CATÓLICA

UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO

Escola Superior de Biotecnologia

IMPREGNATION AND CHARACTERIZATION OF CURCUMIN INTO POLY (METHYL METHACRYLATE) RESOURCING TO SUPERCRITICAL TECHNOLOGY

by

Miguel Luís Meneses Antunes Ferreira da Silva

May/2017

CATÓLICA

UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO

Escola Superior de Biotecnologia

IMPREGNATION AND CHARACTERIZATION OF CURCUMIN INTO POLY (METHYL METHACRYLATE) RESOURCING TO SUPERCRITICAL TECHNOLOGY

Research done at ICMAB/Thesis presented to *Escola Superior de Biotecnologia* of *Universidade Católica Portuguesa* to fulfill the requirements of Master of Science degree in Biomedical engineering

by

Miguel Luís Meneses Antunes Ferreira de Silva

ICMAB-CSIC - Insituto de Ciencia de Materiales de Barcelona - Consejo Superior de Investigaciones Científicas.

Supervisor: Ana Lopez Periago

Co-Supervisor: Concépcion Domingo

May/2017

Dedicatória

Dedico esta tese à minha avó, que sempre transmitiu toda a sua luz para a minha vida, mesmo nos momentos mais escuros.
À minha mãe, pelo amor incondicional que uma mãe tem para dar.
Ao meu Pai, pelo amor parental que sempre mostrou para mim, e para a amizade e o apoio que sempre manifestou para comigo nos momentos certos.
A grandes amigos que sempre à sua maneira me colocaram a moral em cima, não preciso de mencioná-los eles sabem quem são.
A todos os demais que não estão aqui mencionados que contribuíram para finalizar esta etapa importante na minha vida.

Resumo

O seguinte trabalho centra-se no desenvolvimento de uma nova técnica que permite a incorporação de Curcumina em polímeros, técnica essa designada por Tecnologia Supercrítica.
Curcumina é um composto fenólico natural que que com o passar das gerações sempre foi utilizado como uma erva medicinal e como uma especiaria dietética por certas culturas orientais.

O polímero escolhido designa-se por PMMA (Polimetilmetacrilato). A impregnação foi bem-sucedida, revelando que o Dióxido de Carbono no seu estado supercrítico pode parcialmente solubilizar a Curcumina, e tal informação à data desta investigação (outubro de 2015) não se encontrava na literatura.

O Setup ideal dentro do reator supercrítico continha frascos com dois diferentes tipos de PMMA e o aditivo em estudo (Curcumina) noutro frasco em separado. Os parâmetros de processamento para obter uma amostra homogénea ocorreram a 150 Bar, 60 º Celsius e a 500 rpm, durante 72 horas. Ambos os frascos de PMMA e de Curcumina possuíam dentro de si agitadores magnéticos para garantir que a impregnação ocorresse de forma homogénea, e para que o polímero pudesse ter uma plasticização mais estável e previsível.

Dados recolhidos do FTIR revelaram que a integridade do PMMA foi mantida, e aparentemente a Curcumina também não parece ser afetada após processamento, contudo deteção de curcumina dentro da matriz do PMMA não pode ser verificada através de espetroscopia de infravermelho, dado rácios tão baixos de impregnação.

Os resultados de XRD mostraram que a cristalinidade da Curcumina é mantida antes e após processamento supercrítico.

Resultados de HPLC mostraram que a solubilidade parcial da Curcumina em CO_2 Supercrítico permitiu a impregnação do aditivo em PMMA, contudo as quantidades impregnadas foram muito baixas.

A quantidade de Curcumina nas amostras ótimas variou entre [0,5 – 2,42 mg_{curc}/g_{matriz}]. Os resultados revelados nesta investigação reforçam o fato da Tecnologia Supercrítica usando CO_2 como o seu solvente supercrítico poder ser uma poderosa ferramenta na criação de nano formulações contendo Curcumina, ou qualquer outro composto solúvel nele para aplicações de Drug-Delivery, e abrem mais uma possibilidade de nano formulação de sistemas contendo Curcumina, tema este que tem sido alvo de estudo há mais de uma década.

Abstract

The present work focused on developing a novel approach that could incorporate curcumin into polymers resourcing to supercritical technology. The polymer chosen was PMMA (poly methyl methacrylate). Impregnation was successful, hence revealing that carbon dioxide at its supercritical state can partially solubilize Curcumin, and such data at the time of this research (October 2015) couldn´t be reliably found in literature. The optimal setup inside the supercritical reactor comprised vials containing two different types of PMMA and the additive curcumin in a separate vial. The processing parameters to attain an homogeneous sample were at 150 Bar, 60 º Celsius and at 500 rpm, for 72 hours. Both the PMMA and the Curcumin vials had magnetic stirrers inside them to ensure that the additive impregnation could occur in a homogeneous way, and that the polymer could have a more stable and predictable plasticization.

FTIR data revealed that the PMMA integrity is maintained, and curcumin apparently is also unaffected after supercritical processing, however detection of curcumin within PMMA matrix couldn't be verified at such low impregnation yields through infrared spectroscopy.

XRD data showed that curcumin crystallinity is maintained before and after supercritical processing.

HPLC results reveal that the partial solubility of curcumin within supercritical CO_2 allowed the impregnation of the additive in PMMA, although the amounts were very small. Curcumin amounts within the optimal samples ranged from [0,5 – 2,42 mg_{curc}/g_{matrix}].

The results shown in this investigation reinforce that Supercritical technology using CO2 as its supercritical solvent can be a powerful tool in creating nanoformulations comprising Curcumin, or any other compound soluble in it for drug-delivery applications, opening another possibility in the creation of nanoformulations systems comprising Curcumin, subject that has been a target of study for over a decade now.

Agradecimentos

My deepest thanks for the experience and for the kindness of all the people at ICMAB, here I list some of all the people that were kind and supportive to me while I was there:

Ana Lopez;

Concépcion Domingo;

Julio;

Nuria Portolez;

Pedro;

Ezhila Emrit;

Ana Oliveira;

All of the people that helped me and supported me at the university, when the time was right, highlighting Professor João Paulo Ferreira, Paula Castro and Claudia Drummond.

Índice

Listas de figuras

Lista de abreviaturas

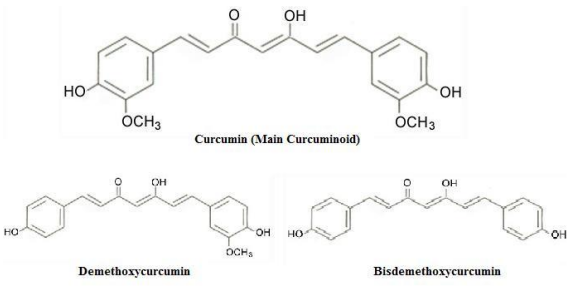
C21H20O6 – Diferuloylmethane, designation for Curcumin
TNF – Tumor necrosis factor alpha
NF-kBeta – Nuclear factor kappa Beta
CO2 – Carbon dioxide
EYPC – Egg-yolk phosphatidylcholine
PLGA – Poly (lactic-co-glycolic acid)
PVA – PolyVinyl Alcohol
PEG – PolyEthylene Glicol
MPEG – Methoxy PolyEthylene Glicol
PCL - PolyCaproLactone
PEO – PolyOxyEthylene (can be also written as POE)
SCCO2 – SuperCritical Carbon Dioxide
SCF – SuperCritical Fluid
Tc – Critical Temperature
Pc – Critical Pressure
PMMA – Poly (Methyl Methacrylate)
ATR – Attenuated Total Reflectance
FTIR – Fourier Transformed InfraRed Spectroscopy
PS – PolyStyrene
PLA – PolyLactic Acid
PP - PolyPropylene
ZnS – Zinc Sulfide
PC - PolyCarbonate
KBr – Potassium Bromide
DTGS – Deuterated Triglycine Sulfate
XRD – X-Ray Diffraction
UV – Ultra Violet
DCM - DiChloroMethane
HPLC – High Pressure Liquid Chromatography
DMSO – DiMethyl Sulfoxide
MeOH – Methanol
CH2O2 – Formic Acid
CH3 - Methyl
CH2 - Methylene
NH/H – Non-Homogeneous / Homogeneous
PET – Poly Ethylene Terephtalate

1. Introduction

1.1 Curcumin overview and state of the art nanoformulations

Curcumin (diferuloymethane) is a natural polyphenol derived from the rhizome of the Curcuma Longa Linn plant. The dried and ground roots of this plant more known as Curcuma or Turmeric have been used as a dye for foods and spices (kurry) in the Far East (1) and also as a natural therapeutic medicine in countries like India and China. The therapeutic properties exhibited by this compound, such as anti-cancer, antioxidant, analgesic, anti-inflammatory, antiseptic, among others, have caused a growing interest in the biomedical field (3). Recent studies (2) show that curcumin has a great potential against several forms of cancer that will be mentioned further. The chemical structure of curcumin is shown in Fig. 1, as well as the derivatives associated with the compound, also known as curcuminoids. The **compound investigated on this study was pure curcumin from Sigma Aldrich ($C_{21}H_{20}O_6$) (Fig. 1 on the left)**. The compound derivatives, as the figure shows, are usually 17% desmethoxycurcumin, and 3 % bisdemethoxycurcumin. The molecule in its pure natural state has the capability on itself to induce the production of a cytokine involved in systemic inflammation called TNF-induced nuclear factor Kappaβ. This cytokine has a very important role in mediating TNFα cytokine, and subsequently, tumorigenesis inhibition (2).

According to Sandurs et al. (4) the compound and its derivatives have different sets of potency for the suppression of tumor necrosis factor (TNFα) - induced nuclear factor Kappaβ (NF-kβ^{II}) activation, whereas pure curcumin has the strongest suppression rate, then desmethoxycurcumin, and the weakest suppression rate is the bisdemethoxycurcumin, which suggests that the methoxy groups have a critical role on the phenyl rings (4).



(Modified from: Changtam C, et al. Curcuminoid analogs with potent activity against Trypanosoma and Leishmania species. Eur J Med Chem. (2010))

Fig. 1 - Pure curcumin and its main derivatives molecular structure (5)

Not only in the TNF suppression, pure curcumin also showed higher efficiency than its derivatives on cardiological and neurological protection, as well as antidiabetic activities (5). Another interesting aspect is the synergy displayed between the three molecules in its therapeutic activity and efficiency, since nematocidal activity individually is lower than the three compounds mixed. Despite the good properties in fighting cancer, curcumin exhibits an enormous potential as a natural therapeutic treatment for many other conditions, related to inflammations, infections and even neurodegenerative diseases such as Alzheimer's or Parkinson's (2).

Having established throughout the years the benefits of Curcumin, from natural therapeutic agent in countries such as India and China to one of the compounds most explored for cancer therapy and other pathological issues in several research fields, different approaches have focused on the efficient extraction of the compound. Turmeric oil was successfully extracted from turmeric (Curcuma longa) using supercritical CO_2 as the processing solvent (6), whereas previous extraction approaches using volatile and hazardous solvents have shown a lower efficiency on the process as well as going against the growing green chemistry pressure in the research field (6).

The impregnation results in this research showed low reactivity of CO_2 towards Curcumin. Nevertheless, it was revealed that CO_2 is capable of partially solubilizing this phenolic compound, something that was unknown at that time, hence opening a new possibility for supercritical Curcumin impregnation using carbon dioxide. However, one of Curcumin biggest setbacks is its low solubility in aqueous systems, mainly due to the presence of methoxy groups, which strongly contribute to the hydrophobicity of this molecule (7).

Curcumin displays a big challenge when it comes to clinical applications, concerning its very low solubility in aqueous solutions, around 0.6 μg/ml and its susceptibility under alkaline conditions is high (8-10), which can cause hydrolysis and consequently biodegradation.

To improve this low bioavailability, there are several approaches, such as the use of adjuvants, formation of curcumin phospholipid complex, liposomal curcumin and other analogous structures. The following section focuses on reviewing some of these curcumin nanoformulations as well as some up-to-date applications.

Liposomes – this nanoformulation poses as one of the main approaches for curcumin bioavailability increase, consisting mainly of a phospholipid layer surrounding an aqueous core,

having the capability to load hydrophobic compounds/drugs at the same time that hydrophilic compounds can solubilize and be loaded in the aqueous core (11,12). On a recent article (13) curcumin was successfully loaded into egg yolk phosphatidyl choline (EYPC) by using an evaporating film technique (common technique using for thin-film deposition or surface coating). The cationic nature of EYPC has induced cell binding and penetration. Another interesting study was the creation of a cationic Liposome-Polyethylene glycol - polyethylenimine complex, with a considerable encapsulation amount of curcumin, around 45% (13). There is a wide variety of research articles in the biomedical field focusing on these particular curcumin nanoformulations, and the encapsulation can be successfully done with a considerable amount of curcumin, the current state of the art focuses on target drug delivery and controlled drug release, and some of these researches have already reached clinical practice (14-15).

The versatility regarding compounds that can be used to combine curcumin is a strong research point when it comes to polymeric nanoparticles. The formation of nanoparticles has already been well defined and reviewed regarding curcumin nanoformulations. There are mainly two strategies for their preparation, either by dispersion of performed polymers or by polymerization of monomers (16).

Many techniques have already been applied for nanoparticle production such as solvent evaporation, dialysis, micro-emulsion, and also supercritical fluid technology. Shaikh et al. (17) developed promising PLGA nanospheres synthetized by the emulsion-evaporation method and using PVA (polyvinyl alcohol) as the surfactant. The resulting nanoparticles had a 15 % loading efficiency, and exhibited a biphasic release behavior of curcumin. First, around 24 % of curcumin was released in the first 24 hours, then 20 % was released for the next 20 days.

Ananda et al. (18) prepared curcumin loaded polycaprolactone - chitosan nanoparticles using the precipitation method. The particles had dimensions ranging between 220 nm and 360 nm, and the size had direct influence with the loading efficiency, where the bigger the particles get the lower the loading efficiency of curcumin was. The controllable size and distribution with further experimentation can allow the desired amount of curcumin to be loaded and further on released in precise quantities.

Some recent research has focused on coupling curcumin to small molecules, mostly amino acids such as proline, glycine, leucine, valine and cysteine, in the attempt to form aminated conjugates. Research led by Tang. Et al. successfully conjugated curcumin with ethylene glycol chains through β-thioester bonds in the presence of intracellular glutathione and

esterases (19). Also through covalent bonding, curcumin molecules were linked to a block co-polymer of methoxy Polyethylene Glycol and poly (lactic acid), known also by PEG and PLA respectively (20).

There is also experimentation done on Dextran, which in other fields such as tissue engineering is used as an extracellular matrix substitute in articular cartilage tissue for chondrocyte cell growth, where by using the coacervation method (a liquid-liquid phase separation driven by electrostatic interactions) curcumin loaded nanoparticles of dextran-sulfate and chitosan were formed with a round shape ranging from 200-220 nm in particle size, being held by electrostatic interaction, with a loading capability of 75 % and 5 % respectively.

This paper shows in detail that both the chemical structure and arrangement of different compounds have a strong influence on the conjugation of the complex molecule of curcumin (21).

Micelles - Composed of amphiphilic block co-polymers that spontaneously form into a round shape with a hydrophobic core generally ranging between 20 nm and 100 nm in size, these complex structures have the ideal morphology for curcumin entrapment. Different materials used in the formation of these complex molecules, such as PLGA-PEG-PLGA (22), MPEG-PCL (23), PEO-PCL (24) have all reported successful encapsulation of curcumin, showing that this approach can be a feasible way for curcumin drug-delivery into biological systems.

Due to the molecular nature of curcumin and its derivatives, the solubility can be highly enhanced in aqueous systems when coupled to amino acids. Conjugates such as glycine, valine cysteine, among many others have been successfully coupled with curcumin (26, 33-35). The conjugation of curcumin to these small molecules has been exploited to both natural and synthetic compounds, and poses as a strong approach to enhance its bioavailability in biomedical applications.

Despite not having as much impact in nanoformulations as the ones mentioned before, not due to its lower or higher inefficiency, but mainly due to the higher complexity inherent to the formulation of viable complex structures with significant amounts of curcumin trapped with controllable release, protein carriers (25, 26, 27), cyclodextrins (28 29), solid dispersions (30, 31, 32) among other miscellaneous formulations (33, 34, 35) have already successfully coupled curcumin with substances and materials present in literature, significantly increasing the compounds stability and bioavailability, strongly preventing the hydrolysis of the compound when it undergoes physiological stress conditions.

All of these nanoformulations were approaches that succeeded in increasing the solubility of curcumin, but in an environment, that doesn't have stress conditions regarding temperature pressure and agitation. Creating biomaterials with these nanoformulations can and has been feasible, but not in a supercritical fluid media. The present work focuses on using supercritical fluid technology to achieve successful impregnation of curcumin into polymeric matrices using carbon dioxide as the fluid, in the attempt of making a viable drug-delivery device comprising curcumin. There is very little information regarding the behavior of curcumin under supercritical CO_2 as well as information regarding its solubility when in contact with CO_2 over its critical temperature and pressure points.

Curcumin in its chemical structure incorporates several functional groups. The aromatic rings (phenol) are binded by two unsaturated functional groups. The diketones (Fig. 2) strongly contribute to the stabilization of the molecule. These aspects among many others that have been minuciously described in literature (35), have shown that to uncover its full potential for therapeutic applications, there is a strong need to fight the setback of the insolubility of curcumin in aqueous solution, as well as its susceptibility to become inactivated or altered.

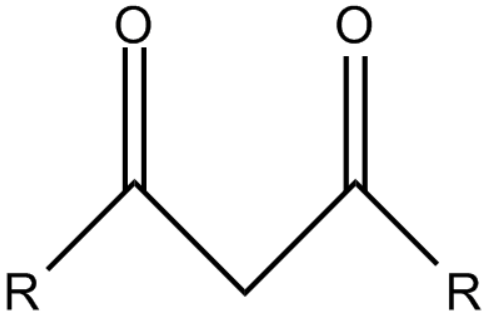


Fig. 2 – Structure of a diketone (140)

The same goes for Curcumin behavior in supercritical media. What happens when curcumin undergoes supercritical processing? Is there any solvation from the CO_2? Does the supercritical fluid causes or induces any biochemical alteration to the molecule and its derivatives? The present work focuses on answering these questions, and the following section focuses on supercritical technology principles and applications and describes in depth the principles and issues regarding impregnation procedures in supercritical media.

1.2 Supercritical technology

1.2.1 Industrial developments

Supercritical fluid technology has made an enormous progress in the past two decades. The beginning of this technology started in Germany, and initially the purpose of this new processing technique was applied for extraction only. The first big applications that achieved an industrial scale focused on extraction of caffeine from coffee beans and the extraction of hops resins (36). Initially there was no competition in the market, which allowed German industries to extend to smaller applications, like certain food processing niches such as aromas, colorants and diet lipids (37). Nowadays the competition has started to appear, and there are already full large scale supercritical extraction industries in the United States, and also some medium-scale industries spread around some countries in Europe (36).

There is currently a wide variety of applications, such as dry cleaning (37), food processing (38), the primordial and already mentioned extraction (39, 40), formation of polymer particles (41), impregnation of additives into polymers (42) or dyes into textiles (43), power generation (44, 45), and more recently, this highly tunable solvent is posing as a promising alternative for product sterilization under mild conditions (46). Not only these but many other $SCCO_2$ approaches are in progress, implemented in certain research institutes to fill in small niches and can be found all over the world in broad areas, either in pharmaceutical, biomedical or other fields that demand research and material processing (47).

There is no way to refute the versatility and strong potential of this technique, however, from the applications and strides in research to implementation in society activities at an industrial scale, the list becomes quite small.

The current ongoing industrial applications throughout the world are noticeable and most of the industries mentioned below are still up and running. Starting from the larger scale industries, Coffee beans and oil extraction facilities are operating at large scales mainly in the United States for some years now, and paint manufacture resourcing to processes such as polymer reticulation and particle atomization are also fully industrialized using SC technology, also in the U.S. (48).

Medium size facilities in France have been using for more than a decade supercritical fluid extraction and processing applied to food ingredients and for the treatment of cosmetic products (49).

In eastern countries, China and South Korea have been resourcing to this technology for the preparation of pharmaceuticals, and have already reached an industrial level due to the high efficiency pharmaceutical processing has when compared to conventional solvents processing. India is also making fast progress in implementing at an industrial level SC technology mainly for extraction of spices and aromas on large agricultural productions (50).

On a smaller scale, but still with important contribution for the society, mainly in the medical field, there are small scale industrial applications in performing bone delipidation for human grafts (51).

There has been also a growing interest in implementing supercritical technology using water as the supercritical substance by inducing its oxidation for hazardous waste destruction (51).

These approaches on supercritical fluids have been growing over the past decade, and constantly new processes using this technology are discussed, reviewed and presented on the annual European Meeting on Supercritical Fluids (EMSF)

1.2.2 Supercritical fluids

Supercritical technology translates into a series of mechanical equipment that ensures that a certain substance undergoes a change on its physical state when its matter is pushed to temperatures beyond those of its critical point. When boiling doesn't occur, the rise of temperature and pressure eventually induce a state where distinction between liquid and gas can no longer be made (52). This was first found by Charles Cagniard de latour when evaluating the discontinuities of the sound of a ball inside a cannon filled with various fluids at different temperatures (53).

No matter which substance, if it has a critical point, when it surpasses its critical temperature (Tc) and pressure (Pc), it attains unique properties. As a fluid, it has a high density, and as a gas it has a very low viscosity, and it is possible to pass from gas to a liquid or the other way around, without crossing the gas-liquid barrier, as the figure 3 shows for carbon dioxide. The solvation capability of these SC fluids for solid matter, such as polymers is very high, due to the high density that these fluids have (54).

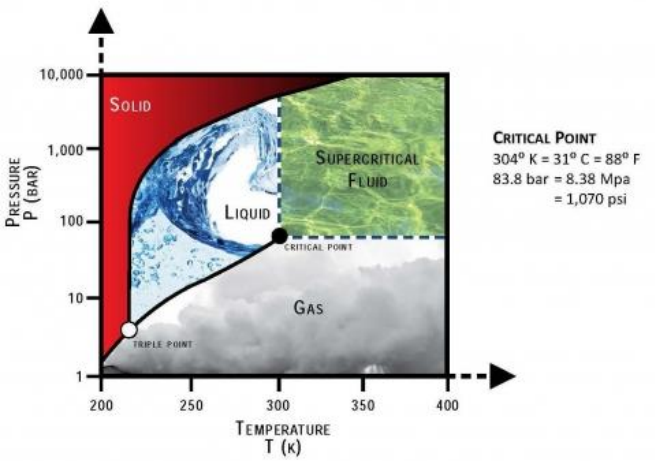


Fig. 3 – Carbon dioxide state with increase temperature and pressure

There are a wide variety of substances that can be used for supercritical processing, since many substances despite their molecular weight and chemical structure, have a critical point. However, their nature and their structural arrangement influences the critical point, displaying different temperature and pressure values among them required to match that state.

The smallest change in one of these parameters can cause the biggest spike in the density of the supercritical fluid. By having no phase boundary, there is no surface tension. These tunable solvents are, without a doubt, marking their territory as a sustainable technology for the future, by using mainly the "green" solvent CO_2 as the supercritical fluid.

There are many substances that can be employed as supercritical fluid, but in general, the substance used for this is CO_2. The growing interest in using CO_2 as the main SC substance arised from its low toxicity and environmental impact when used for chemical extractions. Not only that but the fact that CO_2 critical point can be achieved at relatively mild temperature and pressure conditions (Critical temperature – 304.25 K, 31.10 °C, 87.98 °F; Critical pressure - 72.9 atm, 7.39 MPa, 1.071 psi) only increased the viability of this substance for SC processing, since it increases the range of materials that can be used without risk of denaturation, or any type of change. Of also great importance we have CO_2 low reactivity with other molecules, and the selective solubility we can achieve by varying the pressure.

The following table shows different substances that exhibit supercritical properties when their critical temperature and pressure is reached.

Table 1. Substances that can reach supercritical states and respective critical conditions to attain it (Reid et al. 1987).

Solvent	Molecular weight	Critical temperature	Critical pressure	Critical density
	g/mol	K	mPa (atm)	g/cm^3
Carbon dioxide (CO_2)	44.01	304.1	7.38 (72.8)	0.469
Water (H_2O)	18.02	647.3	22.12 (218.3)	0.348
Methane (CH_4)	16.04	190.4	4.60 (45.4)	0.162
Ethane (C_2H_6)	30.07	305.3	4.87 (48.1)	0.203
Propane (C_3H_8)	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C_2H_4)	28.05	282.4	5.04 (49.7)	0.215
Propylene (C_3H_6)	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH_3OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C_2H_5OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C_3H_6O)	58.08	508.1	4.70 (46.4)	0.278

Attending to the supercritical fluid in the thermodynamics field, there are a few aspects that need to be considered to understand what happens with materials when dissolved or swelled in these supercritical fluids, and what makes this solvent (CO_2) in a supercritical state so special when compared to conventional solvent usage.

Reinforcing what was mentioned before, Supercritical Fluids are gases, slightly above the temperature and pressure of their vapor-liquid boundary critical point. When this SC state is achieved, the boundary between liquid and vapor becomes indistinguishable, and the solvent acquires unique characteristics. Small changes in pressure and temperature cause significant variations of the density of the fluid, which translates into a strong and tunable solvent power for the dissolution of other substances. When we are at gas-like densities, the power of dissolution is drastically slow, the molecules of the matter we want to dissolve (solid or liquid) don't interact with the fluid and dissolution doesn't occur. On the other hand, when we work at liquid-like densities, the SC fluid molecules have the capability to surround the material molecules, forming a kind of cluster around it, bringing them to the gas phase. Based on this behavior, we can have as the scheme on figure 3 shows, either a high ratio of dissolution of the target molecule, or an induced precipitation of the substance we place in the supercritical media.

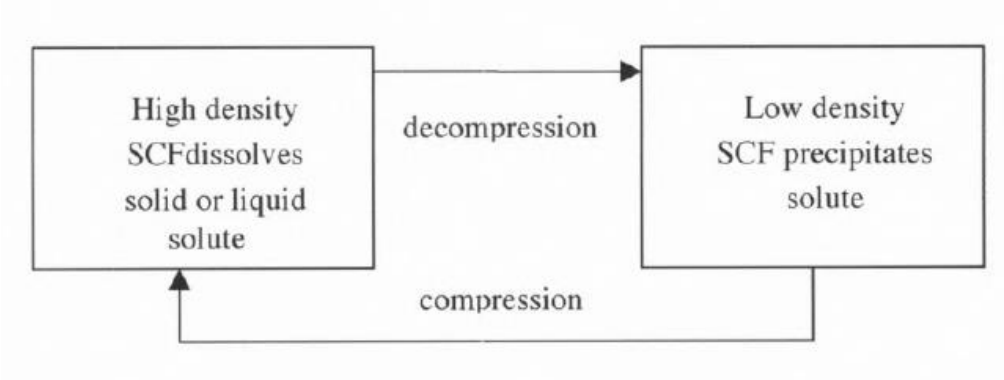


Fig. 4 - Diagram showing the behavior of matter when under high or low densities in a supercritical media (138)

By having the ability to tune the power of the SC solvent we can either dissolve all the solutes we place on a SC media, or simply achieve more selectivity on the solutes we want to dissolve by slightly lowering the density in the liquid-phase density (55). Taking the phase behavior mentioned briefly above into consideration, the challenge for a thermodynamic researcher is to fully understand the different varieties of the phase behavior, since there are a wide number of phases and compositions that can be achieved, and to understand and fully interpret the phase and the systems compositions when any process reaches an equilibrium between the fluid and the processed substance is a core issue for any supercritical application (56).

1.2.3. $SCCO_2$ in polymer processing

As it is mentioned before, the most common fluid used in supercritical technology is Carbon dioxide, due to the wide availability for it, and its nontoxicity. After any form of processing, synthesis, fractionation extraction and so on, the CO_2 can be easily removed from a system by simple depressurization, and the residue after certain processes such as impregnation is practically absent, justifying why pharmaceutical and food industries are more interested in this processing technique as an alternative to other conventional technologies. The physical properties inherent to this molecule are very attractive not only for synthesis, but also for polymer processing in material science, hence making it the worldwide most used supercritical solvent most used.

To use $SCCO_2$ efficiently as a processing solvent in polymeric applications, one of the first aspects that has to be well understood is the **Solubility** of supercritical CO_2 in polymeric materials. Information wise, there is vast literature with information regarding specific polymer

solubility when above their Tg and Tm. Tg represents the temperature point at which the material passes from a glass like texture to a soft, rubbery state, hence being called the “glass transition temperature”. When it comes to Tm, it is the melting point of the material. The information provided in literature regarding the solubility data, the Tg and Tm and specific polymer behavior, is of utmost importance for polymer processing applications such as polymer blending, microcellular foaming and viscosity analysis. In these applications, it is important to have a concrete knowledge of SC phase behavior, to avoid phase separation (which as mentioned before has direct influence in polymer dissolution or precipitation), and to keep the CO_2 concentration below the solubility boundaries (57).

In the case study regarding the main material that curcumin was impregnated on, PMMA – Poly (methyl methacrylate) is the polymer in study. Previous work has uncovered that the interactions that induce polymer dissolution of PMMA are due to the carbonyl groups of poly (methyl methacrylate), and this was verified by Fourier transform infrared spectroscopy (FT-IR) (58).

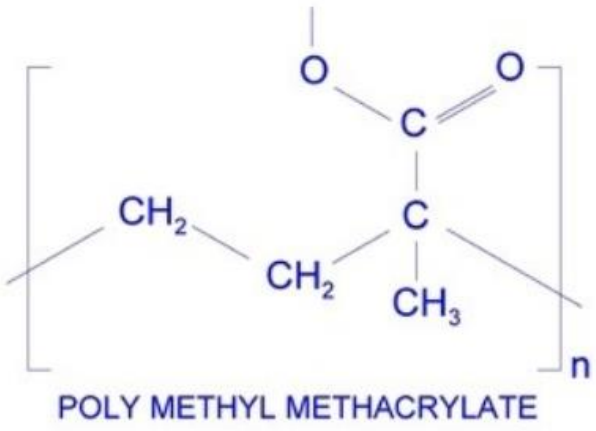


Fig. 5 – Molecular structure of PMMA (Slideshare,www.slideshare.net)

In further detail, a research focusing on the solubility of PMMA and PS over a wide range of pressures was made to understand the influence of carbonyl groups in polymer solubility. The pressure range was performed at three different temperatures, and results showed that not only the carbonyl group interactions is significantly higher above the critical point, but also the sorption capacity above the critical point is lower with increasing

temperature. According to Shieh et al. (59) this might happen due to the exothermic sorption process leading to lower sorption capacity with rising temperatures.

In general, for solubility measurements there are well defined methods that can quantify CO_2 solubility in polymers, and those are as follows:

- Phase separation method, mainly for low viscosity polymers easy to mix. Here, the polymer is exposed to the target gas pressure, and then the samples are taken from this polymer-rich phase, and from the CO_2-rich phase. The quantified amount of CO_2 in the polymer-rich phase reveals the solubility of CO_2 in the polymer, although this is only feasible for polymers with low viscosity;
- Pressure Decay Method, this method is feasible to attain reliable data because when a polymer and CO_2 are in a closed system, the polymer sorption into the solvent causes a pressure reduction over time until equilibrium is reached, hence through specific markers of pressure reduction the solubility can be correlated and estimated.
- Gravimetric method, the solubility is determined based on the weight difference between a CO_2 sample without polymer, and a CO_2 sample with sorbed polymer inside.
- Chromatographic method, where by following the principles of basic chromatography, here the measurements of solubility are done with a polymer microfilm as the stationary phase and the CO_2 as the mobile phase, and by measuring the specific retention volume of a tracer within the polymer, the solubility can be determined.

Despite these being the main methods for CO_2 solubility into polymers, there are defined theoretical approaches to accurately model solubility data, that have been the base to carry out more developed and accurate solubility estimation models, such as the Lattice fluid theory (60), the Cubic equations of state (61, 62) and off-lattice theory (63).

Another aspect that poses as a strong obstacle for polymer processing is the viscosity of the polymer. High molecular weight polymers are very hard to process in conventional solvents when they have a considerable viscosity (57), therefore the usage of CO_2 comes at a great advantage over the usage of conventional solvents, since it can cause polymer plasticization at relatively low temperatures and pressures. This plasticization can be verified by the Tg reduction, which consequently induces the viscosity reduction.

1.2.4. Supercritical technology in polymer processing: state of the art applications

A polymer is defined by a large molecule constituted by repeated units of the same monomer. They have a broad range of applications, therefore natural or synthetic polymers have important applications and utility for everyday life. As a synthetic example, polystyrene is a plastic polymer used in a worldwide scale for various applications. Natural polymers such as polypeptides, nucleic acids, and sugars have fundamental functions in practically all living organisms (64).

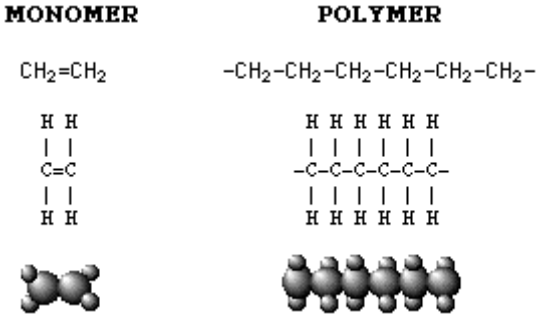


Fig. 6 - Repeated units of an ethane monomer that with an initiator forms the n-ethane polymer (Lapeer, 2010)

In the context of polymer processing using supercritical technology the following applications and possibilities using the supercritical technology will be described below.

For polymer melts, that is polymers that can melt when in supercritical media and go back to their original state without changes in their function and morphology, through supercritical processing they can either be modified, blended with other polymers or with organic/inorganic matrices (composites), foam formation and particle synthesis.

For **polymer modification**, the usage of carbon dioxide facilitates the diffusion of monomers and catalyst or initiators into the polymer matrices, mainly due to the homogeneous penetration power of supercritical CO_2. When compared with conventional solvents, CO_2 allows polymer modification of polymer melts without causing their degradation, which often occurs when volatile, hazardous solvents are used. Most polymer modifications done focus on chemical grafting into polymers, to attain properties that the polymers by themselves don't have. Successful researches have focused on modifying Poly (propylene) by chemical grafting it with monomers in order to attain better polymer hydrophilicity. Monomers such as methyl

acrylate (65), methyl methacrylate (66) and styrene (67), are examples of successful chemical grafts done on poly (propylene), and the thermal properties that arise from these experiments not only showed promising characteristics due to the chemical grafting, but also due to the plasticizing effect of CO_2. Other researches focused on additions reactions for chemical grafting have also been conducted and the feasibility of the process is reinforced by the selective dispersion that CO_2 ensures in addition reactions, where in certain cases even the fragile crystalline structure of the polymer in study is maintained throughout the whole process (68).

For **polymer blends** formation, the process is based on mixing two (or more) immiscible polymers with each other, with or without reaction among them. The non-reactive manner starts by placing two immiscible polymers in an SC media, ensuring that they reach their molten state, and when this molten state is reached two phases are formed. One phase is dispersed (in the form of droplets) whereas the other is continuous. Once again it is important to note that the viscosity of the polymers targeted for blending influences the process, this means that in the formation of blends, the viscosity ratio among them will determine the size of the droplets of the polymer dispersed into the matrix of the other (69). Investigations reported successful blends on several types of materials. PMMA is one of them. The CO_2 dispersion in the minor phase (the PMMA droplets phase) ensures a high yield of polymer transfer of PMMA into a matrix of polystyrene (PS) (70). Several researches focused on polymer blending showed positive results (71-74) and general conclusions show that the viscosity reduction (57) of the minor components targeted for blending induce a higher momentum transfer from the major (more viscous) component, which causes the minor polymer to break up into small droplets, which is the case of the PMMA mentioned above.

Polymer composites are another category for polymer processing, and the impregnation processing implies this application, since a polymer composite is formed by incorporating an organic or inorganic material into a polymeric matrix. Over the past few years researching the optimal mechanisms for polymer blending, it has been clear that the improved dispersion of one component into the other improves the results when comparing to conventional approaches. This principle is also applied in the formation of polymer composites. With higher yield, maintained structure, and unaltered polymer function, several polymer composites have been formed by dispersing particles throughout the polymer matrix, such as PMMA with clay particles (75), PLA, PLGA and PCL with calcium hydroxyapatite (76), PP and ZnS particles (77), among other polymer composites reported in literature.

One of the most revolutionary applications using $ScCO_2$ is the **microcellular foaming** of polymers, and the versatility of the materials that can be foamed can be applied to a vast number of areas, from medical applications (78) to high density circuits (79). This procedure can be achieved in two separate ways. One is **batch foaming**, where initially a polymer in a powder form is placed in contact with $SCCO_2$, with no stirring. Naturally and already described in Section **2.2**, the CO_2 that manages to be dissolved into these small particles will reduce their glass transition temperature (Tg) and their melting temperature (Tm), inducing the molten state of the polymer. Foam is formed with controlled depressurization at different sets of temperatures, and controlling temperature and pressure has direct effect on foam porosity, and reported work (80-83) has shown that increasing pressure with decreasing temperature enhances the cell number and decreases cell size (84-88).

The other way is **continuous microcellular foaming**. When we are in batch foaming, a single-phase polymer/CO_2 solution occurs and its controlled solely by the diffusion of CO_2 into a stationary polymeric matrix. The disadvantage here is that stationary processing results in low diffusion rates, consequently there is a need for long processing time in order to attain considerable diffusion into the polymer matrix which can be expensive. With continuous processing this slow diffusion can be surpassed and the diffusion of CO_2 into the matrix is significantly faster, allowing rapid mixing in a short amount of time. For this rapid mix to occur, extrusion techniques have been developed to handle viscous materials (89-92). The growing interest in controlling the foam shape for specific applications is also quite feasible due to the advantages of CO_2 in viscosity reduction, low processing temperature and short cycle times (93).

A growing approach for polymer applications using $SCCO_2$ is the **particle formation**. The most common methods for particle production are rapid expansion of supercritical solutions (RESS), Solution enhanced dispersion by supercritical fluid (SEDS), aerosol solvent extraction system (ASES) and the particles from gas-saturated solutions (PGSS). These are the main techniques where CO_2 poses as the active solvent. The gas antisolvent precipitation (GAS), supercritical antisolvent precipitation and the precipitation by compressed antisolvent (PCA) are the ones where CO_2 plays the role of an antisolvent.

$ScCO_2$ acts as an antisolvent when it has a partial or total miscibility with the solvent present in the media, but has

no solubility at all with the solute. A two-step process takes place, the CO_2 rapidly diffuses into the solution and the main solvent dissolves into the $SCCO_2$, causing a decrease in solvation

power and sub saturation of the solute, hence precipitation of the solute occurs (94).

All of these techniques are rapidly being approached and refined, since they thrive on the advantages that CO_2 has at particle size control, particle distribution and morphology. Not only that but the fact that there is no need for solvent recovery and treatment of solvent emissions or residues, $SCCO_2$ particle formation made an exceedingly more attractive alternative in modern science.

Another application to atend to, more related to synthesis instead of polymer processing is **polymerization in supercritical CO_2**. The polymerization in SC media has caught the attention of investigators to produce all sorts of polymers, such as polyamides (95), polyesters (96), polycarbonates, etc. (97). More recent research showed that CO_2 can act as a solvent and as a co-monomer at the same time (98,99). The final yield depends logically on temperature, pressure, reaction time, and solubility of the catalyst/initiator in SCCO2. However, CO_2 assisted polymerization brings the advantage of mass transfer uptake, since the viscosity reduction in SC media decreases the mass transfer resistance in polymeric reactions (due to the general viscosity of the polymer). With this said, and taking into account that viscosity reduction ratio highly depends on the solubility of CO_2 in the polymer, this solvent will directly determine the molecular weight and properties of the produced polymer.

1.2.5. Supercritical fluid impregnation in polymers

Among all the supercritical fluid applications described in this thesis, there is one common aspect that in material science makes carbon dioxide an impactful and advantageous solvent when compared to conventional volatile hazardous solvents, and that is its plasticizing effect towards polymers. However, this process is only feasible if the solute is soluble in the supercritical fluid, and if there is a favorable partition coefficient so that the target polymeric matrix can be charged with enough solute (100).

Kikic et al. (101) wrote a review regarding feasible impregnation applications with promising results, such as additive/drug impregnation into polymers (102), impregnation of dyes into textiles (103,104), chelate complexes into polymers (105), among many other studies. The data extracted from papers present in this review (101) show that the interactions

with supercritical CO_2 and the different systems that are going to be processed (polymer and solute) depend on two major points, the solubility of the solute in the supercritical fluid, and the modifications that can occur in the polymeric matrix after the solute becomes trapped in it.

However, for this entrapment we have two different sets of mechanisms based on the solute-polymer affinity. If the solute has low affinity for the polymer, the solute is dissolved in the supercritical fluid, hence loaded into the polymer matrix and depressurization ensures the trapping of the desired solute. The only negative aspect is the irregular dispersion of the solute within the polymeric matrix. On the other hand, when the solute has a high affinity to the polymeric matrix, a considerable partition coefficient with the polymer and the fluid phase can be attained, as well as a homogeneous dispersion. This represents a tremendous potential in impregnating drug molecules into polymeric matrices, mainly for drug-delivery applications (101).

State of the art applications for impregnation are in constant development, mainly research for controlled drug delivery. Supercritical impregnation systems have shown to have a more stable behavior in molecular structure and drug-release behavior when compared to conventional immediate drug release (106). For biomedical applications, almost 40 % of impregnated drugs for human body insertion are made of materials that are water insoluble. Methods to overcome this setback are in constant development nowadays. A good alternative to overcome this is the increase in surface area of the material, consequently enhancing the rate of dissolution. The usage of aerogels is a promising alternative to improve the rates of dissolution of additives/drugs in the human system, due to their very low density and high porosity (107). An aerogel is a solid material with high porosity, gel-derivative, whose liquid part is replaced by a gas. The material processing is designated by sol-gel, and the processing involves an initial phase, where a precursor is added to a solution, forming the designated solution. Afterwards, a polymerization occurs, causing the formation of a gel-like structure. In a final step the gel can be dried (107), acquiring its very low density properties. For producing a high surface area in aerogel production SCF is the most effective method. A very common material for aerogel production is silica.

These aerogel structures are frequently used as catalysts (108), carriers of active substances (109), insulators (110) and for microelectronic applications (111). Being aerogel one of the most advantageous form of material for drug-delivery, and impregnation one of the most efficient methods for drug/additive insertion into a polymeric matrix. Many strides

with new compounds and novel approaches have recently been achieved in the aerogel impregnation area.

Giray et al. (112) impregnated ketoprofen, an anti-inflammatory drug used in the treatment of acute and chronic rheumatoid arthritis and osteoarthritis, into hydrophilic and hydrophobic silica. The solubility of this active compound in CO_2 was reported to be 0.2 wt.% at 40 ºC and 180 Bar. The impregnation reached 25% in the hydrophilic silica, whereas the hydrophobic showed lower values, around 16%. Similar impregnation procedures have been done using silica as the target matrix, such as Griseofulvin (antifungal drug) for skin and nails fungal infection, achieving a total loading of 11.2 wt.% (113,114,115). Ibuprofen was also used as a model drug for impregnation analysis on silica hydrogels (116,117,118). More recently, metal particles have been widely impregnated onto materials, to overcome the difficulties over control in particle dimensions, size and distribution. The Supercritical fluid deposition method (SCFD) is a feasible alternative for other conventional methods where the parameters mentioned above cannot be properly controlled, such as micro emulsion (119), ion-exchange, etc. (120,121).

PMMA was the polymer used for the impregnation processes in this research. This material is a rigid thermoplastic widely used in the market, since it is very resistant, light and highly shape tunable. Also, the recycling of processed acrylates is economically viable, hence being used at an industrial scale in several areas. The versatility of the compound and the low inflammatory response in the human system, has caught the interest of many fields, where some of them such as lens replacement in the treatment of cataracts (122), orthopedic industry (123), among others, use this material on a regular basis. Several other applications resources to this material, although not for research purposes, more due to the basic transparent look of the material, where the appearance and the properties of it pose as an interesting type of material to build all sorts of eccentricities (124).

When it comes to its relevance in the biomedical field, the range of investigations that gave use to this material is vast and versatile, hence the interest of supercritical processing using this acrylate. In more recent research, aside the fact that there are several papers that have already reviewed PMMA behavior in supercritical media (125), a recent impregnation approach for ophthalmic drug delivery uses PMMA as the template matrix, with an impregnated additive mentioned below. The patient undergoes an eye surgery, and the risk of inflammation is considerable, although with the use of this controlled released system, the data in the article revealed that the drug-release system was capable of releasing for 3

months the impregnated additive Flurbiprufen, strongly reducing the inflammation risks after the operation (126).

The usage of PMMA for microcellular foaming was achieved by Settnes et al. where the formation of a PMMA graphene oxide was successfully achieved by having supercritical CO2 acting as the foaming agent (127). This research revealed that the controllable cell size, distribution and the physical properties of the foam only reinforced the potential of microcellular foam investigations allready validated in the past, not only with PMMA (128), but also with other polymers such as Polystyrene (PS) (129), Polycarbonate (PC) (130), Polypropylene (PP) (131) and Poly lactic acid (PLA) (132).

Not only these structures reveal good potential for clinical applications, but recent investigations have showed the strong possibility in using certain nanoparticles to enhance specific properties for the target study. The usage of nanosilica (133) and graphene (134) are common choices to enhance the properties of a foam without altering the properties of the polymer (135).

The impregnation of PMMA has been widely used for different circumstances, involving different additives regarding the application in question, however, S. Diankov et al. (136) worked on a drug that has a strong importance for standard daily medical treatment, and that is salicylic acid (o-HBA), a common drug used for pain relief, due to trauma, surgery, or disease associated symptoms. With this being said, the o-HBA was successfully impregnated in a matrix of PMMA, with a total uptake of 27mg/$g_{polymer}$, and the optimal conditions were achieved at 40 º C, and 12, 16 and 20 MPa, with concentrations of HBA ranging from 0.05 to 0.7 mg/g. The reproducibility was below 3.2% of the solute uptake.

The feasibility of this particular impregnation process revealed in this master report, using curcumin as the drug and PMMA as the matrix, was analyzed in this paper, as well as the possible structural changes that the matrix and the additive underwent. The main objective here was understanding if Curcumin can be dissolved in Supercritical CO_2, and if so, to make a homogeneous drug-matrix system and characterize its stability and functionality.

2 Materials and methods

2.1 Materials

Provided by good fellow and Bonar Polymers respectively, $PMMA_{GF}$ and $PMMA_{BP}$ were the materials used as the matrices for the impregnation procedure.

The Curcumin used was pure Curcumin from Sigma Aldrich, stored at 4 ºC.

$PMMA_{GF}$ Poly (methyl methacrylate) from Good Fellow

$PMMA_{BP}$ Poly (methyl methacrylate) from Bonar Polymers

The PMMA used and processed in supercritical media was in the form of beads ($PMMA_{BP}$) or in the form of rods ($PMMA_{GF}$). Both PMMAs had a molecular weight of around 300,000 g mol^{-1}.

The chemicals comprised in the impregnation process, for loading analysis included Acetone (99.5%) from Fluka, 37% (w/w) hydrochloric acid, Dichloromethane and ethanol (all from Merck, a.r.). Carbon dioxide (99.995%) was supplied by air products, Carburo metálicos group.

For cromatographical purposes the eluent used was methanol (Fluka, 99.8%) and formic acid (Fluka, 98%), and the organic solvent used for quantification was Dimethyl sulfoxide (DMSO, 99.1%).

2.2 Methods

2.2.1. Synthesis of Curcumin

Pure Curcumin was synthesized, following 1964 Pabon's method for Curcumin and related compound synthesis (137). Minor adaptations were performed and are described below. An initial solution was made by having 100 ml of ethyl acetate added to 0.50 g of boron trioxide (B2O3, Sigma-Aldrich) and mixed. To this solution 9.20 g of terbutylborate (Aldrich, 99.0%) and 3.0 g of vanillin (0.40 mol) were added. The resulting solution was stirred at 400 rpm for 30 minutes at 40 º C, for three hours and then left overnight at room temperature. The day after a solution of 0.50 g of n-butylamine (Sigma Aldrich) and 10.0 ml of ethyl acetate were added dropwise.

To this resulting mixture, after a yellowish color starts to appear, reaction catalyzer was added (tributylamine) then left at room temperature with stirring for 72 hours. According to Pabon's method the amount of catalyzer added should be on a 1:10 ratio of total solution.

After the addition of catalyst, a solution of 30 ml of hydrochloric acid (0.4 M) was diluted from concentrated HCl (37%) and added to the mixture. From this point a phase separation process was performed (Figure 5). According to Pabon et al. method, it is recommended to use ethyl acetate to induce layer separation, although the approach on this synthesis process was different. To the mixture 50 ml of dichloromethane was added, and later on, 50 ml of water were added, then again 50 ml of dichloromethane, in order to attain a more stable and efficient separation phase and, consequently, a higher yield. Using a Sartorius filter and a filtering setup, 0.834 g of curcumin were filtered off the solution and afterwards, washed with methanol.

Fig. 7 - Phase separation

Fig. 8 - Pure crystallized curcumin

The pure Curcumin obtained, as the figure 6 shows, resembles small needles. Morphologically the product obtained was highly crystalline, meaning that despite the fact that the synthesis was a success, and the product at hand was highly pure (crystallized Curcumin), it was impossible

to use it for Supercritical processing since Supercritical Fluids have a direct influence in altering the crystallinity substances in this particular state (138).

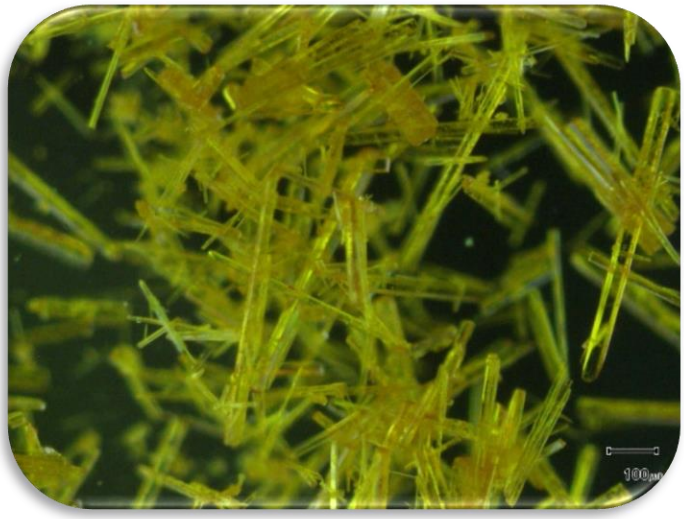


Fig. 9 – Synthetized curcumin in crystalline form

2.3. Supercritical Impregnation equipment and procedure

The impregnation process in $SCCO_2$ was performed in a high-pressure system. The batch system is displayed on the image below (Fig. 7). The system had three reactor pathways, and the ones used were a tubular reactor of around 120 ml of total volume, and a wider reactor with a visualization chamber and a volume of around 200 ml. The system is composed by a CO_2 bottle (GASIN, air products group), a cryostat (LAUDA Ecolinex Star edition RE106), a syringe Pump (Teledyne ISCO D-series model 260D) coupled with a pump controller (D-series Pump controller). For this research, two reactors were used, a tubular reactor vessel (Autoclave engineers certified, 2900 PSI, 600 F, 2005) and a wide reactor vessel with an observation chamber (Thar Designs, Inc. USA). The heater used was a PID Engs. Tech equipment, coupled with monitoring internal and external temperature parameters.

The high pressure isolated equipment (Fig. 7) was ensured through tubing, needle valves (Autoclave engineering) and ball valves (Parker). Pressure indicators of the system (Nuova firma) and pressure indicator of the reactor (Mc Daniel controls) were placed in several sections of the Supercritical equipment, and in case of high risk situations, rupture disks (Oseco, HALMA group company) were installed in key points of the setup for safety measurements regarding the pressure capacity that the supercritical system can withstand.

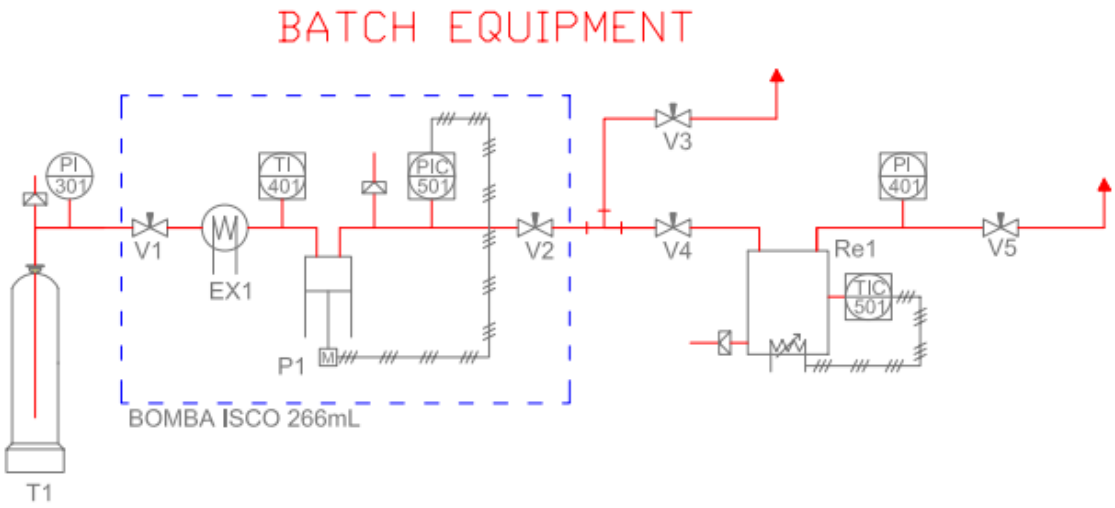


Fig. 10 – Schematic of the batch Supercritical system

Fig 11 – equipment used for the supercritical impregnation process

PMMA beads were wrapped in a flat paper set up with 0.45 Micrometer pore size, and the curcumin was placed in a vial and a stirrer was placed in the reactor. From this point, in every trial, when the set up was ready inside the reactor, the system was then pressurized using

the syringe pump and heated using a resistance around the reactor surface until desired pressure and temperature were attained. The same approach was done several times until the ideal parameters (Temperature, Pressure, Stirring, Co-solvent) could be found for Curcumin impregnation on PMMA. The optimal samples obtained were reimpregnated from previous trials. The setup schematic is shown in the following figure (Fig. 9), and the last optimal setup of the windowed reactor is also shown in the image on the right (Fig. 10). All of the vials had stirrers inside, and on the right image PMMA particles were directly placed on the vials, no paper setup was used for the impregnation procedure on the optimal samples.

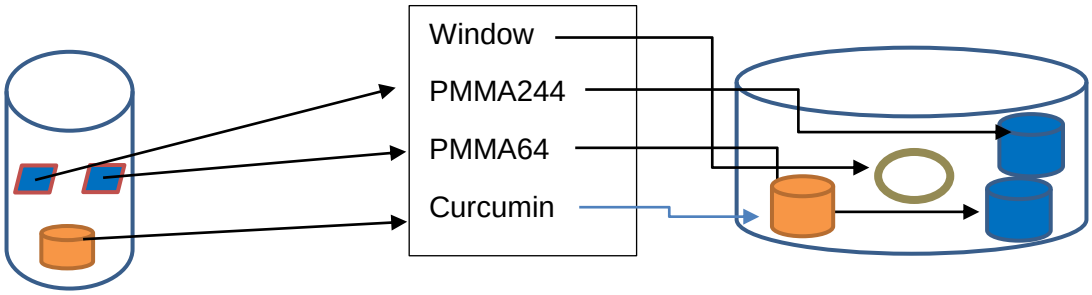


Fig. 12 – Setup of SC tubular reactor

Fig. 13 - Setup of the optimal samples on the windowed reactor

2.4. Characterization

Processed PMMA in supercritical media underwent through characterization techniques to provide structural and biochemical data of the processed samples.

The interactions between the polymer and Curcumin were analyzed with ATR-FTIR spectroscopy. For FTIR, pellets of potassium bromide (KBr) comprising the sample were made by using a PerkinElmer presser. The data obtained was registered using a Bruker Optics Equinox 55 spectrometer, with a Golden Gate ATR accessory from Specac Ltd. And a deuterated triglycine sulfate (DTGS) detector, with a resolution of 2 cm^{-1}.

For optical evaluation and characterization an Olympus BX51 Microscope was used coupled with a Nikon camera and a replacement burner (Olympus). The microscope was equipped with a fluorescent device (Olympus U-RFL-T), an image management/control machine (Olympus DP20), and a computer wiring interface (VOSTRO) model from dell.

Curcumin and processed polymers were subdued to XRD analysis for evaluation of crystallinity before and after supercritical processing. Analysis was performed in a powder diffractometer (Siemens).

2.5 UV analysis

After impregnation of curcumin into both PMMA's, ultraviolet analysis was performed in order to provide an estimation of the amount of curcumin impregnated into the polymeric matrix. The analysis was performed using a Cary 5000/Varian device with a Varian Ibérica S.L. spectrometer. The solvent used for Curcumin calibration curve was DCM. Around 50 mg of sample were added and placed into vials, 5 ml of DCM were added, and after being centrifuged and filtered, the absorbance at 261.6 nm and 417 nm were determined and registered in the equipment. Due to similar purposes and more reliability, the quantitative data used for discussion came from HPLC analysis, due to its precision and more reliable procedure. Nevertheless, UV data is displayed in the **annex I** of this paper.

2.6 HPLC data

For accurate estimation of the amount of curcumin impregnated in the PMMA matrix, HPLC was performed using Agilent technologies 1100 series and 1200 series equipment. Separation was done with the coupled C18 column of the equipment. A calibration curve was made with DMSO and curcumin with concentrations ranging from 1 ppm to 20 ppm from a mother solution of $10mg_{curcumin}/10ml_{DMSO}$. For separation purposes MeOH 67% and CH2O2 33% were used as the mobile phase solvents, and the retention time for all samples were approximately 7.5 minutes.

3 Results/Discussion

For the first trial, it became clear that CO2 in its supercritical state displays a partial solubility with curcumin, due to the yellowish color that PMMA acquired after the supercritical processing. Such data couldn't be found in literature.

Hence, further trials and experimentation focused on optimizing the supercritical process to attain a homogeneous impregnation. The **annex III** in the annex shows all the trials regarding processing parameters, setup arrangement and usage of co-solvent.

However, the interactions between supercritical CO2 and curcumin are still in an initial state when it comes to its understanding, and very few research has focused on analyzing the solubility of curcumin into CO2, as well as the possibility to impregnate this additive in polymeric matrices using supercritical technology.

3.1 Electronic microscope data

Microscopic images were taken to the samples after impregnation. It is clear that the aggregated micro particles are homogeneously impregnated. An interesting aspect that was observed is the core of the PMMA transmitting the light that comes from beneath, showing how troublesome it can be with this polymer to attain a homogeneous plasticization. This can be justified due to the nature of the PMMA material. PMMA transmits light up to 92% in its natural form (84). And the samples observed in the images are after Supercritical processing. Giving that PMMA is a strong polymer (rigid matrix highly dense and cross-linked), the plasticization of the material doesn't occur homogeneously when in supercritical media. It first plasticizes around the surface, and gradually plasticizes to the core of the matrix, justifying also the difficulty in obtaining homogeneous samples. The swelling of SCCO2 in this material requires a lot of time, and as the image shows, curcumin is easily impregnated first in the periphery part of the material and gradually gets impregnated further into the core of the matrix, showing that the impregnation not only depends on the solubility of Curcumin with supercritical CO2, but also on the time required for plasticization and swelling of the polymer.

Microscopic images were taken to the MAS-004 samples in order to observe the homogeneity. It is noticeable that a big portion of the PMMA aggregated micro particles are homogeneous regarding the curcumin impregnation.

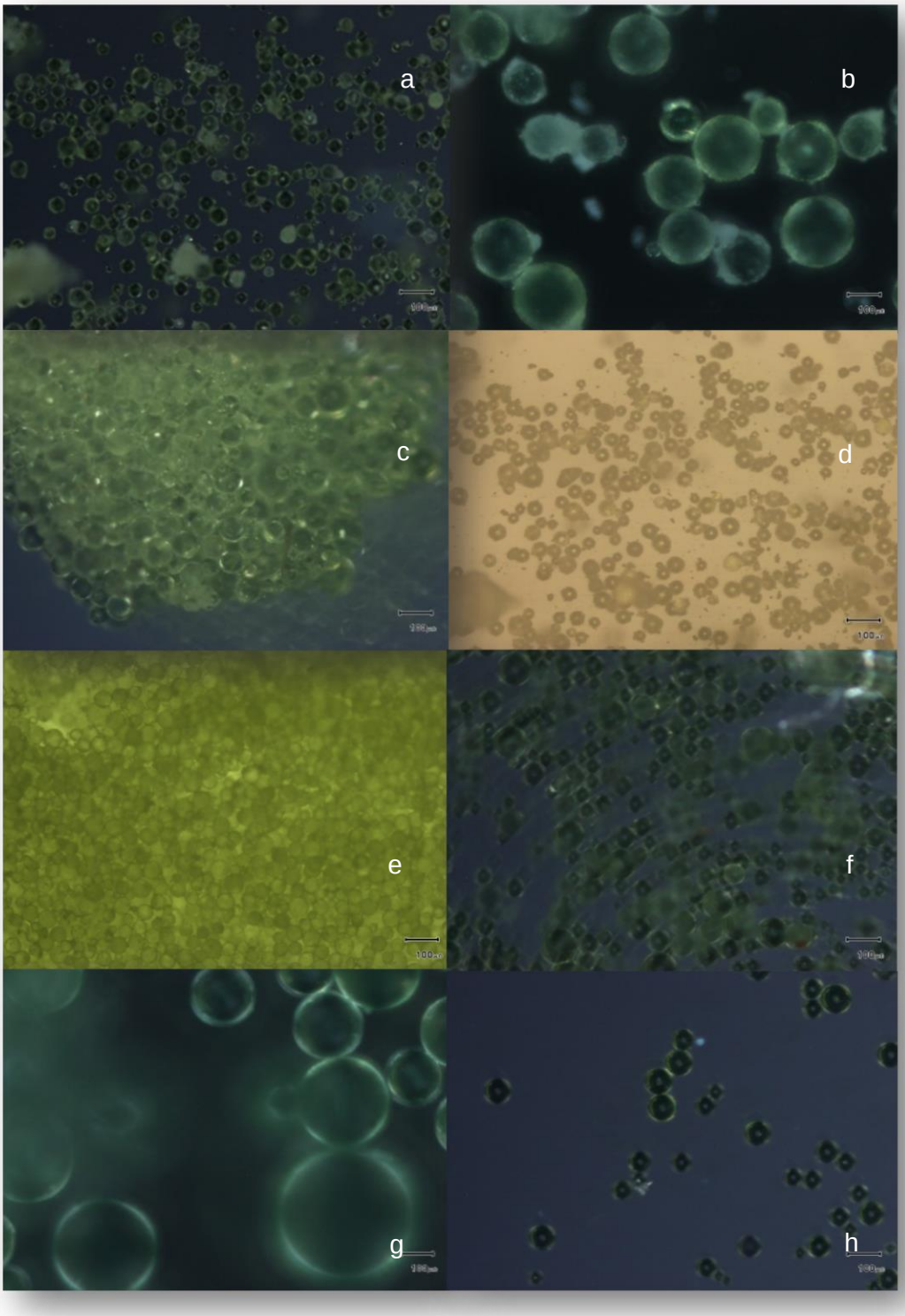


Fig. 14 – PMMA 64 (a-d) and PMMA 244 (e-h) impregnated with curcumin

3.2 ATR/FTIR characterization

Characterization of the samples after SC processing were first evaluated through ATR and FT-IR. Both have the same application in this case, to analyze the interactions between our polymer (PMMA), and the impregnated additive (curcumin), however, FT-IR penetrates through the whole sample, whereas ATR only ranges in 20 micrometers in depth (ideal for materials with high porosity). PMMA is a non-crystalline polymer with a highly cross-linked matrix, therefore usage of FTIR should provide more information regarding structural changes and interactions.

After the first impregnation, the sample was first analyzed with ATR spectroscopy, to see if there was any sign of curcumin present in the sample, to unravel any structural change in the material, and to see if Curcumin structure remained intact.

As the ATR chromatogram below shows (Fig 15), there are characteristic peaks of PMMA in the chromatogram that are identical for the processed PMMA and the PMMA in its natural state. Given that ATR is more useful to porous structures, which is not the case, FT-IR was performed. Nevertheless, the ATR spectra was enough to show that PMMA didn't suffer any structural/chemical change, as well as no presence of curcumin typical peaks within the impregnated matrix spectra.

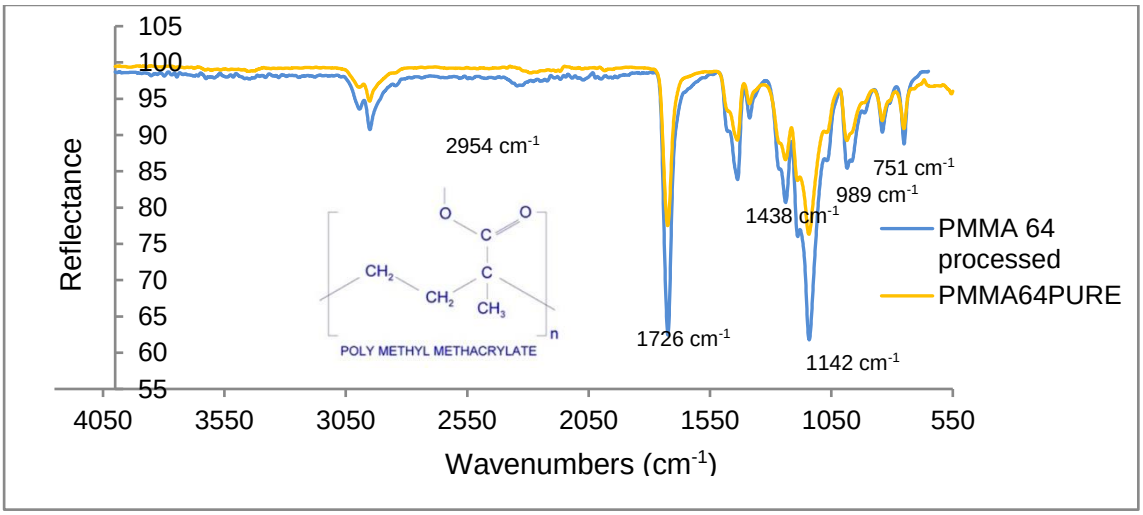


Fig. 15 - ATR of pure and processed polymer

The same steps were made for processed and non-processed curcumin (Fig. 16). After analyzing the spectra, it shows that pure Curcumin from Sigma-Aldrich didn't reveal any new interactions, or molecular denaturation/alteration.

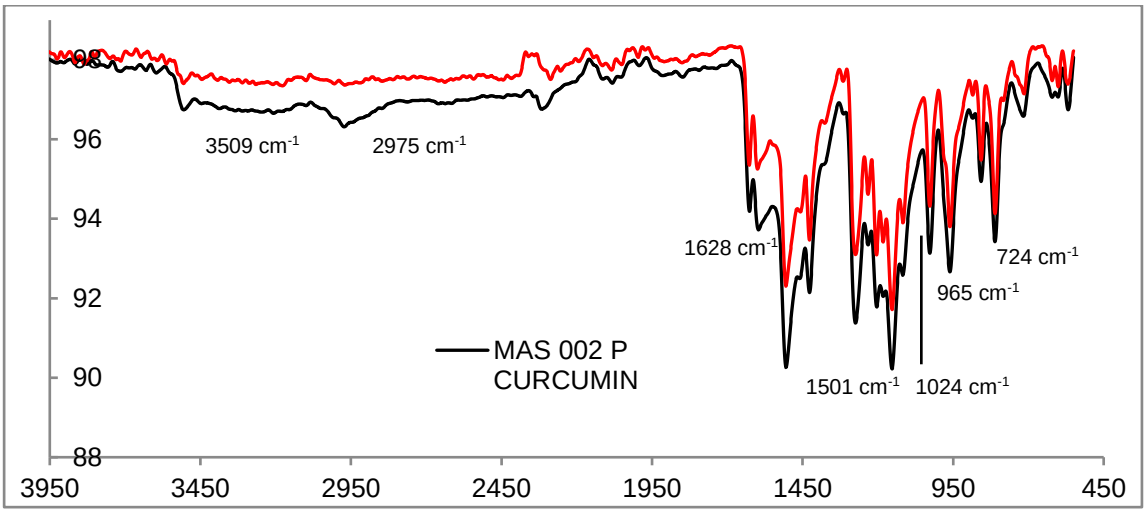


Fig 16 – ATR of pure and processed Curcumin

To analyze the interactions between curcumin and the PMMA polymer, as well as the presence of the additive within the polymeric matrix, Potassium Bromide pellets were prepared and FTIR was performed. For the first three trials, the data chosen was from the second trial MAS-002 (fig 17), for better observation of the peaks.

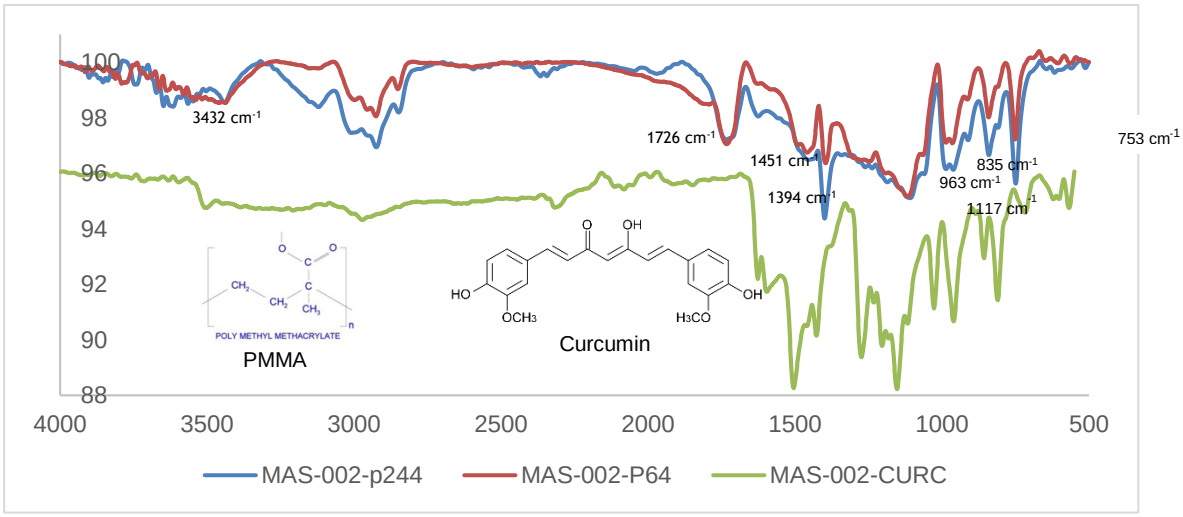


Fig 17 – FTIR of processed curcumin and samples from MAS-002 trial

3.2.1 PMMA FTIR characterization (MAS-002)

By analyzing the images above, it can be observed that at 835 cm-1, 963 cm-1 and at 1117 cm-1 we have typical PMMA vibrations that can be found if the polymer is in a normal state.
Two typical vibrations can be found at 753 cm-1 and at 1394 cm-1, which are associated to the alpha-methyl groups. A distinct absorption band between 1095 and 1203 can be observed, associated with the C-O-C stretching vibration.
Around the 1451 cm-1 region, it can be observed the bending vibrations of the C-H bonds of the CH3 and CH2 groups.
Two weak absorption bands at 1726 cm-1 and 3432 cm-1 can also be found in the spectra, associated to –OH stretching and bending vibrations. The presence of these peaks show that we have a typical characterization of macromolecular PMMA.

3.2.2 Curcumin Characterization

Regarding the ATR spectra shown in Fig. 16, results indicate that curcumin quantities are not significant enough to be shown in the spectra of the MAS-002 processed samples, and we can verify the absence of structural changes for both the additive and the polymer. The presence of –OH can be evidently found at the sharp peak shown in the spectra at 3509 cm^{-1}. At 1628 cm^{-1} there is a predominance of C-C and C-O stretching behavior. At 1501 cm^{-1} a strong peak can be found due to the aromatic ring stretching vibrations. At 1024 cm^{-1}, like the PMMA there is a C-O-C peak.
Trans –CH vibrations can be found at 965 cm^{-1} and cis -CH vibration of aromatic ring can be found at 724 cm^{-1}.

Regarding the FTIR analysis, the presence/absence of curcumin inside the polymer cannot be verified, because the amount of Curcumin that we can impregnate using supercritical CO_2 as the solvent was very small, as HPLC results will show. This means that even if characteristic curcumin peaks were not observed in the PMMA spectra, the curcumin is present, just not in enough quantity to be detected. Matching peaks observed between the processed polymer and

curcumin are associated with similar atomic stretching / bending behavior, such as C-O-C peak, -OH vibrations etc...

The FTIR analysis was conclusive when it comes to the integrity of the polymer (PMMA) and the additive (curcumin) becoming unaltered before and after supercritical processing. When it comes to the impregnated Curcumin to be detected within the polymer processed sample, the obtained FTIR results don't reveal such data.

3.3 XRD

X-ray diffraction is a technique that allows the identification of the molecular structure of molecules. In depth analysis using this technique can provide us the exact structural arrangement of any molecule, because the incident monochromatic ray beam is diffracted in several directions, and using Bragg's equation to calculate the possible directions of the diffracted beams, we can estimate a structure of our molecule.

In this investigation, XRD was used for detection of Curcumin in the processed PMMA, and confirmation of structural integrity of our additive. Since PMMA is an amorphous compound and curcumin is a crystalline one, the diffraction (Fig. 18) is very distinct between them both. Crystalline compounds reveal very sharp peaks in XRD, whereas amorphous molecules don't display sharp peaks. Therefore, the possibility of observing sharp peaks in the impregnated samples could indicate the presence of curcumin.

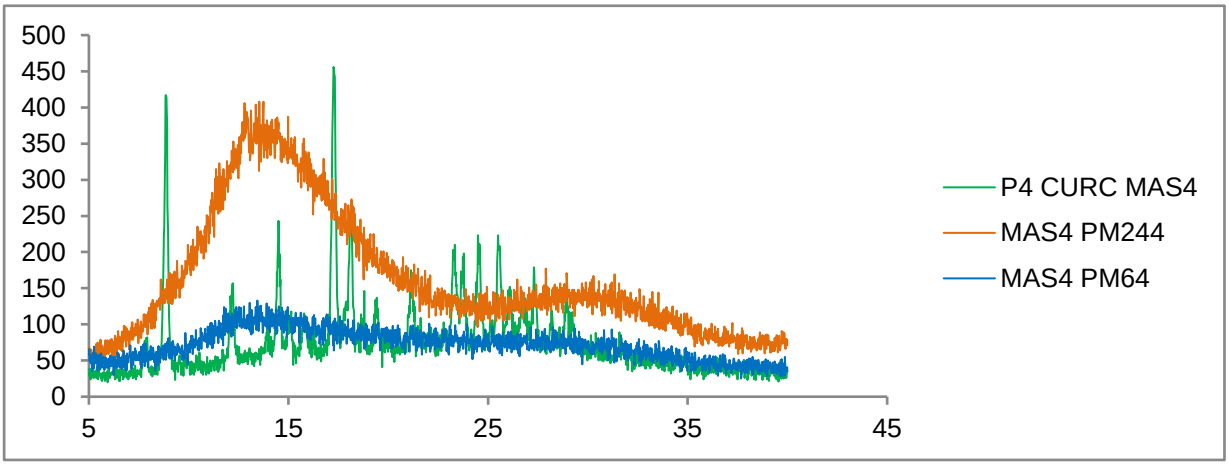


Fig. 18 XRD data of processed Curcumin and first homogeneous samples

The processed samples did not display any crystalline behavior, showing that curcumin impregnated amounts are not significant enough to be detected. However, the crystalline structure of pure curcumin after being processed alone in $SCCO_2$ doesn't appear to be affected in supercritical media, meaning that the undetected crystalline peaks of curcumin are not displayed due to very low impregnation amounts into the samples. It is to note though that crystalline compounds under supercritical media generally undergo structural changes, not only that but supercritical technology has been used to enhance crystal growth (138).

3.4 Determination of Curcumin in impregnated samples (HPLC analysis)

The processed samples underwent through two quantification approaches, UV analisys and HPLC. In terms of reliability of results and accuracy, the data chosen was HPLC. As mentioned before UV procedure and results can be found in the **annex I**.

The optimal samples selected for HPLC measurements were the reimpregnation of MAS-010 and MAS-011, each PMMA type was impregnated in the reactor with an observation chamber (window) with two separate vials with a stirrer inside, to ensure homogeneous results. The samples taken for HPLC quantification were the reimpregnation trials of both PMMA 244 and PMMA 64 (MAS-012 and MAS-013).

A calibration curve of Curcumin (Fig. 19) was previously made with concentrations ranging from 0.1 ppm to 20 ppm. 10 mg of curcumin were solubilized in 10 ml of DMSO, the solvent used for this HPLC analysis, and from this mother solution dilution were performed until the desired concentrations for the calibration were achieved and quantified (Fig. 20).

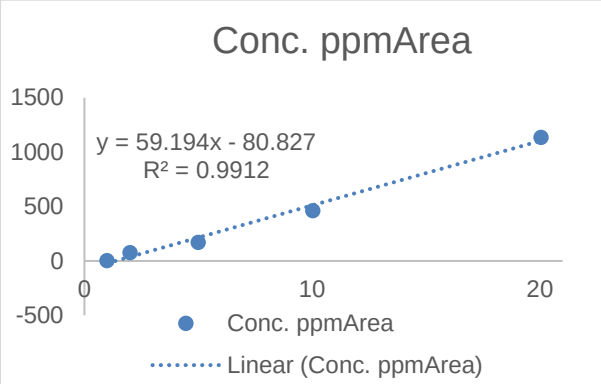


Fig. 19 - Calibration Curve of Curcumin

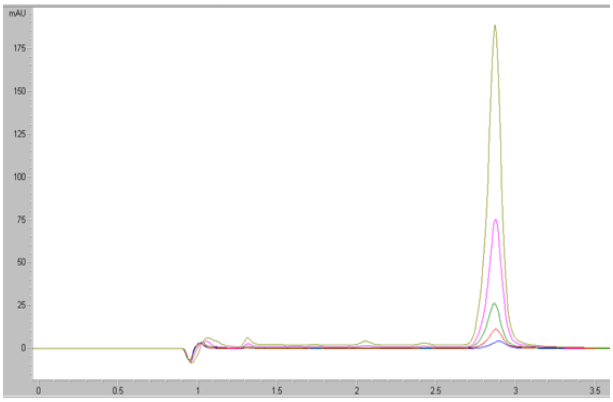


Fig. 20 - Standards of Curcumin

The image on the right (Fig. 20) represents the separation peaks of different concentrations of the DMSO/Curcumin solution, for the calibration curve. It is to note that aside the main peak, there are concentration dependent side peaks that were not supposed to be shown in this chromatogram. The plotted chromatogram displays the dilutions performed of pure curcumin. The small side peaks observed can be justified either by structural changes (denaturation such as hydrolysis, side reactions, etc.) that might have been caused with the use of DMSO, or simply by the fact that curcumin is recommended to be storage at -20 ºC, although it was storaged in a fridge and exposed several times to room temperature for experimentation purposes. Despite these possible factors, curcumin is very unstable in solution form, and its stability is stronger in acidic Ph, the more we increase the Ph the more unstable it becomes, since the increase of Ph induces dissociation of the molecule. Light is also a strong factor in curcumin stability, where the absence makes it more stable. What we see in the chromatogram can be ferulic acid or vanillin, which are byproducts of curcumin degradation (138).

The DMSO is a polar aprotic solvent, which logically has a stronger solvating effect on polar substances rather than apolar. The solvating power of this solvent was effective towards curcumin, however, PMMA wouldn’t fully dissolve.

To overcome this setback and try to fully solubilize curcumin, Dichloromethane was used to dissolve the remaining mass of the samples, and consequently to release the remnants of the impregnated curcumin. After the samples were fully dissolved in DCM, nitrogen was used to evaporate the remaining DCM from the HPLC vials (Fig. 21). This HPLC approach to quantify curcumin within the samples with DMSO revealed that despite DMSO being a solvent with very good characteristics for polymer dissolution, it might show a certain reactivity with

curcumin, hence a side reaction might have occurred in MAS 13 sample, for the impregnated PMMA64, which might have caused the orange color shown in the image below.

Fig. 21 - HPLC vials after DCM evaporation; Left to right (13 P244; 12 P64; 12 P244; 13 P64)

Using 67% of Me OH and 33% of Formic acid in the mobile phase, the results obtained are shown in the table below:

Samples	Sample mass (mg)	g/gmatrix	PPM
MAS-012 P64	10.13 mg	0.002681	21.448
MAS-012 P244	10.05 mg	0.000527	4.2183
MAS-013 P64	10.08 mg	0.001961	15.689
MAS-013 P244	10.11 mg	0.001057	8.4550

Regarding the HPLC results, data obtained shows that the impregnation trials that were homogeneously obtained could retain between 0.5 $mg_{curcumin}/g_{matrix}$ to 2.42 $mg_{Curcumin}/g_{matrix}$, which matches to a wt.<1%, proving that despite the partial solubility that supercritical CO_2 has with curcumin, the results obtained show that there is indeed a low yield of impregnation, therefore curcumin can be partially solubilized and trapped into the PMMA polymeric matrix, although at very slow rates.

Variations in pressure and temperature allow us to control the density of the fluid, making $SCCO_2$ a liquid-like fluid that exhibits solvent properties towards polymeric materials, such as

PMMA. In this fluid phase, where the polymer is swelled by the supercritical fluid, drug solubility in supercritical media and the affinity of the additive towards the polymer are key aspects to obtain high impregnation yields. The reason for curcumin to be slightly solubilized in this Supercritical impregnation process is unknown. However, in general Supercritical CO_2 has bigger solvating potential towards **apolar polymers than pola**r. Even though PMMA is apparently polar since it has an ester in its molecule, it mainly consists of a non/polar backbone (such as a hydrocarbon backbone), hence in overall the polymer is apolar, displaying hydrophobic properties. Fluorine compounds are highly solubilized by this Supercritical Fluid, and many nanoformulations have focused on coupling curcumin with fluorine compounds to form conjugates that can display hydrophilic properties, this suggest way higher impregnation yields if curcumin was coupled to a fluorine compound.

The qualitative analysis such as FTIR and XRD indicates that no apparent structural changes occurs to both PMMA and Curcumin when they undergo supercritical processing. When using these techniques not just for structural analysis but also for detection of the presence of curcumin, the results were negative, which means as mentioned before, that curcumin impregnated quantities might not be significant enough to be detected either in FTIR and XRD. PMMA molecule doesn't seem to change its structure, which is to be expected since PMMA has low reactivity in general with solvents. The HPLC results show that Curcumin impregnation can be obtained with Supercritical CO_2, but also that there can be a partial denaturation when it comes down to curcumin derivatives. This is a stride in supercritical research since data regarding curcumin solubility in supercritical media is absent or very residual in present times.

Based on trial comparison, the best impregnation samples were MAS-012 and MAS-013, which were both reimpregnation of MAS-010 and MAS-011 respectively, with a yield percentage of < 2%. The samples were homogeneous and the standards to attain such homogeneous sample revolved around 150 Bar, 60 ºC, constant stirring at 500 rpm between 24 - 48 h. There were two types of PMMA used, as mentioned before, PMMA microparticles and PMMA rod. The main focus was on the micro particles, however, curcumin was impregnated on two different trials with PMMA in the form of rods and the depressurization factor was a strong aspect in obtaining a highly porous impregnated rod (rapid depressurization) or a small size pore matrix (slow depressurization). Results clearly suggest that for a dense matrix polymer such as PMMA, the time required for the matrix to fully plasticize from surface to the core of the micro particles is a strong factor for the swelling and possibility to homogeneously incorporate the pure curcumin into the matrix.

In overall this study provided reliable information regarding the possibility to solubilize Curcumin and impregnate it into polymeric samples with the usage of supercritical CO2 as the solvent. Since the first trial it became clear that curcumin displays a slight solubility towards supercritical carbon dioxide, opening the possibility to solubilize it and to impregnate it using Carbon dioxide in its supercritical state. It is important to understand that these first steps toward curcumin applications resourcing to supercritical CO_2 are limited for now not in a qualitative way, but in a quantitative way, since FTIR showed no apparent structural changes, XRD showed no loss of crystallinity of the curcumin molecule, but quantitatively, both UV and HPLC data showed that impregnation yields occur at a very low percentage, of around < 2% curcumin per gram of Matrix, taking in consideration that all the mass losses and the sample loss associated with systematic errors can occur. Such errors are associated with for instance, sample loss during any phase where it needed to be solubilized at an 100% rate, the fact that some solvents don't have the desired affinity towards our turmeric additive, hence complicating certain quantitative data, such as peak areas calculations of the HPLC provided data, or effective obtention of data for the UV absorbance measurements, which can be strongly influenced during sample preparation.

The quantitative data presented on this thesis is at an initial state, and this novel nanoformulation of curcumin poses as a new approach in the biomedical, chemical and Supercritical field to explore.

Although far from being ideal to develop an efficient drug delivery system comprising curcumin, there are a few variables to take into consideration for better results. Important aspects such as different setup parameters (Cosolvent, Temperature, Pressure, Agitation, etc.) would enhance curcumin solubility. Working around these variables to attain a more stable and favourable terciary phase system for our aditive, will improve its stability with the supercritical system and consequently, its affinity towards the polymeric matrix. The affinity enhancement would logically cause more quantities of our Pure Curcumin inside of our polymeric carrier, increasing curcumin yield on the final sample. Such approach will surely suggest that the quantities impregnated can be significantly higher. This supercritical approach towards solubilizing Curcumin with Ethanol, Supercritical CO_2 into PMMA, is a novel approach to explore, among many others towards Curcumin's bioavailability enhancement. This "green chemistry" technique is of utmost importance when it comes to using this substance for all the

ongoing clinical trials, because no matter the conditions and the pathologies, the prevention of infection and inflammation must be avoided.

4. Conclusion

In this study, we focused on using PMMA as a polymer carrier for curcumin impregnation, where the main interest for this approach arises from the potential that curcumin has shown for medical treatments over the past decade, such as cancer, infection, inflammation, neurological diseases, lifestyle related diseases (heart failure diabetes, atherosclerosis, alcoholism, etc.). The process studied here was made possible through the usage of a supercritical system at ICMAB, belonging the Solid-State Chemistry group, that has been improving and adapting the mechanical system throughout the years with the several research already done in the supercritical field. The usage of an isolated reactor with real time capability of altering the processing parameters regarding an impregnation procedure allowed a homogeneous impregnation of Curcumin. The obtained samples obtained a low, yet very favourable result regarding Curcumin impregnation, opening a new approach towards a novel nanoformulation of this additive.

This Master thesis report represents a small portion of the beginning of a new alternative focused on creating drug-delivery systems comprising Curcumin, through Supercritical Technology. The results show a promising alternative that with the obtention of higher impregnation yields, if properly optimized and investigated, could reach its desired therapeutic effect towards all the applications based on the capabilities that this compound can offer, such as diseases involving tissue inflammation, immunological infection, cancer treatment, neurological disorders, among several others forms of lifestyle related diseases.

Referencias

1 Pabon, H. (1964). A synthesis of curcumin and related compounds. Recueil Des Travaux Chimiques des Pays-Bas, 83, 379.

2 Naksuriya, O., Okonogi, S., Schiffelers, R. M., & Hennink, W. E. (2014). Curcumin nanoformulations: A review of pharmaceutical properties and preclinical studies and clinical data related to cancer treatment. Biomaterials, 35(10), 3365–3383.

3 Sandur, S. K., Pandey, M. K., Sung, B., Ahn, K. S., Murakami, A., Sethi, G., … Aggarwal, B. B. (2007). Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis, 28(8), 1765–1773.

4 Sandur, S. K., Pandey, M. K., Sung, B., Ahn, K. S., Murakami, A., Sethi, G., … Aggarwal, B. B. (2007). Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis, 28(8), 1765–1773.

5 Nutrabusiness, 11/2014 http://www.nutrabusiness.com/wpcontent/uploads

6 Gopalan, B., Goto, M., Kodama, a, & Hirose, T. (2000). Supercritical carbon dioxide extraction of turmeric (Curcuma longa). J Agric Food Chem, 48(6), 2189–2192.

7 Saleh A. Mohamed, Reda M. El-Shishtawy, Omar A. M. Al-Bar & Ahmed R. Al-Najada (2016): Chemical modification of curcumin: Solubility and antioxidant capacity, International Journal of Food Properties

8 Kurien BT, Singh A, Matsumoto H, Scofield RH. Improving the solubility and pharmacological efficacy of curcumin by heat treatment. Assay Drug Dev Techn 2007.

9 Tønnesen HH, Másson M, Loftsson T. Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. Int J Pharm 2002.

10 Wang YJ, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY, et al. Stability of curcumin in buffer solutions and characterization of its degradation products. J Pharm Biomed 1997.

11 Karewicz A, Bielska D, Loboda A, Gzyl-Malcher B, Bednar J, Jozkowicz A. Curcumin containing liposomes stabilized by thin layers of chitosan derivatives. Colloid Surf B 2013.

12 Lin YL, Liu YK, Tsai NM, Hsieh JH, Chen CH, Lin CM, et al. A lipo-PEG-PEI complex for encapsulating curcumin that enhances its antitumor effects on curcumin-sensitive and curcumin-resistance cells. Nanomed Nanotechnol 2012.

13 Shaikh J, Ankola D, Beniwal V, Singh D, Kumar M. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci 2009.

14 Barenholz Y. Doxildthe first FDA-approved nano-drug: lessons learned Journal Control Release 2012.

15 Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 2009.

16 J. Prasad Rao, Kurt E. Geckeler. (2011), Polymer nanoparticles: Preparation techniques and size control parameters, Progress in Polymer Science, Volume 36, issue 7, July 2011, 887-913

17 Shaikh J, Ankola D, Beniwal V, Singh D, Kumar M. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared

18 Anand P, Nair HB, Sung B, Kunnumakkara AB, Yadav VR, Tekmal RR, et al. Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability in vivo. Biochem Pharmacol 2010.

19 Manju S,SreenivasanK.Conjugationofcurcuminonto hyaluronicacidenhances its aqueous solubility and stability. J Colloid Interface Sci 2011.

20 Yang R, Zhang S, Kong D, Gao X, Zhao Y, Wang Z. Biodegradable polymer-curcumin conjugate micelles enhance the loading and delivery of low-potency curcumin. Pharm Res 2012.

21 Anitha A, Deepagan V, Divya rani V, Menon D, Nair S, Jayakumar R. Preparation, characterization, in vitro drug release and biological studies of curcumin loaded dextran sulphate-chitosan nanoparticles. Carbohyd Polym 2011.

22 Song Z, Feng R, Sun M, Guo C, Gao Y, Li L, et al. Curcumin-loaded PLGAePEGe

PLGA triblock copolymeric micelles: preparation, pharmacokinetics and distribution in vivo. J Colloid Interface Sci 2011.

23 Lu Y, Park K. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. Int J Pharm 2013.

24 Ma Z, Haddadi A, Molavi O, Lavasanifar A, Lai R, Samuel J. Micelles of poly(ethyleneoxide)-b-poly(Ɛ-caprolactone) as vehicles for the solubilization, stabilization, and controlled delivery of curcumin. J Biomed Mater Res A 2008.

25 Kim TH, Jiang HH, Youn YS, Park CW, Tak KK, Lee S, et al. Preparation and characterization of water-soluble albumin-bound curcumin nanoparticles

with improved antitumor activity. Int J Pharm 2011.

26 Kratz F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. J Control Release 2008.

27 Esmaili M, Ghaffari SM, Moosavi-Movahedi Z, Atri MS, Sharifizadeh A, Farhadi M, et al. Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application. LWT Food Sci Technol 2011.

28 Yadav VR, Prasad S, Kannappan R, Ravindran J, Chaturvedi MM, Vaahtera L, et al. Cyclodextrin-complexed curcumin exhibits anti-inflammatory and antiproliferative activities superior to those of curcumin through higher cellular uptake. Biochem Pharmacol 2010.

29 Yallapu MM, Jaggi M, Chauhan SC. Poly (b-cyclodextrin)/curcumin self-assembly: a novel approach to improve curcumin delivery and its therapeutic efficacy in prostate cancer cells. Macromol Biosci 2010.

30 Alam MA, Ali R, Al-Jenoobi FI, Al-Mohizea AM. Solid dispersions: a strategy for poorly aqueous soluble drugs and technology updates. Expert Opin Drug Del 2012.

31 Okonogi S, Oguchi T, Yonemochi E, Puttipipatkhachorn S, Yamamoto K. Physicochemical properties of ursodeoxycholic acid dispersed in controlled pore glass. J Colloid Interface Sci 1999.

32 Giri TK, Kumar K, Alexander A, Ajazuddin, Badwaik H, Tripathi DK. A novel and alternative approach to controlled release drug delivery system based on solid dispersion technique. B-FOPCU 2012.

33 Mohanty C, Sahoo SK. The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. Biomaterials 2010.

34 Anuchapreeda S, Fukumori Y, Okonogi S, Ichikawa H. Preparation of lipid nanoemulsions incorporating curcumin for cancer therapy. J Nanotechnol 2012.

35 Zanotto-Filho A, Coradini K, Braganhol E, Schröder R, de Oliveira CM, Simões-Pires A, et al. Curcumin-loaded lipid-core nanocapsules as a strategy to improve pharmacological efficacy of curcumin in glioma treatment. Eur J Pharm Biopharm 2013.

36 Michel Perrut, 2000, Supercritical Fluid Applications: industrial Developments and Economic Issues

37 Bott, T. R.; King, M. B.; 1993, Supercritical fluid processing of Food and Biomaterials; Blackie A&P: London, ISBN 0-7514-0184-6

38 Mendiola, J.A.; Herrero, M.; Cifuentes, A.; Ibañez, E. (2007). "Use of compressed fluids for sample preparation: Food applications". Journal of Chromatography A 1152 (1-2): 234–246

39 U.S.EPA Method 3560 Supercritical Fluid Extraction of Total Recoverable Hydrocarbons

40 U.S.EPA Method 3561 Supercritical Fluid Extraction of Polycyclic Aromatic Hydrocarbons

41 Yeo, S.; Kiran, E. (2005). "Formation of polymer particles with supercritical fluids: A review". J. Supercrit. Fluids 34: 287–308.

42 Duarte A., Casimiro T., Aguiar A., Luísa A, Duarte C., "Supercritical Fluid polymerisation and impregnation of molecularly imprinted polymers for drug delivery", Journal of Supercritical Fluids, 2006

43 Truc T., Charles L., Charles A., Sergei G., "Supercritical fluid impregnation of different azo-dyes into polymer: in situ UV/Vis spectroscopy study"

44 "THE EFFECT OF TEMPERATURE ON THE sCO2 COMPATIBILITY OF CONVENTIONAL STRUCTURAL ALLOYS"(PDF). The 4th International Symposium - Supercritical CO2 Power Cycles.

45 J. Parks, Curtis. "Corrosion of Candidate High Temperature Alloys in Supercritical Carbon Dioxide" (PDF). Ottawa-Carleton Institue for Mechanical and Aerospace Engineering.

46 A.White, D. Burns, T.W. Christensen, "Effective terminal sterilization using supercritical carbon dioxide", J. of Biotechnology

47 Cansell, F., Aymonier, C., & Loppinet-Serani, A. (2003). Review on materials science and supercritical fluids. Current Opinion in Solid State and Materials Science, 7(4-5), 331–340.

48 Proceedings of the 4th International Symposium on Supercritical Fluids, Sendai, May 11-14, 1997; Saito, S., Arai, K., Eds

49 Proceedings of the 5th Meeting on Supercritical Fluids, Nice, France, Mar 23-25, 1998; Perrut, M., Subra, P., Eds.; Vol. 2, ISBN 2-905-267-28-3.

50 Proceedings of the 5th International Symposium on Supercritical Fluids, Atlanta, GA, April 8-12, 2000; Eckert, C., Teja, A., Eds.

51 Proceedings of the 5th Meeting on Supercritical Fluids, Nice, France, Mar 23-25, 1998; Perrut, M., Subra, P., Eds.; Vol. 2, ISBN 2-905-267-28-3.

52 Petrucci, Ralph H., William S. Harwood, F. Geoffrey Herring, and Jeffry D. Madura. "The Critical Point." General Chemistry: Principles & Modern Applications, ninth Edition. New Jersey: Pearson Education, Inc., 2007. 478-479

53 "Charles Canigard de la Tour." Wikipedia. 29 May 2008

54 Hardy, James K. "Supercritical Fluids." Chemical Separations (2005) 27 May 2008

55 D. Ctiouchi, D, GOurgouillon, M. Courel, J. Vital, M, Nunes da Ponte "The influence of phase behaviour on reactions at supercritical conditions: the hydrogenation of a-pine- ne" Ind. Eng. Chem. Research, 2001. 40, 2551

56 Bolmatov, D.; Brazhkin, V. V.; Trachenko, K. (2013). "Thermodynamic behaviour of supercritical matter". Nature Communications

57 Nalawade, S. P., Picchioni, F., & Janssen, L. P. B. M. (2006). Supercritical carbon dioxide as a green solvent for processing polymer melts: Processing aspects and applications. Progress in Polymer Science (Oxford), 31(1), 19–43.

58 Fried JR, Li WJ. High-pressure FT-IR studies of gas-polymer interactions. J Appl Polym Sci 1990; 41:1123–31.

59 Shieh, Y., & Liu, K. (2002). Solubility of CO 2 in Glassy PMMA and PS over a Wide Pressure Range: The Effect of Carbonyl Groups, (2), 107–113.

60 Sanchez IC, Lacombe RH. An elementary molecular theory of classical fluids. pure fluids. J Phys Chem 1976; 80:2352–62.

61 Zhong C, Masuoka H. A new mixing rule for cubic equations of state and its application to vapour-liquid equilibria of polymer solutions. Fluid Phase Equilib 1996; 123:59–69.

62 Peng DY, Robinson DB. A new two-constant equation of state. Ind Eng Chem Fundam 1976; 15:59–64.

63 Chapman WG, Gubbins KE, Jackson G, Radosz M. New reference equation of state for associating fluids. Ind Eng Chem Res 1990; 29:1709–21.

64 McCrum, N. G.; Buckley, C. P.; Bucknall, C. B. (1997). Principles of polymer engineering. Oxford; New York

65 Dong Z, Liu Z, Han B, Pei X, Liu L, Yang G. Modification of isotactic polypropylene films by grafting of methyl acrylate using supercritical CO2 as a swelling agent. J Supercrit Fluids

2004; 31:67–74.

66 Liu Z, Song L, Dai X, Yang G, Han B, Xu J. Grafting of methyl acrylate onto isotactic polypropylene film using supercritical CO2 as a swelling agent. Polymer 2002;43: 1183–8.

67 Liu Z, Dong Z, Han B, Wang J, He J, Yang G. Composites prepared by the polymerization of styrene within supercritical CO2-swollen polypropylene. Chem Mater 2002; 14:4619–23.

68 Friedmann G, Guilbert Y, Catala JM. Modification chimique de polymeres en milieu CO2 supercritique Greffage de groups isocyanate-isopropyle sur une chaine de poly(ethylene-co-alcool vinylique). Eur Pol J 2000; 36:13–20.

69 Favis BD, Chalifoux JP. The effect of viscosity ratio on the morphology of polypropylene/polycarbonate blends during processing. Polym Eng Sci 1987; 27:1591–600.

70 Elkovitch MD, Lee LJ, Tomasko DL. Effect of supercritical carbon dioxide on morphology development during polymer blending. Polym Eng Sci 2000; 40:1850–61.

71 Watkins JJ, McCarthy TJ. Polymerization of styrene in supercritical CO2-swollen poly(chlorotrifluoroethylene). Macromolecules 1995; 28:4067–74.

72 Edward K, Lesser AJ, McCarthy TJ. Composites prepared by the anionic polymerization of ethyl 2-cynoacrylate within supercritical carbon-dioxide-swollen poly(tetrafluoroethylene-co-hexafluoropropylene). Macromolecules 2000;33:4506 see also pages 8192–8199.

73 Li D, Han B. Impregnation of polyethylene(PE) with styrene using supercritical CO2 as the swelling agent and preparation of PE/polystyrene composites. Ind Eng Chem Res 2000; 39:4506–9.

74 Chang Y, Xu Q, liu M, Wang Y, Zhao Q. Supercritical CO2-assisted synthesis of poly(acrylic acid)/nylon1212 blend. J Appl Polym Sci 2003;90:2040–4

75 Wang Y, Liu Z, Han B, Zhang J, Jiang T, Wu W, et al. Synthesis of polypropylene/ZnS composite using the template prepared by supercritical CO2. Chem Phys Lett 2003;381:271–7.

76 Wang JD, Mandel FS, Popov VK, Howdle SM. Solvent-free manufacturing of synthetic bone composites. Ceram Trans 2000;107:97–104.

77 Garcia-Leiner M, Lesser AJ. Polymer-clay nanocomposites prepared in supercritical carbon dioxide. Polym Prepr 2003; 88:92–3.

78 Devine JN, Kemmish DJ, Wilson B, Griffiths I, Seargeant KM. Biocompatible polymeric materials for medical devices. (UK) WO:2002000275, 2002.

79 Farrar PA. Method of forming foamed polymer insulators for use in high density circuits. (USA) US:2002168872, 2002.

80 Baldwin DF, Park CB, Suh NP. A microcellular processing study of poly(ethylene terephthalate) in the amorphous and semicrystalline state. Part II: cell growth and process design. Polym Eng Sci 1996; 36:1446–53.

81 Mizumoto T, Sugimura N, Moritani M. CO2-induced stereo complex formation of stereoregular poly(methyl methacrylate) and microcellular foams. Macromolecules 2000;33: 6757–63.

82 Lee S-J, Kim M-S, Chung JG. Preparation of porous polycarbonates membranes using supercritical CO2 enhanced miscibility. J Ind Eng Chem 2004; 10:877–82.

83 Asandei AD, Erkey C, Burgess DJ, Saquing C, Saha G, Zolnik BS. Synthesis of PLGA foams by high temperature supercritical CO2 expanded ring opening copolymerization of D, L-lactide and glycolide. Polym Prepr 2004; 45:1014–5

84 Barry JJA, Gidda HS, Scotchford CA, Howdle SM. Porous methacrylated scaffolds: supercritical fluid fabrication and in vitro chondrocyte responses. Biomaterials 2004;25: 3559–68.

85 Park CB, Baldwin DF, Suh NP. Effect of the pressure drop on cell nucleation in continuous processing of microcellular polymers. Pol Eng Sci 1995; 35:432–40.

86 Mooney DJ, Baldwin DF, Suh NP, Vacanti JP, Langer R. Novel approach to fabricate sponges of poly (D, L-lactic-co-glycolic acid) without the use of organic solvents. Biomaterials

1996; 17:1417–22.

87 Japon S, Leterrier Y, Manson JE. Recycling of poly (ethylene terephthalate) into closed cell foams. Polym Eng Sci 2000;40: 1942–52.

88 Shutov FA. Integral/structural polymeric foams: technology, properties and applications. Berlin Heidelberg: Springer-Verlag; 1986.

89 Michaeli W, Heinz R. Foam extrusion of thermoplastics polyurethanes(TPU) using CO2 as a blowing agent. Macromol Mater Eng 2000;284–285:35–9.

90 Han X, Zeng C, Lee J, Koelling KW, Tomasko DL. Extrusion of polystyrene nanocomposite foams with supercritical CO2. Polym Eng Sci 2003; 43:1261–75.

91 Siripurapu S, Gay YJ, Royer JR, DeSimone JM, Spontak RJ, Khan SA. Generation of microcellular foams of PVDF and its blends using supercritical carbon dioxide in a continuous

process. Polymer 2002; 43:5511–20.

92 Han X, Zeng C, Koelling KW, Tomasko DL, Lee J. Continuous microcellular polystyrene foam extrusion with supercritical CO2. Polym Eng Sci 2002;42:2094–106.

93 Jacobsen K, Pierick D. Injection molding innovation: the microcellular foam process. Plast Eng 2001; 57:46–51.

94 Garc, C. A., Concheiro, A., & Alvarez-lorenzo, C. (2014). Processing of Materials for Regenerative Medicine Using Supercritical Fluid Technology.

95 Bin Z, Chen M, Liu W. Effect of co-solvents on the polymerization of acrylamide in supercritical CO2. Polym Prepr 2004; 45:512–3.

96 Odell PG, Hamer GK. Polycarbonates via melt transesterification in supercritical carbon dioxide. Polym Prepr 1997; 38:470–1.

97 Gross SM, Flowers D, Roberts G, Kiserow DJ, DeSimone JM. Solid-state polymerization of polycarbonates using supercritical CO2. Macromolecules 1999; 32:3167–9.

98 Mang S, Cooper AI, Eamon CM, Chauhan N, Holmes AB. Copolymerization of CO2 and 1,2-cyclohexene oxide using a CO2-soluble chromium porphyrin catalyst. Macromolecules

2000; 33:303–8

99 Hwang Y, Jung J, Ree M. Terpolymerization of CO2 with propylene oxide and e-caprolactone using zinc glutarate catalyst. Macromolecules 2003; 36:8210–2.

100 Kazarian SG, Martirosyan GG. Spectroscopy of polymer/drug formulations processed with supercritical fluids: in situ ATR-IR and Raman study of impregnation of ibuprofen into PVP. Int J Pharm 2002; 232:81–90.

101 Kikic, I., & Vecchione, F. (2003). Supercritical impregnation of polymers. Current Opinion in Solid State and Materials Science, 7(4-5), 399–405.

102 Guney O, Akgerman A. Synthesis of controlled-release productsin supercritical medium. AIChE J 2002; 48:856–66.

103 Shim JJ, Choi JH, Ju JH, Son BK, Ahn JM, Kim BH, et al. Dyeing of polyester, aramid and polypropylene fibers in supercritical CO2. In: 6th International Symposium on Supercritical

Fluids, 2003. p. 2101–6.

104 VeugelersW JT, Witkamp GJ. Textile dyeing in supercritical carbon dioxide. In: 6th International Symposium on Supercritical Fluids, 2003. p. 2119–24.

105 Said-Galiyev E, Nikitin L, Vinokur R, Gallyamov M, Kurykin M, Petrova O, et al. New chelate complexes of copper and iron: synthesis and impregnation into a polymer matrix from solution in supercritical carbon dioxide. Ind Eng Chem Res 2000;39: 4891–6.

106 - Kikic I, Sist P (2000) Applications of supercritical fluids to pharmaceuticals: controlled drug release systems. Supercrit Fluids 366:291–306

107 Pap, G., Scarlett, T. J., Investigator, P., Caneba, G., & Trotter, K. (2016). Potential Applications of Supercritical Carbon Dioxide Extraction and Impregnation for the Stabilization and Conservation of Industrial Heritage Artifacts.

108 Pajonk GM (1991) Aerogel catalysts. Appl Catal 72:217–266.

109 Ulker Z, Erkey C (2014) An emerging platform for drug delivery: aerogel based systems. J Control Release 177:51–63.

110 Baetens R, Jelle BP, Gustavsen A (2011) Aerogel insulation for building applications: a state-of-the-art review. Energy Build 43:761–769.

111 Contolini RJ, Hrubesh LW, Bernhardt AF (1993) Aerogels for microelectronic applications: fast inexpensive, and light as air. Lawrence Livermore National Lab, Livermore

112 Giray S, Bal T, Kartal AM, Kizilel S, Erkey C (2012) Controlled drug delivery through a novel PEG hydrogel encapsulated silica aerogel system. J Biomed Mater Res A 100A:1307–1315.

113 Smirnova I, Suttiruengwong S, Arlt W (2005) Aerogels: tailor- made carriers for immediate and prolonged drug release. KONA Powder Part J 23:86–97

114 Smirnova I, Tu¨rk M, Wischumerski R, Wahl MA (2005) Comparison of different methods for enhancing the dissolution rate of poorly soluble drugs: case of griseofulvin. Eng Life Sci

5:277–280

115 Smirnova I, Suttiruengwong S, Seiler M, Arlt W (2004) Dissolution rate enhancement by adsorption of poorly soluble drugs on hydrophilic silica aerogels. Pharm Dev Technol 9:443–452

116 Caputo G (2013) Fixed bed adsorption of drugs on silica aerogel from supercritical carbon dioxide solutions. Int J Chem Eng.

117 Caputo G, Scognamiglio M, De Marco I (2012) Nimesulide adsorbed on silica aerogel using supercritical carbon dioxide. Chem Eng Res Des 90:1082–1089.

118 Alnaief M, Antonyuk S, Hentzschel CM, Leopold CS, Heinrich S, Smirnova I (2012) A novel process for coating of silica aerogel microspheres for controlled drug release applications. Microporous Mesoporous Mater 160:167–173.

119 Yu KMK, Yeung CMY, Thompsett D, Tsang SC (2003) Aerogel-coated metal nanoparticle colloids as novel entities for the synthesis of defined supported metal catalysts. J Phys Chem B 107:4515–4526.

120 Martinez S, Vallribera A, Cotet CL, Popovici M, Maritn L, Roig A, Moreno-Manas M, Molins E (2005) Nanosized metallic particles embedded in silica and carbon aerogels as catalysts in the Mizoroki–Heck coupling reaction. New J Chem 29:1342–1345

121 Baumann TF, Fox GA, Satcher JH, Yoshizawa N, Fu R, Dresselhaus MS (2002) Synthesis and characterization of copper-doped carbon aerogels. Langmuir 18:7073–7076.

122 Apple, David J (2006). Sir Harold Ridely and His Fight for Sight: He Changed the World So That We May Better See It. Thorofare NJ USA: Slack.

123 Miller (1996). Review of Orthopedics (4 ed.). Philadelphia: W. B. Saunders. p. 129

124 http://www.basscat.com/

125 Kazarian, S. G. (2000). Polymer Processing with Supercritical Fluids, 42(1), 78–101

126 Rita, A., Duarte, C., Luisa, A., Vega-gonz, A., Subra-paternault, P., Gil, M. H., … Duarte, C. M. M. (2007). Supercritical fluid impregnation of a biocompatible polymer for ophthalmic drug delivery, 42, 373–377. http://doi.org/10.1016/j.supflu.2007.01.007

127 Settnes, M., Power, S. R., & Lin, J. (n.d.). Preparation of PMMA / graphene oxide microcellular foams using supercritical carbon dioxide.

128 Zhhang H B, Yan Q, Zhheng W G, He Z Xnd Yu Z Z 2011 Touggh grapheneе-polymermicrocellular foams for electromagneetic interferennce shielding, ACS Appll. Mater. Interfaces 3918-24

129 Yang J T, Wu M J, Chen F, Fei Z D and Zhong M Q 2011 Preparation, characterization, and supercritical carbon dioxide foaming of polystyrene/graphene oxide composites J. Supercrit. Fluids 56 201-7

130 Chen H, Guan R, Zhao J Z, Jiang S F, Ke Z and Zha S W 2013 Microcellular foaming of plasticized thin PC sheet I. Effects of processing conditions J. Wuhan Univ. Technol. Mater. Sci. Ed. 28 235-42

131 Zhang P, Zhou N Q and Li. B 2007 Effects of process variables on microcellular structure and crystallization of polypropylene foams with supercritical CO2 as the foaming

agent-a study of microcellular foaming of polypropylene Polym. Plast. Technol. Eng. 46 885-91

132 Zhai W T, Ko Y, Zhu W L, Wong A S and Park C B 2009 A study of the crystallization, melting, and foaming behaviors of polylactic-acid in compressed CO2 Int.J. Mol. Sci. 12 5381-97

133 Ji G Y, Zhai W T, Lin D P, Ren Q, Zheng W G and Jung D W 2013 Microcellular foaming of poly (lactic acid)/silica nanocomposites in compressed CO2: Critical influence of crystallite size on cell morphology and foam expansion Ind. Eng. Chem. Res. 52 6390-8

134 Yang J T, Wu M J, Chen F, Fei Z D and Zhong M Q 2011 Preparation, characterization, and supercritical carbon dioxide foaming of polystyrene/graphene oxide composites J. Supercrit. Fluids 56 201-7

135 Lee L J, Zeng C C, Cao X, Han X M, Shen J and Xu G J 2005 Polymer nanocomposite foams, Compos. Sci. Technol. 65 2344-63

136 Diankov, S., Barth, D., Vega-gonzalez, A., Pentchev, I., & Subra-paternault, P. (2007). Impregnation isotherms of hydroxybenzoic acid on PMMA in supercritical carbon dioxide, 41, 164–172.

137 Pabon, H. (1964). A synthesis of curcumin and related compounds. Recueil Des Travaux Chimiques des Pays-Bas, 83, 379.

138 Degradation studies of curcumin - Ashokkumar, P., Unichem, M., & Mishra, P. A. (2015).

139 Rosaler, J. (2015). Studies in History and Philosophy of Modern Physics Local reduction in physics. Studies in History and Philosophy of Modern Physics, 50, 54–69.

140 Australian Government Department of Health, National Industrial Chemicals, Notification and assessment scheme.

141 Beheshti S. (July, 2016) Nanocurcumin for Prostate Cancer Patients Undergoing Radiotherapy, University of Medical Sciences, Tehran, Iran

142 - American cancer society – colon rectal cancer page - http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-treating-chemotherapy

143 – Baylor Research Institute, March 2016 Curcumin in Combination With 5FU for Colon Cancer

144 Nicholas LaRusso, November 2016 Study Evaluating the Safety and Efficacy of Curcumin in Patients With Primary Sclerosing Cholangitis, Mayo Clinic, Europharma, Inc Verified November 2016 by Mayo Clinic

145 VA Office of Research and Development, July 2016 Curcumin and Yoga Therapy for Those at Risk for Alzheimer's Disease

146 Goldstein, B., October 2016 Adjunctive Curcumin for Symptomatic Adolescents With Bipolar Disorder: Brain and Body Considerations, Sunnybrook Health Sciences Centre

147 Naksuriya O. January 2014 Curcumin Nanoformulations: A Review of Pharmaceutical Properties and Preclinical Studies and Clinical Data Related to Cancer Treatment Biomaterials 35 (10), 3365-3383.

Annex I – UV analysis

Regarding the focus on this thesis, the quantification of the impregnated amount of curcumin into the PMMA polymeric matrix, two quantitative analysis were performed for estimation of impregnated amount into PMMA. Since HPLC is the most reliable data for quantification purposes, the UV analysis is shown in this annex.

UV/Vis spectroscopy was performed in several samples as table **X** shows, to attain an estimation of how much quantity of Curcumin was impregnated in the polymeric matrix. Using a calibration curve for curcumin that correlates the absorbance value with curcumin concentration, the amounts of Curcumin impregnated into PMMA polymeric matrices could be calculated.

The samples that went through UV analysis are shown in table 2.

Table 2. Processed samples for UV analysis

	ABS	Volume (ml of DCM)	Mass (mg)	[C] – (Molar)	[C] - (g/gMatrix)	[C] - (mg/gMatrix)
MAS12-P64	1.16967	5 ml	52.5	$2.274 * 10^{-5}$	0.001675	1.675
MAS12-P244	0.9781	5 ml	50.8	$2.274 * 10^{-5}$	0.001398	1.398
MAS13-P64	2.04779	5 ml	51.7	$2.274 * 10^{-5}$	0.002947	2.947
MAS13-P244	0.57028	5 ml	48.2	$2.274 * 10^{-5}$	0.000807	0.807
MAS4-P64	0.33819	5 ml	49.0	$2.274 * 10^{-5}$	0.000471	0.471

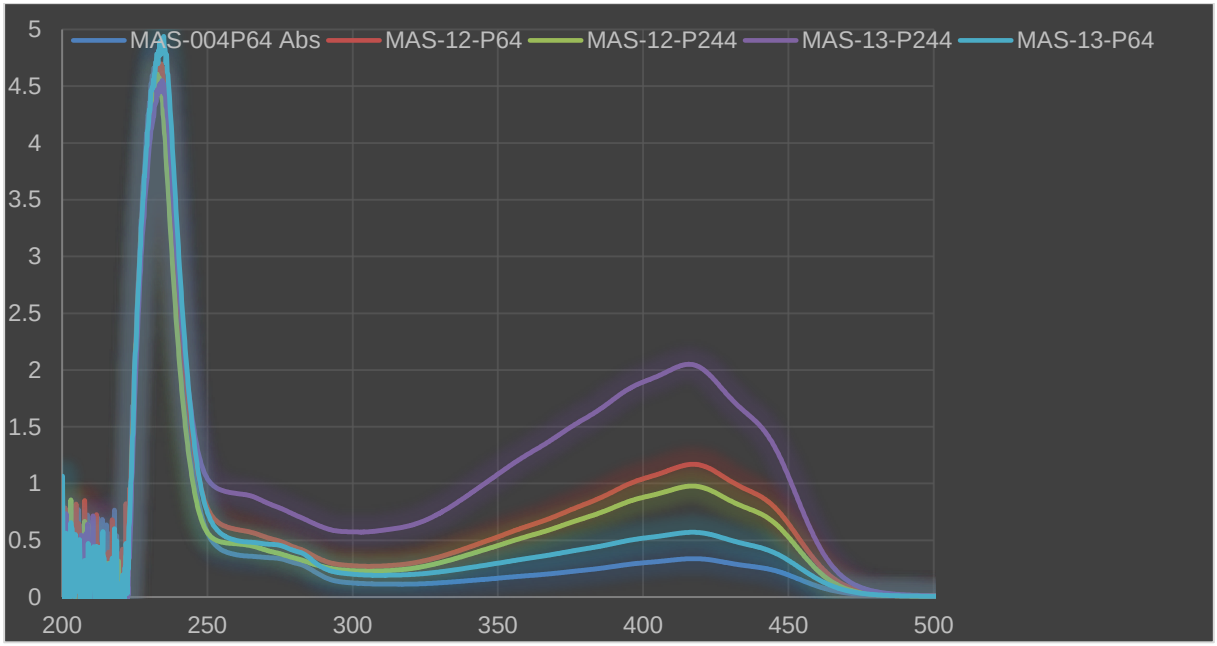


Fig. 23 - UV absorbance chromatogram

When comparing the data to the HPLC analysis, we can clearly see some discrepancies regarding the quantification values. Although the amounts are within a somewhat similar order of magnitude, the values of the samples are not the same between UV and HPLC. The chosen data was HPLC for obvious reasons, mainly the precision and the less occurrence of errors during its procedure.

UV quantification not only can have inherent errors regarding the solvent quantities, sample quantities, or any kind of systematic errors regarding the equipment, and solubilisation issues related to the impregnated polymeric matrix not fully dissolving in the DCM and consequently releasing the impregnated curcumin. Hence, the data present on the table above can show us that Curcumin was successfully impregnated in the samples. Several samples were initially quantified in terms of Curcumin yield through UV, although the first homogeneous sample (MAS-004) and the optimal samples were the ones highlighted.
The amounts impregnated ranged from [0.471 mg/g_{matrix} –2.947 mg/g_{matrix}], and the data obtained was based on a calibration curve previously made by the Solid State Chemistry group from ICMAB for quantification purposes of Curcumin based on absorbance at its specific wavelength, 417 nm.

When looking to the UV spectra, we have two distinct peaks absorbing at two different wavelengths, one at 261 nm and the other at 417 nm where the first corresponds to the PMMA absorption peak, and the second one matches the Curcumin one.
The provided data gave us a glimpse regarding the quantities of Curcumin within the polymer samples, giving enough to proceed to HPLC for more accurate results.

Annex II - (PET trials)

A polymeric textile was brought in to ICMAB to explore processing possibilities using supercritical technology. The textile taken was made of Silk, with monofilaments of PET - Poly (ethylene terephthalate). Previous research performed by my Portuguese Supervisor, Ana L. Oliveira, in the 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, focused on using this textile for Bone tissue regeneration, mainly in the craniofacial complex.

As table Y shows, a brief trial comparison was done to understand the homogeneity of the sample when supercritical state is attained, and the influence of the process, mainly temperature in the physical integrity of the textiles.

Table 2.

	Pressure	Temperature	Time	Stirring
PETS1	130	30	48	500
PETS2	130	60	48	500
PETS3	200	30	48	500
PETS4	200	60	48	500

To these textiles, ATR and DSC were used to understand what happened after supercritical processing. No specific peaks were detected regarding the textile structure

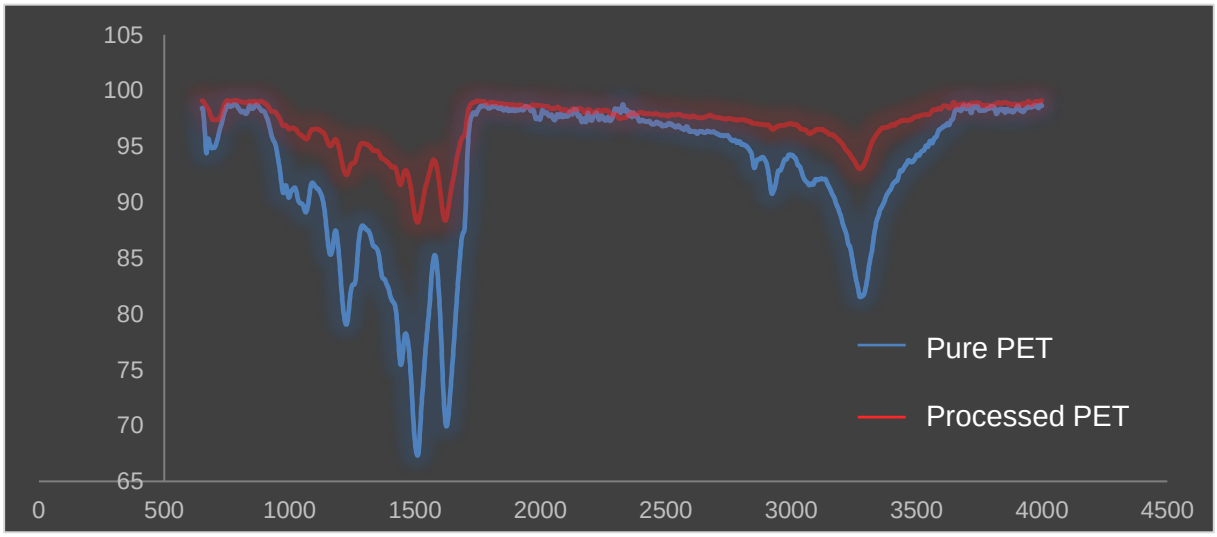


Fig. 24 - ATR of the processed and non-processed textiles

The following plots asserts to a technique used to evaluate the Tg and Tm changes that might have possibly occurred on the material, however this data was discarded from the thesis main topic, hence being shown here in the annex.

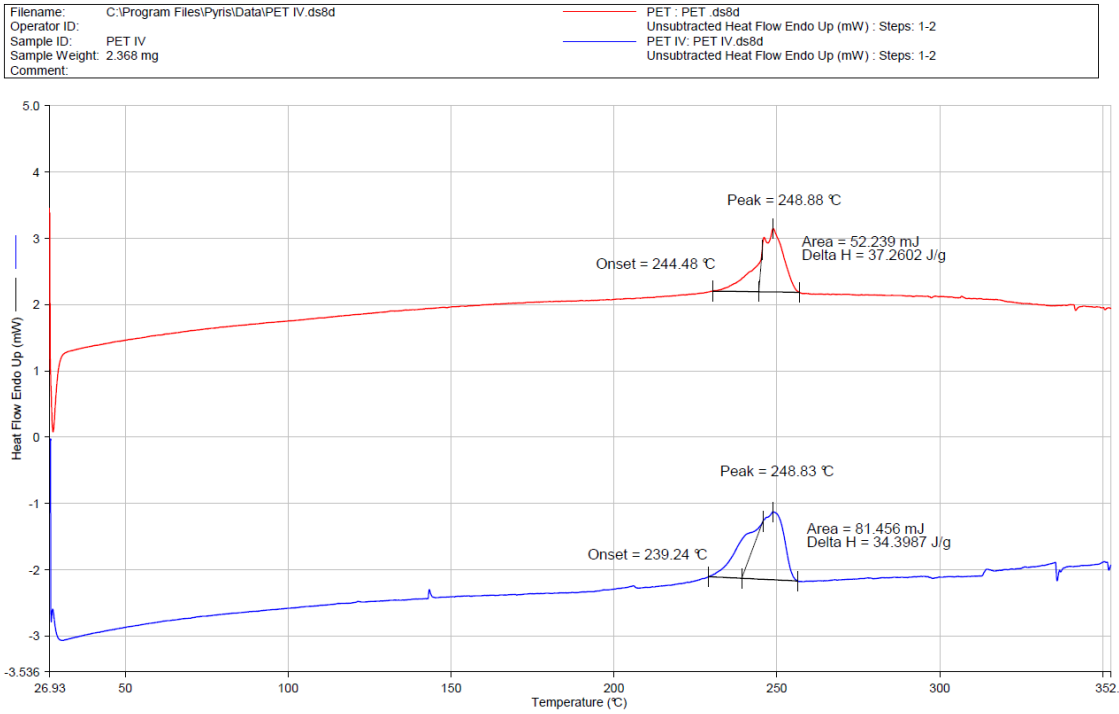


Fig. 25 – Differential Scanning Calorimetry (DSC) of the PETS4 before processing (Red), and after processing (Blue)

Annex III – All setups

	Pressure (Bar)	Temperature (ºC)	Stirring (rpm)	Time (hours)	Co-solvent (ml)	Notes
MAS-001	150	60	500	48	none	Not homogeneous (NH)
MAS-002	140	60	500	48	none	NH
MAS-003	150	60	500	48	none	New setup: flat paper; NH
MAS-004	150	40	500	48	none	Homogenous (H)
MAS-005	150	40	500	48	none	Big amounts of PMMA (NH)
MAS-006	150	40	500	96	none	Big amounts of PMMA (NH)
MAS-007	150	40	500	48	Ethanol (6 ml)	PMMA plasticized w paper
MAS-008	150	40	500	24	Ethanol (6 ml)	Cellulose fibers still plasticized
MAS-009 (Textiles)	140	60	500	24	none	Impregnated in PET only
MAS-010	150	60	500	72	none	For analisys big amounts (NH-almost)
MAS-011	140	40	500-550	24	Ethannol (2 ml)	No plasticization of paper+PMMA
MAS-012 (reimpg. of MAS-010)	150	60	500	24	Ethanol (2ml)	homogeneous
MAS-013 ()	140	40	500	48	2 drops <1%	homogenous

Annex IV – Operating Protocoç

Supercritical processing with tubular reactor protocol

Supercritical Processing: equipment processing;

1-Check all valves, make sure all valves closed to attain an isolated system;

2-Turn on the pump and fill it to pressurize system later on;

3-Reactor setup;

3.1-Always place the samples inside the reactor;

3.2-Put the blanket around the reactor ;

3.3-Place magnetic stirrer;

3.4- Make a schematic of the setup;

4-How to pressurize tubular reactor: set a lower pressure than target pressure (standard is 15 bar lower ex: 135 bar to get 150 bar on reactor);

Set ____ A____P value_____ press RUN;

5-Open CO2 valve, mind the P.I. 1 to control the amount of pressure and CO2 in your system;

6-Open V1 and wait for pressure to stabilize between CO2 and V2;

7- Turn V3 towards tubular reactor;

8-Open V2 and wait for pressure to settle throughout the system until it reaches V4;

9- After pressure stabilized throughout the whole system at bottle pressure, open V4;

10- With V4 open wait until the reactor is filled with CO2, after it is filled close V4 and isolate the reactor (V5 aand V6 closed as well);

10.1-Close CO2 bottle valve

11- After reactor is filled, turn on reactor Temperature indicator and heater and wait for T to reach temperature;

12- Once the reactor is filled, temperature is stabilized and the whole system is at bottle pressure except the reactor, next step is to pressurize the system using the pump settings;

13-BEFORE using the pump, check if V1 is closed, check if V2 is closed before setting the new pressure and make sure V3 is turned towards the tubular reactor;

14- After the pressure has been properly set in the pump, slowly open V2 needle valve so that the system until V4 gets pressurized;

15-Once you have the target pressure until V4, open V4 and wait for reactor to reach the target pressure (note that the temperature of the reactor will drop a few degrees due to the Carbon dioxide);

16- Close V2 valve;

17- Once Target pressure is reached, isolate reactor by closing V4 valve and making sure there are no leaks in V5 and V6 valves;

18-After closing the reactor valves around the reactor, make sure all the valves of the system are properly closed;

Notes: Reaction going at constant T and P (isolated segment of the system)

After step 18 press stop button in the pump

Turn on the stirring

Depressurize the other segments of the system (the pathway is optional, choose the shorter and safest one always, (IMPORTANT) but there is only one way to empty the tubular reactor)

19 - Starting with the segment between CO2 bottle and V1, slowly open autoclave valve to remove all the remaining pressure in this segment. Make sure V1 is closed;

20 – With V1 and V2 closed set pump option to refill to depressurize the pump;

21 – Once pump has depressurized, compensate the pressurized segment between V2 and V4 by SLOWLY opening V2 valve with V3 turned towards tubular reactor. In this step, note that V4 is ALWAYS closed;

22 – After the depressurization has stabilized between the V1 –V2 segment and V2-V4 segment, slowly open V1 so that the remaining CO2 exits the AE valve;

22.1 – Before opening V1 valve make sure that V2 is open, V3 is towards tubular reactor, V4 and CO2 bottle valve are closed;

Reactor depressurization

23- After reaction is finished, and all previous steps were followed to depressurize the other segments of the system, make sure that V4 is firmly closed and SLOWLY open V5 (the only pathway to depressurize the reactor);

24 – There are 2 exit valves on the reactor, only use V5 needle valve, SLOWLY open it enough until the exit tube causes smooth bubbling on the water beaker;

25 – Wait for reactor temperature to cooldown a bit so that you can remove the heater;

26 – Unplug the termopar;

27- Unplug the reactor of the supercritical setup;

28- Remove the samples from the reactor, along with the magnetic stirrer;

29 – Clean all the materials, shut down the pump and the cryostat, in case no more experiments are happening during the day;

Notes: - during the experiments control the air flux inside the hotte, due to the amounts of carbon dioxide in the atmosphere when valves are open to the outside;

- Pressure flow is always towards where there is less pressure, play around the SC system segments to ensure that the pressure flow pathway is the right one;

- In case you leave a reaction overnight place a note in the glass with your name and cellphone number as well as your supervisors name and phone number so that coworkers can be aware of the experiment;

Annex V – Curcumin clinical practice

Clinical Status of Curcumin in applied medical research field

Taking the analysis of the results into account, there is a strong potential in using Supercritical CO2 as a solvent alternative for polymer swelling and creation of controlled-release drugs. Not only that but also the fact that the final product can be obtained residue-free, without any kind of contamination, poses as an enormous advantage for these kinds of medical applications, where aseptic conditions are necessary, and the purity of certain substances are extremely valuable to any kind of clinical application.

currently there are phase II studies of nanocurcumin vs Placebo for the treatment of patients with prostate cancer, in the attempt to confirm and verify the suppression of tumour/cancer growth factors, caused by a not very well understood interaction or effect of the phenolic rings with the cancer cells (141). The presence of these phenolic rings in the supercritical processing of curcumin was confirmed through FTIR showing that Supercritical processed curcumin can have the same kind effect of these phase II studies using nanocurcumin. This study has been verified on July 2016 and there are still ongoing recruits for human patients with prostate cancer (141).

Another clinical trial regarding cancer is being performed, not for prostate but for colon cancer, which already has a somewhat therapeutic treatment using 5-FU, a chemical named 5-Fluorouracil often given with the vitamin-like drug leucovorin (also called folinic acid) for chemotherapy purposes (142). This novel pilot study that also started in 2016 is based on coupling curcumin with this 5-FU drug, in the attempt to improve not only the efficiency of the treatment but also the recovery enhances from chemotherapy procedures, although there is still a long way to go for this clinical trial to display any accurate conclusions regarding human recovery (143). The usage of the Supercritical impregnation method to couple curcumin to an existing drug could provide better improvements in patients, most likely due to the absence of contaminations in drug-delivery systems, as well as unaltered structure and function of Curcumin.

These are a couple of the dozens of clinical trials ongoing in the year 2016 regarding the usage of curcumin, not only for cancer therapy, but also for diseases such as sclerosis cholangitis (144), Alzheimer's (145), and even behavioural disorders such as bipolar behaviour into young students (146), among many others. These approaches are up to date and undergoing clinical trials, whether they are in pilot studies, or already in phase II studies with human patients undergoing clinical experimentation.