles or powders, as it can produce fine particles of a wide range of inorganic, organic and polymeric materials (11). Drugs such carbamazepine, ibuprofen, paclitaxel, and nabumetone, amongst others, have been micronized by RESS (1, 12-14).

The RESS methodology promotes the bioavailability of drugs (15, 16) by creating particles with narrow size distributions and increased surface area (17), and is simultaneously considered a mild and green technology as it does not produce residual solvents or in case solvents / gases are used, they can be recycled and re-utilized (2). This RESS methodology has specific features that need to be adequately explored in order to extract the highest potential of this technique.

In the case of the supercritical solvent processes such as the rapid expansion of supercritical solutions (RESS), the solute is directly dissolved in the supercritical phase forming a supercritical solution, which is expanded by passing through a nozzle. This expansion, caused by a rapid decrease of the pressure and temperature of the supercritical solution, leads to an abrupt decrease of the solute's solubility. In consequence, the existing equilibrium between the solute and the solvent ends, leading to the formation of very fines particles of solute by precipitation (18).

### 1.2.1. Concept

The RESS technique uses the solvating power of fluids in the supercritical state for dissolving solid materials that cannot be dissolved at atmospheric conditions of pressure and/or temperature.

The particles manufactured by RESS are very fine (from nanometer to micrometer sized particles) and present very narrow size distributions due to the very

fast crystallization of the dissolved solute. After the dissolution of the solute in the supercritical fluid, occurs a very fast change of the supercritical fluid to the gas-like properties: the expansion. This abrupt change originates a high super-saturation and, consequently, the particle formation (19). Experimental conditions such as the extraction pressure, the extraction temperature, the initial concentration of the solute, and the configuration of the noozle, amongst others, have an influence in the final properties of the manufactured particles with this technique (20).

### 1.2.2. Solvents used in RESS

The solvents used in RESS as supercritical fluids are gases pressurized and heated above their critical point (higher pressure and temperature), exhibiting simultaneously gas-like and liquid-like properties. Therefore, these fluids in supercritical state exhibit densities close to liquids and viscosities close to gases or vapors (11).

Carbon dioxide ($CO_2$), trifluoromethane ($CHF_3$) and propane ($C_3H_8$) are gases commonly used in the dissolution / extraction of materials (21). The carbon dioxide is the most commonly used fluid as it is inexpensive and easily recyclable. Additionally, $CO_2$ presents a relatively low critic temperature and a moderate critic pressure, leaves no residues and is not flammable.

### 1.2.3. Particle formation

The particles are formed during the expansion of the solution, in which there is a fast nucleation. When the supercritical solution is transferred between the extraction and the expansion chambers, there is an abrupt change of pressure, together with a change of temperature, to the atmospheric conditions. There is an immediate removal of the solvent, which changes from the liquid to the gaseous

state, leading to a high super-saturation and an immediate transformation of the solubilized drug to the solid state through nucleation (22, 23).

### 1.2.4. Process parameters influencing the properties of the particles

Various process parameters have influence in the final properties of the particles manufactured by RESS, such as the extraction temperature, the extraction pressure, the pre-expansion temperature, the spray distance, post-expansion pressure, and the nozzle configuration (length, diameter and geometry) (3, 9, 13, 14, 24-26).

## 1.3. Extrusion and co-extrusion

Extrusion is defined as the process of forming a new product (designated as the extrudate) with uniform shape and density, by forcing a material or a mixture of materials, under controlled conditions, through a die or orifice. The new product acquires the shape of the extrusion die**.** Co-extrusion consists on the simultaneous extrusion of two or more materials (or mixtures of materials) in order to create a multi-layered extrudate (27).

### 1.3.1. Applications

The extrusion and co-extrusion techniques have been widely applied in various industries such as the food, ceramics, plastics, metals, and electronics industries (28-38), but also in biomedical, nutraceutical and pharmaceutical applications (39-41).

In the pharmaceutical field, the wet and hot-melt extrusion processes are commonly applied in the manufacture of drug products. However, these processes present some limitations, namely the use of solvents in the formulations (e.g. water) and/or changes on temperature during manufacturing (hot melt). Due to these limitations, certain drugs cannot be processed by extrusion, as they are solvent and/or heat sensitive. Thus, the possibility of performing extrusion without the use of solvents and changes in temperature during processing represents a step forward in the field, allowing a wider application and turning this technology into a green process (42).

Various pharmaceutical dosage forms have been manufactured through the extrusion or co-extrusion processes, namely pellets (43), granules (44), tablets (45, 46), transdermal or transmucosal films (47), implants (48), and periodontal inserts (49).

These dosage forms are suitable for administration via different routes, which vary from the oral, transdermal, transmucosal, and transungual routes of administration (48).

### 1.3.2. Types of extrusion

There are several types of extrusion and co-extrusion – wet or paste, dry or solvent-free, hot-melt or melt, cold, and solid lipid extrusion – depending on the inclusion of solvents, on the temperature changes during processing and on the materials that are used (50). Additionally, the processes of extrusion can be categorized according to the operation mode: discontinuous (e.g. ram extrusion) or continuous (e.g. screw extrusion) processes.

#### 1.3.2.1. Wet extrusion

The wet extrusion or co-extrusion, also known as paste extrusion, requires the formation of a homogeneous mixture of powdered materials (dry mixing), which is wet massed with the addition of a liquid binder (e.g. water). Afterwards, the moistened / wet mass is pressed through a die to form the extrudates, which can be further processed. Wet extrusion is widely applied in the pharmaceutical field especially in the manufacture of pellets (51).

#### 1.3.2.2. Solvent-free extrusion and solid lipid extrusion

The formulations for solvent-free extrusion or co-extrusion, also designated as dry extrusion, do not include any liquid excipients or solvents as extrusion aids. In general, the solvent-free extrusion is a two-step process starting with the blending of solid materials, which afterwards are directly fed into the extruder chambers for processing. Solvent-free extrusion is advantageous when delivering

drugs sensitive to moisture or solvents, reducing the possibility of occurring degradation of the drug and further change of properties (42).

The solid lipid extrusion or co-extrusion is a kind of solvent-free extrusion, in which powdered lipids are mixed with drugs and extruded at a temperature below the melting ranges of the lipids. Therefore, the lipids do not melt but only soften, and the drug is dispersed in it (42, 52).

#### 1.3.2.3. Hot-melt extrusion

In hot-melt extrusion or co-extrusion, also known as melt extrusion, a powder blend of drug(s) and carrier(s) is heated, melted and pressed through the die. Neither water nor solvents are used. Therefore, degradation of the drugs due to hydrolysis is avoided. When the intention is to deliver thermo-sensitive drugs, the use of the hot-melt extrusion process needs to be appropriately evaluated in order to prevent any degradation or loss of properties due to the effect of temperature increase (53).

Different dosage forms have been manufactured via hot-melt extrusion, such as pellets, granules, tablets, implants, suppositories, films, amongst others (54). Nowadays, the hot-melt extrusion technique has been especially used for increasing the solubility of polymers.

#### 1.3.2.4. Cold extrusion

In cold extrusion or co-extrusion there are no changes of temperature during processing, which is advantageous when delivering drugs that are thermo-sensitive. After an appropriate mixture of the excipients and drugs, the extrudable mass is fed into the extruder chamber for further extrusion at room temperature (42).

#### 1.3.2.5. Discontinuous extrusion

Discontinuous extrusion or co-extrusion, in which are mainly used ram or piston extruders, is generally used for laboratory purposes or for extruding specialized materials that require a strict control. In ram extrusion, the displacement of the ram generates high pressures, forcing the extrudable masses to cross the dies. The materials are feed to a barrel (which is heated in the majority of the cases) and forced through the die being transformed into a solid product with a specific and desired shape. The feeding of the materials and extrusion of masses is not continuous (55).

#### 1.3.2.6. Continuous extrusion

Continuous extrusion or co-extrusion, in which are mainly used screw extruders, is used for industrial purposes. These extruders present one, two or multiple rotating screws, which continuously push the extrudable masses inside a heated barrel towards an extrusion screen. The screw extrusion presents a high throughput rate, allows an easy cleaning and the usage of different screen types (56).

### 1.3.3. Extruders

In general, all types of extruders – the ones classified as screw-, gravity- and piston-type extruders, and also the ones classified as sieve and basket, roll, and ram extruders – include a part for transporting and feeding the extrudable masses into the extrusion chambers, and a screen or die through which the materials are pushed, shaping the extrudates (57).

The ram extruders include the following components: a stainless steel barrel, consisting of a reservoir for the extrudable materials; different dies with different shapes and/or dimensions, which are installed in the base of the barrel; and

a stainless steel piston, or ram, that fits loosely into the barrel. The ram extruders allow the non-continuous extrusion of mixtures of materials and operate in two stages: firstly, the extrudable mass is inserted into the barrel and consolidated; secondly, a load is applied to the ram, forcing the extrudable mass to cross through the die and produce the extrudates.

If required, the ram extruders can be instrumented with a load cell, allowing recording of the applied loads and the ram displacements during processing. The moving speed of the ram (extrusion rate) can be adjusted.

### 1.3.4. Shapes of extrudates

The extrudates and co-extrudates can have different shapes depending on the features of the extrusion dies used. The most common ones are the rod or cylindrical and tubular shapes (58-61). However, there is also the possibility of manufacturing extrudates with a laminar shape (55).

### 1.3.5. Materials for extrusion

A vast range of materials can be used for preparing extrudates and co-extrudates. The selection of materials, their combination and proportions in the formulations, has an impact on the properties of the extrudates. In some cases, specific materials are preferred with the aim of obtaining extrudates with specific and desired properties. For example, the change or substitution of a single excipient in a formulation may result in a change of the release rate for the same drug (62).

In the extrusion or co-extrusion processes the materials are used with different purposes or functions. In general, all formulations include fillers, binders, lubricants and disintegrants. However, if needed, surface-active agents, pH adjusters, release modifiers or cosolvents, amongst others, can also be included to

modify the properties of extrudates. Additionally, solvents are often used as extrusion aids, especially water, which is the most commonly used solvent in extrusion (55).

In some cases, it is necessary to include complementary materials with similar functions (e.g. a combination of two or more binders). Additionally, the proportions of the materials can influence and define their main roles in the formulation. For example, lactose is considered filler if present in a fraction higher than 50%, while is considered disintegrant when lower than 10%.

Fillers are mainly used to increase bulk. Lactose, dicalcium diphosphate, mannitol, and starch and its derivatives, are examples of fillers.

Binders are used to assure mass cohesiveness during processing and contributing for the integrity of the extrudates after manufacture. Some of them absorb and retain water facilitating the wet extrusion process and improving further spheronization. Microcrystalline cellulose, polyvinylpyrrolidone, gelatin, starch, hydroxypropylmethylcellulose, methylcellulose, hydroxypropylcellulose, sodium carboxymethylcellulose, carbomer, and chitosan have been used as binders.

Lubricants improve the ability of the mixtures of materials for extrusion, and glidants facilitate the movement of the extrudable masses over the surfaces of the extruders, contributing for the success of the extrusion process. Light mineral oil, sodium stearyl fumarate, colloidal silicon dioxide, hydrogenated castor oil, and glycerol behenate, amongst others, are used as lubricants or glidants.

Disintegrants (e.g. croscarmellose sodium and sodium starch glycolate) accelerate the release of drugs from extrudates by facilitating the destruction of the extrudates and co-extrudates when in contact with water.

As referred previously, the extrudates can be used for controlling the release of drugs as it is possible to obtain immediate (42), sustained (63) or retarded (47) releases. Also, it is possible to target the release of a drug (48). This control of

release is a powerful advantage of the dosage forms manufactured via extrusion or co-extrusion.

Powdered cellulose and carrageenans have been used as extrusion aids producing extrudates with fast drug releases (64, 65). Chitosan and carbomer are examples of materials used to promote a sustained release (release modifiers).

Lipids and lipid-based materials have also been included in formulations for extrusion, especially due to their binding properties, but also as lubricants. Waxes, mono- and diglycerides, polyglycolised glycerides, amongst others, allow the manufacture of extrudates. These lipid materials have been applied in the process of extrusion avoiding changes in temperature and avoiding the inclusion of solvents for facilitating the extrusion process. The use of lipid materials for extrusion has been considered very promising (42).

#### 1.3.5.1. Materials for extrusion at room temperature, in the absence of solvents

The majority of the commonly used excipients for extrusion and co-extrusion do not allow the processing at room temperature and in the absence of solvents.

Lipid-based materials with low melting ranges such as fatty acids, mixtures of fatty acids with polyethylene glycols, mixtures of triglycerides with low and high melting ranges, and glycerides, have been tested and proved to be promising for extrusion at these conditions (66-68).

#### 1.3.5.2. Formulations for extrusion

The process of creating formulations suitable for extrusion consists, in the majority of the cases, a trial and error exercise. The main objective to achieve when combining the different materials is to obtain a cohesive and plastic extrudable

mass, which preserve its homogeneity during the entire extrusion procedure. Additionally, the mixture of materials must have a suitable flowability during processing, be non-adhesive and produce rigid extrudates that preserve their shape after manufacture and during storage.

### 1.3.6. Process of extrusion and co-extrusion

Extrusion, as well as co-extrusion, allows the manufacture of pharmaceutical dosage forms with quality. After the identification of the composition of the formulation and the proportions of the components, it is necessary to define appropriate operational and processing parameters in order to obtain products with the desired properties. Also, the extrusion process must be well characterized and described, contributing for the definition of the best operational conditions. Additionally, several other pharmaceutical operations, such as milling or sieving, amongst others, might be applied simultaneously, contributing for the success of the extrusion process.

#### 1.3.6.1. Operational parameters and equipment features

When developing an appropriate formulation, it is important to define the most adequate operational parameters in order to obtain the necessary processing conditions. The extrusion rate, the temperature of processing and the design and features of the die(s) have a decisive influence and impact on the quality of extrudates.

Higher extrusion rates may promote the cohesion of the materials instead of producing excessively soft extrudates, which break easily and present major quality defects. Also, higher extrusion rates may increase the stability of the structure of the obtained extrudates. However, high extrusion rates may originate or

facilitate the appearance of severe surface imperfections or defects, such as the sharkskin (69).

The temperature of processing and of the equipment, that might be slightly different, also influences the final properties of the manufactured extrudates. The increase on temperature may determine a partial or complete melting of the materials, promoting a more efficient mixing over processing. Additionally, the cooling process may also have an impact on the properties of the extrudates, as changes in temperatures always change the properties of the materials and, in consequence, of the manufactured products.

The design, shape and characteristics of the extruding dies also have a decisive influence in the final properties of the extrudates and co-extrudates. Specific shapes of the die may produce products that are not sufficiently consistent, or that contain surface defects resulting from an irregular flow of the extrudable masses inside the extrusion chambers and/or the occurrence of an excessive friction on the extruder walls and surfaces.

#### 1.3.6.2. Characterizing the extrusion process (extrusion profiles)

During the extrusion and co-extrusion processes it is possible to build up an extrusion profile with the applied loads to the extrudable masses in function of the displacement of the ram. The obtained extrusion profiles allow characterization of the process of extrusion.

A typical extrusion procedure originates profiles with three different regions or stages: the compression, the steady-state and the forced flow stages. In the compression stage occurs a large change in the displacement of the ram but a small change in the load applied to the extrudable mass, as the ram is descending and consolidating the materials into a plug with the minimum volume and highest density. At this point, there is an abrupt increase in the load and a minimal change in

the displacement, while the material density remains unaffected. The compression stage ends when the pressure applied to the extrudable mass is sufficient for the material start the flow through the die. The beginning of the flow is followed by the steady-state flow period in which the force required for maintaining the extrusion process remains constant as the displacement increases. At the end of the process, when the steady-state stage and the extrusion conditions are no longer possible to be maintained, there is a forced flow stage. In this stage, there is an increase in the force with the displacement caused due to the close approach of the ram to the die surface.

Generally, the extrusion procedures that produce extrudates with the desired quality, present long steady-state stages, with constant and small variations of forces applied during that period.

#### 1.3.6.3. Other manufacturing operations involved

The manufacture of extrudates requires the use of technological operations that complement the extrusion process itself, contributing for the quality of the extrusion process and of the obtained products.

In the majority of the cases, the milling and/or sieving of the starting materials are operations essential for allowing the further preparation of quality extrudates, as the control and calibration of the particle size of the starting materials might have an impact in the properties of the final products.

Additionally, the mixing operations and proper homogenization of the materials composing the extrudable masses is essential for guaranteeing the homogeneity of the manufactured extrudates.

In some cases, the drying and organic solvent removal operations can also be indispensible for preparing extrudates with the desired features after manufacture and in a long-term perspective.

### 1.3.7. Characterization and properties of extrudates and co-extrudates

The characterization and assessment of the extrudates and co-extrudates is important for understanding the impact of the operating conditions on the properties of final products. Several properties can contribute for describing the main features of a certain extrudate, namely the physical and mechanical properties.

#### 1.3.7.1. Surface

The surface of extrudates and co-extrudates must be homogeneous, uniform and smooth. The existence of any surface imperfections or defects can cause fragmentation.

The sharkskin defect is considered one of the most typical surface defects of extrudates. The shark-skinned extrudates present similar features than a ridge-like spiral structure, which occurs in a transversal direction to the extrusion flow. The sharkskin defect is caused by flow instabilities during extrusion at the entry and/or at the exit of the die, and can be resolved or minimized by adjusting the rate of extrusion (70).

Extreme sharkskin normally results in fragmentation or complete breakage of the extrudates. Additionally, extrudates can present rough surfaces, representing less severe defects, which also have an impact on the quality of the extrudates. Shark skinning is promoted by short die lengths and by low extrusion rates. Therefore, an increase in the length and/or in the extrusion rate can improve quality of extrudates and result in smooth surfaces.

Also, the surface quality of extrudates and the occurrence of surface defects depends on the components included in the formulations. Therefore, surface defects can be controlled from the very beginning, during the formulation development stage. In general, the inclusion of binding agents improves the quality of the extrudates by reducing the possibility of occurrence of surface defects.

#### 1.3.7.2. Thickness

The measurement of the thickness at different regions of the extrudates and co-extrudates provides information over the regularity of its shape. The thickness must be regular and uniform throughout the entire extrudates.

The extrudate composition, the features of the extrusion die and of the extruder walls, and of the extrusion procedure, namely the extrusion rate and temperature, may influence the thickness of the extrudates and co-extrudates, as it may influence the expansion of the materials right after extrusion, when exiting the extrusion die. This expansion phenomenon must be considered and studied for all extrusion formulations and procedures.

#### 1.3.7.3. Density and porosity

When the extrudable materials are subjected to high and intense pressures or extrusion loads, there are changes in the density and porosity of the extrudates and co-extrudates. Therefore, the density and porosity of the manufactured products must be controlled over the extrusion procedure and must remain unchanged over storage, so that the extrudates properties are maintained.

#### 1.3.7.4. Mechanical properties

As referred previously, when the extrudable masses are subjected to high extrusion loads, there are observed changes in the extruded materials that have an impact in the final extrudates.

The assessment of the mechanical properties of the extrudates and the analysis of its evolution over time is very important for concluding over the quality of the obtained products.

The bending strength, the deformation, the stiffness, and the Young's modulus of elasticity always bring very relevant data about the extrusion process

and, more importantly, complement all the physical information collected of each extrudate.

#### 1.3.7.5. Thermal behavior

The analysis of the thermal behaviour of both starting materials and obtained extrudates is important for concluding over the overall properties of the extrudates. The raw materials composing the formulations will have a decisive influence on the final products, as their properties will shape the properties of the extrudates. Therefore, a careful and complete study of the properties of each raw material available is essential when designing the formulations in order to select the most adequate materials for obtaining products with the specific and desired features.

Additionally, the assessment of the thermal behaviour of the extrudates after the manufacture and over storage contributes with important information for concluding over the physical and mechanical properties of the extrudates. In the majority of the cases, the changes identified in the thermal behaviour of a certain extrudate, correlate with changes in the physical and/or mechanical properties of the extrudates.

### 1.3.8. Further processing of extrudates

Extrudates and co-extrudates can be further processed into solid dosage forms such as pellets, tablets (prepared from pellets), suppositories, implants and others (54, 71).

The manufacture of pellets of uniform shape, size and density, containing one or more drugs, has been considered the most important application for extrusion and co-extrusion. The pelletization process involves a sequence of operations: blending of powdered materials, wetting of the mass by adding a liquid

binder, extruding (or co-extruding) by pressing the moistened mass through an extrusion die, spheronizing with a fast-rotating friction plate and drying. The extrusion of a wet mass originates an intermediate spaghetti-like product, which is spheronized to yield a spherical product (50).

The obtained pellets can be coated. In general, the coating is applied for guaranteeing a sustained release of the drug(s) or for targeting the drug(s) to specific absorption regions of the gastrointestinal tract (e.g. enteric-coated or colon targeted delivery) (51). Also, the obtained pellets can be transformed into tablets (72).

## 1.4. Model drugs

The model drugs selected for this study – coumarin and levothyroxine – were specifically used in certain parts of the laboratorial work and, at a later stage, the coumarin particles manufactured via RESS were included in the formulation of an extrudate for combining the benefits of both technologies.

### 1.4.1. Coumarin

Coumarin is a naturally occurring substance, which is part of several plants and essential oils, such as tonka beans, sweet clover, oil of cassia, and lavender, amongst others (73).

Coumarin (1,2-benzopyrone) is a white crystal with an odor similar to vanilla at room temperature (molecular weight: 146.15 g/mol; melting point: 68-70 ºC; boiling point: 297-299 ºC). Coumarin is composed of an aromatic ring fused to a condensed lactone ring. Coumarin is freely soluble in ethanol, chloroform, diethyl ether and oils and is slightly soluble in water (74).

Coumarin is used in medicinal products due to its bronchial dilator, anti-inflammatory and analgesic properties. Also, coumarin has been tested and evaluated for the treatment of cancer, as a HIV inhibitor and in the treatment of lymphedema and venous insufficiency (75, 76).

### 1.4.2. Levothyroxine (free acid)

Levothyroxine (T4) is a synthetic hormone orally administered for the treatment of hypothyroidism and goitre. Levothyroxine has a molecular weight of 776.87 g/mol, a melting point of 231 ºC and is slightly soluble in water (0.105 mg/L at 25 ºC). Levothyroxine has been previously administered through the skin, especially in cosmetic creams to reduce deposits of adipose tissue on skin (77).

# CHAPTER 2

## Characterization of coumarin particles micronized by the rapid expansion of supercritical solutions (RESS) technique

***Adapted from:***

*Influence of process variables on the properties of coumarin particles micronized by supercritical fluids (RESS) to be delivered in a laminar extrudate, G. Oliveira, J. F. Pinto, AAPS PharmSciTech (2015).*

## 2.1. Introduction

In recent years, technologies using fluids in the supercritical state have been increasingly considered in the pharmaceutical field with several applications, especially for the extraction of substances from natural products and for the manufacture of drug particles with small sizes (78, 79). The reduction of the particle size enhances the bioavailability of the drugs, one of the major challenges when developing new pharmaceutical dosage forms, since it results in the increase of the specific surface area of the powdered drugs, promoting their dissolution in the biological fluids (19).

Various strategies and technologies have been applied on improving the dissolution of poorly water-soluble drugs, particularly those related to size reduction of particles (4, 80).

Comparing with other micronizing methodologies – such as the mechanical comminution of larger particles (top-down approach) via dry or wet milling (81), or the controlled association of a molecularly dispersed drug (bottom-up approach) by spray-drying (82), freeze-drying (7) or controlled crystallization (83) – the supercritical fluids (SCF) technologies present important advantages, as they reduce the possibility of occurring undesired phenomena, namely chemical or thermal degradation due to mechanical action (84), occurrence of polymorphic transitions and formation of amorphous states of the drug (85). Furthermore, SCF technologies avoid the usage of large amounts of solvents, eliminating the presence of solvent residues in the final particles or the need for special environmental protection measures.

Lately, the Rapid Expansion of Supercritical Solutions (RESS) technique has been one of the most used SCF technologies in the micronization of drugs such as gemfibrozil (17), diclofenac (86) and nabumetone (14), amongst others. It has been applied in the design of drug particles, as it allows the control of

the manufacturing process by designing the most suitable formulations and processing parameters in order to obtain drug particles with desired attributes, particularly reduced sizes, narrow size distributions and homogeneous morphologies (19, 87).

In the RESS manufacturing processes, the experimental conditions influence the physical and/or chemical properties of the obtained particles (86, 88-90). Understanding the effect of the processing conditions (e.g. operation in a continuous or discontinuous mode) is paramount for designing particles with required features (18).

For this study, coumarin was selected as model drug, as it has been tested as therapeutic agent in various types of cancer (91).

## 2.2. Aim of study

This study aimed at manufacturing and characterization of particles of coumarin with controlled and reduced sizes using the rapid expansion of supercritical solutions (RESS) technology.

## 2.3. Materials and methods

### 2.3.1. Materials

Coumarin from Sigma-Aldrich (Germany) was the starting material for the manufacture of particles with reduced sizes. In the RESS experiments, the gas / solvent used was carbon dioxide ($CO_2$, MW 44.01 g/mol, Air Liquid, Germany).

### 2.3.2. Methods

#### 2.3.2.1. Manufacture of coumarin particles

Prior to micronization, raw coumarin (20 g) was milled in a laboratory size pearl-ball mill (10 cm diameter with 4 balls with a 2 cm diameter) for 120 min (350 rpm at 23 ºC).

The rapid expansion of supercritical solutions (RESS) technique was considered in both the discontinuous mode (experiments 1-10), considering specific initial quantities of coumarin (2, 10, 15 or 20 g), pre-expansion temperatures (40 or 60 ºC), pre-expansion pressures (15-35, 25-35, 15-25 or 35-42 MPa) and post-expansion pressures (0.1, 2.5 or 5.0 MPa), and the continuous mode (experiments 11-15) also considering specific initial quantities of coumarin (25 or 50 g), two flow rates of carbon dioxide (1-2 or 11-12 kg/h) and different expansion periods (0.5, 4.0 or 5.0 h) (Table 2.1).

Experiments were carried out in a multipurpose pilot unit for high-pressure micronization (SITEC-Sieber Engineering AG, Maur / Zürich, Switzerland). For further description on the equipment's operation the reader is referred to the work by Türk et al. (19).

In the discontinuous mode of operation, coumarin was placed in the extraction chamber (volume of 730 $cm^3$) before heating (pre-expansion temperature) and the internal pressure increased (pre-expansion pressure) by injecting carbon dioxide into the chamber. Both temperature and pressure were maintained for a specific period of time (extraction time) allowing the dissolution of coumarin. Then, the solution of coumarin in $CO_2$ was transferred through a nozzle (d = 150 µm) to the expansion chamber, set at defined conditions (post-expansion temperature and pressure), allowing the collection of particles in a collector. To prevent the decrease of pressure in the extraction chamber below the critical pressure, the process of

transferring was interrupted at the minimum pre-expansion pressure to prevent solubility problems of coumarin in supercritical carbon dioxide. The pressure in the extraction chamber (internal pressure) was then increased to the initial pre-expansion pressure by injecting fresh carbon dioxide into the chamber, completing one expansion cycle.

In the continuous mode of operation, coumarin was placed in the extraction chamber (volume of 730 $cm^3$) and the internal temperature and pressure increased. Once both predefined temperature and pressure were reached in the extraction chamber, the supercritical solution of coumarin in carbon dioxide was immediately and continuously transferred through the nozzle (d = 150 µm) to the expansion chamber set with a specific temperature and pressure allowing the continuous collection of particles in a glass collector. The pressure in the extraction chamber was kept constant by the continuous injection of fresh carbon dioxide. The operation was carried out under constant pressure and temperature conditions for a specified period of time with continuous production of particles (Table 2.1).

The quality of the process of manufacturing particles was evaluated based on the yield of the process, defined as the ratio between the mass of collected coumarin particles after production and the initial mass of raw coumarin used.

#### 2.3.2.2. Characterization of particles

The size and size distribution of coumarin particles was determined by laser diffractometry (Mastersizer 2000, Hydro S dispersion unit, Malvern Instruments, UK). The samples were dispersed in a saturated aqueous solution of magnesium sulphate (filtered supernatant) to prevent dissolution of coumarin. Measurements (n=3) were carried out considering the refractive indexes of materials (water: 1.33; coumarin: 1.66), obscuration limits (between 0.5-10 %), stirring speed of suspension (1750 rpm), intensity of ultrasounds (50 %, 10 s before each measurement),

background measurement time (10 s), sample measurement time (10 s), delay between measurements (10 s) and number of measurement cycles (10 cycles).

The median ($d_{50}$), percentiles ($d_{10}$ and $d_{90}$) and span [$(d_{90}-d_{10})/d_{50}$] were considered for the characterization of size and size distribution of each population of particles manufactured. Particles of coumarin were also observed by optical microscopy (BX51 Microscope, Olympus Corp., Japan).

The specific surface area of coumarin particles was determined by gas adsorption based on the Brunauer, Emmett and Teller (BET) methodology. Samples (2-3 g, n=3) were accurately weighed and analyzed on a SA 3100 Beckman Coulter system (Beckman Coulter Inc., Germany). The surface area was calculated using the SA-VIEW$^{TM}$ software (2.12 version). The morphology of particles of coumarin (raw material, RESS and milled) were analyzed by optical microscopy (BX51 Microscope, Olympus Corp., Japan) and scanning electronic microscopy (DSM 940, Carl Zeiss, Germany, with a sputter coater, E 5100, Bio-Rad, München, Germany).

The densities of coumarin particles were determined by helium pycnometry (AccuPyc 1330, Micromeritics, USA) at room temperature (23±2 ºC) (n=3).

The coumarin particles were tested for thermal behavior right after manufacture by differential scanning calorimetry (Q200, TA Instruments, USA). Samples (3-6 mg, n=3) were placed in hermetic sealed aluminium pans and tested at 10 ºC/min between 60-80 ºC. Enthalpies of fusion were calculated for each sample.

After manufacture, coumarin particles were analyzed by X-ray diffractometry for amorphicity behavior. Samples were mounted on an aluminium holder and analyzed in a Philips PW1730 diffractometer with automatic data acquisition (APD Philips v.35B). The diffractograms were recorded at room temperature in the range $10°<2\theta<35°$ and data collected in a continuous mode, with a step size of 0.015° ($2\theta$) and an acquisition time of 1.5 s/step.

***Table 2.1: Experimental conditions for discontinuous and continuous modes of operation for the manufacturing of RESS particles.***

| Experiment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial amount of coumarin (g) | 2 | 10 | 15 | 20 | 10 | | | | | | 25 | | | | 50 |
| Extraction time (h) | 3 | | | | | | | | | | NA | | | | |
| Pre-expansion pressure (MPa) | 15-35 | | | | | 15-25 | 15-35 | 15-25 | 15-35 | | 15-25 | | | | |
| Pre-expansion temperature (ºC) | 60 | | | | 40 | 60 | | | | | 40 | | | | |
| Post-expansion pressure (MPa) | 0.1 | | | | | | | | 2.5 | 5 | 4 | | | | |
| Post-expansion temperature (ºC) | 35 | | | | | | | | | | 50 | | | | |
| Expansion cycles | 4 | | | | | | | | | | NA | | | | |
| Flow rate of $CO_2$ (Kg/h) | NA | | | | | | | | | | 1-2 | | | 11-12 | 1-2 |
| Expansion period (h) | NA | | | | | | | | | | 0.5 | 4.0 | 5.0 | 0.5 | 4.0 |

*NA = Not applicable*

#### 2.3.2.3. Analysis of data

Data was analyzed by an ANOVA (one-way analysis of variance) study for a level of confidence of 99 % and complemented by Tukey's HSD test (statistical significance considered for $p < 0.01$).

## 2.4. Results

### 2.4.1. Properties of coumarin particles

Both RESS and milling processes produced particles with reduced sizes but without showing a distinct shape or morphology, although RESS and milled particles (Figure 2.1, A-B) resemble more spherical than the non-treated commercial particles, the latter with markedly edges (Figure 2.1, C).

The median sizes of RESS (between 18.7±0.8 and 61.0±0.7 μm) and milled (85.5±1.9 μm) particles were significantly smaller by comparison with non-treated coumarin (725.1±57.2 μm) (Table 2.2). The span values for different populations of particles (1.5-2.6) revealed that RESS particles were not significantly different than raw coumarin (1.7), whereas milled particles presented always a higher span (2.6) (Table 2.2), i.e., some RESS experimental conditions enabled the production of narrow size distributions whereas milled particles produced particles with wide distributions (Figure 2.2). In line with these observations RESS particles showed a specific surface area between 0.282 and 0.423 $m^2/g$, milled particles of 0.274 $m^2/g$ and non-treated particles 0.175 $m^2/g$ (Table 2.2).

Worth to mention that both RESS and milled particles agglomerated, whereas non-treated particles remained individualized and loose (Figure 2.1, A-C) suggesting that an increase on the surface area and/or modifications due to processing increased their cohesiveness.

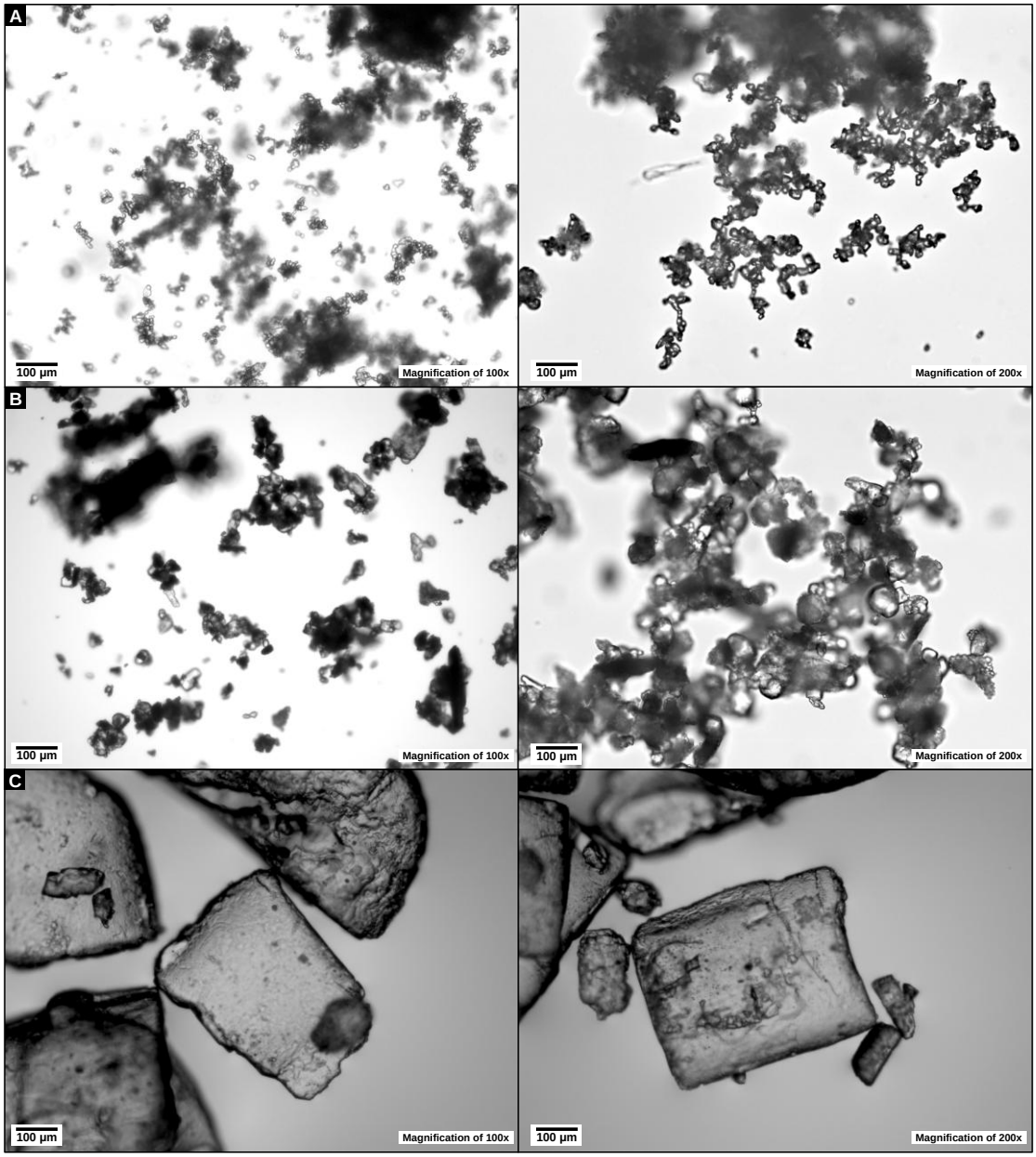



***Figure 2.1: Images of coumarin particles manufactured by RESS (Experiment 13) (A), milled from raw particles (B) and raw commercially available particles (C) obtained by optical microscopy.***

***Table 2.2: Manufacturing yield, amount of coumarin left in the extraction chamber, particle size distribution, density, surface area and enthalpy of fusion of the manufactured coumarin particles.***

| Coumarin sample | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Milled | Commercial |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Manufacturing Yield (%)** | | 10 | 42 | 45 | 35 | 18 | 12 | 17 | 14 | 73 | 60 | 8 | 43 | 70 | 2 | 56 | NA | NA |
| **Coumarin left in extraction chamber (%)** | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 10 | 10 | 21 | 23 | NA | NA |
| **Particle size distribution** [a] | **$D_{10}$ (µm)** | 7.8±0.3 | 11.7±0.8 | 12.2±0.2 | 12.2±0.2 | 16.9±0.1 | 11.7±0.1 | 14.5±0.2 | 22.5±1.0 | 12.3±0.9 | 18.2±0.4 | 12.3±0.2 | 9.7±0.1 | 12.2±0.1 | 9.9±0.3 | 10.7±0.9 | 32.2±0.8 | 185.3±15.6 |
| | **$D_{50}$ (µm)** | 18.7±0.8 | 24.8±1.2 | 32.0±0.7 | 32.9±0.6 | 39.2±0.5 | 26.8±1.2 | 33.3±1.8 | 61.0±0.7 | 37.5±1.0 | 55.4±0.4 | 33.3±1.2 | 26.3±0.7 | 25.8±0.4 | 25.4±2.5 | 38.0±1.8 | 85.5±1.9 | 725.1±57.2 |
| | **$D_{90}$ (µm)** | 57.0±4.6 | 51.7±0.7 | 86.0±4.0 | 79.9±2.6 | 89.3±4.6 | 60.0±6.1 | 80.8±5.1 | 140.4±4.2 | 90.4±1.9 | 134.5±1.4 | 88.5±10.1 | 73.2±1.8 | 52.1±1.1 | 47.2±8.4 | 101.6±3.0 | 251.2±14.8 | 1436.0±45.9 |
| | **Span ($D_{90}$-$D_{10}$) /$D_{50}$** | 2.6 | 1.6 | 2.3 | 2.1 | 1.8 | 1.8 | 2.0 | 1.9 | 2.1 | 2.1 | 2.3 | 2.4 | 1.5 | 1.5 | 2.4 | 2.6 | 1.7 |
| **Density** [a] **(g/cm$^3$)** | | NA | 1.34±0.00 | 1.36±0.00 | 1.35±0.00 | 1.34±0.00 | 1.36±0.01 | 1.33±0.00 | 1.35±0.00 | 1.32±0.01 | 1.34±0.02 | 1.27±0.01 | 1.31±0.01 | 1.32±0.01 | 1.34±0.00 | 1.30±0.01 | 1.39±0.00 | 1.39±0.00 |
| **Surface area (m$^2$/g)** | | ND | 0.353 | 0.301 | 0.307 | 0.339 | 0.413 | 0.345 | 0.423 | 0.333 | 0.377 | 0.362 | 0.282 | 0.375 | ND | 0.332 | 0.274 | 0.175 |
| **Enthalpy of fusion (mJ/mg)** | | 128.2 | 118.4 | 129.2 | 131.1 | 123.6 | 130.7 | 125.2 | 129.3 | 130.8 | 120.5 | 99.7 | 130 | 129.2 | 127.6 | 129.5 | 128.7 | 143.2 |

[a] *n = 3*
*ND = Not possible to determine (not enough amount of particles for analysis).*
*NA = Not applicable*

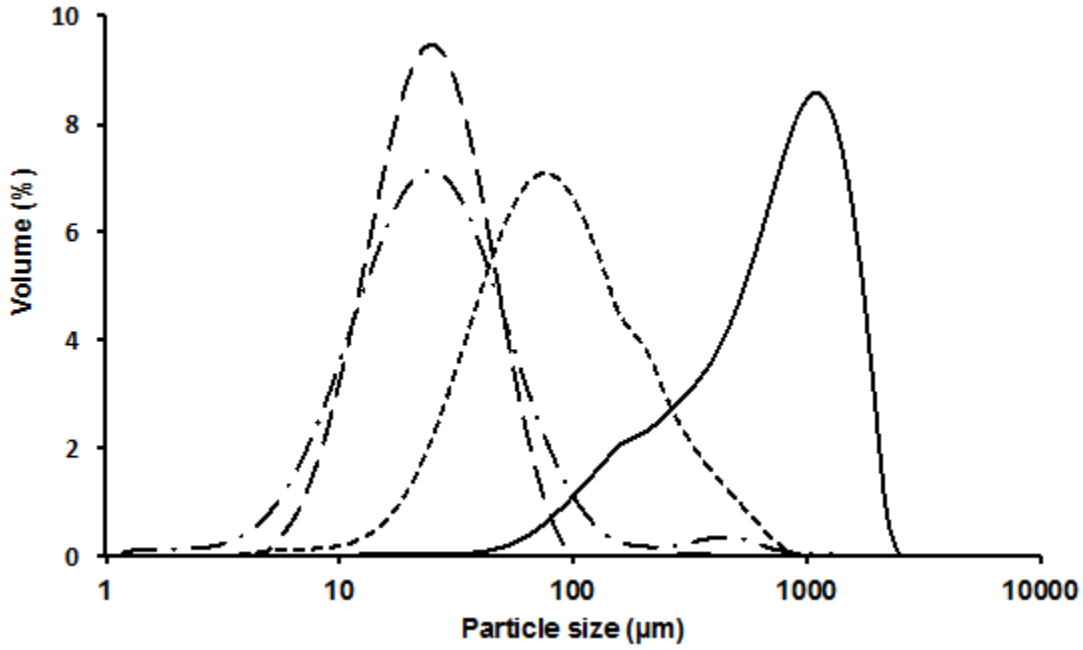



***Figure 2.2: Particle size distributions of non-processed coumarin particles (——), after milling (. . . .) and from the RESS experiments 2 (- - -) and 13 (— · —).***

Coumarin particles manufactured by RESS were less dense (densities between 1.273±0.008 and 1.358±0.004 g/cm$^3$) than milled (1.389±0.001 g/cm$^3$) and non-treated (1.394±0.001 g/cm$^3$) particles (Table 2.2), suggesting that packing of molecules in the RESS production was lower than the packing in original particles which was not modified by milling.

The analysis of the X-Ray diffractograms showed changes on the diffraction angles of some peaks, with lower intensities in the treated particles (Figure 2.3) suggesting that amorphization occurred in the same level. However, it must be pointed out that coumarin is prone to crystallize / recrystallize, thus, it was not surprising that milled and RESS particles presented a high degree of crystallinity and there were no significant differences in their diffractograms, meaning that all treated particles presented similar amorphous contents, although higher than non-treated particles.

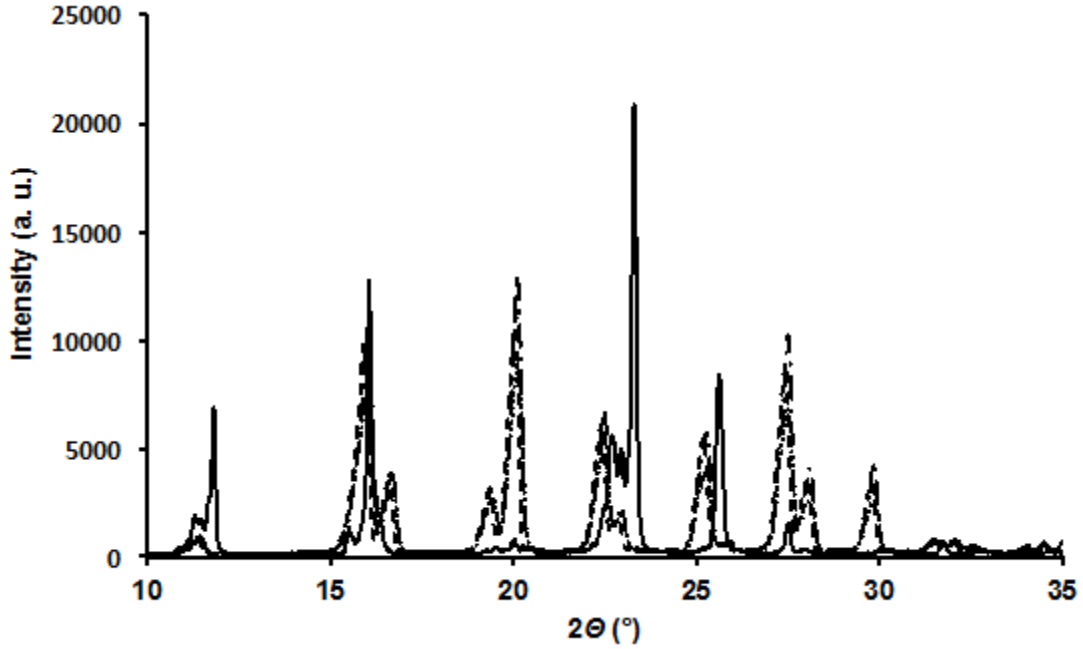



***Figure 2.3: X-ray diffractograms of non-processed coumarin particles (▬▬), after milling (▪▪▪▪) and from the RESS experiments 2 (▬ ▬ ▬) and 13 (▬ ▪ ▬).***

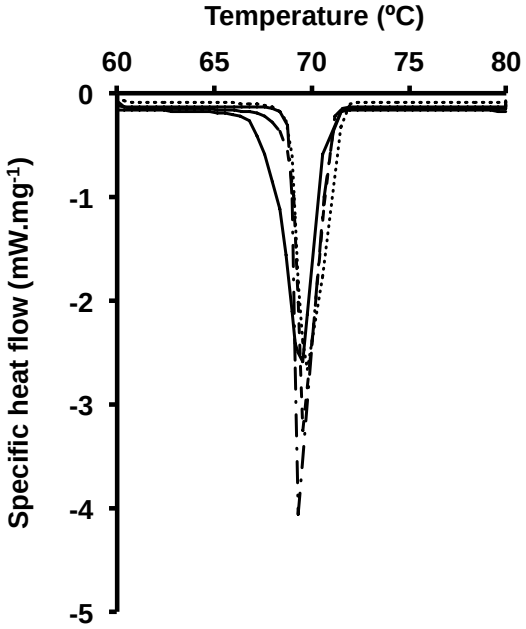



***Figure 2.4: Thermal behavior of non-processed coumarin particles (▬▬), after milling (▪▪▪▪) and from the RESS experiments 2 (▬ ▬ ▬) and 13 (▬ ▪ ▬).***

The analysis of the thermograms (Figure 2.4) and enthalpies of fusion of all types of particles (Table 2.2) did not reveal any relevant changes in their thermal behavior resulting from milling and RESS techniques, as mentioned previously.

### 2.4.2. RESS experimental conditions

The yields of the manufacturing processes tended to increase with the increase of the amount of solute placed in extraction chamber at the beginning of the process. Additionally, it was observed that the yields reached maximum values with high values of post-expansion pressure and that, at the same time, large fractions of solute were retained in the expansion chamber and, consequently, were not transformed into particles. The processing conditions might have originated a dead volume in the expansion chamber rather than in the extraction chamber, where there was confirmed the complete extraction of the solutes.

Regarding the particles manufactured by the discontinuous flow mode (Experiments 1-10), the amount of solute (coumarin) in the extraction chamber influenced the size of the particles, as there was an increasing trend of the median size with the increase of the initial amount of coumarin: particles with median sizes of 18.7±0.8 µm (2 g, Experiment 1), 24.8±0.6 µm (10 g, Experiment 2), 32.0±0.7 µm (15 g, Experiment 3) and 32.9±0.6 µm (20 g, Experiment 4) were collected (Table 2.2).

Similarly, when the pre-expansion temperature increased from 40 to 60 ˚C the populations of particles obtained presented median sizes of 39.7±0.5 (40˚C, Experiment 5) and 24.8±1.2 µm (60˚C, Experiment 2), respectively, with very similar size distributions (Table 2.2). An increase of the temperature in the extraction chamber was reflected by a higher dissolution and low viscosity of the supercritical solution.

In a different set of experiments when the pre-expansion pressure was higher than 25 MPa, particles were formed with higher median sizes (between 33.3±1.8 μm, Experiment 7 and 61.0±0.7 μm, Experiment 8) by comparison to experiments in which the pre-expansion pressure did not exceed 25 MPa (26.8±0.4 μm, Experiment 6) (Table 2.2).

When the pressure in the expansion chamber increased from 0.1 to 2.5 or 5.0 MPa increased the median size of particles from 32.9±0.6 μm (Experiment 2) to 37.5±1.0 μm (Experiment 9) and to 55.4±0.4 μm (Experiment 10), respectively (Table 2.2).

The influence of the processing conditions on the amplitudes of the particle size distributions (spans) of the coumarin particles manufactured in the discontinuous mode was neither clear nor revealed relevant trends: changes on the initial amount of coumarin (span of 2.6/2 g, 1.6/10 g, 2.3/15 g and 1.6/20 g), pre-expansion temperature (span of 1.6/40 ºC and 1.5/60 ºC) and pre-expansion pressure intervals (span 1.8/15-25 MPa, Experiment 6, 2.0/25-35 MPa, Experiment 7, and 1.9/35-42 MPa, Experiment 8) did neither affect nor determined any changing trend in the size distribution amplitudes. However, an increase of post-expansion pressure gave wider size distributions (span of 1.6 for 0.1 MPa, Experiment 2 by comparison with 2.1, Experiment 9 and 2.1 for 5.0 MPa, Experiment 10) (Table 2.2).

Regarding the continuous mode of operation, the manufacturing yields ranged from 2 up to 70%, depending on the experimental conditions: higher expansion periods, smaller amounts of coumarin in the extraction chambers and low carbon dioxide flow rates (Experiment 13) delivered high quantities of product, by opposition to low expansion time and high carbon dioxide flow rate (Experiment 14). It is also interesting to point out the fact that large percentages of materials were left in the extraction chamber (not observed for the discontinuous mode of operation),

which are directly related to small manufacturing yields. Thus, one can assume that low yields were dependent on the dissolution of coumarin in the extraction chamber.

The increase of the initial amount of coumarin did increase the median sizes of particles: 26.3±0.7 µm (25 g) and 38.0±1.8 µm (50 g) (Table 2.2). However, an increase in the flow rate of carbon dioxide produced particles with smaller median sizes – 33.3±1.2 µm for 1-2 kg/h (Experiment 11) and 25.4±2.5 µm for 11-12 kg/h (Experiment 14) (Table 2.2) – and an increase on the expansion time also formed particles with smaller median sizes: 33.3±1.2 µm (0.5 h, Experiment 11), 26.3±0.7 µm (4.0h, Experiment 12) and 25.8±0.4 µm (5.0 h, Experiment 13) (Table 2.2).

The particles manufactured in the continuous mode presented similar size distributions (spans) in comparison with particles obtained commercially and via the discontinuous mode of operation (Table 2.2).

As mentioned previously, all other properties of particles prepared by RESS, namely the morphology, density, surface area, thermal behavior and crystallinity, did not present significant differences with the exception of particles produced according to Experiment 11 for which the density and the enthalpy of fusion were lower than other articles, suggesting the formation of different particles.

In the discontinuous flow experiments, the entire amount of solute was dissolved and transferred to the expansion unit, as there was not detected any coumarin in the extraction unit at the end of the process (Table 2.2). However, in all continuous flow experiments, a fraction of solute was not dissolved, as there were calculated fractions of unused solute between 10 and 60% (Table 2.2). Also, the manufacturing yields of the RESS experiments varied: there were registered low (2-18 %), intermediate (35-45 %) and high (56-73 %) manufacturing yields (Table 2.2).

## 2.5. Discussion

The results obtained proved that both micronizing methodologies – bottom-up (RESS) and top-down (milling) approaches – allowed the manufacture of particles with a significant reduction in size. As expected, this reduction on particle size resulted in increased surface areas of the powders and in increased agglomeration ability of particles, which is also promoted by heat generation in some cases (e.g. milling) (90, 92).

The formation of particles through these processes of micronization occurred by different mechanisms, depending on the technology used. The formation of particles via milling in a ball mill resulted from the breakage of the original coumarin particles due to the impact of the pearl balls on the raw coumarin particles and due to the collision of the particles with each other and with the walls of the mill into smaller particles. However, the formation of particles via RESS occurred through a mechanism of fast nucleation of the solute from the supercritical solution during the expansion of the latter in the expansion chamber (13). The differences of temperature and pressure between the extraction and expansion chambers were high, particularly the decrease of pressure, leading to an abrupt super-saturation of the solute in the carbon dioxide, which was rapidly transformed into the solid state as the carbon dioxide changed to gas leaving the chamber rapidly. These abrupt changes resulted in the formation of solid particles with reduced sizes (13).

The different mechanisms of particle formation are the grounds to justify the production of particles with different properties, namely different morphologies, particle size distributions or densities. In the case of milled particles, the mechanisms of the process justified the production of particles showing a more irregular morphology and higher densities by comparison to the particles formed by RESS. Milling is also a random process, in which the reduction on particle size depends on many variables (e.g. crystal homogeneity or energy of milling) while the

RESS process depends more linearly on the process and materials variables, thus the latter produces particles with lower size distributions.

The reduction of crystallinity of all micronized particles, when compared to the original particles, probably resulted from the creation of amorphous regions mainly at the surface of the particles (case of the milled particles) or from the abrupt or extremely fast process of particle growth that occurs in the supercritical technologies (case of the RESS particles), impacting the formation of the crystalline structure of the particles. However, in both processes there was a preservation of the crystalline properties and structure of the particles, as their amorphous / crystalline contents and thermal behaviors were similar, likely dependent on the high ability of coumarin to crystallize. Both processes were successful in manufacturing particles with reduced sizes, thus, higher surface areas than raw material particles.

When analyzing more judiciously the two RESS processes used in the study – the discontinuous and the continuous modes of operation – it was possible to confirm that some of the experimental conditions affected the properties of the particles. In the discontinuous flow experiments lower fractions of solute used at the start originated more concentrated supercritical solutions of coumarin, which consequently created particles with larger sizes when the solution was expanded due to a higher super-saturation (89). On the other hand, the effect of different processing temperatures in the extraction chamber (pre-expansion temperature) on coumarin revealed that the temperature did not influence the size distribution of particles, as both temperatures allowed the production of supercritical solutions with equivalent saturations. Changes in the pressure of the extraction chamber (pre-expansion pressure) failed to show a clear influence on the size of particles whereas lower post-expansion pressures originated particles with smaller median sizes. The pressure gradient between the extraction and expansion chambers was far more critical for the

precipitation of solid particles than the temperature gradient, which was small between the two chambers.

In the continuous mode of operation, the continuous flow of the supercritical solution in the RESS apparatus, together with the inexistence of an initial period of dissolution for coumarin might have changed the impact on the supercritical solution concentration on the size of the particles, as there was not a stabilization of the supercritical solution in the chamber and the dissolution of the solute had to occur over the entire manufacturing period.

The particles manufactured in the continuous mode presented wider size distributions, likely due to the use of a higher flow rate of carbon dioxide. High flow rates of carbon dioxide produced supercritical solutions with lower concentrations than solutions kept in the extraction chamber for different periods of time circulating more rapidly in the equipment. Consequently the time of contact of the carbon dioxide in supercritical state with the dissolving solute was smaller, which might support these observations.

Additionally, longer periods of contact between the supercritical carbon dioxide and the solute resulted in higher amounts of coumarin dissolved in the solvent over the time of experiment, as shown by the low yields obtained for low expansion periods and high fractions of coumarin remaining in the extraction chamber. As observed for experiment 11, in which the expansion period was 0.5 h, a high amount of coumarin was not dissolved and remained in the extraction chamber by the end of the experiment.

In the case of the discontinuous flow experiments, the time allowed for dissolution (3 h) was sufficient for dissolving the entire coumarin. However, the continuous mode revealed a loss of efficiency in the extraction. Shorter expansion periods and higher initial amounts of solute might justify this loss of coumarin

(remaining in the extraction chamber), as the expansion period had not been enough for the entire consumption of the solute.

The experiments in which higher initial amounts of solute, post-expansion pressures and expansion periods were used, presented the highest manufacturing yields. An increase in the expansion time might minimize or even eliminate the loss of raw coumarin left in the extraction chamber.

From the data collected, it was evident that both approaches considered for the RESS technique could have been used to improving the dissolution of drugs by the reduction of their particle size and increase on the surface area, increasing their solubility and dissolution rates (19). The continuous flow experiments were more productive than the discontinuous flow experiments as the yield of particles obtained was higher.

## 2.6. Conclusions

The RESS technique was successfully used in manufacturing particles of coumarin with reduced sizes (between 20-60 µm, mean values) and high surface areas. Both RESS procedures – the discontinuous and the continuous flow operation modes – and their specific experimental conditions influenced the properties of the manufactured particles, especially the size and size distribution.

Both RESS operating modes delivered micronized particles but optimization was paramount to reach high production yields, small particles size, smooth surfaces and less dense and more amorphous particles than the ones produced by milling.

# CHAPTER 3

## Characterization of laminar extrudates manufactured at room temperature in the absence of solvents

***Adapted from:***

*Characterization of laminar extrudates manufactured at room temperature in the absence of solvents for the delivery of drugs, G. Oliveira, M. A. Wahl, J. F. Pinto, International Journal of Pharmaceutics 454 (2013) 90– 98.*

## 3.1. Introduction

Extrusion is a technology widely applied in various fields such as plastic processing, ceramics, food and pharmaceutical industries (57). In the pharmaceutical field, the wet and hot-melt extrusion processes are commonly applied in the manufacture of medicines. However, these processes present some limitations, namely the use of solvents in the formulations (e.g. water) and/or changes on temperature during manufacturing. Due to these limitations, certain drugs cannot be processed by extrusion, as they are heat and/or solvent sensitive, thus, the possibility of performing extrusion without the use of solvents and changes in temperature during processing represents a step forward in the field, allowing a wider application and turning this technology into a green process (42).

Extrudates can have different shapes. The most common ones are the rod or cylindrical (59) and tubular (61) shapes. However, there is also the possibility of manufacturing extrudates with laminar shape, which might be suitable for oral or topical delivery of drugs (55). Furthermore, extrudates with a laminar shape manufactured in the absence of solvents at room temperature can be regarded as a green technology.

Only a small number of excipients have been reported as suitable for the manufacture of extrudates at room temperature in the absence of solvents. Binders and lubricants such as lipid-based excipients with low melting ranges like fatty acids (61) and mixtures of fatty acids with polyethylene glycols (PEG) have been processed under these conditions (66). Equally, combinations of triglycerides with low and high melting ranges allow the manufacture of extrudates at moderate temperatures (e.g. 40 ºC), not affecting the stability of the extrudates (67).

Glycerides, such as glyceryl palmitostearate, glyceryl trimyristate or glyceryl dibehenate, can also be used in the manufacture of extrudates with different

properties due to their chemical and physical complexity, which lead to distinct melting, crystallization, polymorphic and other physical differences (68).

It follows that the mechanical properties of extrudates manufactured from solid lipid-based excipients need to be properly assessed as these extrudates may undertake important physical changes throughout storage (93). Lipid-based excipients with a heterogeneous composition are more sensitive to formulation and processing variations, leading to more evident changes in their structural stability throughout time (aging), which can have an impact on the product characteristics, such as the release behavior of the drug (93-95). The release of drugs from extrudates with such components can be modified with the inclusion of hydrophilic materials (e.g. PEG), by changing the surface area of the extrudates (67), by a criteria selection of specific lipid-based excipients (e.g. different melting ranges) or by considering the use of appropriate combinations of these to manufacture controlled release solid dosage forms (96).

## 3.2 Aim of study

This study aims to evaluate the extrusion ability of materials by laminar extrusion at room temperature in the absence of solvents and the potential of the new dosage form to deliver drugs, as well as, to assess the stability of the properties of extrudates manufactured over storage.

## 3.3. Materials and methods

### 3.3.1. Materials

The materials selected for this study included several solid lipids, namely, glyceryl palmitostearate (Precirol™ ATO 5, Gattefossé, France; melting range: 69-74ºC), glyceryl trimyristate (Dynasan™ 114, Sasol, Germany; melting

range: 53-57ºC), glyceryl dibehenate (Compritol™ 888 ATO, Gattefossé, France; melting range: 55-58ºC), hard fat (Witocan™ 42/44, Sasol, Germany; melting range: 42-44ºC) and mixtures of polyethylene glycol with glycerides (Gelucire™ 33/01, Gattefossé, France; melting range: 33-35ºC). These materials are mixtures of different lipid components (Table 3.1). Additionally, a mixture of polydimethylsiloxane with silicon dioxide (Simethicone™ Q7-2243 LVA, Dow Corning, Belgium) was included in all formulations and lactose (SpheroLac™, Meggle Pharma, Germany) was considered as the diluent. The model drug was milled coumarin particles (0-90, 250-355 and 710-1000 µm), obtained from coarse coumarin (Sigma-Aldrich, Munich, Germany) by milling the latter in an analytical grinder (A10 Yellow Line, IKA, Königswinter, Germany).

### 3.3.2. Methods

#### 3.3.2.1. Preparation of extrudates

The excipients in each formulation (Table 3.2) were mixed in a planetary mixer (Kenwood Chef, Hampshire, UK) for 15 minutes and placed in the extrusion chamber. The manufacture of the laminar extrudates was carried out using an in-house built ram extruder (Lurga, Sacavém, Portugal) with a specific die (1x40mm rectangular cross section) designed to prepare extrudates with a laminar shape (Figure 3.1).

The ram extruder was adjusted to a universal testing machine (LR 50K, Lloyds Instruments, Leicester, UK) fit with a load cell that allowed collection of the applied force and displacement data from the process. Extrudates were manufactured at different extrusion rates (100, 300 and 500 mm/min), cut into squares (40*40*1mm) immediately after production and stored in a desiccator (23 ºC / 30 % RH).

***Table 3.1: Composition and melting ranges of the lipid-based excipients.***

| Lipid-based excipients | Fatty acids chain length [a] | Melting range (ºC) | Observations [a] |
|---|---|---|---|
| Polyethylene glycol glycerides | $C_{10}$ to $C_{18}$ (saturated fatty acids) | 33 – 35 | - |
| Hard fat | $C_{10}$ to $C_{18}$ (saturated fatty acids) | 42 – 44 | - |
| Glyceryl trimyristate | $C_{14}$ (saturated fatty acids) | 53 – 57 | - |
| Glyceryl palmitostearate | $C_{18}$ | 55 – 58 | Mixtures of mono (19.1 %), di (53.2 %) and tristearate (27.4 %) |
| Glyceryl dibehenate | $C_{22}$ | 69 – 74 | Mixtures of mono (17.8 %), di (53.0 %) and tribehenate (26.8 %) |

[a] *According to the information provided by the suppliers.*

***Table 3.2: Composition of the formulations considered in the study (%).***

| Formulation | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Polyethylene glycol glycerides | 10 | 10 | 4 | 4 | 4 | 4 |
| Hard fat | - | 30 | - | 33 | 33 | 33 |
| Glyceryl trimyristate | 30 | - | 33 | - | - | - |
| Glyceryl dibehenate | - | 30 | - | 33 | 33 | 33 |
| Glyceryl palmitostearate | 30 | - | 33 | - | - | - |
| Polydimethylsiloxane – silicon dioxide mixture | 20 | 20 | 20 | 20 | 20 | 20 |
| Lactose | 10 | 10 | 10 | 10 | 5 | - |
| Coumarin | - | - | - | - | 5 [a] | 10 [a] |

[a] *Three different laminar extrudates were manufactured for each formulation containing particles of coumarin with three different size ranges: 0-90, 250-355 and 710-1000 µm.*

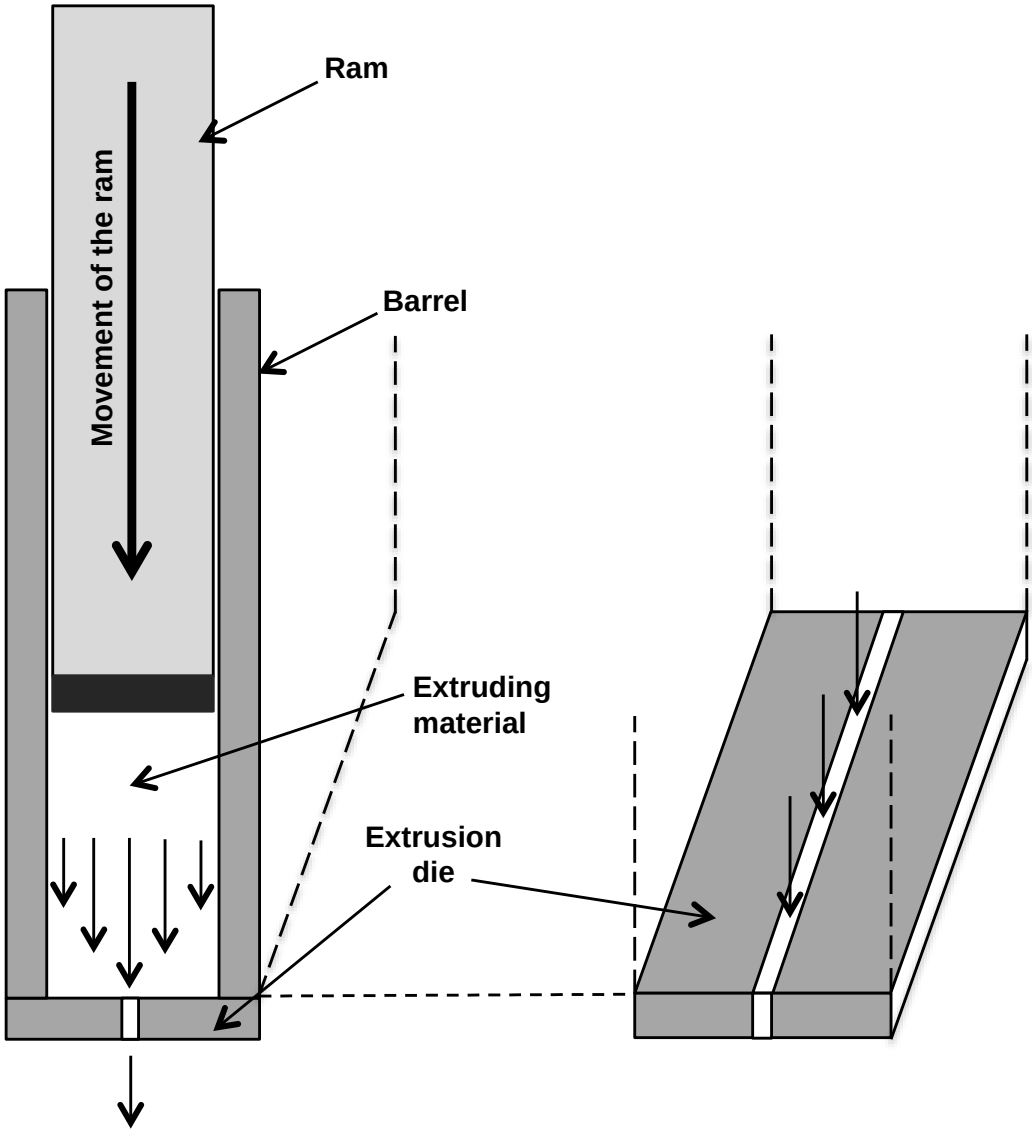



***Figure 3.1: Schematic representation of the ram extruder used to manufacture the laminar extrudates.***

### 3.3.2.2. Characterization of extrudates

#### 3.3.2.2.1. Physical characterization

The extrusion force at steady state ($F_{ss}$) was calculated for each extrusion cycle as the mean of the force points obtained between 70 and 100 mm of the piston's displacement. The dimensions (measured with a caliper) of the extrudates manufactured were the thickness (n=10), measured at five distinct points to assess the uniformity of each specimen, the width and length of each extrudate (originally cut with 40*40*1mm). The densities of both raw materials and extrudates (at 0, 1, 2, 3, 7, 10 and 20 days after production) were determined in a helium pycnometer (AccuPyc 1330, Micromeritics, USA) at room temperature (23 ºC) (n=3). The expected densities of the extrudates were calculated from the densities of the raw materials weighted for their fractions in the formulations. The latter have allowed the calculation of the porosities of the extrudates, based on the relative decrease on the densities of the extrudates, according to the following equation:

$$Porosity\ (\%) = 1 - \frac{apparent\ density}{theoretical\ density} \times 100 \qquad \textbf{Eq. 3.1}$$

Both the raw materials and extrudates were evaluated for potential changes due to heat by differential scanning calorimetry (DSC, Q200, TA Instruments, New Castle, USA). Samples (3.0-6.0 mg, n=3) were placed in hermetic sealed aluminium pans and tested at 10 ºC/min (20-160 ºC). Each sample of extrudates was analyzed at days 0, 1, 3, 7, 10 and 20, after manufacture.

Extrudates (n=3) were characterized for mechanical properties, namely by a flexure test at 1, 2, 3, 7, 10 and 20 days after manufacture by a three-point bend rig test (TA.XT Plus, Stable Microsystems, Godalming, UK) at both axial and normal directions of extrusion. Force was applied to the laminar extrudates and

both force and displacement were recorded to allow calculation of the bending strength, stiffness, deformation and Young's modulus of elasticity (97).

The release of coumarin from extrudates was assessed by dissolution tests 7 days after their production (Eur. Ph. 2.9.3., n=3, paddle method, 50 rpm, phosphate buffer, pH=6.8, AT7 Sotax, Switzerland). The quantification of coumarin was carried out by UV spectroscopy at 307 nm (U-2000 Spectrophotometer, Hitachi, Japan). To describe the mechanisms of release of coumarin from the extrudates, three mathematical models were considered – zero order, Higuchi and Korsmeyer-Peppas – and their suitability determined, based on their fitting to the data assessed by the calculated coefficients of correlation (n=3). The efficiency of dissolution of coumarin was determined for all extrudates (98) and the release rates were calculated as the best correlation obtained from two separate parts of each curve (between 0-3 hours and 4-8 h, following a preliminary analysis of data). A statistical analysis was performed based on a one-way analysis of variance (ANOVA) with a 99% level of confidence and Tukey's HSD test. Statistical significance was considered for $p < 0.01$.

## 3.4. Results and discussion

### 3.4.1. Manufacture of extrudates

As described previously (42), several powdered lipids have been used as extrusion binders and lubricants, particularly when solvents are to be avoided. Those powdered lipids (glyceryl trimyristate, glyceryl palmitostearate and glyceryl dibehenate) have been proven as alternative materials to the commonly used excipients in wet and hot-melt extrusions (93).

The analysis of the extrusion profiles provided information on the ability of the formulations to produce extrudates with good quality. Typically,

extrusion profiles present three different stages: the compression stage, the steady state flow stage and the forced flow stage (57). Long and uniform steady state stages were obtained with materials with good extrusion properties and appropriate processing conditions (e.g. the right extrusion rate), leading to good quality of the extrudates. If uneven extrusion profiles occur, the quality of the resulting extrudates is in general poor and various surface defects can be observed (57).

In this work all extrusion profiles presented typical features (Figure 3.2): the initial displacement registered a residual extrusion force (compression stage) until it reached an abrupt increase of the force indicating the beginning of the process of extrusion, which was followed by the steady state flow stage. For all extrusion rates, the mean extrusion forces registered at the steady state flow ($F_{ss}$) stage were below 6000 N, and constant over the manufacture of extrudates, prior to the onset of the forced flow stage, which proved the satisfactory extrudability of all formulations and the production of high quality extrudates (99).

All mixtures based on different formulations allowed the manufacture of extrudates with laminar shape (rectangular cross section) at different extrusion rates. Also the different lipid-based extruded masses behaved equally to changes in the extrusion rate. It was observed that for formulations A to D (Table 3.2) an increase in the extrusion rate corresponded to an increase on the extrusion forces at steady state (Table 3.3) and, at the same time, the extrusion profiles became more irregular. These observations were expected and resulted from an increase of the shear stress at the wall of the die as a reaction to the increase of the ram speed reflected by a lower lubrication ability of the formulation to compensate such increase on shear stress. Furthermore, a decrease on laminar flow near the die wall shall not be excluded (100).

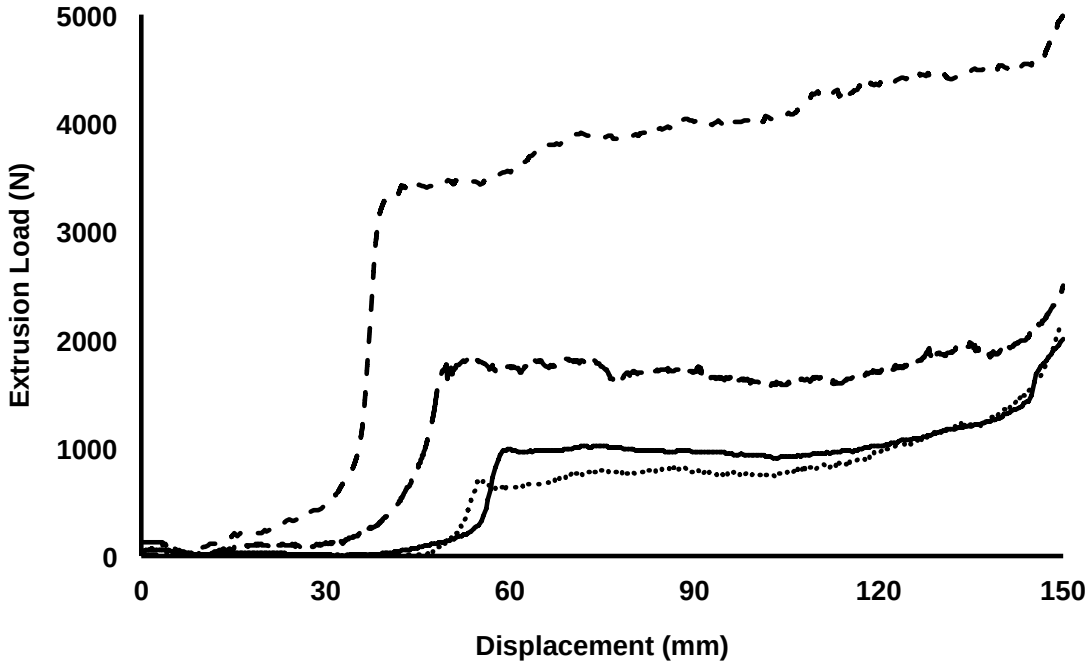



***Figure 3.2: Extrusion profiles of laminar extrudates from formulations A (——), B (· · · ·), C (- - -) and D (— —), manufactured at 300 mm/min.***

***Table 3.3: Mean extrusion force (N) at steady state ($F_{ss}$) of laminar extrudates obtained from formulations manufactured at different extrusion rates.***

| Extrusion Rate (mm/min) | Formulation A [a] | Formulation B [a] | Formulation C [a] | Formulation D [a] |
|---|---|---|---|---|
| **100** | 746 ± 203 $^{r}$ | 389 ± 17 $^{r}$ | 4429 ± 203 $^{r}$ | 932 ± 78 $^{r}$ |
| **300** | 1296 ± 142 $^{s}$ | 1000 ± 356 $^{r}$ | 4566 ± 820 $^{r}$ | 1228 ± 222 $^{r,s}$ |
| **500** | 1504 ± 68 $^{t}$ | 3239 ± 1341 $^{r}$ | 5576 ± 130 $^{r}$ | 4749 ± 531 $^{s}$ |

[a] *For each column, the means with different letters in the indices are significantly different (ANOVA; $p < 0.01$; Tukey's HSD; n=3).*

In general, the manufacture of laminar extrudates from formulations A and B, which contained a higher content of polyethylene glycol glycerides (10%), required lower $F_{ss}$, by comparison with the ones observed for formulations C and D (only 4%), turning evident that polyethylene glycol glycerides promoted an easier extrusion. This might have occurred due to the lowest melting range of polyethylene glycol glycerides (33-35ºC) resulting from the composition of the material, which is a mixture of saturated fatty acids with a high variety of chain lengths (between 8 and 18 atoms of carbon) and, consequently, softened (i.e., for the purpose of this work became less viscous), thus, turning the mixture more fluid at lower forces (66).

Additionally, laminar extrudates obtained from formulation C, which contained two lipid excipients with a melting range within the ones for the other excipients (glyceryl palmitostearate: 53-57ºC; glyceryl trimyristate: 55-58ºC), required higher $F_{ss}$ by comparison with the ones observed for formulation D extrudates, which included a mixture of two lipid-based excipients, one with a higher melting range (glyceryl dibehenate: 69-74ºC) but another with a lower melting range (hard fat: 42-44ºC). The effect of the simethicone and lactose in the extrudability of the masses was not relevant based on the differences observed, as their contents were kept constant throughout the experiments. This suggests an interaction between the liquid simethicone and lactose, a material that shall remain in the solid state over the process of extrusion. One can suggest that simethicone provided some lubrication for the extrusion of lactose particles.

The mixture of glyceryl dibehenate and hard fat improved the extrudability of the masses, whereas the inclusion of both glyceryl palmitostearate and glyceryl trimyristate produced a mass with higher difficulty to be extruded reflected by the higher forces observed at steady state. An increase in the length of the carbon chains of the fatty acid corresponds to an increase in the melting range of the lipid and, thereby, to a decrease on the extrudability of the masses (66).

Saturated fatty acids with longer chains interacted more strongly and, thereby, required higher forces for extrusion whereas lipid-based excipients containing a high variety of saturated fatty acids were easier to extrude and required lower forces, suggesting an easier softening, if not melting, of the component with the lowest melting range. Additionally, these melting ranges can vary within the same lipid material as a function of the possible existence of different polymorphic forms (66). Thus, the melting ranges of excipients is paramount for this type of process of extrusion, as the composition of each lipid-based material included in the formulations, especially the length of the carbon chain of the saturated fatty acids, plays a major effect on the process of extrusion. Thus, appropriate lipid-based excipients and their mixtures must be carefully selected to achieve both a satisfactory process and product (42).

For the manufacture of extrudates including coumarin particles, only one of the four initial formulations (A – D) was selected and also only one extrusion rate for it's processing. Formulation D was elected for further analysis and inclusion of the drug, as it allowed the manufacture of extrudates without any visual defect (see section 3.3.2) and minor changes of the mechanical properties over time of storage (see section 3.3.6). Furthermore, the extrusion rate of 300 mm/min was selected to further develop our study and, therefore, was used in the manufacture of extrudates from formulations E and F, though all extrusion rates were suitable for the manufacture of the extrudates.

The extrusion profiles obtained for different manufacturing cycles at 300 mm/min with formulations E and F, containing coumarin particles with three different size ranges, were equivalent to the ones obtained for formulation D. However, the extrusion forces at steady state ($F_{ss}$) registered for these extrudates (Table 3.4) revealed that the formulations containing coumarin particles, independently of their sizes, required slightly lower extrusion forces at steady state

when compared with formulations that included lactose only. Therefore, one can say that coumarin facilitated the process of extrusion, when comparing to lactose formulations. A possible explanation for this observation can be based on the fact that lactose and coumarin particles have different melting ranges (143-148 and 68-70 ºC, respectively), which might justify the lower resistance of coumarin particles to extrusion that might have soften under the application of the extrusion load.

***Table 3.4: Influence of coumarin particle size on the mean extrusion force (N) at steady state ($F_{ss}$) for the extrusion of excipients into laminar extrudates (obtained from formulations E and F, manufactured at 300 mm/min, n=3).***

| Coumarin size range (µm) | Formulation E [a] | Formulation F [a] |
|---|---|---|
| **0 – 90** | 1013 ± 187 [r] | 1127 ± 166 [r] |
| **250 – 355** | 1077 ± 141 [r] | 869 ± 86 [s] |
| **710 – 1000** | 1334 ± 198 [r] | 844 ± 124 [s] |

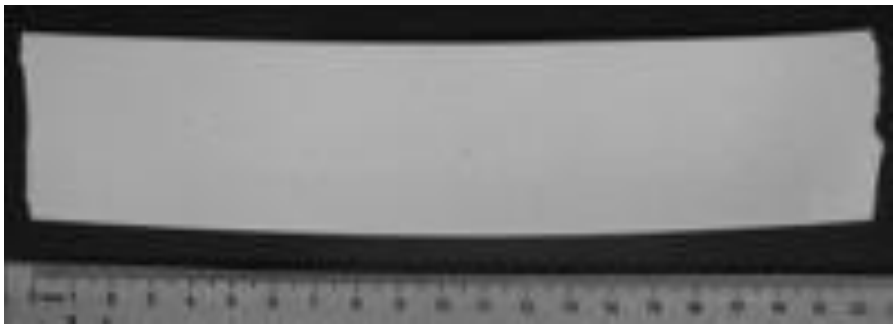
[a] *Means with different letters (indices) are significantly different (ANOVA; p<0.01; Tukey's HSD; n=3)*

### 3.4.2. Visual inspection of extrudates

The quality of the extrudates surface, namely a homogeneous and smooth surface, can be critical for successfully achieving the intended applications for a specific extrudate, namely reproducible dissolution rate of carrying drugs. Due to extrusion flow instabilities at the entry and/or at the exit of the die, extrudates often present some distortions and surface imperfections that can be visually identified: sharkskin defect, roughness or surface scratches and some structural defects such

as surface cracks or fractures, variations of shape, lack of homogeneity due to a deficient mixing or incorporation of air during extrusion, excessive softness or brittleness, amongst others (70). Extrudates obtained from formulations A, C and D, at all extrusion rates, were visually similar and did not present any surface defect (Figure 3.3.a). However, extrudates B were excessively soft and presented a severe sharkskin defect (Figure 3.3.b), likely due to elongation stresses created at the die exit (69, 70), resulting in their exclusion from further consideration in other experiments.

**a)**



**b)**

***Figure 3.3: Example of laminar extrudates with no surface defects (formulations A, C, D, E and F) (a) and with surface defects (formulation B) (b).***

Extrudates including particles of coumarin (formulations E and F) presented a visual aspect similar to formulation D, as the partial or complete substitution of lactose by coumarin did not change the properties of the extruded masses and, consequently, did not have an impact on the process of extrusion or on the quality of the extrudates.

### 3.4.3. Thickness of extrudates

All extrudates (A – F) presented a thickness of approximately 1.6 mm. There were not relevant differences observed between the thicknesses of different extrudates and neither the extrusion rate nor the inclusion of coumarin particles influenced the final thickness of the extrudates.

For a 1 mm width die, extrudates thicknesses were as high as 1.6 mm, representing a relaxation of 60%. Afterwards, all extrudates kept their thicknesses and no changes were detected over storage. The materials on converging to the die were submitted to stresses that were total or partially released on exiting the die. This relaxation can be assumed to be constant as there is no effect seen by the different formulations. The assumption can be made on the grounds that, contrary to elastic materials, which react on removing the force by the ram, plastic materials conserve their shape in consequence of the application of a deforming force (101).

### 3.4.4. Density and porosity of extrudates

A decrease on the densities of extrudates A and C over time of storage was observed, whereas extrudates D maintained their density. Concomitantly, the porosities of extrudates A and C increased with time while extrudates D maintained their porosity or presented a slight decrease. These results suggest that extrudates with a lower content of polyethylene glycol glycerides (which are composed by a mixture of saturated fatty acids with a wide range of chain

lengths) are more likely to keep their densities and porosities over time of storage. Additionally, the extrudates that included lipid-based excipients with less variety of chemical composition and saturated fatty acids with similar carbon chain lengths were less likely to show variability of the density / porosity over time of storage, as these excipients promote the stabilization of the structures in comparison with other mixtures and more complex lipid-based excipients (95).

The decrease of density and consequent increase of porosity, especially observed for extrudates A and C was in line with our previous observations about the manufacture and thickness of extrudates. Right after the extrusion, the extrudates expanded and their thicknesses were much higher than expected, due to an abrupt decrease of the pressure applied to the materials inside the extrusion chamber, in comparison with the pressure after the die exit. Although changes in the dimensions on the extrudates were not realized, the observed decrease in the densities might have been associated with the expansive behavior after extrusion: laminar extrudates expanded (their volume increased while the mass remained unchanged), resulting on a decrease of the density. This process of expansion might have been more prolonged and more intense for extrudates obtained from formulations A and C, rather than laminar extrudates from formulation D due to the previously referred composition differences, particularly an increase of both hard fat and glyceryl dibehenate fractions on the formulation at the expenses of polyethylene glycol glycerides.

This trend was observed for extrudates manufactured at all extrusion rates (100, 300 and 500 mm/min). However, higher extrusion rates produced less dense and more porous extrudates. Figure 3.4 presents the density and porosity over time of storage of extrudates A (data from extrudates C and D is not shown).

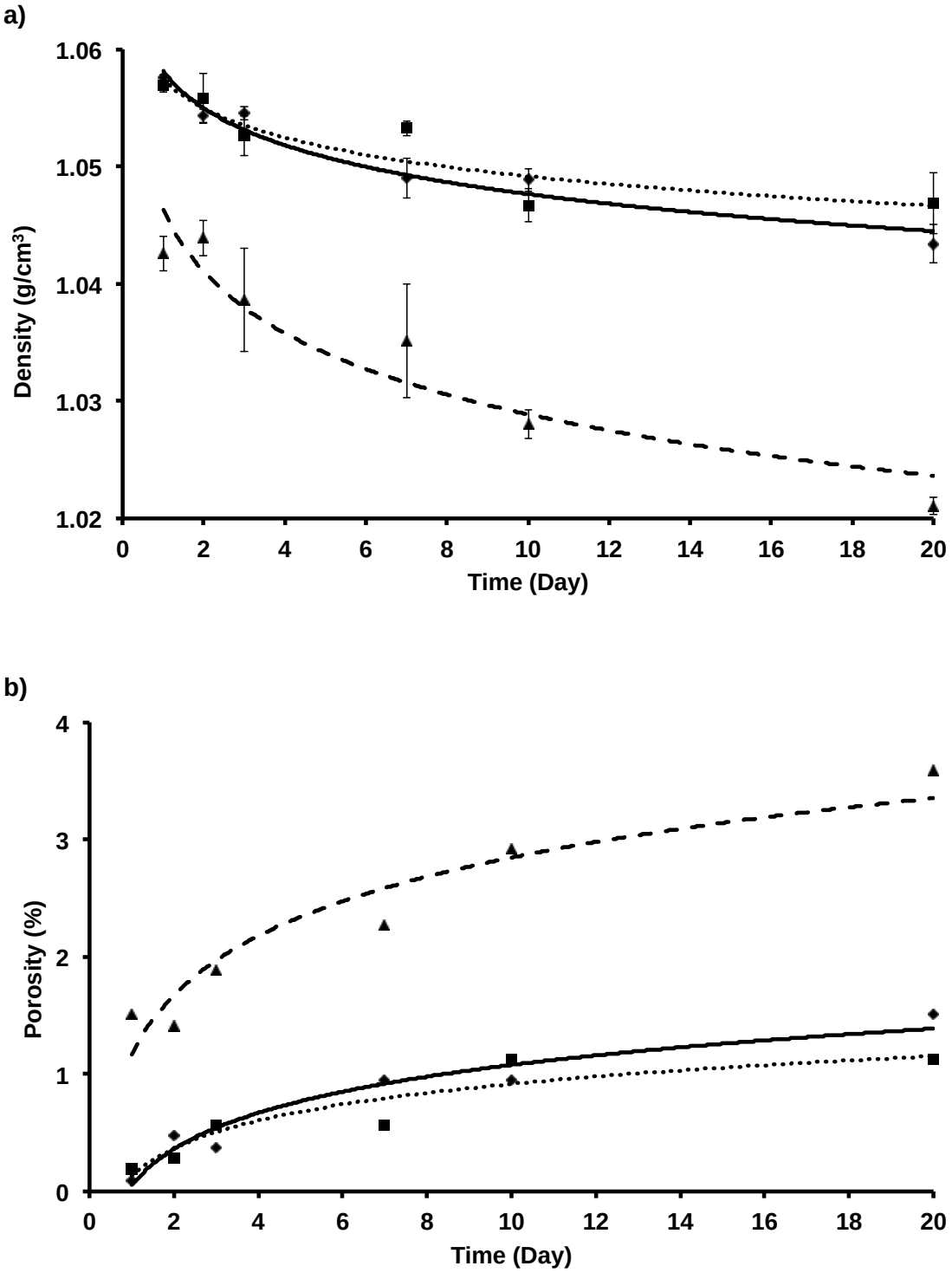



***Figure 3.4: Density (a) and porosity (b) over storage of extrudates A obtained at different extrusion rates: 100 (—◆—), 300 (· ·■· ·) and 500 (– ▲ -) mm/min.***

The difference between the pressures applied to the extrudable mass inside the extrusion chamber and at exiting the extrusion die was relevant and increased with the extrusion rate. Therefore, the materials could have suffered a more severe expansion phenomenon due to this pressure difference, resulting in less dense and more porous extrudates.

These changes of density and porosity over time, more evident for formulations A and C than for formulation D, were related to the changes of the mechanical properties observed for all tested laminar extrudates (see section 4.6, Chapter 3) and testify the aging phenomena that occurs with extrudates mainly composed of lipid materials (102).

### 3.4.5. Thermal behavior of raw materials and of extrudates over time

The changes on the thermal behavior and enthalpies of fusion observed for both extrudates D and F over time (Figure 3.5 and Table 3.5) strongly suggest and support the occurrence of an aging phenomenon, which was expected to occur in extrudates mainly composed by lipid materials. For both extrudates, the enthalpy of fusion increased over time suggesting a stabilization of the structure of the extrudates due to changes of the materials, particularly the lipid materials, which transform themselves into more stable forms (102).

***Table 3.5: Enthalpies of fusion (J/g) of extrudates D and F (coumarin 0-90 µm) over time of storage.***

| Formulation | Day 1 | Day 3 | Day 7 | Day 10 | Day 20 |
|---|---|---|---|---|---|
| **D** | 104.4 | 107.6 | 130.9 | 139.6 | 142.3 |
| **F** | 111.6 | 125.2 | 151.6 | 174.1 | 198.8 |

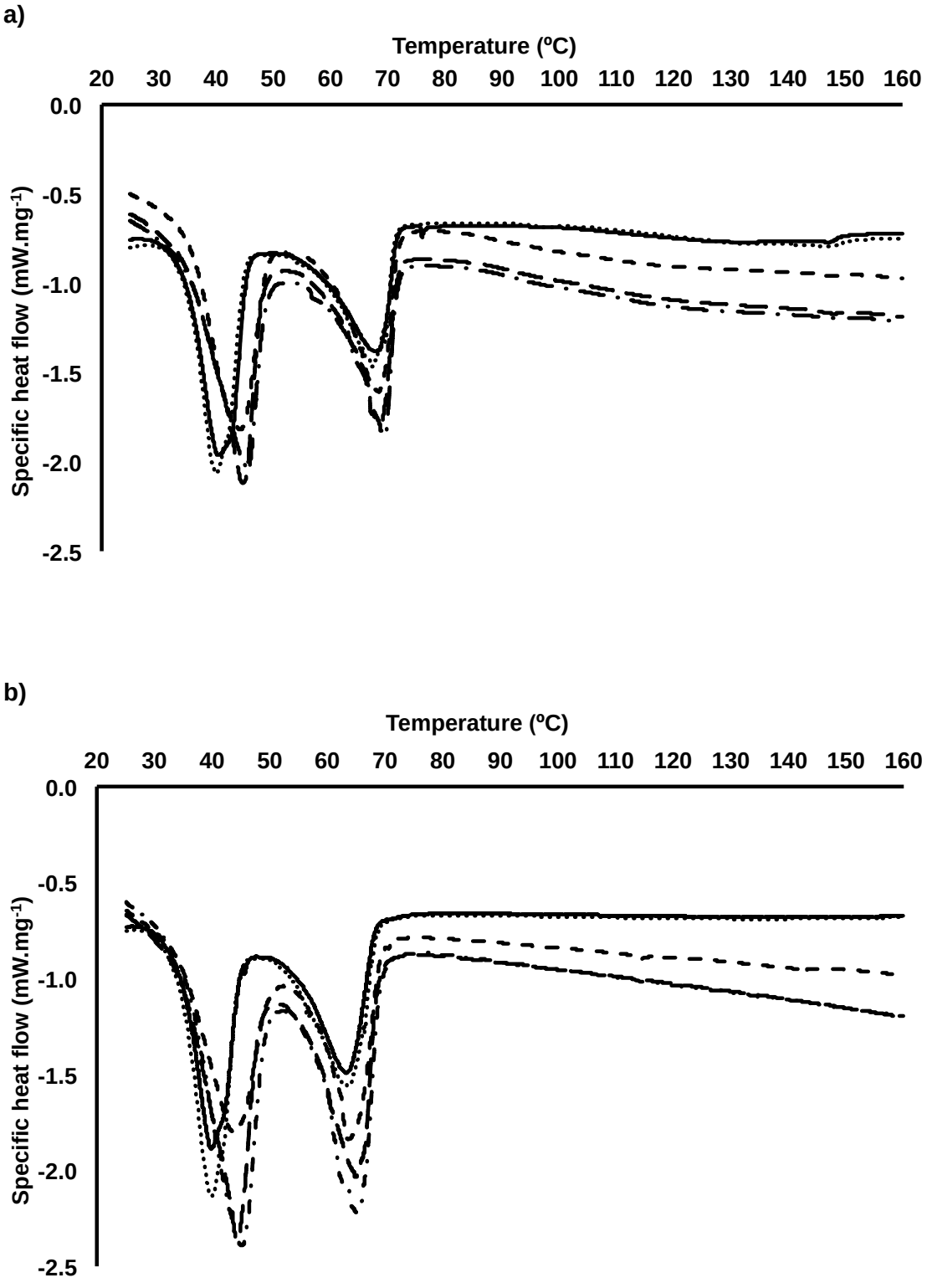



***Figure 3.5: Thermograms of extrudates obtained from Formulation D (a) and Formulation F (coumarin 0-90 µm) (b), and analyzed over time of storage: 1 (——), 3 (· · · ·), 7 (- - -), 10 (— —) and 20 (— · —) day.***

The comparison between the thermal behaviors of the individual raw materials and the extrudates after their mixture and extrusion was not as clear as one would anticipate. Over the process of extrusion, materials were subjected to high pressures and shear forces and interacted in a non-immediately clear way. Consequently, one can expect that the thermal behaviors of the extrudates were not a simple sum of the thermal behaviors of the single materials but a far more complex result of the effects. These effects were likely to happen at both molecular and supra molecular levels of the extrusion process on the materials, reducing the evaluation of the outcome to a qualitative assessment.

Those effects resulted from the high forces applied to the mixtures of powders during extrusion and from the generated friction of the extruding materials on the surfaces of the extruder and between particles, have determined an increase on the temperature that led to changes on the solid state of the materials. Afterwards, these materials tend to stabilize and may undertake changes in their structure to a greater state of stability (102). These observations can anticipate and contribute for the explanation of the changes of the mechanical properties observed for all laminar extrudates.

### 3.4.6. Mechanical characterization of extrudates

The use of glycerides and triglycerides in the manufacture of pharmaceutical dosage forms presents important limitations related to their chemical complexity, which can create a variety of crystalline forms, including metastable forms with consequences on the properties and performance of the dosage forms manufactured (66). The mechanisms responsible for these changes are either the conversion of the triglycerides from unstable or metastable to more stable polymorphic forms, or the conversion from the amorphous to the crystalline state of

the glycerides. Altogether they are the basis of the ageing phenomena observed with these materials (102).

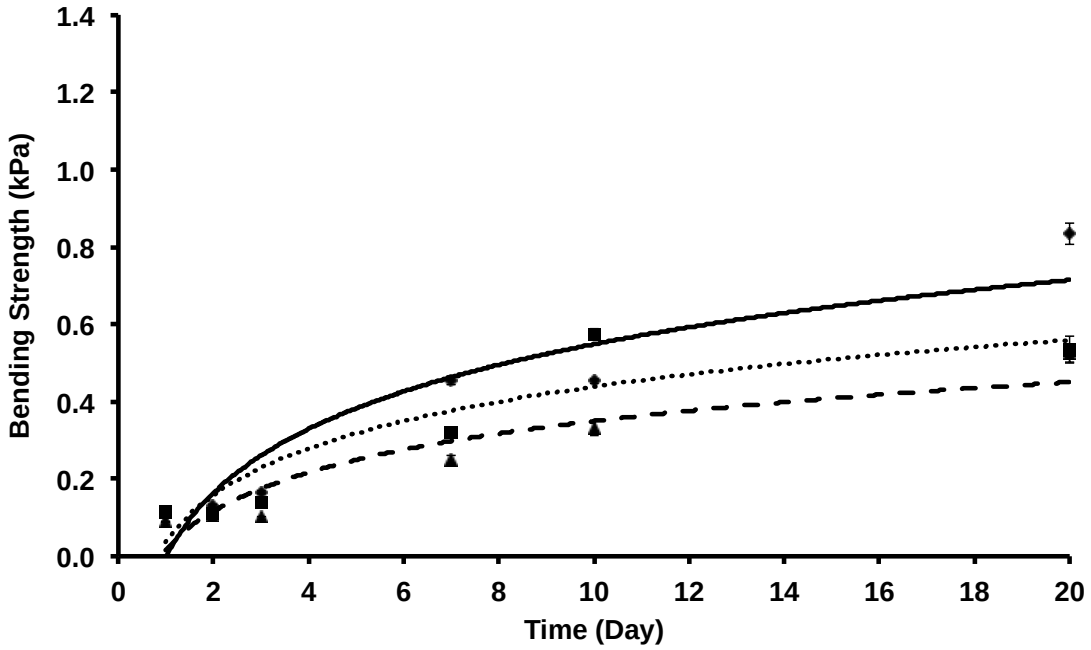
The occurrence of polymorphic changes during storage in dosage forms, mainly made of lipids have been correlated with changes on their mechanical properties over time (e.g. tensile strength) (103).

Figure 3.6 presents the changes of the mechanical properties of extrudates A over time of storage (for simplification data from extrudates C and D is not shown, as it presented similar patterns). Extrudates manufactured at different extrusion rates (100, 300 or 500 mm/min) presented similar changes and all observations were equivalent for all formulations regarding the modifications of the mechanical properties over time of storage. The overall pattern of the different extrudates can be explained by the stabilization over time of storage. The similarity between formulations suggests that different combinations of lipids ended up on the same final behavior. Variations due to extrusion rate suggest that the lowest rate (100 min/mm) allowed the materials to acquire a higher stability in spite of higher shear stresses, as discussed previously.

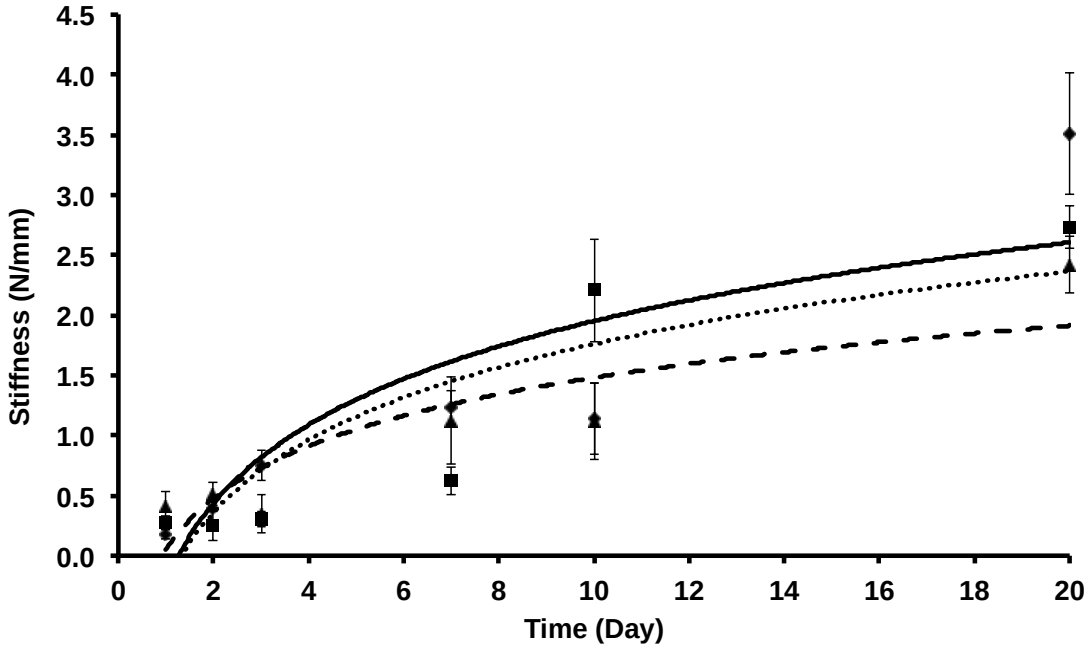
Extrudates A and C presented wider changes of the bending strength over time when compared with laminar extrudates D, reflecting their lower ability to resist to the deformation produced from an applied load. However, extrudates D showed a higher variability for the bending strength in the first seven days after extrusion before reaching a plateau. Extrudates A and C presented an increasing bending strength, which did not stabilize with time, as observed for laminar extrudates D.

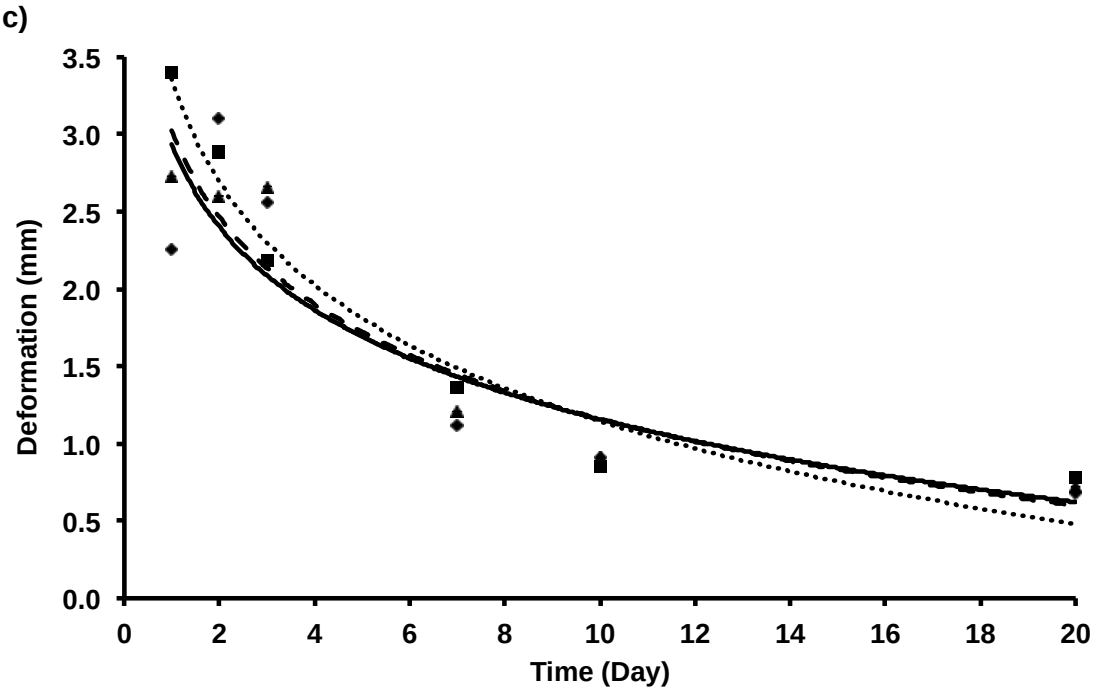
Extrudates A and C presented a wider increase of stiffness over time, reflecting their increased resistance to deformation in response to an applied force (rigidity), whereas laminar extrudates D presented a narrow and small decrease of stiffness, reaching a stable status.

a)


Bending Strength (kPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (Day)

b)


Stiffness (N/mm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (Day)



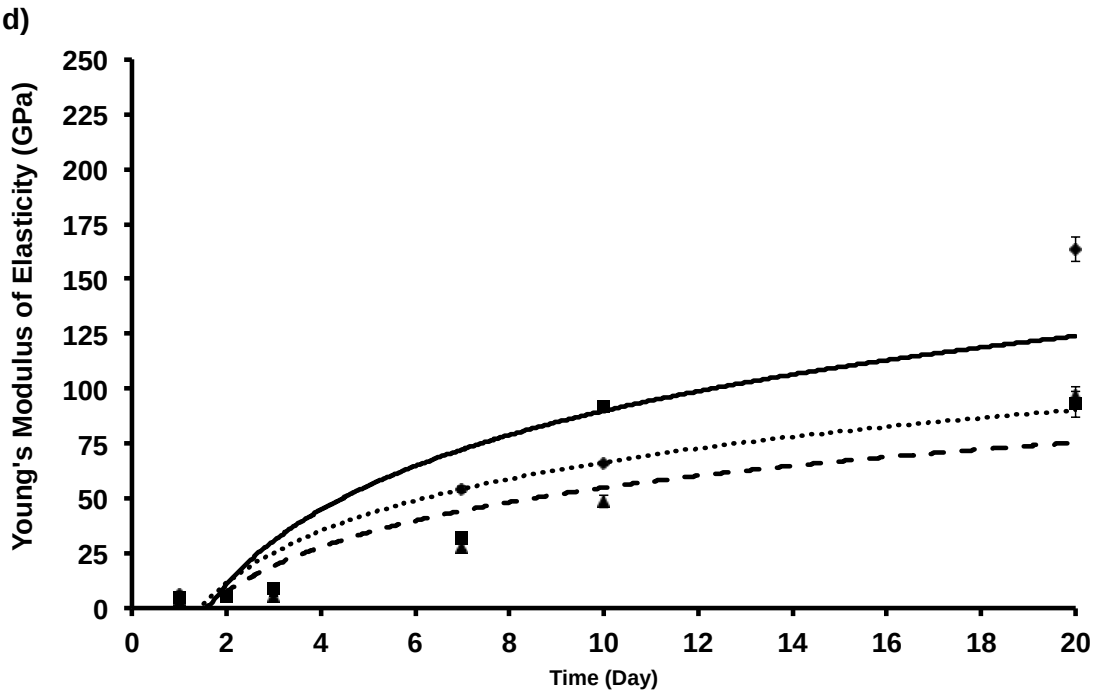




***Figure 3.6: Bending strength (a), stiffness (b), deformation (c) and Young's modulus of elasticity (d) of extrudates A, obtained at different extrusion rates: 100 (—♦—), 300 (· ·■· ·) and 500 (– ▲ -) mm/min.***

When a force was applied in their middle section, extrudates A presented a strong decrease of deformation over time, in comparison with extrudates C, which had a mild decrease, and with extrudates D that maintained stable deformation behavior during storage.

While the elasticity kept decreasing after manufacture for extrudates A and C (increasing Young's modulus of elasticity), laminar extrudates D presented a mild increase of elasticity over time, reaching a maximum after 10 days of storage.

Altogether, the mechanical tests considered, different on the properties studied, reflected a coherent behavior of the extrudates regarding their structures. Hamdani *et al.* concluded that, in general, the chemical and physical complexity of the lipid based materials leads to a complex and difficult to predict behavior of the products manufactured with these materials (e.g. physical modifications, polymorphism, crystallization or melting) (68).

Khan and Craig stated that triglycerides could present a partially amorphous layered structure, which gradually crystallizes during storage (aging) (104). This process of crystallization occurred with a rate dependency on the number of carbon atoms in the fatty acid chains: the longer the fatty acid chain, the longer would be the crystallization time. Therefore, the composition of extrudates A and C, including 60-66 % of glyceryl trimyristate and glyceryl palmitostearate (two glycerides with fatty acids with similar and medium carbon chain lengths – $C_{14}$ and $C_{18}$), can explain the increase on the bending strength and stiffness and decrease on the elasticity. These two materials could form stronger molecular interactions between their molecules, leading these extrudates to build up more stable structures when compared with laminar extrudates D. Extrudates D, made of 66 % of hard fat and glyceryl dibehenate (two lipids with significant differences between the chain lengths of their fatty acids – $C_{10}$ to $C_{18}$ and $C_{22}$, respectively), might explain the instability observed in the first days after extrusion and the lower bending strength and stiffness

and the mild increase of the elasticity, resulting in a weaker structure. The comparison of either extrudates C or D, with extrudates A, which presented the highest fraction of polyethylene glycol glycerides in the formulation (mixture of saturated fatty acids with the highest variability of chain lengths, $C_{10}$ to $C_{18}$) can explain the wider changes verified in the mechanical properties of the former extrudates, as polyethylene glycol glycerides are less resistant to the application of stress, undertaking wider mechanical changes. For instance, two days after extrusion, extrudates A manufactured at 300 mm/min deformed by 2.6 ± 0.0 mm, whereas extrudates C and D only deformed by 0.8 ± 0.0 and 1.3 ± 0.3 mm, respectively.

All these results support the hypothesis that changes observed on the mechanical properties of the extrudates were closely related to the type of lipids in the formulations. The lipid materials progressively changed their molecular interactions in order to achieve a more stable state and these physical changes of the raw materials were reflected on the stability of the mechanical properties of the laminar extrudates. Thus, the increase on the bending strength and stiffness were consistent with these changes and aging phenomena, as the materials tend to reorganize after the high stress applied over extrusion. Furthermore, the stabilization of the extrudates structure after extrusion led to a progressively lower mobility of the molecules, which justifies the lower elasticity of the laminar extrudates and, consequently, the lower ability of the extrudates to deform. The lipids had different behaviors after extrusion, due to their molecular composition and ability to cope with the stresses applied in the process of extrusion. Different mixtures of lipid excipients and, particularly, different lengths of the carbon chains of the saturated fatty acids, resulted in different molecular interactions in the laminar extrudates structure and, consequently, in different mechanical properties. Consequently, extrudates needed

to have their properties stabilized over a defined time of storage to allow a reproducible performance, particularly on drug release.

The process of manufacture had also an impact on the structure of the extrudates and influenced their properties. As an example, the friction of the extruding mass on the walls of the extrudate and die can influence the polymorphic behavior of the lipid materials during processing and then storage. Due to this friction, a thin molten layer was likely to be present on the surface of the extrudates, which started to re-solidify when the extrudates have exited the die (66).

### 3.4.7. Release of coumarin from extrudates

The raw materials included in the formulations can greatly influence the release patterns of a drug from the extrudates and, consequently, it was expected that various mechanisms were involved in the release of the drug, namely drug dissolution and diffusion, swelling and erosion of the matrix, or the occurrence of a combination of two or more of these processes (51). Lipid materials can act as matrix formers, influencing and controlling the release of the drug (105). Thereby, broad ranges of release patterns could be obtained by varying the composition of the lipid matrix (95).

As hydrophobic materials were present in the extrudates manufactured in the present study, a slow release rate of the drug might have been expected, as described in other studies (106). The dissolution of the drug and/or lactose at the surface of the extrudates might have increased the porosity of extrudates, from which the drug was slowly released as observed previously for glyceride bases (107). No evidence was found for swelling or erosion of the extrudates, as the extrudates remained intact and did not disintegrate throughout the entire time of the dissolution test.

In this study, extrudates including different percentages of particles of coumarin with different size ranges were manufactured (formulations E and F). None of these extrudates released the entire quantity of coumarin as both formulations (E and F) released only up to 75% of coumarin in 8 h (Figure 3.7).

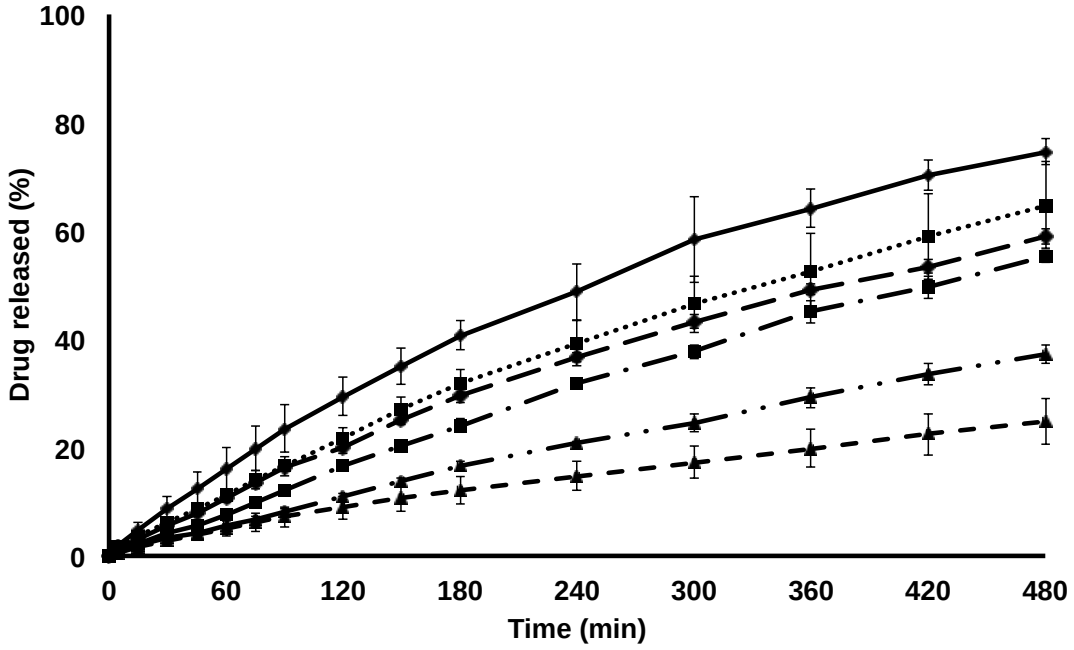



***Figure 3.7: Drug release profiles of laminar extrudates that included coumarin particles with sizes less than 90 µm (5%: ▬▬; 10%: ▬ ▬), between 250-355 µm (5%: ·····; 10%: ▬·▬) or between 710-1000 µm (5%: ▪ ▪ ▪; 10%: ▬··▪).***

It was observed that a decrease in the median size of the coumarin particles determined an increase in the release rate of coumarin, both for laminar extrudates including 5 and 10 % of coumarin (Table 3.6), as smaller particles have a higher surface area, accelerating the dissolution of coumarin into the dissolution medium. Additionally, an increase in the percentage of coumarin from 5% to 10%

also increased drug release rates, when comparing extrudates including coumarin particles with the same size range. Although potentially controversial, these results strongly suggest that differences in the median sizes of drug particles led to differences in the release rate of drugs from laminar extrudates composed mainly by lipid-based excipients. As these lipid-based formulations form a solid matrix, the particle size of the drug played an important role the release rate by affecting the structure of the extrudates, as previously described previously for other dosage forms (108).

***Table 3.6: Dissolution efficiency, release rates of coumarin from extrudates seven days after manufacture and coefficients of determination ($R^2$) and release exponents (n) obtained from Korsmeyer-Peppas dissolution model.***

| Fraction of coumarin (%) | Coumarin Particle Size (µm) | Dissolution efficiency (%) [a] | Release rate ($mg.h^{-1}$) [a] | | Korsmeyer-Peppas | |
|---|---|---|---|---|---|---|
| | | | 0 – 3 h | 4 – 8 h | $R^2$ | *n* |
| **5** | 0 – 90 | 45.7 ± 3.7 $^{m}$ | 8.6 ± 1.0 $^{r,u}$ | 3.8 ± 0.8 $^{x}$ | 0.995 ± 0.005 | 0.75 ± 0.05 |
| | 250 – 355 | 36.9 ± 4.2 $^{n}$ | 6.5 ± 0.6 $^{r}$ | 3.8 ± 0.8 $^{x}$ | 0.997 ± 0.002 | 0.79 ± 0.06 |
| | 710 – 1000 | 14.2 ± 2.4 $^{p}$ | 2.6 ± 0.6 $^{s}$ | 1.5 ± 0.4 $^{y}$ | 0.999 ± 0.001 | 0.75 ± 0.17 |
| **10** | 0 – 90 | 34.1 ± 0.9 $^{n}$ | 11.7 ± 0.5 $^{t}$ | 6.3 ± 0.3 $^{z}$ | 0.997 ± 0.001 | 0.75 ± 0.01 |
| | 250 – 355 | 30.0 ± 1.1 $^{n}$ | 9.3 ± 0.5 $^{t}$ | 6.7 ± 0.7 $^{z}$ | 0.997 ± 0.001 | 0.87 ± 0.03 |
| | 710 – 1000 | 20.0 ± 0.9 $^{p}$ | 6.4 ± 0.5 $^{u}$ | 4.9 ± 0.8 $^{x,z}$ | 0.999 ± 0.000 | 0.87 ± 0.05 |

*[a] For each column, the means in the dissolution efficiency and release rates with different letters in the indices are significantly different (ANOVA; $p < 0.01$; Tukey's HSD; $n = 3$).*

It was also verified that the release rate of coumarin from the extrudates decreased with time. For all formulations, the release rate in the first 3 hours of the dissolution was always higher than the release rate observed between 4 and 8 hours of the dissolution test (Table 3.6).

To better describe the mechanisms involved in the release of coumarin from these extrudates, several mathematical models could have been applied (98).

In this study, the zero order and the Higuchi models presented unsatisfactory $R^2$ values, whereas the Korsmeyer-Peppas model presented $R^2$ values close to 1 (Table 3.6) and was accepted as a relevant descriptor of the release of the drug. As the *n* parameter of the Korsmeyer-Peppas model had values between 0.57-0.93 for all laminar extrudates, coumarin must have been released by diffusion and, concomitantly, by structural alterations of the extrudates such as relaxation of the matrix, also described as an anomalous transport / non-Fickian model (105). In general, the Korsmeyer-Peppas model was the one that better fitted the release profiles obtained. However, some laminar extrudates, especially those including 10% of coumarin, also presented high $R^2$ values for the zero order model, suggesting that coumarin is released almost without affecting the dimensions and/or structure of the extrudates.

## 3.5. Conclusions

The manufacture of extrudates with laminar shape at room temperature in the absence of solvents is a promising technology to manufacture new dosage forms for delivery of drugs. Our study revealed that the raw materials, mainly the lipid-based excipients, and consequently the extrudates, undertook an aging process materialized in changes in the mechanical properties, density and thermal behavior, similar to the aging phenomena previously observed for glycerides

and triglycerides. Different formulations with different components or proportions gave laminar extrudates with different mechanical properties. The bending strength, stiffness, deformation, elasticity and density of the manufactured laminar extrudates changed over time depending on the composition and on the extrusion conditions.

The characteristics of the materials, especially the variety of glycerides and fatty acids that compose the lipid-based excipients, had an important influence in the mechanical properties of the laminar extrudates. These properties need to be stabilized over time, so that the expected performance of the dosage form is not altered.

Laminar extrudates including different percentages of drug and with drug particles with different size ranges presented distinct release profiles. Small coumarin particles revealed higher release rates from laminar extrudates when comparing with larger drug particles. Laminar extrudates composed mainly by lipid-based excipients had a retardation effect in the release of the drug. Therefore, the drug release rates can be modified just by changing the composition of the formulations and/or by changing the size of the drug particles that are included in the laminar extrudates.

# CHAPTER 4

## Delivery of drugs from laminar co-extrudates manufactured by a solvent-free process at room temperature

***Adapted from:***

*Delivery of Drugs from Laminar Co-Extrudates Manufactured by a Solvent-Free Process at Room Temperature, G. Oliveira, M. A. Wahl, J. F. Pinto, Journal of Pharmaceutical Sciences (2014) 103(11):3501-10.*

## 4.1. Introduction

Co-extrusion involves the separate preparation of two or more mixtures of excipients with drug(s), which are conveyed separately into a die where they come together as a co-extrudate with a pre-defined number of layers and shape (e.g. laminar, tubular, rod or cylindrical) (27), depending on the extruder features, particularly on the extrusion die (109, 110). In the last decade the technology of co-extrusion has been applied in several areas namely in the ceramics, food, metallurgy and polymer industries and, also, in the manufacture of medical devices and pharmaceutical products (111).

In drug delivery, multi-layered extrudates can be used on modulating the release of drugs either by producing a multi-modal release (60) or by allowing a higher fine-tuning ability on controlling the release of a drug (112) by imposing different release kinetics to each layer, depending on the composition of individual layers (113). Furthermore, co-extrusion can be considered for the combined delivery of drugs in the same dosage form (even if incompatible), which can be present in different layers (27). Traditionally, extrusion and co-extrusion of materials is accomplished by either using a solvent [e.g. wet masses (114)] or in the absence of solvents [e.g. hot (27) or cold melt extrusion (110)].

Lipid-based materials such as fatty acids, mixtures of fatty acids with polyethylene glycols, triglycerides and glyceride bases (e.g. glyceryl palmitostearate, glyceryl trimyristate or glyceryl dibehenate), have been proven as suitable excipients for extrusion at room temperature (42, 61, 67, 93), which can be considered a green technology. However, the use of these materials represents an important challenge to the formulator, as their composition, particularly their chemical and physical variability and complexity, can greatly influence the properties of the extrudates due to their ability to crystallize or suffer polymorphic changes (68). Therefore, a deep knowledge of the properties of these lipid-based starting materials is essential for

better predicting and understanding the properties of the extrudates manufactured and their stability (i.e. aging over storage (68)). For instance, a higher fraction of lipid-based materials with a heterogeneous composition can lead to more relevant changes on the physical stability of the extrudates over time with a negative impact on their performance, particularly on the release rate of the drug (68).

## 4.2. Aim of study

This study aimed at the manufacture of laminar extrudates and co-extrudates (with one, two or three layers) in the absence of solvents and at room temperature with the potential for the delivery of a model drug by the oral or topical routes of administration, and to assess the stability of their properties over storage.

## 4.3. Materials and methods

### 4.3.1. Materials

Glyceryl dibehenate (Compritol$^{TM}$ 888 ATO, Gattefossé, France), hard fat (Witocan™ 42/44, Sasol, Germany), a mixture of polyethylene glycol with glycerides (Gelucire™ 33/01, Gattefossé, France), a mixture of polydimethylsiloxane with silicon dioxide (Simethicone™ Q7-2243 LVA, Dow Corning, Belgium) and lactose (SpheroLac$^{TM}$, Meggle Pharma, Germany) were used as starting materials for the manufacture of the extrudates and co-extrudates. Coumarin particles (0-90 µm) milled from coarse coumarin (Sigma-Aldrich, Germany) in an analytical grinder (A10 Yellow Line, IKA, Germany) were sieved (Vibratory Sieve Shaker, AS 200 digit, Retsch GmbH, Germany) before consideration as model drug.

### 4.3.2. Methods

#### 4.3.2.1. Manufacture of extrudates and co-extrudates

For each extrudate or co-extrudate layer, the materials (Table 4.1) were mixed in a planetary mixer (Kenwood, Hampshire, UK) for 15 min and placed in the respective extrusion chamber, according to the type and position of the layers of each sample (Table 4.2). The manufacture of laminar extrudates and co-extrudates was carried out using a ram extruder (Lurga, Portugal) with a fixed design of chambers and dies (Figure 4.1), at defined extrusion rates, which correspond to specific mass flow rates (Table 4.2). The ram extruder was fixed to a universal testing machine (LR 50K, Lloyds Instruments, UK) fit with a load cell allowing recording of the force applied and displacement of the ram. The extrudates and co-extrudates obtained were cut into squares (40x40mm) immediately after exiting the extrusion die and stored in a desiccator (23 °C / 30 % RH) prior to characterization.

***Table 4.1: Density of raw materials and composition of the individual laminar extrudate layer.***

| Materials | Density (g/cm$^3$) | Layer (%) | |
|---|---|---|---|
| | | A | B |
| **Polyethylene glycol glyceride** | 0.944 ± 0.001 | 4 | 4 |
| **Hard fat** | 0.999 ± 0.001 | 33 | 33 |
| **Glyceryl dibehenate** | 1.021 ± 0.000 | 33 | 33 |
| **Polydimethylsiloxane – silicon dioxide mixture** | 0.956 ± 0.000 | 20 | 20 |
| **Lactose** | 1.543 ± 0.001 | - | 10 |
| **Coumarin** | 1.380 ± 0.001 | 10 | - |

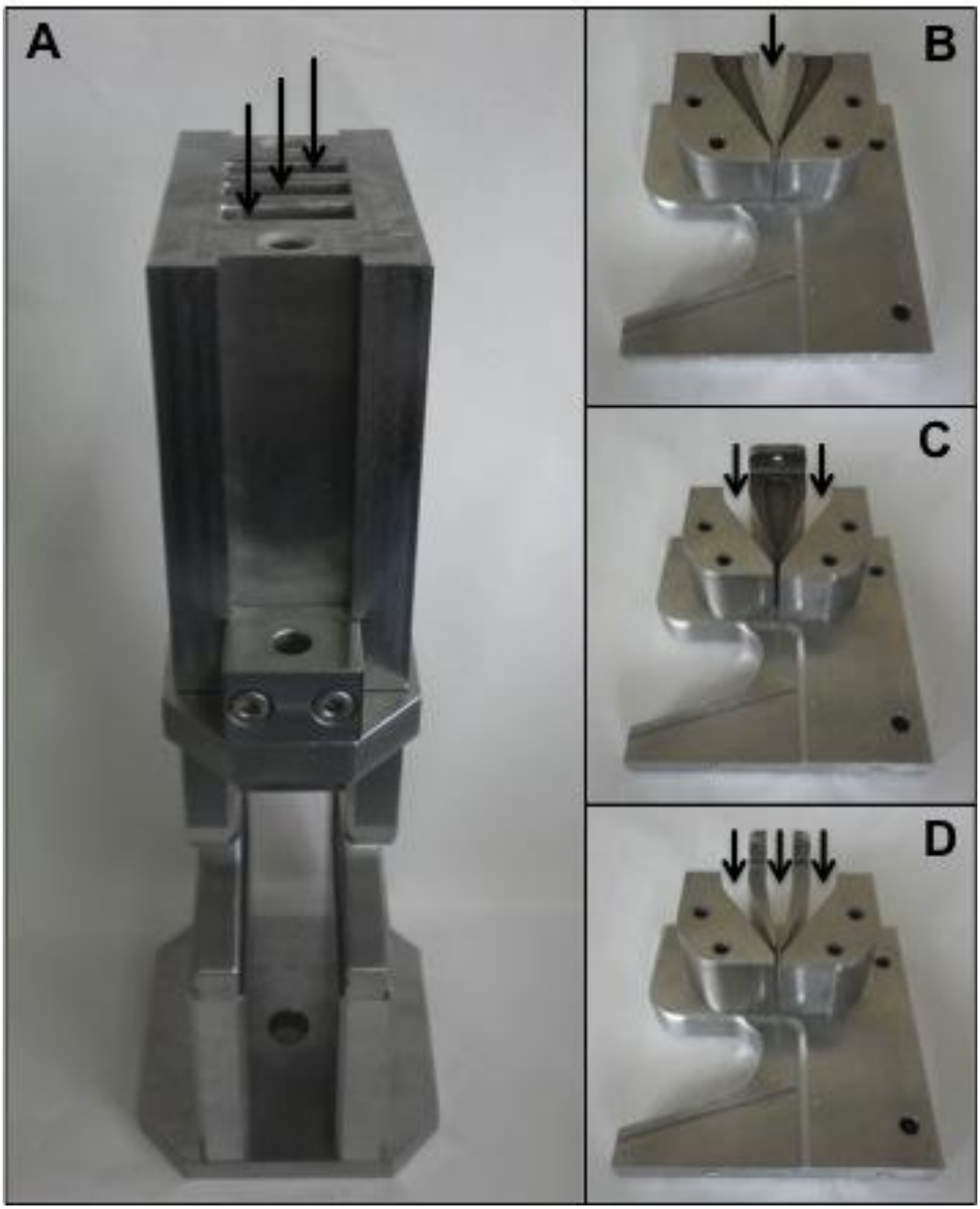



***Figure 4.1: Extruder (a) and extrusion dies used in the manufacture of extrudates with one layer (b), two layers (c) and three layers (d).***

***Table 4.2: Composition of laminar extrudates and co-extrudates, extrusion rates and mass flow rates.***

| Extrudate | Number of layers | Type and order of layers | Extrusion rate (mm/min) | Mass flow rate (g/min) |
|---|---|---|---|---|
| **I** | 1 | A | 100 | 61 ± 3 |
| **II** | 1 | A | 300 | 174 ± 8 |
| **III** | 1 | A | 500 | 299 ± 7 |
| **IV** | 2 | A + B | 300 | 349 ± 12 |
| **V** | 3 | B + A + B | 300 | 448 ± 11 |

#### 4.3.2.2. Characterization of extrudates

##### 4.3.2.2.1. Extrusion profiles and force at steady state ($F_{SS}$)

For each extrudate and co-extrudate the extrusion force at steady state ($F_{ss}$) was calculated as the mean of the force values obtained between 100 and 130 mm of the displacement of the ram.

##### 4.3.2.2.2. Morphological evaluation

Extrudates and co-extrudates were visually inspected to detect any surface defect due to poor process control or formulation inadequacy and observed (surface and cross section) by scanning electron microscopy (SEM) using a DSM 940 scanning electron microscope (Carl Zeiss, Germany). Previously, the samples were coated with gold in a Sputter Coater (E 5100, Bio-Rad, Germany).

#### 4.3.2.2.3. Thickness of extrudates

The thickness of cut extrudates was measured using a caliper (n=10) at five predefined and distinct points to assess thickness uniformity.

#### 4.3.2.2.4. Density and porosity of extrudates

The densities of both starting materials and extrudates (at 1, 3, 8, 15, 30, 60 and 90 days after manufacture) were determined by helium pycnometry (AccuPyc 1330, Micromeritics, USA) at 23±2 °C (n=3). The expected densities of the extrudates, calculated from the densities of the starting materials weighted for their fractions in the formulations, allowed the calculation of the porosities of the extrudates (94).

#### 4.3.2.2.5. Mechanical characterization of extrudates

Extrudates and co-extrudates (n=3) were characterized for mechanical properties at 1, 3, 8, 15, 30, 60 and 90 days after production, by a flexure test: a three-point bend rig test (TA.XT Plus, Stable Microsystems, UK) at perpendicular direction of extrusion. Force was applied to extrudates and both force and displacement were registered to allow calculation of the bending strength, stiffness, deformation and Young's modulus of elasticity (97).

#### 4.3.2.2.6. Thermal behavior of extrudates

The extrudates were evaluated for potential changes due to heat by differential scanning calorimetry (DSC, Q200, TA Instruments, USA). Samples (3.0-6.0 mg, n=3) were placed in hermetic sealed aluminium pans and tested at 10 °C/min (20-180 °C). The enthalpies of fusion were found for each sample analyzed (1, 8, 30, 60 and 90 days after manufacture) and for all starting materials.

#### 4.3.2.2.7. Release of coumarin from extrudates

The release of coumarin from extrudates was assessed by a dissolution test 30 days after production (Eur. Pharm., n=3, paddle method, 50 rpm, phosphate buffer, pH=6.8, AT7 Sotax, Switzerland) and samples quantified by UV spectroscopy at 307 nm (U-2000 Spectrophotometer, Hitachi, Japan). The efficiency of dissolution of coumarin was determined for all extrudates for characterizing the complete release of the drug (98). To provide a deeper understanding of the release of coumarin, the *Korsmeyer-Peppas* mathematical model was considered and applied separately to two periods of release (0-360 and 360-1440 min), as 360 min was the time point that better emphasized the differences on the drug release at the beginning and at the end of the process of dissolution.

All statistical analysis was performed based on a one-way analysis of variance (ANOVA) with a 99% level of confidence and Tukey's HSD test. Statistical significance was considered for $p < 0.01$.

## 4.4. Results

### 4.4.1. Extrusion profiles and force at steady state ($F_{SS}$)

The extrusion profiles registered when manufacturing all extrudates and co-extrudates presented the main features associated with a typical extrusion profile, namely the compression, the steady state flow and the forced flow phases (57). Formulations A and B (monolayers) also presented these features. Single layer extrudates (I to III) were easier to obtain than the multilayer ones (IV and V), and the higher the extrusion rate, the higher the $F_{SS}$ (Table 4.3). Looking at the $F_{SS}$ values one could observe the smoothness of the curves reflecting the ease of materials on extruding.

### 4.4.2. Morphological evaluation of extrudates

*Visual inspection:* All extrudates and co-extrudates were approved as no defects or surface irregularities were detected by visual inspection. Figure 4.2.A exemplifies an extrudate (formulation IV, A+B) with surface, length and width similar to the others manufactured. Additionally, it was not possible to distinguish any discontinuity line or irregularity between the different layers of extrudates IV and V (A+B, B+A+B, respectively).

*Scanning electron microscopy:* Scanning electron microscopy (SEM) has shown that all extrudates presented smooth surfaces (only minor surface defects were detected at low magnifications), confirming the visual observations and the good quality of extrudates (Figure 4.2).

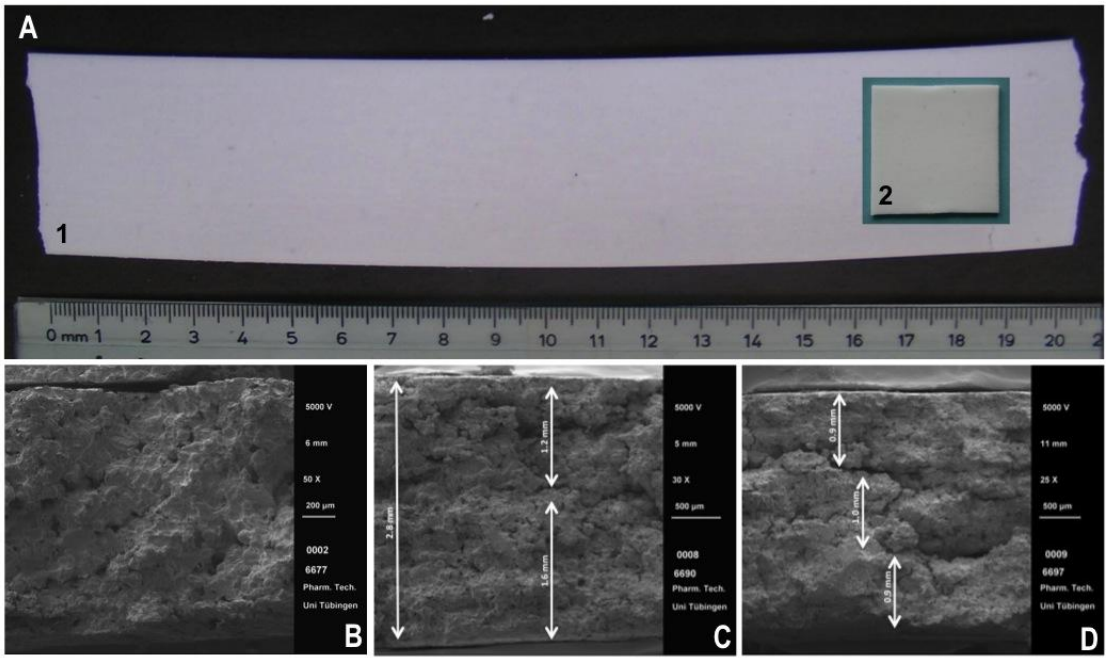



***Figure 4.2: Co-extrudate type A+B (a) after exiting the die (1) and after cut in a 40x40x1.5 mm sample (2, insert not to scale), and SEM photos of sections of (B) single extrudate (extrudate II, A), (C) co-extrudate with two layers (extrudate IV, A+B) and (D) co-extrudate with three layers (extrudate V, B+A+B).***

***Table 4.3: Extrusion force at steady state ($F_{ss}$), thickness and enthalpies of fusion of extrudates, dissolution efficiency (after 960 min of release) and 'n' parameter and coefficients of determination ($R^2$) obtained from the Korsmeyer-Peppas model.***

| | | Extrudates I | Extrudates II | Extrudates III | Co-extrudates IV | Co-extrudates V |
|---|---|---|---|---|---|---|
| **$F_{ss}$ (N) [a,b]** | | 2293 ± 37 [s] | 3876 ± 126 [r] | 4302 ± 116 [r] | 9987 ± 287 [t] | 21739 ± 419 [u] |
| **Thickness (mm) [c]** | | 1.51 ± 0.03 | 1.52 ± 0.03 | 1.53 ± 0.04 | 2.93 ± 0.03 | 2.94 ± 0.03 |
| **Enthalpies of fusion (J/g)** | **Day 1** | 160.9 | 131.9 | 128.0 | 116.8 | 142.4 |
| | **Day 8** | 154.3 | 152.1 | 149.8 | 148.1 | 133.3 |
| | **Day 30** | 150.3 | 130.6 | 149.2 | 153.8 | 142.6 |
| | **Day 60** | 146.4 | 139.4 | 142.4 | 148.9 | 139.3 |
| | **Day 90** | 146.3 | 158.8 | 147.4 | 137.1 | 132.2 |
| **Dissolution efficiency (%) [d]** | **Day 1** | 41.3±1.0 [r,1] | 42.0±0.8 [r,1] | 41.0±1.6 [r,1] | 24.2±0.6 [s,1] | 14.9±0.2 [t,1] |
| | **Day 3** | 40.9±0.4 [r,1] | 41.9±1.1 [r,1] | 41.2±0.4 [r,1] | 24.6±2.2 [s,1] | 14.8±0.2 [t,1] |
| | **Day 8** | 39.6±0.9 [r,s,1] | 42.0±0.4 [r,1] | 38.4±0.5 [s,1,2] | 24.7±1.2 [t,1] | 14.3±0.3 [d,1,2] |
| | **Day 15** | 40.1±0.4 [r,1] | 39.9±0.2 [r,1,2] | 38.6±0.4 [r,1,2] | 22.9±0.9 [s,1] | 12.9±0.4 [t,3] |
| | **Day 30** | 39.5±1.0 [s,1] | 36.5±0.2 [r,3] | 38.6±0.6 [r,s,1,2] | 23.9±0.7 [t,1] | 14.0±0.2 [u,2] |
| | **Day 60** | 40.0±0.6 [s,1] | 36.5±1.2 [r,3] | 36.4±1.5 [r,2] | 22.4±0.8 [t,1] | 12.5±0.1 [u,3] |
| | **Day 90** | 36.8±0.4 [r,b,2] | 38.3±0.6 [r,2,3] | 35.5±0.5 [s,2] | 23.0±0.2 [t,1] | 12.3±0.2 [u,3] |
| ***n*** **(Korsmeyer-Peppas)** | **0-360 min** | 0.65 ± 0.01 | 0.66 ± 0.01 | 0.67 ± 0.01 | 0.63 ± 0.01 | 0.99 ± 0.01 |
| | **360-1440 min** | 0.67 ± 0.02 | 0.66 ± 0.01 | 0.66 ± 0.01 | 0.56 ± 0.01 | 0.83 ± 0.01 |
| **$R^2$ (Korsmeyer-Peppas)** | **0-360 min** | 0.999 ± 0.001 | 0.998 ± 0.001 | 0.999 ± 0.000 | 0.998 ± 0.000 | 0.999 ± 0.000 |
| | **360-1440 min** | 0.999 ± 0.000 | 0.999 ± 0.000 | 0.999 ± 0.000 | 0.998 ± 0.001 | 0.998 ± 0.000 |

[a] *n=3*
[b] *The means of $F_{SS}$ with different letters in the indices are significantly different (ANOVA; $p < 0.01$; Tukey's HSD; n = 3).*
[c] *n=10*
[d] *The means of dissolution efficiency with different letters in the indices (for each row) and the means of dissolution efficiency with different numbers in the indices (for each column) are significantly different (ANOVA; $p < 0.01$; Tukey's HSD; n = 3).*

The analysis of the SEM images showed that the identity of individual particles was not entirely lost, with some pores within the extrudates, suggesting that the melting of the particles did not happen. Also, the different layers of co-extrudates IV and V could be seen in Figure 4.2 (particularly 4.2.C and 4.2.D), which depicts a cross sectional cut of mono-, bi- and trilayer extrudates.

### 4.4.3. Physical characterization of extrudates

#### 4.4.3.1. Thickness

The thicknesses of all mono-, bi- and trilayer extrudates have shown an increase of about 50% over the expected values (Table 4.3). Monolayer extrudates used the central chamber of the extruder (1 mm thick), whereas bi- and trilayer extrudates used other chambers (2 mm thick). The thicknesses were constant over time.

#### 4.4.3.2. Density and porosity

All extrudates presented densities slightly lower than expected, which decreased over time, whereas the porosities increased over storage, with the exception of co-extrudate IV that presented a stable porosity from day 30 onwards (Figures 4.3 and 4.4).

Co-extrudates V (three layers) have shown the highest density, whereas monolayer extrudates I and III (manufactured at 100 and 500 mm/min, respectively) were the less dense. The porosities presented reciprocal changes to densities. Furthermore, the density and porosity were not expected to be affected by eventual dissolution of one component in another because no volume reduction was observed, once all components of the formulation were mixed together.

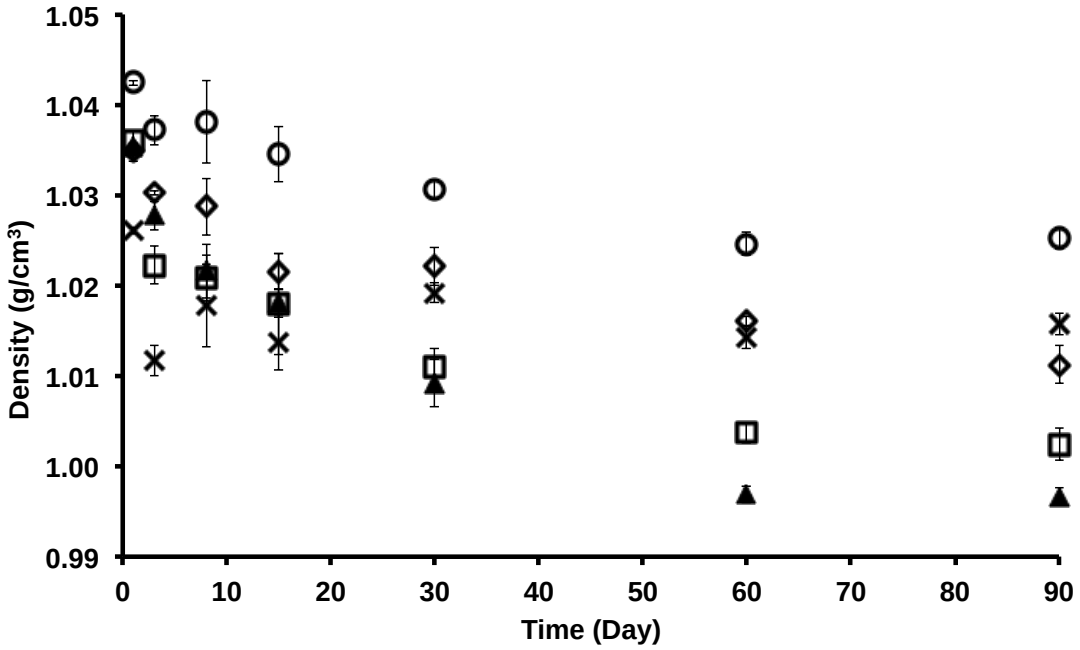



***Figure 4.3: Density of the extrudates over time from formulations I (□), II (◇), III (△), IV (X) and V (○).***

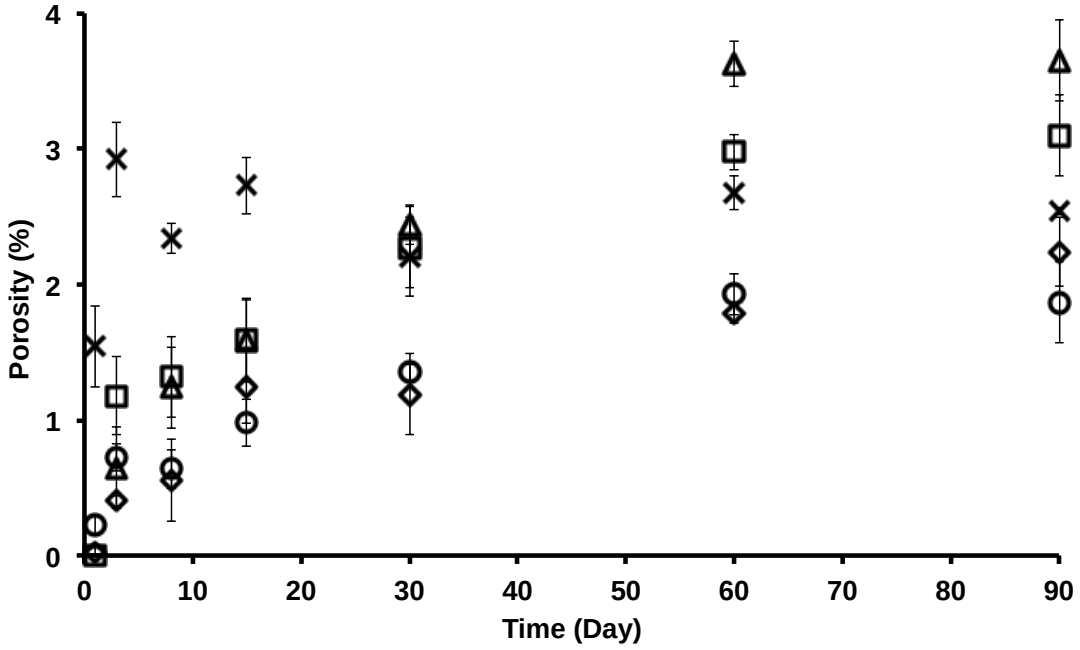



***Figure 4.4: Porosity of the extrudates over time from formulations I (□), II (◇), III (△), IV (X) and V (○).***

The changes observed were more evident in the first days after production, along with certain instability, but from day 30 onwards the variations on these properties were minimal, suggesting a stabilization of structures over time.

Note: expected densities of 1.034 g/cm$^3$ (extrudates from formulations I, II and III), 1.042 g/cm$^3$ (extrudates from formulation IV) and 1.045 g/cm$^3$ (extrudates from formulation V) were considered for the calculation of porosity.

#### 4.4.3.3. Mechanical characterization

The bending strength of extrudates and co-extrudates increased up to 4-fold from day 1 to day 90 of storage (Figure 4.5): extrudates I, 0.20±0.00 to 0.79±0.03 kPa; extrudates II, 0.22±0.01 to 0.94±0.02 kPa; extrudates III, 0.19±0.00 to 0.84±0.06 kPa; extrudates IV, 0.26±0.00 to 1.07±0.05 kPa; extrudates V, 0.21±0.01 to 0.93±0.03 kPa.

Similarly, extrudates I (1.41±0.20 o 0.69±0.06 mm) presented the highest change on their deformation ability during storage, whereas extrudates II (1.10±0.13 to 0.62±0.05 mm) and III (1.22±0.18 to 0.66±0.05 mm) presented an intermediate shift and co-extrudates IV (0.78±0.06 to 0.48±0.04 mm) and V (0.81±0.21 to 0.47±0.06 mm) only a slight change (Figure 4.6). The overall decrease to half values of deformation reflects a stabilization of the products. The presence of one or more layers was reflected by a decrease on deformation, although direct relationships were hard to identify. After day 30 of storage, the changes observed were residual.

There was a significant increase in the stiffness of co-extrudates IV and V (two and three layers) in the first 15 days after manufacture (2.16±0.13 to 7.70±0.90 N/mm and 1.77±0.50 to 7.75±0.71 N/mm, respectively), comparing to extrudates I, II and III (one layer), in which the stiffness suffered a slight increase in the same period of storage (0.24±0.05 to 1.10±0.33 N/mm, 0.33±0.05 to 1.32±0.16

N/mm and 0.25±0.04 to 1.58±0.13 N/mm, respectively) before stabilizing over time (Figure 4.7).

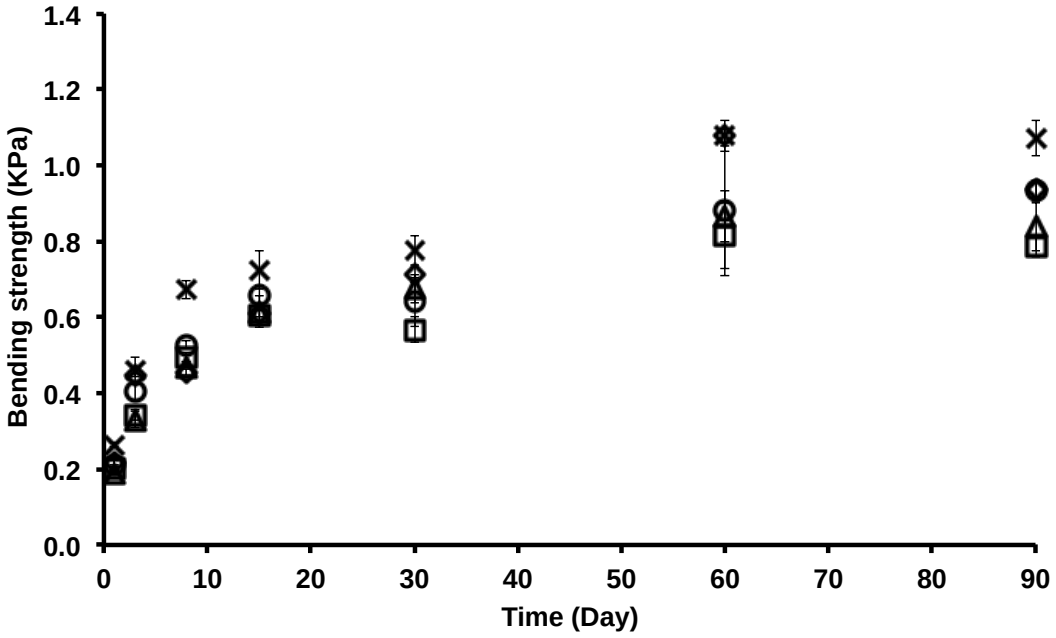



***Figure 4.5: Bending strength of the extrudates over time from formulations I (□), II (◇), III (△), IV (X) and V (○).***

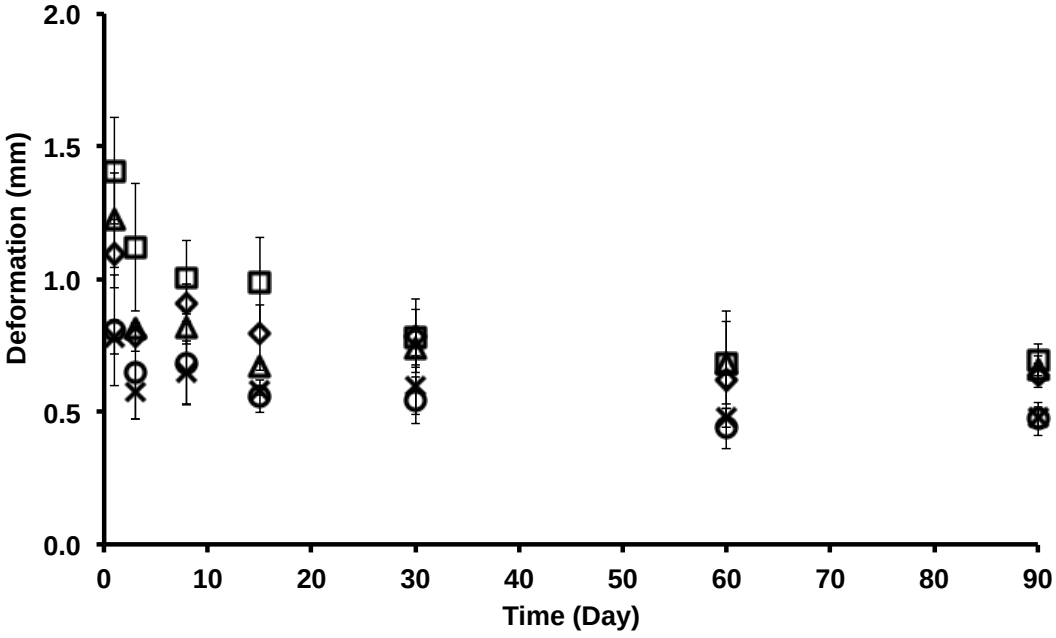



***Figure 4.6: Deformation of the extrudates over time from formulations I (□), II (◇), III (△), IV (X) and V (○).***

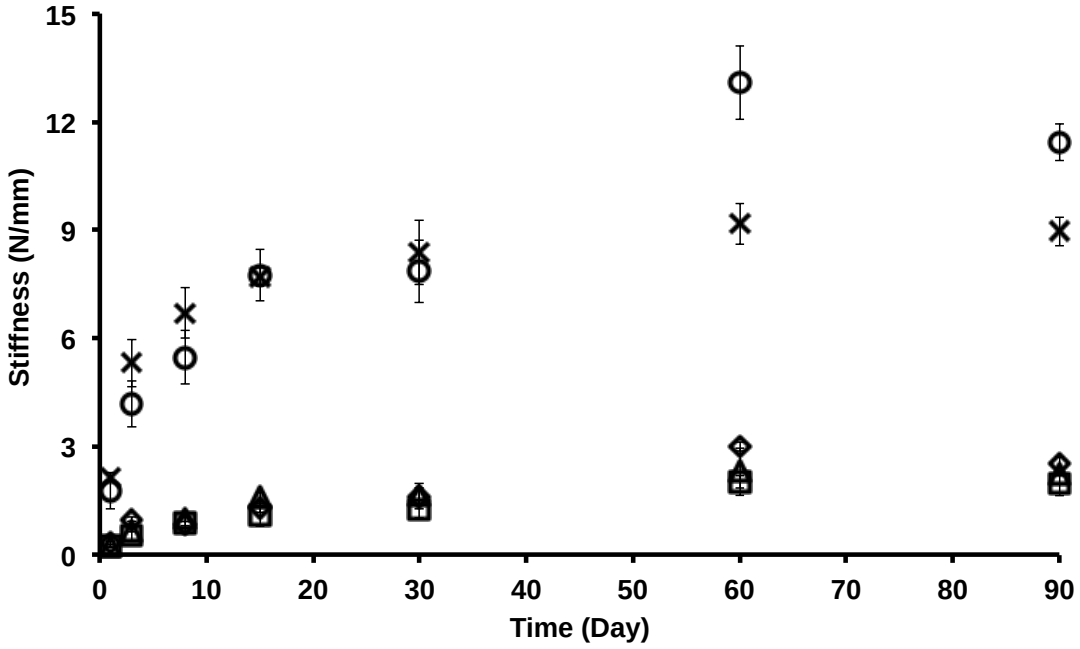



***Figure 4.7: Stiffness of the extrudates over time from formulations I (□), II (◇), III (△), IV (X) and V (○).***

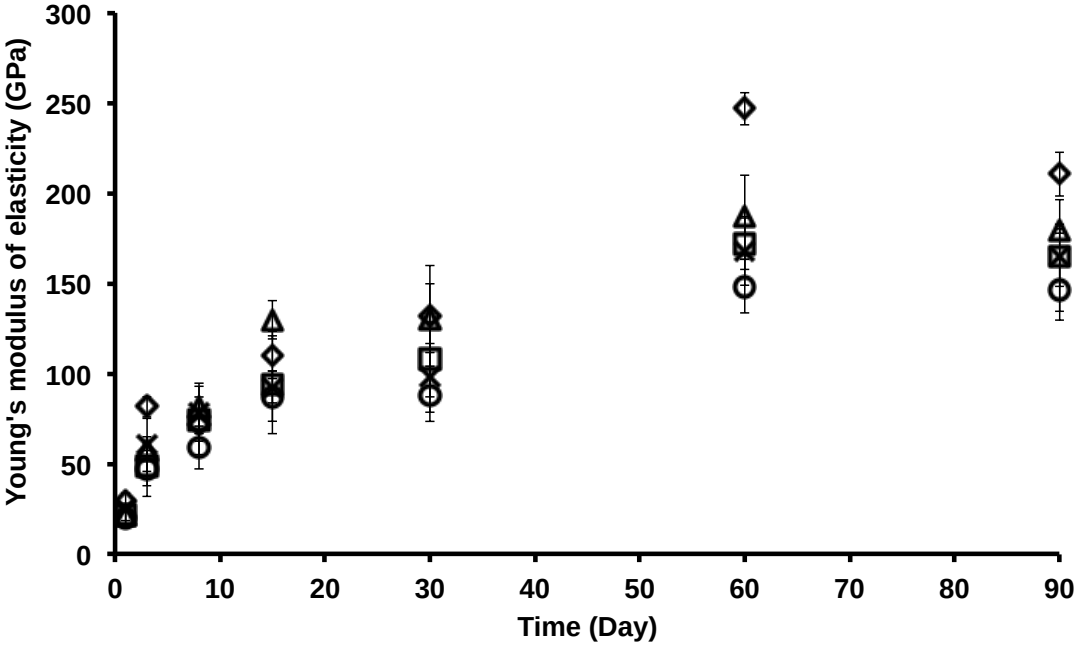



***Figure 4.8: Young's modulus of elasticity of the extrudates over time from formulations I (□), II (◇), III (△), IV (X) and V (○).***

All extrudates and co-extrudates lost their elasticity over time (the Young's modulus of elasticity increased). As expected, the highest shift on elasticity occurred in the first 15 days after manufacture (Figure 4.8). This loss of elasticity was higher for extrudates II (29.0±4.7 to 210.5±12.1 GPa). Extrudates I, III and IV presented an intermediate decrease of elasticity over storage (21.5±5.3 to 165±30.9 GPa, 22.3±3.6 to 179.0±5.2 GPa and 25.0±1.6 to 165.4±17.1 GPa, respectively), whereas extrudates V had the lowest shift (20.0±5.4 to 146.3±16.7 GPa).

To the author best knowledge, there are not comparative values for these properties to enable comparisons and the assessment of the quality of the extrudates.

These patterns of modification for all mechanical properties were more evident and intense in the first days of storage until day 15, after which stabilization seems to have occurred. These results are in a reasonable agreement with the densities and porosities, whereby stabilization started between days 15 and 30 of storage.

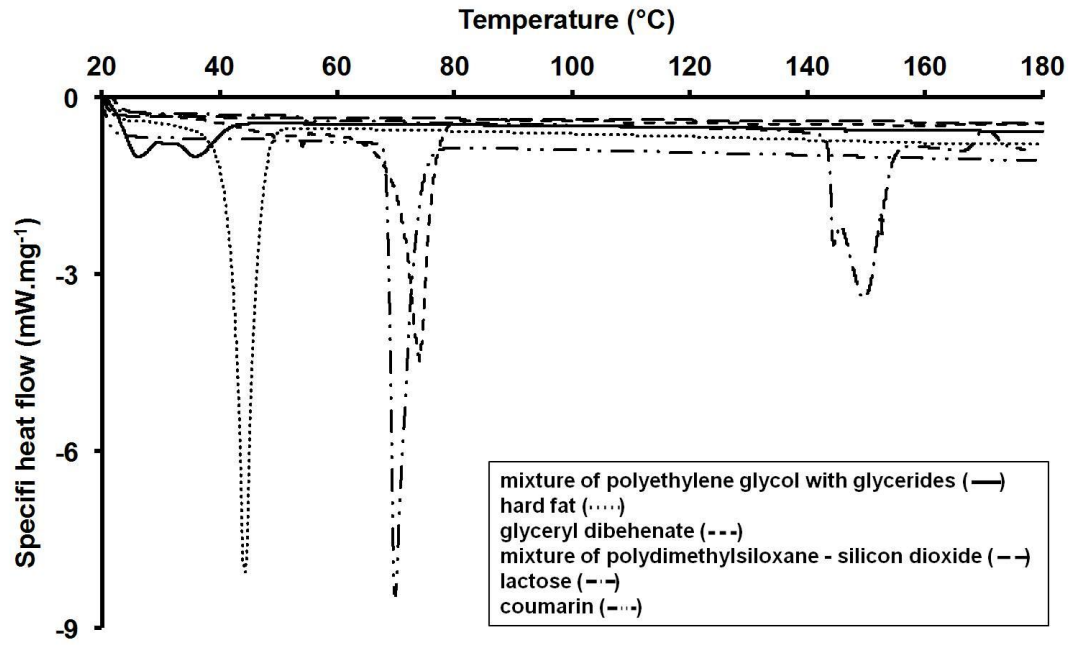
#### 4.4.3.4. Thermal behavior

Figure 4.9.A considers the thermal behavior of each starting material prior to manufacture for a better understanding of the thermal properties of the manufactured extrudates, as each material and its proportion in the formulation directly influences the thermal behavior of the final products.

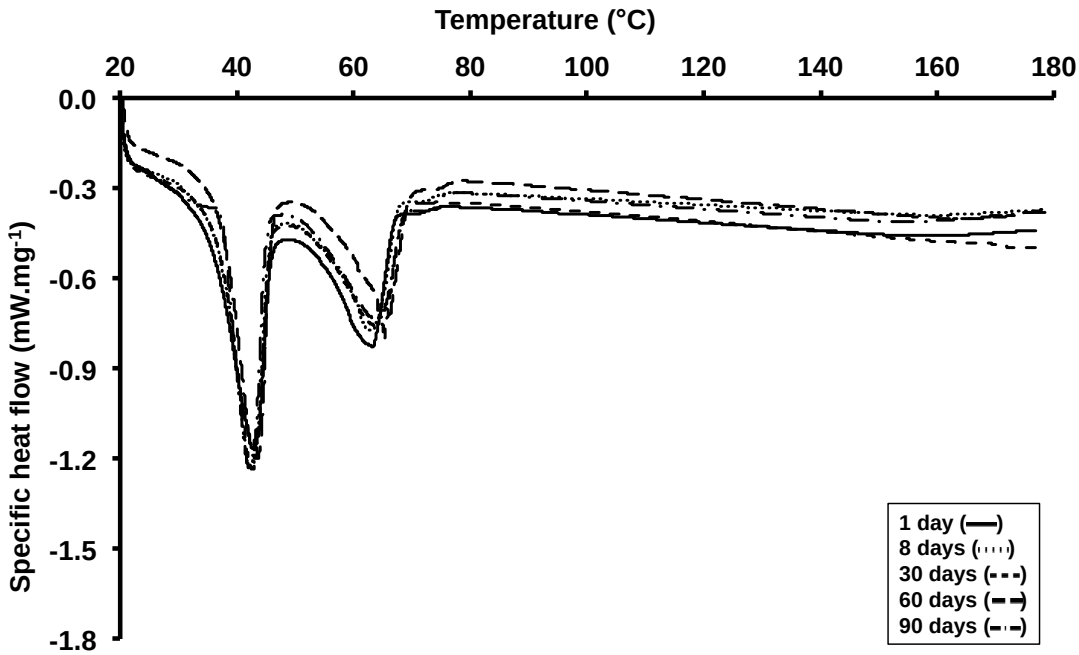
Extrudates and co-extrudates were analyzed over storage and presented modifications of thermal behavior (Figure 4.9.B-F), which were highlighted by different enthalpies of fusion over time (Table 4.3). It was neither possible to identify clear trends of increase / decrease of the enthalpies of fusion over time, nor directly correlate these results with other properties of the extrudates. However, it

was evident that changes of the mechanical properties, densities and porosities of the extrudates were concomitant with the changes observed in thermal behavior.

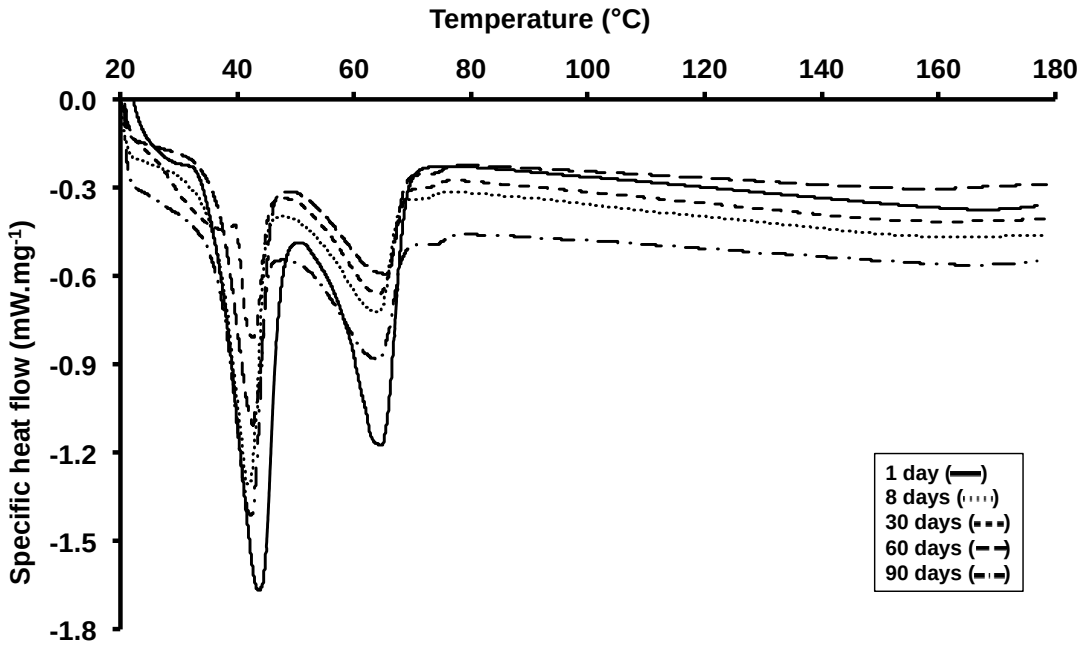
**a)**



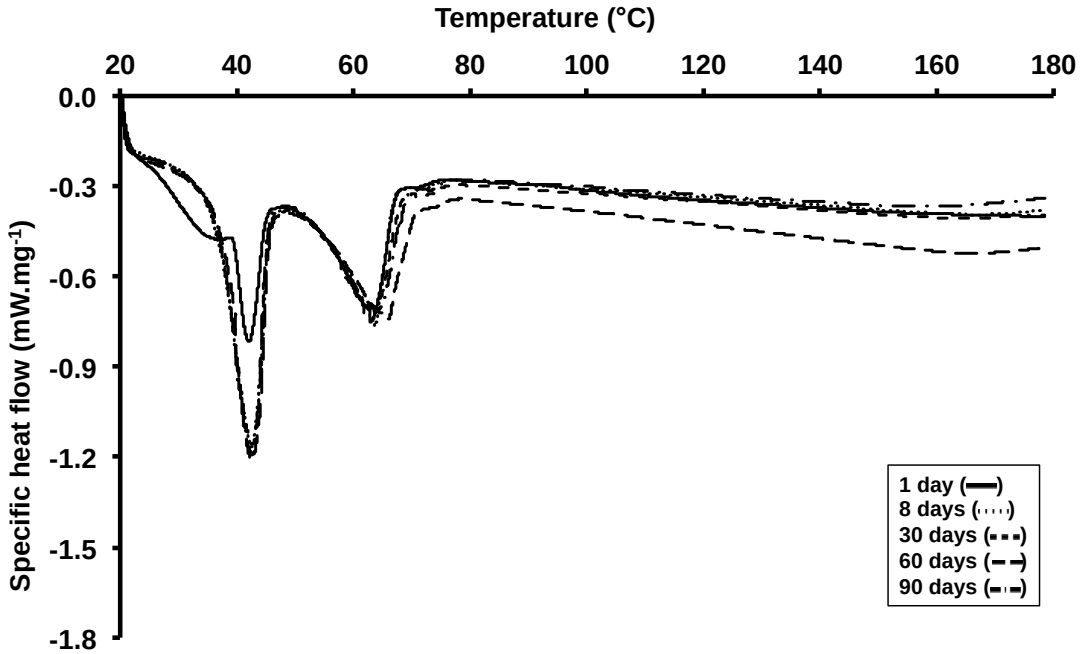

**b)**



**c)**


Temperature (°C)
20
40
60
80
100
120
140
160
180
0.0
-0.3
-0.6
-0.9
-1.2
-1.5
-1.8
Specific heat flow (mW.mg-1)
1 day
8 days
30 days
60 days
90 days

**d)**


Temperature (°C)
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Specific heat flow (mW.mg-1)
1 day
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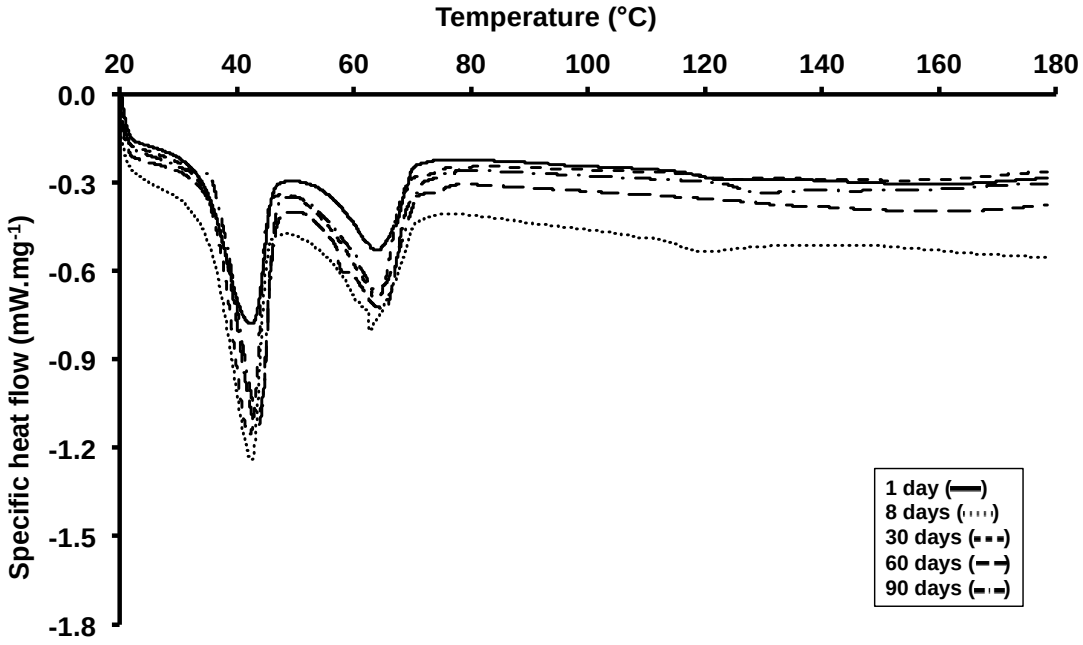
30 days
60 days
90 days

e)




f)

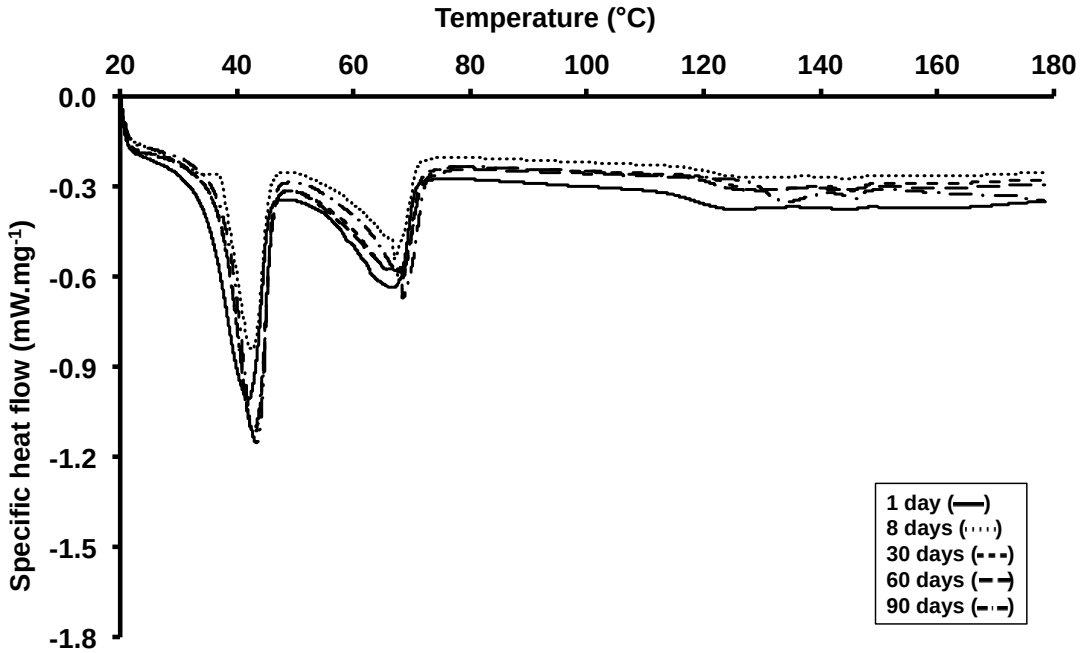



***Figure 4.9: Thermograms of the raw materials used in the study (a) and of the extrudates obtained from formulations I (b), II (c), III (d), IV (e) and V (f), analyzed over storage time.***

#### 4.4.3.5. Release of coumarin

As anticipated, the release of coumarin from extrudates with one layer (I, II and III) was faster than from co-extrudates IV (two layers) and V (three layers), which presented the intermediate and the slowest release of coumarin, respectively (Figure 4.10).

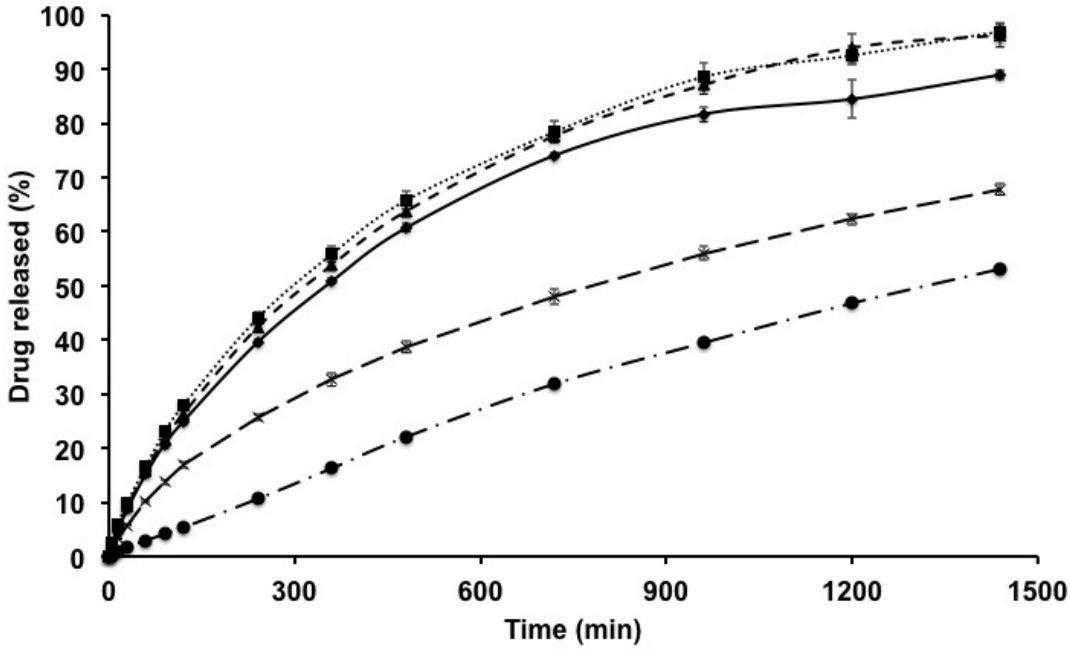



***Figure 4.10: Drug release profiles obtained 30 days after manufacture of extrudates I (·· ■ ··), II (—◆—), III (– ▲ -), IV (—x—) and V (-·⬮·-).***

The fractions of coumarin released after 24h of dissolution from extrudates stored for 30 days were higher for extrudates with one layer (I: 97.0±1.2 %; II: 89.0±0.8 %; III: 96.3±2.2 %), by comparison to co-extrudates with two

(67.9±1.1 %) or three layers (53.1±0.1 %). The study considered the dissolution 30 days after manufacture but the observations were similar for all other time points.

For better characterizing the release of coumarin, the dissolution efficiencies (ratio between the area under the dissolution curve and the total area for a complete dissolution of coumarin) were considered. Table 4.3 summarizes the dissolution efficiencies for the different extrudates and times of storage. It was observed that the number of layers and the time of storage affected the release of coumarin: the higher the number of layers and the longer the time of storage is, the lower is the dissolution efficiency.

The obtained release profiles were divided in two different regions: the initial phase of release (0-360 min) and the later period (360-1440 min). Different values calculated from the *Korsmeyer-Peppas* equation (Table 4.3) suggested that distinct mechanisms were governing the release of the drug simultaneously, with an emphasis of one over the others at the beginning (<360 min) followed by a change afterwards.

In the first period (0-360 min), extrudates I to IV presented an intermediate *n* parameter of the *Korsmeyer-Peppas* model (0.63 to 0.67) suggesting that coumarin was released by a combination of two mechanisms: by diffusion and by changes in the structure of the extrudate, such as its relaxation , described as an anomalous transport / non-Fickian model (107). However, co-extrudates V presented a *n* parameter close to 1 (zero order).

In the second period (360-1440 min), the mechanisms of release of coumarin from extrudates I to III (one layer) did not change (similar *n* parameters). However, it was detected a decrease in the *n* values calculated for extrudates IV (0.56) and V (0.83), suggesting changes in the mechanisms of release: coumarin was released from extrudates IV (bilayer) mainly by diffusion ($n \approx 0.5$) and from extrudates V (trilayer) both by diffusion and by structural changes of the extrudates.

## 4.5. Discussion

Lipid-based excipients allow the manufacture of solid dosage forms in the absence of solvents and without changing the temperature during processing (61, 115). However, lipids present features that may affect the quality of the products, such as the existence of different polymorphic forms, leading to the most stable form on storage (42, 93). Also, in the majority of the cases, lipid-based materials are not pure substances but mixtures of different components, introducing a degree of uncertainty (with lack of predictability) on the properties of the extrudates. Additionally, it is well known that lipid-based materials undertake an aging phenomenon derived from the polymorphic changes of their particles (94) and/or due to structural variations (102). All these characteristics are relevant for understanding the behavior of solid dosage forms composed by such materials and for assessing the properties and quality of the manufactured extrudates.

The formulations of this study were primarily composed by lipid-based materials (70%). One of the lipid materials used – glyceryl dibehenate – is composed of a mixture of mono (17.8%), di (53.0%) and tribehenate (26.8%) (data from the supplier). Also, the other lipids included in the formulations – polyethylene glycol glycerides and hard fat – were composed by saturated fatty acids with various carbon chain lengths (from 10 to 18 atoms of carbon).

The extrudates (I, II and III) and co-extrudates (IV and V) when exiting the extrusion die in its final shape did not have their physical and mechanical properties stabilized, as described previously. As expected, the aging phenomenon observed for the lipid-based materials led to the occurrence of a similar aging process on the extrudates, especially reflected by the changes on their mechanical and calorimetric properties over storage (66). The different enthalpies of fusion registered over time proved that the products suffered physical changes after manufacture with an impact on the overall properties of the extrudates, which were

not stabilized right after extrusion but tended to stabilize over time. An additional accelerated stability study was conducted, in which the extrudates were stored at 40±2 ºC and at 75±5 % of relative humidity, and the analysis of the thermal behavior of each extrudate confirmed these observations and trends.

Therefore, the observed changes in the mechanical properties can be explained by these intrinsic characteristics of the starting materials: after extrusion, the lipid materials suffered changes due to polymorphic transformations, searching for the most stable molecular configuration, as discussed by San Vicente et al. (102). These molecular alterations resulted in stronger interactions between the particles of the extrudates and, therefore, in higher bending strengths and stiffnesses of the structures, as well as in a loss of elasticity and ability to deform. These changes occurred over the storage period and were more evident in extrudates I, II and III, when compared to co-extrudates IV and V. This was probably due to the lower mobility of the particles from the co-extrudates resulting from the higher forces at steady state registered during extrusion. Also, the molecular and structural changes of the extrudates justify the modifications observed for the density and porosity. Both the nature of the starting materials and their proportions in the formulations and the experimental conditions of the extrusion process affected the final properties of the extrudates and co-extrudates.

The initial step of manufacture, in which the starting materials were mixed for obtaining a uniform extrudable mass (or masses), was successful, which was key for obtaining homogeneous products with consistent properties (66). All extrusion profiles presented the features expected when masses are homogeneous and adequate for extrusion. Also, both the increase of the extrusion rate and of the number of layers resulted in the increase of the $F_{ss}$. The pressure of the extrudable masses on the walls of the die was more intense likely due to the higher impact of the particles on the surfaces of the extruder chambers and dies when applied higher

extrusion rates and, also, when two or three different extrudable masses are combined at the same region of the extruder.

The steady state regions of the extrusion profiles testified the good quality of the extrusion process, as those areas were long and uniform, which was essential for achieving products of high quality. Also, the uniform and homogeneous surface features observed for all samples, without any detectable surface defect, testified the success of the mixing. Additionally, the observed homogeneous distribution of the materials throughout the extrudates (including the drug particles) was evident, which is relevant, as it prevents unwanted dissolution consequences (e.g. inappropriate release of the drug) (116).

Similarly, the rate of extrusion considered had an impact on the flow of the extrudable masses through the extrusion chambers particularly at the exit of the die (108). It is well known that the masses are subjected to an intense pressure towards the walls of the extruder chambers and that, when exiting the die, the extrudates can suffer an expansion process (elastic recovery) in their structure, resulting in products with higher thicknesses than expected, when compared to the cross section of the dies (101). Therefore, the registered thicknesses of the extrudates were not surprising as they correspond to this structural expansion. Worth to point out that the expansion observed was similar for all extrudates and co-extrudates (approximately 50 % of the die cross section) and did not compromise the surface quality. In a process of extrusion both materials and process affect the increase on extrudate thickness. But in the present research this was not observed. One can suggest that an increase on extrusion rate had an impact on the temperature of extruding materials locally with a decrease on their viscosity. The overall effect became independent of the process. Also, the low standard deviations obtained suggested that swelling of extruded materials was constant over time, which is certainly related to the materials rather than to the process conditions. This

increase of the dimensions / volume of the extrudates may also justify the changes of density and porosity over time, as there were not detected losses of mass over storage.

It is important to mention that the flow of the different masses through the extrusion chambers and dies, especially when the same mass was extruded with different extrusion rates (comparison between extrudates I, II and III) or when two different masses were conveyed towards a single die (comparison between co-extrudates IV and V), expectedly exert distinct forces at steady state. It suggests that the interaction between the particles of the starting materials occurred in different ways or extensions, depending on the extrusion rates and on the number of layers of the extrudates (69). However, this intimate interaction between the starting materials did not result in a complete loss of identity of the individual particles and, also, the applied extrusion forces did not result in a complete melting of the materials (117). One cannot exclude the fact that particles of materials with higher melting ranges remained in the solid state during the entire process or simply a poor heat transfer occurred. In this regard, cold extrusion seems to be closer to a granulation process than to a hot-melt extrusion. Also, the observation and identification of well-defined lines dividing the layers of co-extrudates IV and V suggested that the materials neither melt nor occurred a complete adhesion of the layers, as could be expected or desired, meaning that the melting did not occur or was incomplete at the point of merging the layers in the die.

As referred previously, the mechanical properties registered and their changes over time were also different for all five formulations, even if the changing trends of the properties were comparable. Increasing extrusion rates resulted in increasing forces at steady state applied to the extrudable masses, which were pulled towards the walls of the extrusion chambers and die with higher intensities

and, at the same time, the particles of the raw materials interacted more intimately between each other.

Also, the flow of a single extrudable mass through the extrusion apparatus can occur at simultaneous different speeds (27). In the laminar flow, the inner part of the extrudable masses, which is not in direct contact with the walls of the extrusion chambers or die, travels at a higher speed, when compared with the outer layers or portions of the mass, which interact with the surfaces of the extruder and suffer a decrease in the speed due to friction forces (101). These differences in the speeds of the masses have impact on the properties of the extrudates and on the stability of those properties. The higher the extrusion rate, the higher is the difference of extrusion speed of the inner and outer portions of the extrudable masses. Consequently, the stabilization of the structure of the extrudates can be increasingly compromised with the increase of the extrusion rate. For example, the sharkskin defect is well described and associated with the interaction of the extrudable masses and the walls / surfaces of the extruder. The higher the extrusion rate, the higher is the probability of occurring the sharkskin or other surface defects (69). In our case, the flow of the masses was adequate as there were not identified any surface defects. However, the differences of mechanical properties identified for extrudates I, II and III, which include the same materials and proportions but differ in the rate of extrusion, resulted from the phenomenon described before.

The use of lipid-based materials as main components of the formulation also determined a retardation of the release of the drug, as referred in previous studies (61, 115). The matrices formed through the extrusion of these materials did neither dissolve nor disintegrate in the dissolution media and, therefore, the release of the drug took more time. Additionally, co-extrudates with two and three layers presented a physical barrier (single or double) to the release of coumarin as one of the layers of co-extrudates IV (the one without coumarin) and both outer

layers of co-extrudates V consisted of extra obstacles to the diffusion of coumarin, by increasing the distance of the drug particles to the dissolution medium. It was observed that the higher the number of layers, the slower the release of coumarin.

Furthermore, the changes observed in the mechanical properties of the extrudates over time due modifications of the lipid-based materials and/or structural changes of the extrudates affected the release of the drug, reflected by changes on the dissolution efficiencies over storage, with a trend for decrease. The lower mobility of molecules in the structure of extrudates, reflected by increasing bending strengths and stiffnesses and decreasing elasticity and ability to deform, contributed to a progressive decrease of the dissolution efficiencies over time. The drug release profiles of all extrudates stored at 40±2 ºC and at 75±5 % of relative humidity (accelerated stability study) and registered at different time points confirmed these observations.

According to the *n* parameter calculated from the *Korsmeyer-Peppas* model, the release of coumarin from extrudates I, II and III followed a combination of mechanisms throughout the entire dissolution: both the diffusion of coumarin from extrudates and structural modifications of the matrix contributed to the release. However, while the release of coumarin from extrudates IV followed the same combination of mechanisms in the first period of dissolution (0-360 min), the release occurred mainly due to the diffusion of coumarin rather than due to structural changes of the extrudates in the second period (360-1440 min). Also, the release of coumarin from extrudates V occurred mainly due to structural alterations of the extrudates such as relaxation in the first period of dissolution but in the second period, the release of coumarin was also due to the diffusion of coumarin from the matrix. Therefore, in the beginning of the study, the dissolution experimental conditions had an impact on the structure of the extrudates (which was not visually detected), which afterwards contributed to the release of coumarin.

## 4.6. Conclusions

This study has proved the possibility of successfully manufacturing extrudates and co-extrudates with a laminar shape, in the absence of solvents and without changing the temperature during processing. Additionally, the study proved the possibility of producing co-extrudates with more than one layer (two or three).

The data generated proved that minor changes on both the processing conditions and fractions of raw materials in the formulations affected significantly their properties, particularly the release rate of coumarin. Furthermore, extrudates and co-extrudates composed by different layers (one, two or three) presented not only different release rates of the drug (the increase in the number of layers resulted in a decrease of the release rate) but also different release mechanisms over time.

The assessment of the properties over storage revealed that all extrudates and co-extrudates presented good surface quality and adequate physical properties. However, these physical properties changed over time: the porosity, bending strength and stiffness increased, whereas the density, deformation and elasticity decreased, for all extrudates. This observed ageing effect was constant for each formulation allowing the consideration of these types of extrudates to increase the flexibility on tailoring the release of drugs and simultaneous delivery of non-compatible drugs, by comparison to conventional dosage forms.

# CHAPTER 5

## Release of coumarin from laminar extrudates containing coumarin particles manufactured by RESS

***Adapted from:***

*Influence of process variables on the properties of coumarin particles micronized by supercritical fluids (RESS) to be delivered in a laminar extrudate, G. Oliveira, J. F. Pinto, AAPS PharmSciTech (2015).*

## 5.1. Introduction

Once the drug particles are manufactured, it is important to design delivery systems appropriate to carry, stabilize and deliver such particles without modifying their properties.

As described in the previous chapters, the laminar extrusion of lipid based materials proved to be a promising technology for delivering drugs without affecting the characteristics of their particles. These extrudates are manufactured in the absence of solvents and without significant changes in temperature during processing and, by using low forces (i.e., low shear), help on keeping the original properties of the particles to be delivered.

## 5.2. Aim of study

To assess the release of coumarin from laminar extrudates manufactured at room temperature and in the absence of solvents, containing particles of coumarin manufactured by RESS.

## 5.3. Materials and methods

### 5.3.1. Materials

Laminar extrudates were prepared from glyceryl dibehenate (Compritol™ 888 ATO, Gattefossé, France), hard fat (Witocan™ 42/44, Sasol, Germany), a mixture of polyethylene glycol with glycerides (Gelucire™ 33/01, Gattefossé, France), a mixture of polydimethylsiloxane with silicon dioxide (Simethicone™ Q7-2243 LVA, Dow Corning, Belgium) and coumarin.

## 5.3.2. Methods

### 5.3.2.1. Preparation of laminar extrudates with coumarin

The starting materials for each formulation of extrudate (Table 5.1) were weighted and mixed for 15 min in a planetary mixer (Kenwood Chef, UK). The mixtures were placed in the chamber of a ram extruder (Lurga, Portugal) with a die designed to prepare extrudates with laminar shape (1*40mm rectangular cross section). The extruder was adapted to a universal testing machine (LR 50K, Lloyds Instruments, UK) allowing recording of both force applied to the ram and its displacement during extrusion of materials. The extrusion force at steady state ($F_{ss}$) was calculated as the mean value of data recorded between 80 and 140 mm displacement of the ram. Laminar extrudates were manufactured at a rate of 300mm/min, cut into squares (40*40mm) immediately after production to facilitate their characterization and stored in a desiccator (23ºC / 30% RH).

***Table 5.1: Composition of the laminar extrudates.***

| Materials | Formulation (%) | |
|---|---|---|
| | A | B |
| **Polyethylene glycol glyceride** | 4 | 4 |
| **Hard fat** | 33 | 33 |
| **Glyceryl dibehenate** | 33 | 33 |
| **Polydimethylsiloxane – silicon dioxide mixture** | 20 | 20 |
| **RESS coumarin (obtained from Experiment 13 *)** | 10 | - |
| **Milled coumarin** | - | 10 |

** See Chapter 2.*

#### 5.3.2.2. Characterization of materials and products

The densities of the excipients before extrusion and of the extrudates (1, 3, 5, 8, 15, 30, 60, 90, 120, 150 and 180 days after manufacture) were determined by helium pycnometry (AccuPyc 1330, Micromeritics, USA) at room temperature (23±2 ºC) (n=3). The expected densities of extrudates were calculated from the densities of raw materials according to their fractions in the formulations. The porosities of extrudates were calculated from the densities of the extrudates, according to the following equation:

$$Porosity\ (\%) = 1 - \frac{apparent\ density}{theoretical\ density} \times 100 \qquad \textbf{Eq. 5.1}$$

Extrudates were observed by naked eye for detection of surface defects through and after processing, and their thickness was measured at different regions with a caliper (n=10). The extrudates were also analyzed by scanning electron microscopy (SEM) and images of the surface and of cross section regions were obtained using a DSM 940 scanning electron microscope (Carl Zeiss, Germany). Samples were coated with gold in a sputter coater (E 5100, Bio-Rad, Germany).

Laminar extrudates were tested for thermal behavior by differential scanning calorimetry (Q200, TA Instruments, USA). Samples (3-6 mg, n=3) were placed in hermetic sealed aluminium pans and tested at 10 ºC/min (heating intervals: 60-80 ºC for particles; 20-180 ºC for extrudates). The enthalpies of fusion were calculated for each sample. The extrudates were tested over storage (1, 30, 90 and 180 days).

Extrudates were analyzed by X-ray diffractometry. Samples were mounted on an aluminium holder and analyzed in a Philips PW1730 diffractometer with automatic data acquisition (APD Philips v.35B). The diffractograms were

recorded at room temperature in the range 10°<2θ<35° and data collected in a continuous mode, with a step size of 0.015° (2θ) and an acquisition time of 1.5 s/step. Extrudates were analyzed over storage (at 1, 30, 90 and 180 days).

Extrudates (n=3) were tested for their mechanical properties (1, 3, 5, 8, 15, 30, 60, 90, 120, 150 and 180 days after manufacture) by a three-point bend rig test (TA.XT Plus, Stable Microsystems, UK) at perpendicular direction of extrusion. Force was applied to extrudates and both force and displacement were recorded allowing calculation of bending strength, stiffness, deformation and Young's modulus of elasticity.

The release of coumarin from extrudates was studied through a dissolution test (Eur. Pharm., paddle method, 50 rpm, phosphate buffer, pH=6.8, AT7 Sotax, Switzerland) at different time-points over storage (1, 30, 90 and 180 days), testing different parts of each extrudate (n=3). The coumarin released was quantified by UV spectroscopy at 307 nm (U-2000 Spectrophotometer, Hitachi, Japan). The *Korsmeyer-Peppas* mathematical model was applied to study the mechanisms of release of coumarin from extrudates. The model was applied to the first 16 h of dissolution**.** The dissolution efficiency was determined over storage time (1, 3, 8, 15, 30, 60, 90, 120, 150 and 180 days) according to the following equation (98):

$$ED = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100\% \qquad \textbf{Eq. 5.2}$$

where y is the drug percent dissolved at time t.

Analysis of data: data was analyzed by an ANOVA study (one-way analysis of variance) for a level of confidence of 99% and complemented by Tukey's HSD test (statistical significance considered for $p < 0.01$).

## 5.4. Results

### 5.4.1. Manufacture of extrudates

Laminar extrudates containing either milled or RESS particles of coumarin presented similar extrusion profiles (Figure 5.1) and extrusion forces at steady state within the same range (Table 5.2).

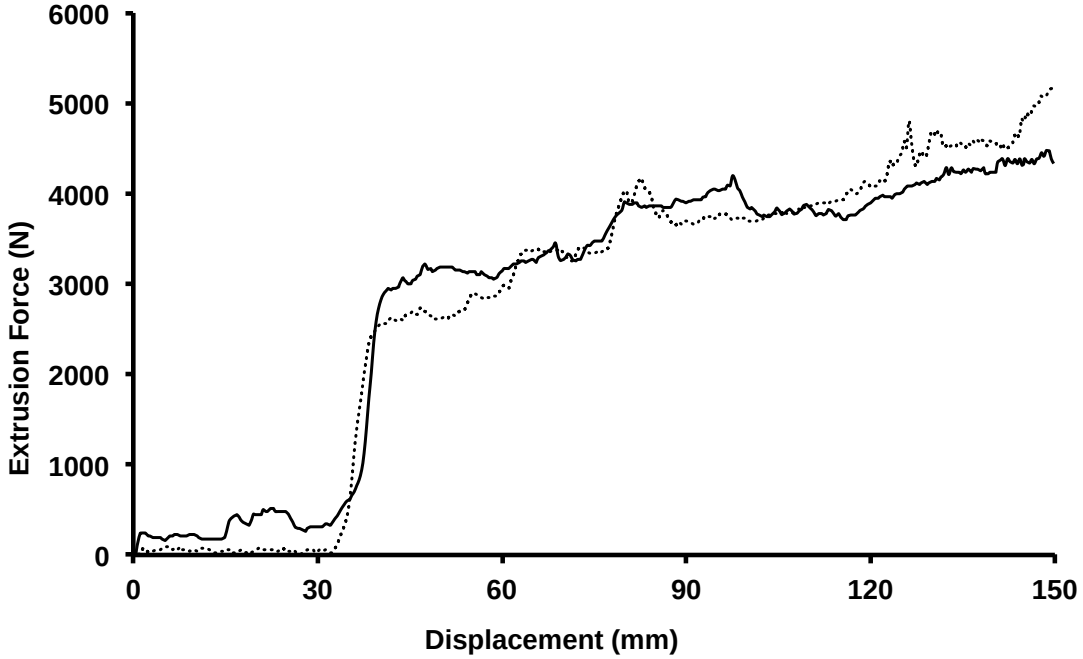



***Figure 5.1: Extrusion profiles obtained after extruding formulation A (.....) and formulation B (——).***

### 5.4.2. Visual inspection of extrudates

The visual inspection confirmed that the extrudates presented good surface characteristics, as the surface was smooth, regular and without defects, and

the distribution of the materials throughout the extrudates was visually homogeneous for both extrudates (Figure 5.2.A).

SEM images captured from both extrudates confirmed the surface regularity and the absence of defects (Figure 5.2.B). Additionally, the SEM image of the cross section of the extrudate (Figure 5.2.C) revealed that at least some particles of the materials did not melt and did not lose their integrity.

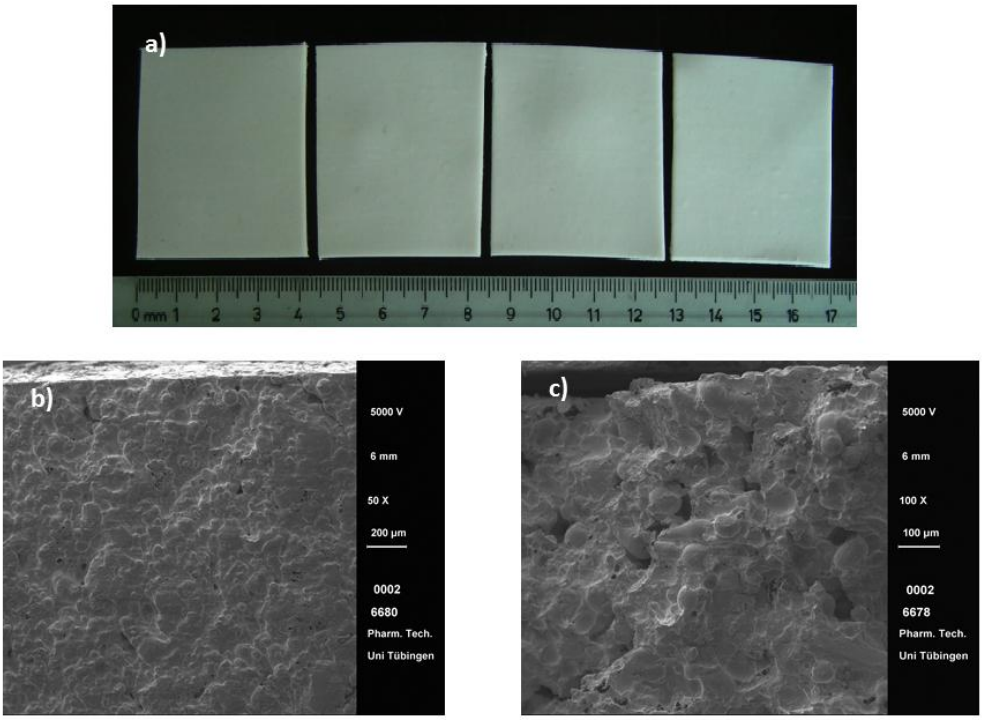



***Figure 5.2: Photo of the laminar extrudate A after exiting the die and after being cut with 40x40 mm (a) and SEM photographs of the surface (b) and of a cross section (c) of extrudates A.***

### 5.4.3. Thickness of extrudates

The thicknesses of extrudates were identical and homogeneous for each extrudate, approximately 50% thicker than the cross section of the die (Table 5.2).

### 5.4.4. Density, porosity and mechanical properties of extrudates

The density, porosity and mechanical properties of extrudates changed over storage: the density, deformation and elasticity decreased over time, whereas the porosity, bending strength and stiffness increased (Figure 5.3, A-F). These changes were more intense on the first days after manufacture but stabilized over time, especially from day 90, onwards.

### 5.4.5. Thermal behavior of raw materials and of extrudates over time

The analysis over time of the thermal behavior confirmed the changes observed in the mechanical properties, density and porosity, as there were detected thermal behavior changes at different days after manufacture (Figure 5.4.A-B). Also, the enthalpies of fusion changed over time (Table 5.2).

Extrudate A presented changes in the amorphous / crystalline content over storage as the X-rays diffractograms presented differences in the peak's intensity at different days after manufacture (Figure 5.5.A). However, extrudate B presented identical X-rays diffractograms and there were not detected any changes in the intensity or diffraction angles of the peaks (Figure 5.5.B). For both extrudates, the peaks corresponding to the excipients were possible to identify, whereas the peaks of coumarin were not detected.

***Table 5.2: Extrusion force at steady state ($F_{ss}$), thickness, enthalpies of fusion and dissolution efficiency from extrudates (960 min of release, over storage).***

| Extrudates | | | A | B |
|---|---|---|---|---|
| **$F_{ss}$ (N)** [a, b] | | | 4043 ± 336 [r] | 3958 ± 165 [r] |
| **Thickness (mm)** [c] | | | 1.52 ± 0.03 | 1.54 ± 0.03 |
| **Enthalpies of fusion (J/g)** | **Day** | **1** | 134.2 | 131.9 |
| | | **8** | 147.8 | 152.1 |
| | | **30** | 146.8 | 130.6 |
| | | **60** | 141.4 | 139.4 |
| | | **120** | 157.7 | 154.3 |
| | | **180** | 154.9 | 149.3 |
| **Dissolution efficiency (%)** [d] | **Day** | **1** | 42.7±1.8 [r,1] | 42.0±0.8 [r,1] |
| | | **3** | 42.3±1.5 [r,1] | 41.9±1.1 [r,1] |
| | | **8** | 39.6±1.0 [r,1,2] | 42.0±0.4 [r,1] |
| | | **15** | 35.5±1.0 [r,1,2] | 39.9±0.2 [r,1,2] |
| | | **30** | 35.3±0.8 [r,1,2] | 36.5±0.2 [r,3] |
| | | **60** | 36.7±1.3 [r,1,2] | 36.5±1.2 [r,3] |
| | | **90** | 39.5±1.3 [r,1,2] | 38.3±0.6 [r,2,3] |
| | | **120** | 37.0±0.1 [r,1,2] | 38.3±1.7 [r,2,3] |
| | | **150** | 34.4±0.9 [r,1,2] | 37.4±0.9 [s,2,3] |
| | | **180** | 29.8±1.1 [r,2] | 31.5±0.9 [s,4] |

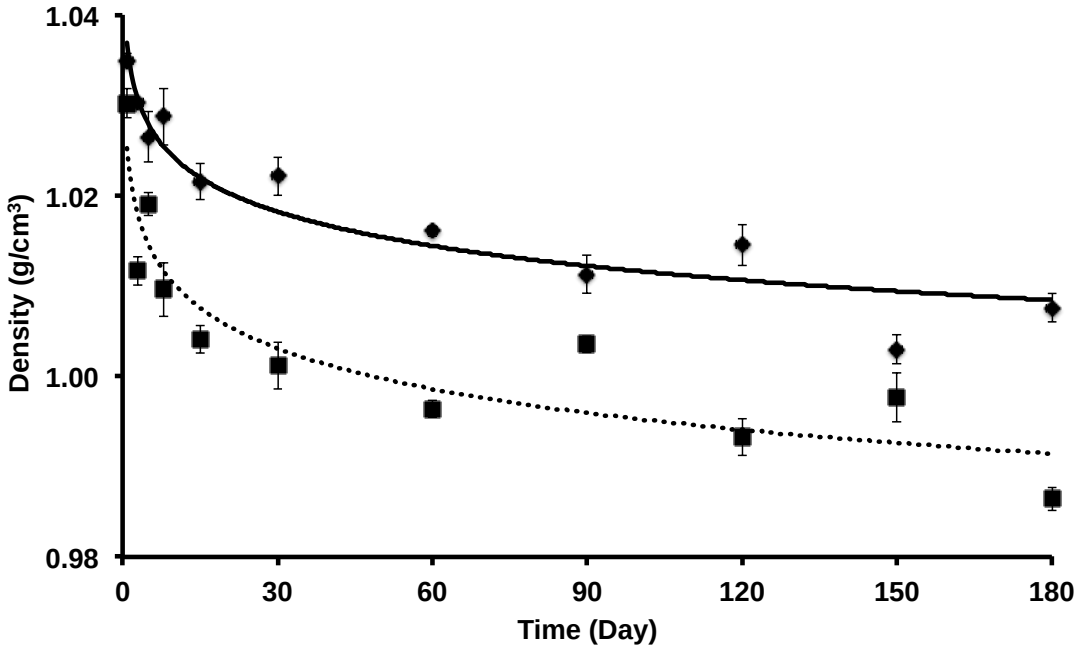
[a] *n = 3*

[b] *The means of $F_{SS}$ with different letters in the indices are significantly different (ANOVA; p < 0.01; Tukey's HSD; n = 3).*

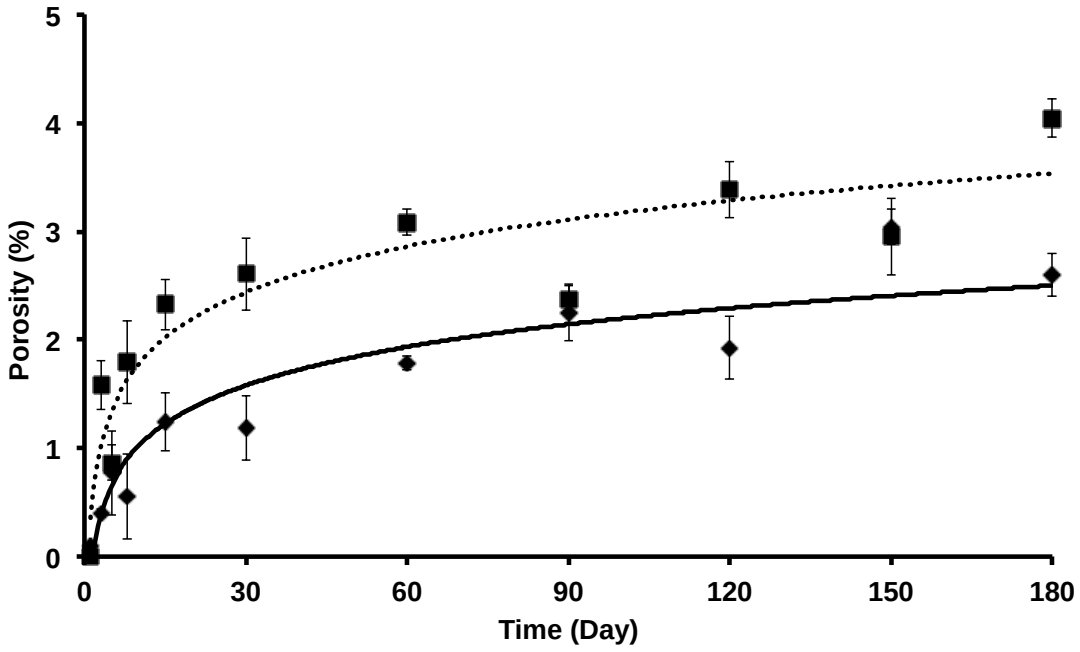
[c] *n = 10*

[d] *The means of dissolution efficiency with different letters in the indices (for each column) and the means of dissolution efficiency with different numbers in the indices (for each row) are significantly different. (ANOVA; p < 0.01; Tukey's HSD; n = 3).*

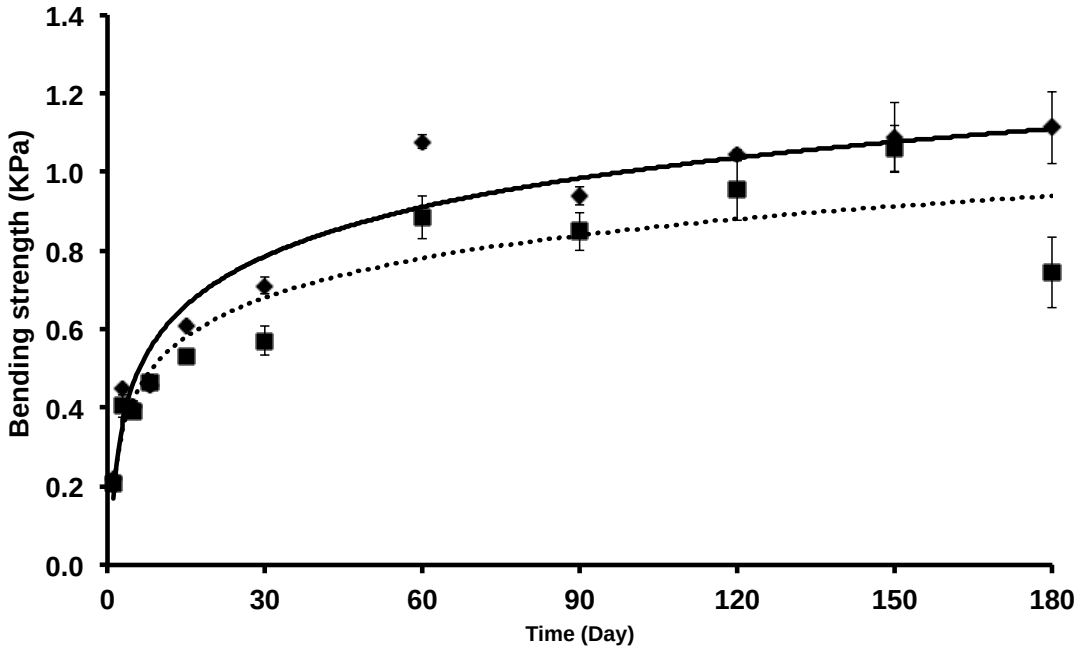
a)


1.04
1.02
1.00
0.98
Density (g/cm³)
0
30
60
90
120
150
180
Time (Day)

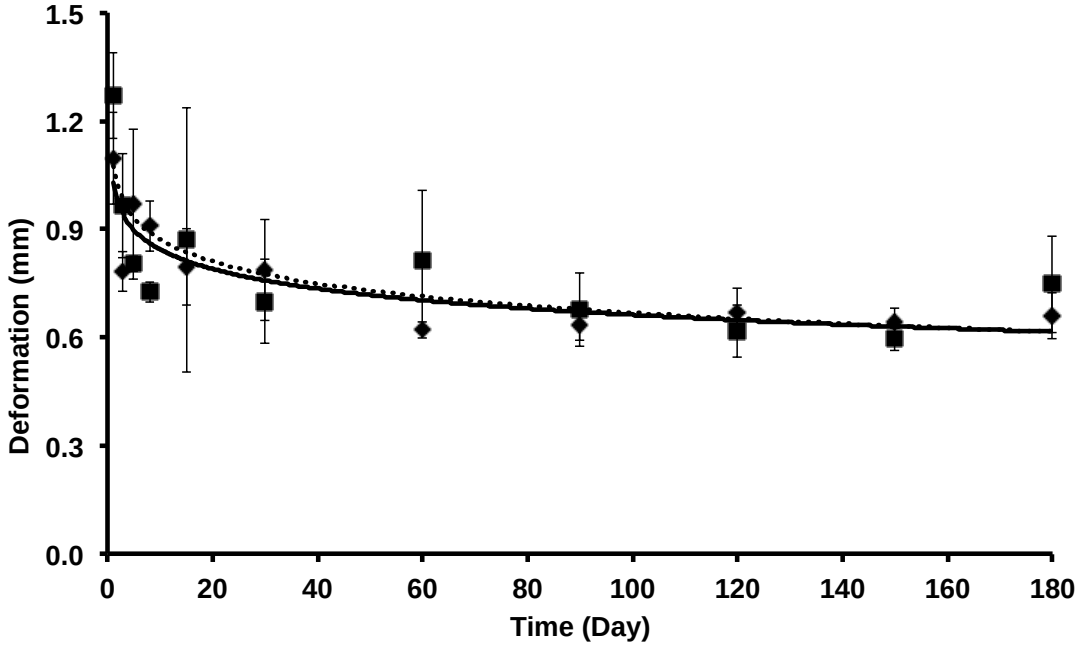
b)

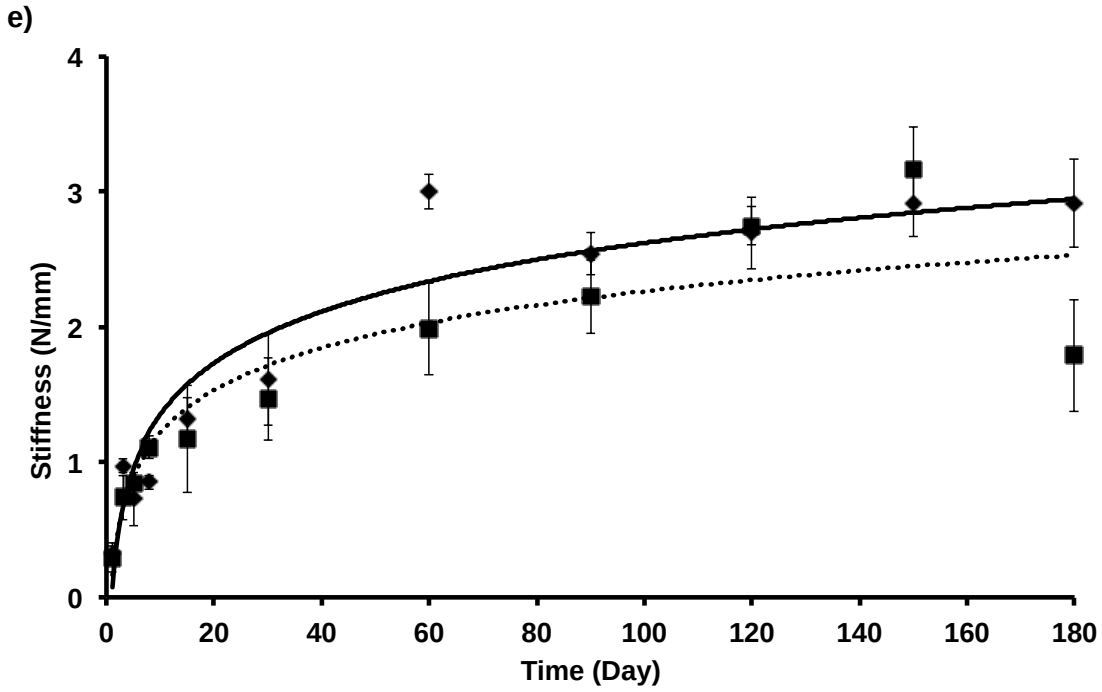

5
4
3
2
1
0
Porosity (%)
0
30
60
90
120
150
180
Time (Day)

**c)**


Bending strength (KPa)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
150
180
Time (Day)

**d)**


Deformation (mm)
1.5
1.2
0.9
0.6
0.3
0.0
0
20
40
60
80
100
120
140
160
180
Time (Day)



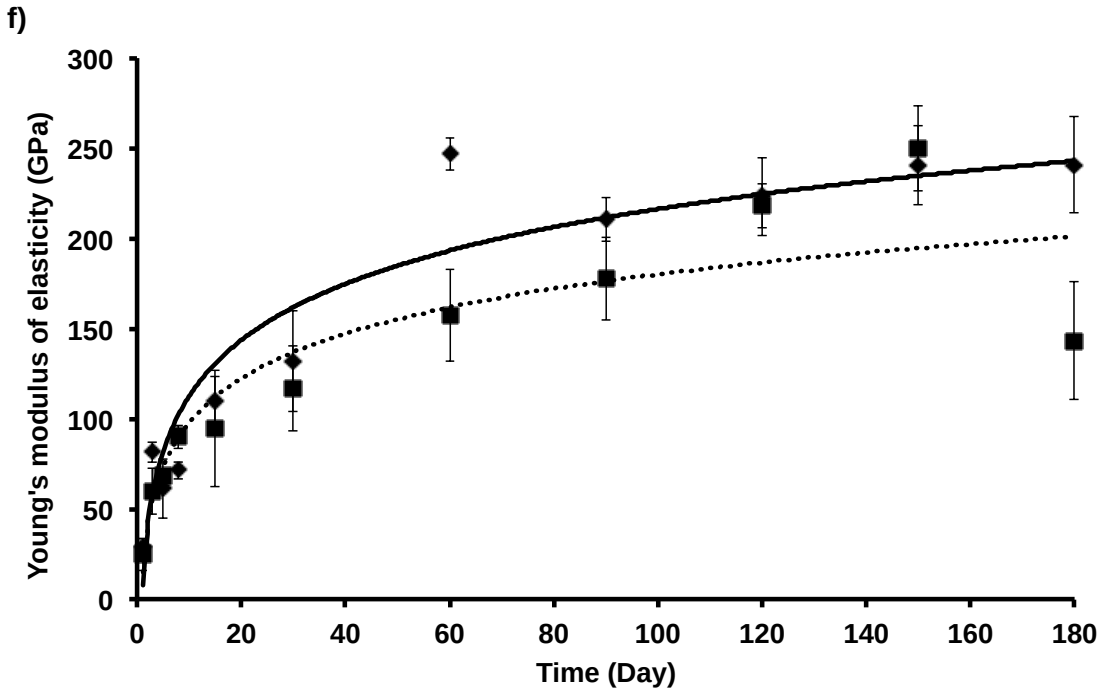




***Figure 5.3: Density (a), porosity (b), bending strength (c), deformation (d), stiffness (e) and Young's modulus of elasticity (f) of laminar extrudates A (·■·) and B (—◆—) over time of storage, with logarithmic fitting curves.***

*[Expected densities of 1.028 g/cm$^3$ (Formulation A) and 1.034 g/cm$^3$ (Formulation B) were considered for the calculation of porosity].*

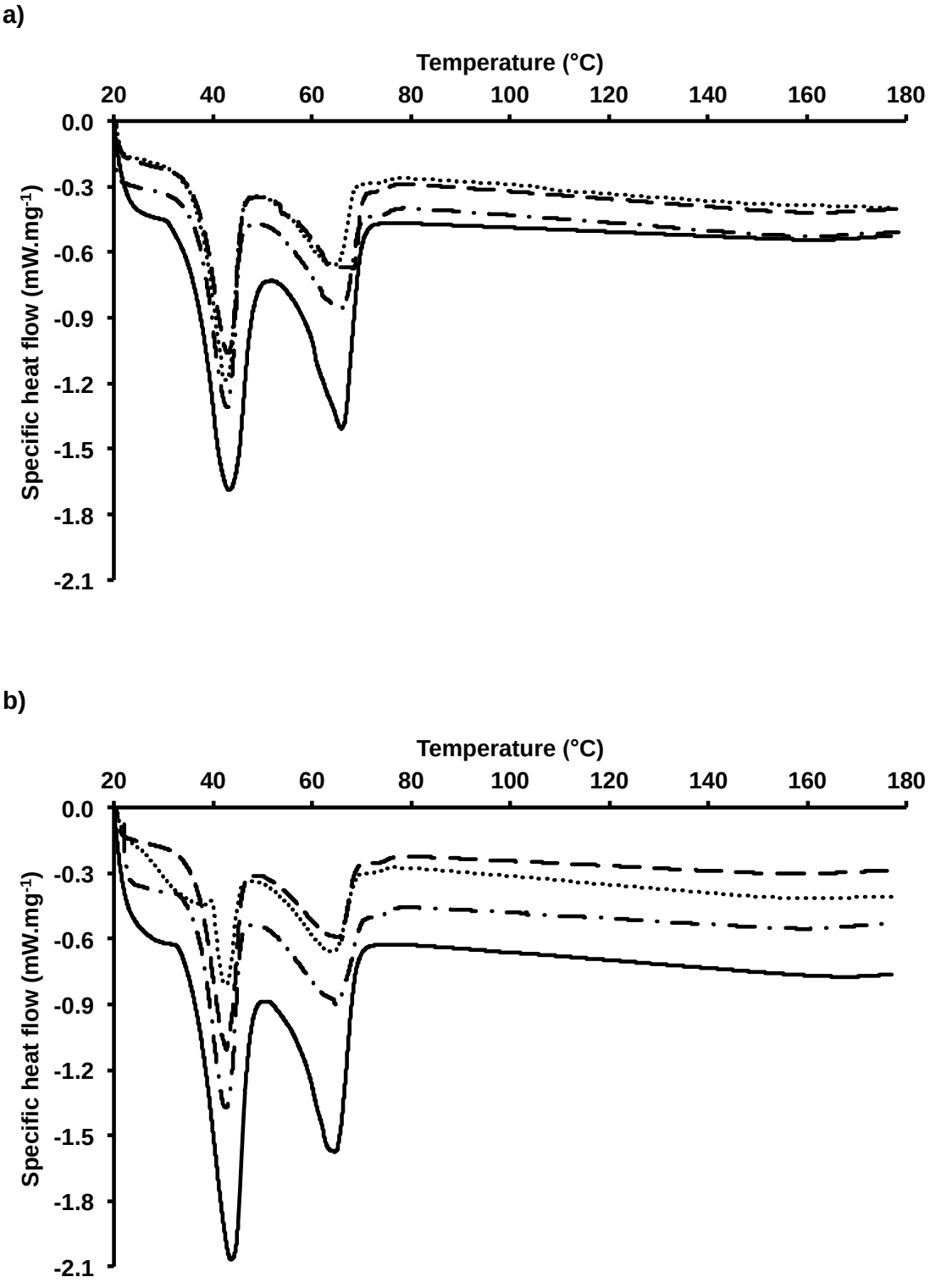



***Figure 5.4: Thermograms of extrudates A (a) and B (b) analyzed over storage: 1 (▬▬), 30 (▪▪▪▪), 90 (▬ ▬) and 180 (▬▪▬) days after manufacture.***

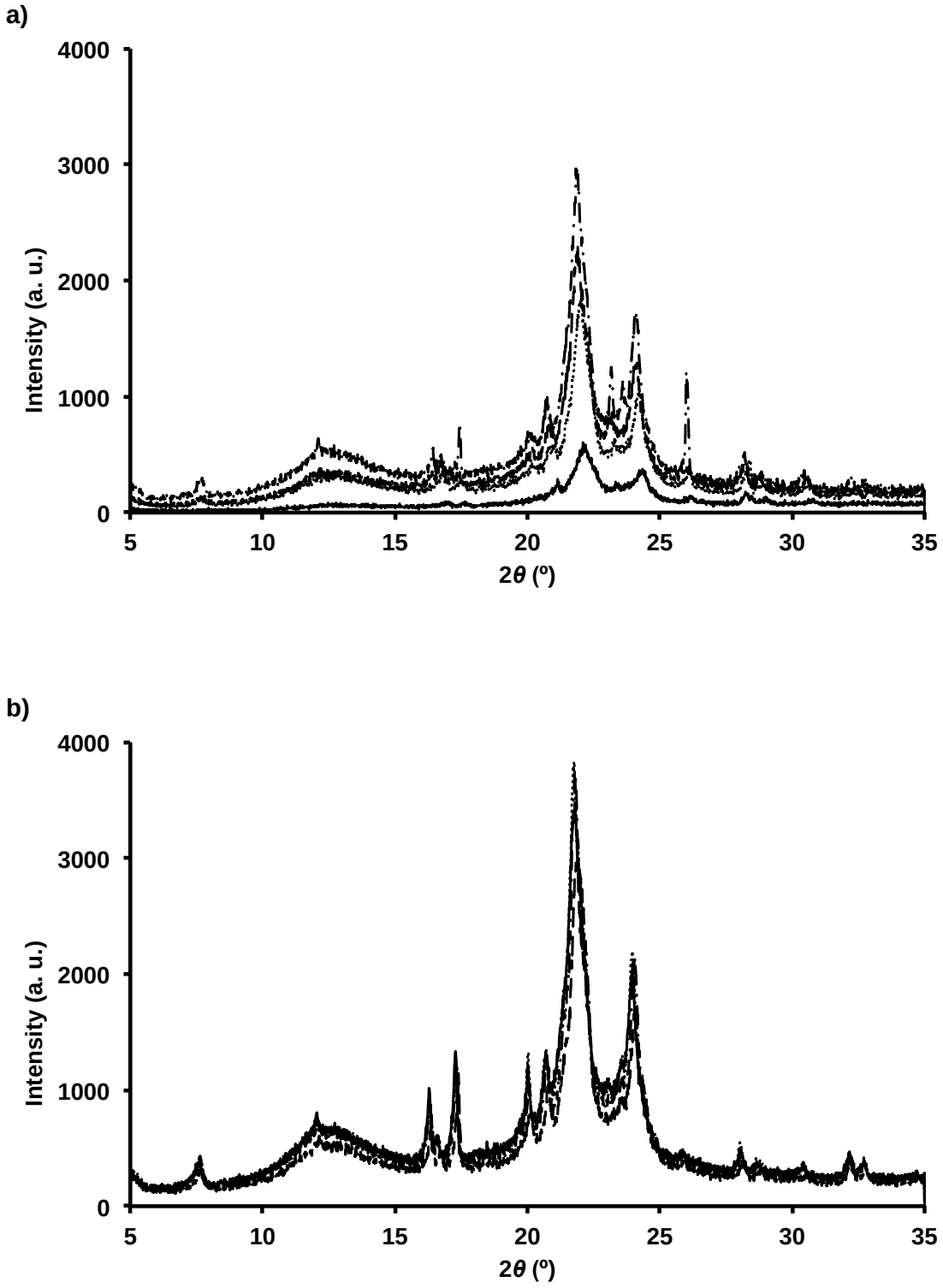



***Figure 5.5: X-ray diffractograms of extrudates A (a) and B (b) analyzed over storage: 1 (▬▬), 30 (▪▪▪▪), 90 (▬ ▬) and 180 (▬▪▬) days after manufacture.***

### 5.4.6. Release of coumarin from extrudates

Coumarin was released almost completely in 24 h from both extrudates and in the same fashion (identical release profiles) (Figure 5.6). The dissolution efficiencies calculated at different time-points changed over storage (Table 5.2): in the first 120 days, the dissolution efficiencies of both extrudates were statistically equal, whereas in the second period (120-180 days) the dissolution efficiencies of extrudates B were higher and statistically different in comparison with extrudates A. Also, when comparing dissolution efficiencies of the same extrudate at different time-points over storage, there were statistical differences: for both extrudates there was a decreasing trend of dissolution efficiency over time.

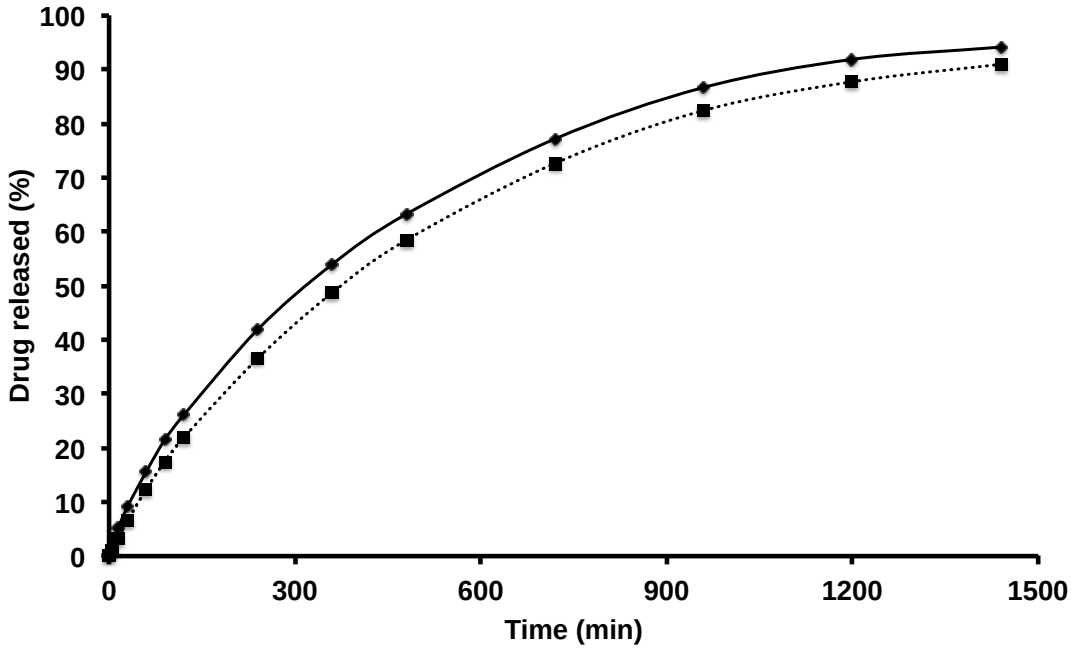



***Figure 5.6: Drug release profiles obtained 90 days after manufacture of laminar extrudates A (·■·) and B (—◆—).***

The release of coumarin from extrudates was analyzed by considering the *Korsmeyer-Peppas* model. The *n* parameters of both extrudates – between 0.50-0.75 (Table 5.3) – revealed a release of coumarin through different mechanisms. The *n* parameter calculated at day 1 was equal or close to 0.50 (ext. A: 0.54; ext. B: 0.50). However, at days 30, 90 and 180, the calculated *n* parameter has shown values between 0.65-0.75.

***Table 5.3: Release exponents (n) and coefficients of determination ($R^2$) obtained from the Korsmeyer-Peppas model (data considered within the interval 0-16 h).***

| Day | Extrudate A | | Extrudate B | |
|---|---|---|---|---|
| | n | $R^2$ | n | $R^2$ |
| 1 | 0.54 ± 0.01 | 0.998 ± 0.001 | 0.50 ± 0.01 | 0.999 ± 0.000 |
| 30 | 0.69 ± 0.02 | 0.998 ± 0.000 | 0.65 ± 0.01 | 0.998 ± 0.001 |
| 90 | 0.71 ± 0.01 | 0.999 ± 0.000 | 0.67 ± 0.01 | 0.999 ± 0.000 |
| 180 | 0.75 ± 0.01 | 0.998 ± 0.000 | 0.71 ± 0.01 | 0.999 ± 0.000 |

When comparing the release of coumarin from extrudates manufactured with the same formulation but including different groups of coumarin particles – RESS or milled – the differences were not significant.

## 5.5. Discussion

The particles manufactured by milling and RESS were incorporated in the extrudates for the assessment of the possibility of delivering the drug while preserving the physical properties of the particles. As the excipients in the

formulations for extrusion were the same and the properties of both groups of coumarin particles were not significantly different, it was not surprising that the processes of extrusion were also not different. The extrusion profiles, particularly the steady-state regions, justified the ability of both formulations in producing extrudates with high quality as ascertained by their properties.

The changes of the mechanical properties observed over time for both extrudates were expected and had been previously described in the previous chapters (21). The observed increase of thickness occurred due to a limited elasticity of the materials right after the extrusion process when exiting the die. Additionally, some particles of the materials did not melt and did not lose their morphology, suggesting that the particles of coumarin might have been included in the matrix of extrudates without melting or losing their properties, as obtained after the different techniques of production.

The manufactured extrudates have shown their ability to form a sustained release dosage form of coumarin. The different values for the *n* parameter (*Korsmeyer-Peppas*) obtained for the different releases suggested different mechanisms of release over time, which was already described previously.

Firstly, the release of coumarin occurred mainly by Fickian diffusion from the matrix, which evolved over time to a combination of mechanisms of release: both Fickian diffusion of the drug from the matrix of extrudates concomitantly with structural changes of the matrix (98). This change of the mechanisms governing the release of coumarin from the extrudates were also in good agreement with the changes of the mechanical properties registered for the extrudates over time, particularly with the decrease of elasticity and increase of bending strength and stiffness, decreasing the ability of extrudates for releasing the coumarin. The resultant reduced mobility of the molecules in the extrudates matrices over time also justifies the decreasing of the dissolution efficiency over storage.

## 5.6. Conclusions

Laminar extrudates containing particles of coumarin micronized by RESS were successfully prepared to deliver the drug as a sustained release dosage form. Extrudates have shown adequate mechanical properties with little degree of aging over storage.

# CHAPTER 6

## Oral and transdermal delivery of drugs from laminar extrudates manufactured at room temperature and in the absence of solvents: an *in-vitro* study in excised skin and an *in-vivo* study in rats

## 6.1. Introduction

The manufacture of extrudates has been widely used in the pharmaceutical field, especially in the preparation of pellets for delivering drugs through the oral route of administration (42). However, it is possible to manufacture extrudates with other shapes (e.g. tubular or laminar) (60) allowing the extent of the use of extrudates to other applications, such as the delivery of drugs through the skin (topical or transdermal).

Also, new ways of performing extrusion have been developed in alternative to the conventional processes: wet and hot-melt extrusion. Therefore, the development of an alternative extrusion procedure not requiring changes of temperature during processing and not involving solvents as starting materials, represents a step forward and an innovative way of performing extrusion. This opens the possibility of processing a higher number of drugs through extrusion, namely those that are sensitive to heat or change their properties in contact with solvents.

Laminar extrudates and co-extrudates manufactured at room temperature and in the absence of solvents have proven their ability for carrying drugs that are released from the product *in-vitro*. However, do these drugs reach their target in order to activate the expected therapeutic effects? It is important to prove the extrudate's ability for delivering drugs through specific routes of administration into their therapeutic targets.

Coumarin and levothyroxine (free acid) have been used as model drugs. Coumarin has been referred as a therapeutic option for reducing tissue swelling due to disease or trauma (e.g. edema) and is also effective against protein lymphedemas (118). Additionally, coumarin has been tested in the treatment of certain types of cancer (91). Levothyroxine (free acid) is a drug commonly used for treating thyroid related affections, especially for the hormonal substitution therapies

for treating hypothyroidism. Therefore, levothyroxine is used for chronicle diseases, and is administered therapeutically in a daily basis (119).

## 6.2. Aim of study

To assess the potential of laminar extrudates manufactured at room temperature and in the absence of solvents for delivering drugs through oral or transdermal routes of administration in rats and in excised human skin.

## 6.3. Materials and methods

### 6.3.1. Materials

The laminar extrudates included glyceryl dibehenate (Compritol™ 888 ATO, Gattefossé, France), hard fat (Witocan™ 42/44, Sasol, Germany), polyethylene glycol glycerides (Gelucire™ 33/01, Gattefossé, France) and a mixture of polydimethylsiloxane and silicon dioxide (Simethicone™ Q7-2243 LVA, Dow Corning, Belgium). Coumarin (0-90 μm) milled from coarse coumarin (Sigma-Aldrich, Germany) in an analytical grinder (A10 Yellow Line, IKA, Germany) and levothyroxine free acid (T4) (Peptido GmbH, Germany) used as purchased, were considered as model drugs. Table 6.1 summarizes the composition of the formulations used.

### 6.3.2. Methods

#### 6.3.2.1. Manufacture of laminar extrudates

Extrudates were manufactured with a ram extruder (Lurga, Portugal) with a single chamber and a rectangular die (1x40mm cross section) designed to prepare extrudates with laminar shape. The extruder was adapted to a mechanical

press (LR 50K, Lloyds Instruments, UK) fit with a load cell allowing the collection of the force and displacement data from the process. The raw materials were mixed in a planetary mixer (Kenwood Chef, UK) for 15 minutes and fed manually into the extruder chamber. Extrudates were manufactured at an extrusion rate of 300 mm/min and cut into squares (40x40x1mm) immediately after production and stored in desiccators (23 ºC / 30 % RH).

***Table 6.1: Composition of the laminar extrudates.***

| Raw materials | Formulation I (%) | Formulation II (%) |
|---|---|---|
| **Glyceryl dibehenate** | 33 | 33 |
| **Hard fat** | 33 | 33 |
| **Polyethylene glycol glycerides** | 4 | 4 |
| **Polydimethylsiloxane and silicon dioxide** | 20 | 20 |
| **Coumarin (0-90 µm)** | 10 | - |
| **Levothyroxine (free acid)** | - | 10 |

#### 6.3.2.2. Characterization of laminar extrudates

The extrusion procedure and formulations were equivalent to those previously developed and described in previous chapters. Therefore, the characterization of the extrudates was minimal and focused only the following properties: surface quality (visual examination); extrusion profiles and mean forces at steady-state (calculated as the mean of the single forces obtained between 70 and 100 mm of displacement); thickness (measured with a caliper at different regions;

n=10); density (n=3) at room temperature (25±2 ºC) using a helium pycnometer (AccuPyc 1330, Micromeritics, USA); and porosity, calculated from the theoretical and apparent densities of extrudates:

$$Porosity\ (\%) = 1 - \frac{apparent\ density}{theoretical\ density} \times 100 \qquad \textbf{Eq. 6.1}$$

Note: the theoretical densities of extrudates were calculated from the apparent densities of raw materials, according to their proportions in formulations.

#### 6.3.2.3. *In-vitro* permeation of coumarin and levothyroxine through excised skin

The release of coumarin and levothyroxine from extrudates and its permeation through excised skin was studied in static vertical diffusion cells (Franz cells). The necessary pieces of human skin were collected from a healthy female with 54 years of age subjected to a surgical intervention to reduce abdominal mass, and after ethical approval and informed consent.

The skin pieces were taken from the transportation package and submerged in isotonic phosphate buffer (pH 7.4) at approximately 60ºC and for 30 seconds until thawed. The fat content was carefully removed and the *stratum corneum* (SC) gently separated from the remaining tissue. The SC was visually inspected for any defects and cut into sections large enough to fit on the Franz cells. The obtained SC pieces were then mounted between the donor and receptor compartments of the diffusion cells (permeation area: 1 $cm^2$).

As receptor media, there was used phosphate buffer (pH 6.8) for coumarin and a mixture of ethanol and water (1:1) for levothyroxine. The sink conditions were assured, the media were constantly stirred with a magnetic bar (200 rpm) and the temperature maintained throughout the experiments (32±0.5 ºC). The

samples of extrudates (37 to 58 mg) were evenly applied on top of the SC (donor compartments), which were immediately sealed to prevent solvent evaporation. Tables 6.2 and 6.3 summarize the masses of coumarin and levothyroxine administered in each sample of extrudate.

Samples (800 µl) were collected from the receptor compartments at several time points – 1, 2, 4, 8, 12 and 24 hours – and replaced with an equivalent amount of receptor medium. Coumarin was quantified by UV spectroscopy at 307 nm using a microplate reader (FLUOStar Omega, BMG Labtech, Germany), whereas levothyroxine was quantified by a solid phase chemiluminescent competitive immunoassay method (IMMULITE 2000 Canine Total T4; conversion factor: 1 nmol/L = 12.87 x 1 µg/dL) performed at DNAtech (Portugal). Repeated measures using six replicated cells were used.

**Table 6.2: Volume of receptor medium, doses of coumarin and T4 delivered in each sample of extrudate.**

| Diffusion cell nr. | Volume of receptor medium (mL) | Mass of coumarin administered (µg) | Mass of T4 administered (µg) |
|---|---|---|---|
| **1** | 4.3 | 4030 | - |
| **2** | 4.8 | 3680 | - |
| **3** | 4.4 | 3990 | - |
| **4** | 4.3 | 4460 | - |
| **5** | 4.7 | 4060 | - |
| **6** | 4.3 | 4200 | - |
| **7** | 3.6 | - | 4860 |
| **8** | 3.6 | - | 4939 |
| **9** | 3.6 | - | 4892 |
| **10** | 3.6 | - | 5608 |
| **11** | 3.8 | - | 5035 |
| **12** | 3.7 | - | 5838 |

#### 6.3.2.4. *In-vivo* delivery of levothyroxine

There were used three groups of five randomly divided Wistar male rats (Charles River Laboratories, Spain) (Table 6.3). The rats were used after a 1-week period of acclimatization to the laboratory. All experiments were carried out with permission of the local animal ethical committee and in accordance with the EU Directive 2010/63/UE and Portuguese legislation (Decreto-Lei 113/2003). The experimental protocol was approved by Direção Geral de Veterinária.

***Table 6.3: Groups of rats tested in-vivo and respective routes of administration.***

| Group | Rat nr. | Route of administration |
|---|---|---|
| **A** | 1 – 5 | Not applicable |
| **B** | 6 – 10 | Transdermal |
| **C** | 11 – 15 | Oral |

Rats of group A did not receive any levothyroxine (control group). Rats of group B were shaved and a single piece of extrudate was applied on the skin with the help of an adhesive. These rats were observed for 24 h in order to guarantee that the samples of extrudates remained in contact with the skin throughout the entire study. Rats of group C received 3 to 5 pieces of extrudates with the help of a gastric gavage. The weighs of the rats and the masses of samples of extrudates used, and the amounts of levothyroxine administered to each rat either through oral or transdermal routes are summarized in Table 6.4.

After 24 hours, animals were sacrificed and blood samples were collected by cardiac puncture. Levothyroxine was quantified as described in section 6.3.2.3.

***Table 6.4: Weight of rats, masses of extrudates used in the in-vivo study, and doses of levothyroxine administered.***

| Rat nr. | Mass of rat (g) | Mass of laminate (g) | T4 administered | |
|---|---|---|---|---|
| | | | Mass (µg) | Mass of T4 per skin area (µg/cm$^2$) |
| **1** | 253 | - | - | - |
| **2** | 255 | - | - | - |
| **3** | 264 | - | - | - |
| **4** | 313 | - | - | - |
| **5** | 268 | - | - | - |
| **6** | 303 | 0.501 | 50083 | 12521 |
| **7** | 302 | 0.496 | 49597 | 12399 |
| **8** | 290 | 0.519 | 51880 | 12970 |
| **9** | 282 | 0.505 | 50472 | 12618 |
| **10** | 309 | 0.511 | 51116 | 12779 |
| **11** | 318 | 0.024 | 2410 | - |
| **12** | 285 | 0.022 | 2179 | - |
| **13** | 310 | 0.020 | 2036 | - |
| **14** | 294 | 0.018 | 1805 | - |
| **15** | 314 | 0.024 | 2394 | - |

## 6.4. Results and discussion

### 6.4.1. Manufacture of laminar extrudates

The extrusion profiles (Figure 6.1) registered during the process of extrusion presented the typical features observed when the process originates extrudates with quality, which have been described extensively in the previous chapters.

The registered mean extrusion forces at the steady state flow stage were respectively 4797±61 and 3950±69 N for formulations I and II. As both formulations were identical, differing only in the drug included, it was expected to obtain similar profiles and values of the mean extrusion forces at the steady state. Additionally, these extrusion profiles are also similar with the ones described in previous chapters and recorded for similar formulations (see chapters 3 and 4).

### 6.4.2. Characterization of laminar extrudates

Both extrudates presented a good external appearance, starting with a homogeneous and smooth surface. There were not detected any surface imperfections nor structural defects such as fractures, variations of shape, excessive softness or brittleness. Therefore, the extrudates presented similar properties than the ones tested and characterized in the previous chapters (see chapters 3 and 4).

Laminar extrudates I and II presented, respectively, thicknesses of 1.52±0.03 and 1.54±0.03 mm, densities of 1.03±0.01 and 1.15±0.01 g/cm$^3$, porosities of 0.3±0.1 and 0.4±0.2 %, given the theoretical densities of 1.03 and 1.16 g/cm$^3$.

All these observations confirm the quality of the extrudates used in the *in-vitro* and *in-vivo* studies.

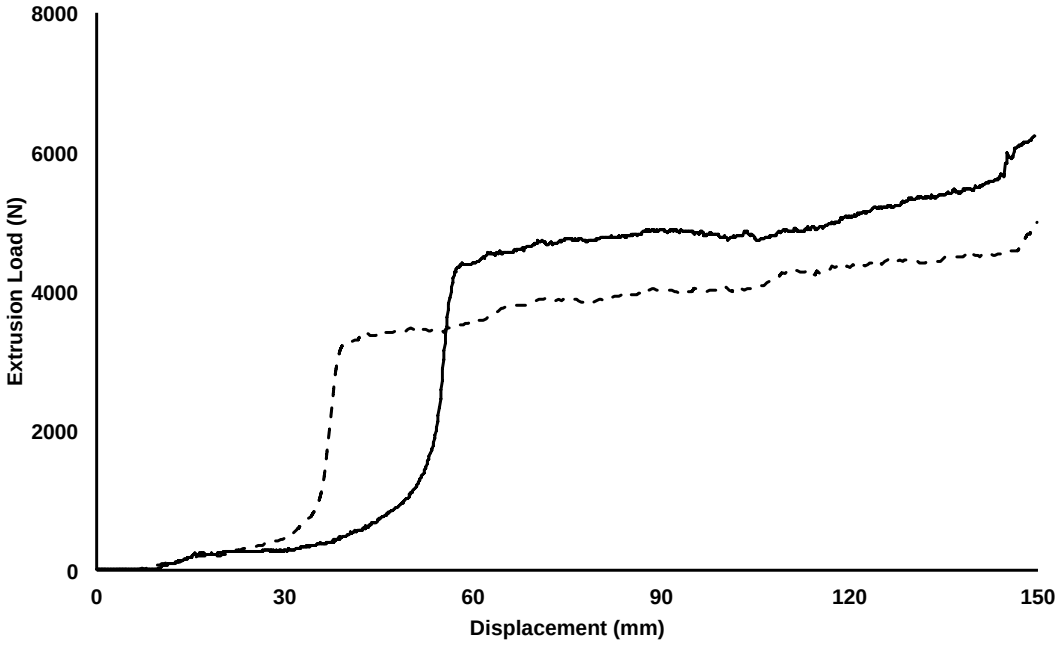



***Figure 6.1: Extrusion profiles of laminar extrudates I (▬▬) and II (▪ ▪ ▪).***

### 6.4.3. *In-vitro* permeation of coumarin and levothyroxine through excised skin

Tables 6.5 and 6.6 summarize the total coumarin and total levothyroxine permeated through the excised skin during the diffusion tests. Both drugs were release by the laminar extrudates and crossed the excised skin (barrier), suggesting the suitability of laminar extrudates for delivering drugs through the skin (permeation).

The data collected from all diffusion cells, confirmed that this diffusion / permeation of coumarin and levothyroxine through the skin presents a high variability. For example, only 2 % of coumarin reached the receptor compartment in diffusion cell nr. 3, whereas up to 18 % of coumarin crossed the excised skin in diffusion cell nr. 4. In any case, only a small amount of coumarin crossed the excised skin in all diffusion cells (between 2 and 18 %). In the case of levothyroxine, there

was not detected any drug at the receptor compartment of diffusion cell nr. 9 after 24h, whereas up to 9.5 % of levothyroxine crossed the excised skin in diffusion cell nr. 8.

***Table 6.5: Total coumarin permeated in each diffusion cell over time.***

| Diffusion cell nr. | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| **Total coumarin permeated (µg / %)** | **1h** | 36 / 1 | 30 / 1 | 33 / 1 | 104 / 2 | 29 / 1 | 11 / 0 |
| | **2h** | 59 / 1 | 35 / 1 | 69 / 2 | 220 / 5 | 49 / 1 | 10 / 0 |
| | **4h** | 127 / 3 | 77 / 3 | 139 / 3 | 599 / 13 | 89 / 2 | 35 / 1 |
| | **8h** | 298 / 7 | 149 / 4 | 59 / 1 | 351 / 8 | 184 / 5 | 106 / 3 |
| | **12h** | 371 / 9 | 215 / 6 | 52 / 1 | 447 / 10 | 207 / 5 | 175 / 4 |
| | **24h** | 656 / 16 | 458 / 12 | 77 / 2 | 794 / 18 | 598 / 15 | 417 / 10 |

***Table 6.6: Total T4 permeated in each diffusion cell over time.***

| Diffusion cell nr. | | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|
| **Total T4 permeated (µg / %)** | **1h** | 4 / 0.1 | 9 / 0.2 | ND | ND | 14 / 0.3 | ND |
| | **2h** | 50 / 1.0 | 85 / 1.7 | ND | ND | 28 / 0.5 | 11 / 0.2 |
| | **4h** | 84 / 1.7 | 175 / 3.6 | ND | ND | 24 / 0.5 | 21 / 0.4 |
| | **8h** | 147 / 4.3 | 288 / 5.8 | ND | 3 / 0.1 | 33 / 0.7 | 45 / 0.8 |
| | **12h** | 187 / 4.8 | 360 / 7.3 | ND | 9 / 0.2 | 52 / 1.0 | 59 / 1.0 |
| | **24h** | 207 / 5.0 | 470 / 9.5 | ND | 18 / 0.3 | 55 / 1.1 | 71 / 1.2 |

*ND = not detected (inferior to 2.95 µg / 0.1 %)*

This high variability might be related with the region of the skin used for each of the diffusion cells, the integrity of the skin, and the ability of each drug for permeating lipid barriers, amongst other factors.

The permeation was prolonged on time and, as referred previously, only small percentages of the drugs reached the receptor compartments.

***Table 6.7: Total levothyroxine (T4) quantified in rat's plasma.***

| Rat nr. | Total T4 in plasma [a] | | | |
|---|---|---|---|---|
| | (nmol/L) | (µg/dL) | (µg) | (%) |
| **1** | 82 | - | - | - |
| **2** | 83 | - | - | - |
| **3** | 83 | - | - | - |
| **4** | 86 | - | - | - |
| **5** | 86 | - | - | - |
| **6** | 892 | 69.3 | 10.4 | 0.02 |
| **7** | 544 | 42.3 | 6.3 | 0.01 |
| **8** | 616 | 47.9 | 7.2 | 0.01 |
| **9** | 428 | 33.3 | 5.0 | 0.01 |
| **10** | 524 | 40.7 | 6.1 | 0.01 |
| **11** | 104 | 8.1 | 1.2 | 0.05 |
| **12** | 664 | 51.6 | 7.7 | 0.36 |
| **13** | 428 | 33.3 | 5.0 | 0.25 |
| **14** | 94 | 7.3 | 1.1 | 0.06 |
| **15** | 50 | 3.9 | 0.6 | 0.02 |

[a] *These values reflect the Total T4 permeated through skin and already subtract the physiological T4.*

### 6. 4.4. *In-vivo* delivery of levothyroxine

The total levothyroxine (T4) quantified in the plasma of each group of study was summarized in Table 6.7. The rats from groups B and C presented levels of levothyroxine in plasma of 685±176 nmol/L and 352±268 nmol/L, which were significantly higher than the natural levels occurring in rats from the control group A (84±2 nmol/L).

These results proved that levothyroxine could be successfully delivered in the blood mainstream through either routes of administration (oral or transdermal).

## 6.5. Conclusions

Laminar extrudates manufactured at room temperature and in the absence of solvents successfully allowed the delivery of coumarin and levothyroxine through excised skin, proving the ability of these extrudates to carry particles of drugs for its delivery through the skin. Additionally, the same laminar extrudates allowed the delivery of levothyroxine to rats through both the oral and transdermal routes of administration, proving their potential for delivering drugs to the blood mainstream.

# CHAPTER 7

## Overall conclusions

## 7.1. Summary of findings

The bibliographic research and, especially, the laboratorial work undertaken during the concretization of this PhD project, produced a set of important findings that are here summarized:

- The RESS technology allowed the manufacture of particles of coumarin with reduced sizes;
- Both RESS operational modes – discontinuous and continuous – and the selection of the experimental conditions for each of the manufacturing procedures influenced the properties of the coumarin particles, especially their sizes and size distribution;
- The processing conditions selected for each RESS experiment influenced the properties of the manufactured particles;
- The extrusion procedure at room temperature and in the absence of solvents allowed the manufacture of laminar extrudates with good quality;
- The manufactured extrudates successfully carried and released drugs, including particles of coumarin manufactured by RESS;
- The laminar extrudates successfully delivered coumarin and levothyroxine through excised skin (*in-vitro*);
- The laminar extrudates successfully delivered levothyroxine through oral and transdermal routes of administration (*in-vivo*).

## 7.2. Achievements

Before beginning the laboratorial work, several objectives and outputs were identified as achievements to accomplish. As already described in the chapters of this thesis, the following objectives were achieved during this study:

- the manufacture of particles of coumarin with reduced sizes by RESS and its characterization;
- the manufacture of laminar extrudates and co-extrudates in the absence of solvents and avoiding changes of temperature during processing, and their characterization;
- the delivery of the manufactured drug particles with reduced sizes within the laminar extrudates prepared.

## 7.3. Verification of the hypothesis in study

All the research and laboratorial work comprised in this thesis was developed with the aim of testing a hypothesis. The proposed hypothesis was *"to produce laminar extrudates and co-extrudates, manufactured at room temperature and in the absence of solvents, containing particles of drugs processed by supercritical fluids"*, in order to overcome some of the obstacles and challenges that permanently arise during the pharmaceutical development.

After considering all these findings, which were accurately described in the previous chapters, it was observed that the work developed for both technologies – rapid expansion of supercritical solutions and extrusion in the absence of solvents and at room temperature – proved that the hypothesis is valid.

## 7.4. Added value

All the work described in this thesis added value to the existing knowledge of the supercritical fluids and extrusion research areas. The findings and outputs described previsouly were innovative as there were explored two different possibilities for engineering or designing drug particles with specific properties via RESS and, additionally, a new way of performing the extrusion procedure avoiding changes of temperature during processing and eliminating the inclusion of solvents in

the formulations, as well as developing the laminar shape for the extrudates, representing a step forward in both fields of research.

The experimental conditions selected for performing the extrusion procedure opened the possibillity for developing further applications, such as the delivery of thermo-sensitive drugs, in comparison to the hot-melt extrusion process, or the delivery of moisture / solvent sensitive drugs, in comparison to the wet extrusion procedure, or the manufacture of extrudates with a laminar shape, in comparison to the commonly used processes of manufacturing pellets.

The reduction of energy consumed in the proposed extrusion procedure in consequence of the elimination of changes of temperature during processing, and the reduction of residual solvents in consequence of the elimination of the solvents from the formulations, could be considered as important contributions for developing new green technologies.

# FUTURE PERSPECTIVES

The scientific research is an activity that never ends. There will always be new theories, new challenges, and new ways of solving the problems. The curiosity of the humanity and the need of constant improvement continuously push the science forward. And the truth will only remain the truth until the moment when a "new truth" arises and replaces the existing knowledge. Thus, a scientific project is not the beginning and never the end.

Therefore, the work developed in this PhD project, regardless of the findings described previously, can be considered as a starting point for new projects as it only addresses a part of the challenges facing the pharmaceutical development and consists of a small contribution for the knowledge in this field.

In order to continue with this work and exploring new perspectives, it would be very interesting to explore the following areas:

- Are there other materials suitable for inclusion in formulations for manufacturing extrudates with a laminar shape, at room temperature and without using any solvent?
- It is possible to deliver two or more drugs using a single laminar extrudate?
- It is possible to deliver the same drug with different release profiles using a single laminar extrudate?
- Can the laminar extrudates be used for delivering drugs through another route of administration rather than the oral and the transdermal?

There are relevant ways of continuing the work developed in this PhD project, contributing for building the knowledge in this field and transforming the laminar extrusion at room temperature and in the absence of solvents in a more relevant technique in the pharmaceutical development field.

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