Current Trends in Biotechnology and Pharmacy
Vol. 18(3) 1904-1912, July 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3.36

Supercritical Fluid for Retama Raetam Porous Film Production: A Strategy for Advancing Drug Dosage

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Abstract

This proposal outlines a novel approach for fabricating porous films designed specifically for therapeutic formulations. These biodegradable films are sourced from Retama Raetam shrub branches through the innovative Supercritical Anti-Solvent (SAS) process. Renowned for their efficacy in managing hypertension and serving as diuretics, these films also excel in enhancing the entrapment of additional active ingredients. Furthermore, they enhance the entrapment of other active ingredients, thereby improving the stability and composition of tablets or capsules. Notably, the resulting film displays an exceptionally narrow and uniform distribution of pore sizes. Employing conditions of 10MPa and 40°C, carbon dioxide is utilized as the antisolvent to fabricate thin films sourced from Retama Raetam. These films boast a median area of approximately 7μm², underscoring their precise and consistent characteristics.

Keywords: Drug delivery, porous film, *Retama Raetam*, SAS process, Supercritical CO_2.

Introduction

Recent studies have shown a significant interest in innovative drug delivery systems. One such innovation is the development of orally disintegrating tablets pills, which turn solid drugs into forms that dissolve fast in the mouth. There is a wide variety of oral dosage forms available, including tablets, chewable tablets, sublingual tablets, capsules, and liquids. Traditional tablets and capsules can be problematic for patients who have difficulty swallowing, whereas liquids can suffer from stability issues and imprecise dosing. An alternative approach to improve drug delivery involves incorporation of active ingredients into a porous film. This biodegradable film has a precisely distributed network of pores, ensuring rapid disintegration upon ingestion and eliminating the need for water consumption. Not all drugs dissolve uniformly within a short time, and patients often need to consume water concurrently, which can be inconvenient.

To address the challenges posed by active ingredients that cannot be combined with biodegradable materials in tablet formulations, the primary goal is to fabricate a porous film that possesses both robust mechanical strength and exceptional flexibility, while maintaining a uniform distribution of fine pores. This porous matrix serves as an ideal medium for the absorption or seamless incorporation of the specific active ingredient in question.

Various methods have been employed to deposit films and multilayers in pharmaceutical applications. These methods include dipping,

centrifugation, coating, and spraying. Dipping involves coating the substrate with a solution containing polycations and polyanions, followed by rinsing Decher et al. (1). The use of coating techniques has been extended to the field of pharmaceuticals, as demonstrated by Lee et al. (2). These adaptations enable precise and uniform deposition of coatings, ensuring accurate drug release profiles and enhancing the overall performance of pharmaceutical formulations. In addition, spray coating has garnered extensive application across various industries. This versatile technique is employed to efficiently apply coatings, both inorganic and organic, to a wide array of substrates.

In addition, the utilization of supercritical fluid technology enables the creation of an exceedingly thin, porous film, further enhancing the efficiency and precision of this manufacturing process. There has been growing interest in crystallization processes that use fluids at supercritical state. In fact, the crystallization using supercritical carbon dioxide (CO_2) has received considerable attention in the pharmaceutical industry, Chakravarty et al. (3). It has converted as the favorite alternative to conventional processes such as evaporative and antisolvent crystallizations. Carbon dioxide presents an array of appealing attributes, primarily owing to its abundant accessibility, cost-effectiveness, eco-friendly nature, widespread recognition as safe, and its relatively low critical temperature, making it an ideal choice for applications requiring gentle operating temperatures, particularly when dealing with heat-sensitive materials. Supercritical crystallization, employed for microparticle production, arises from the substantial supersaturation achieved through rapid depressurization or swift mass transfer between the solution and the supercritical fluid antisolvent. This method finds relevance in the pharmaceutical sector, as indicated by De Marco (4) and also Franco et al. (5). Furthermore, it enables the projection of a thin, porous film with meticulously controlled micropores.

Conventional methods for extracting natural active ingredients from shrub and plant derivatives, such as grinding, spray drying, and solvent evaporation, have limitations when it comes to producing thin deposits that are free from solvents. The presence of solvents in these formulations can potentially lead to inaccuracies in dosing, which is a critical concern in pharmaceutical and related industries.

The RESS (Rapid Expansion of Supercritical Solutions) technique marked the beginning of the search for more efficient extraction techniques. Notably, Ksibi and Subra (6) showed that this method was effective at extracting α- and β- carotene microparticles that were enclosed in lipid films. According to Sharma and Jagannathan (7), this early success encouraged the investigation of additional applications, such as the use of a thin film of ibuprofen produced using also the RESS technique. Morphologies and shapes may be precisely altered with minimal changes to pressure or temperature, which has enormous promise for therapeutic items as highlighted by Ksibi et al. (8).

In sections that follow, we look into the Supercritical Anti-Solvent (SAS) method as a feasible option for generating thin films, particularly in certain thermodynamic conditions. The findings of the SEM analysis of the resulting deposits are also shown. These outcomes demonstrate the pore distribution pattern that is closely narrow. This appears to be a key component with the potential to raise production efficiency for thin, biodegradable films.

Materials and Methods

The Retama Raetam is an abundant shrub used in southern Mediterranean traditional medicine to cure a number of diseases throughout the year as mentioned by Hayet et al. (9). It was used for diabetes, hepatitis, jaundice, skin diseases, rheumatism, fever, and several types of inflammation as noticed by Leon-Gonzalez et al. (10). Moreover, this shrub is employed in Tunisia as a traditional treatment for snake bites, as documented by Saada et al. (11). Moreover, further studies have revealed

that extracts from the seeds of Retama raetam possess diuretic and hypoglycemic properties. Consequently, these seed extracts have the potential to lower plasma triglyceride levels, as demonstrated by Maghrani et al. (12).

Several authors have noted the utilization of *Retama Raetam* as a homeopathic resource. Edziri et al. (13) highlighted the antimicrobial, antioxidant, and antiviral properties found in the flower extracts of *Retama Raetam* Forssk. Furthermore, Eddouks et al. (14) underscored the antihypertensive and diuretic effects observed in the aqueous extract of *Retama Raetam* Forssk. leaves, which were investigated in both normotensive and spontaneously hypertensive rats as mentioned by Eddouks et al. (14).

Numerous research efforts have been dedicated to the comprehensive exploration and characterization of various elements derived from the *Retama Raetam* shrub. These investigations encompass a wide range of aspects related to the plant's composition and properties. One notable study conducted by Hayet et al. (9) investigated deeply into the intricate phenolic composition found within *Retama Raetam* flower oil. This research shed light on the specific phenolic compounds present in the oil, elucidating their potential applications and benefits. In fact, Oil from flower contains a total of 50 components mainly nonanal, linalool, myrcene and many others polyaldehydes and terpenes with a low percentage. In a separate study, El Yadini et al. (15) provided a detailed account of the composition of *Retama raetam* monosperma stems and seeds found in the arid expanse areas and across the Middle Atlas in Morocco. This research not only highlighted the nutritional and chemical makeup of these seeds but also contributed to our understanding of the plant's adaptability to challenging environmental conditions.

Beyond these, several other research endeavors have focused on unraveling the intricate constituents found within different genus of *Retama Raetam*, Leon-Gonzalez et al., (10). These investigations aim to unravel the plant's complex chemical profile, potentially uncovering novel compounds with diverse applications in various fields, from agriculture to medicine.

Furthermore, recent research has unveiled the antimicrobial and antioxidant potential of essential oils derived from Retama raetam, indicating their suitability for applications in the food and pharmaceutical sectors. Notably, a prior investigation conducted by our research team has underscored the antioxidant and antimicrobial attributes of this extract, as reported in Rejab and Ksibi (16).

Figure 1- Retama Raetam shrub flowers and seeds

Our primary emphasis lies in the application of eco-friendly natural solvents and the development of innovative technology systems for utilizing supercritical fluids, separation processes, and material production. As an illustration, eco-conscious chemical methods that involve carbon dioxide, with traits closely resembling those of organic solvents in their supercritical state, provide both ease of handling and eco-friendliness

Supercritical fluid technology is a promising route for the extraction, separation and shaping of divided solids including the SAS process which leads to homogeneous precipitation and recrystallization of materials into microscale size with generally narrow distribution. More-

over, materials regenerated from vegetable matrices using SAS supercritical fluids offer an appealing approach for recrystallizing numerous organic compounds sourced from plants. This method is particularly advantageous for substances that are challenging to fragment or recrystallize, such as extremely long-chain pharmaceuticals that necessitate the production of uniform ultrafine particles with consistent morphology, De Marco (4). The use of the SAS technique allows an accurate control of the crystallization process, resulting in very small and uniform particles, Rejab and Ksibi, (17). Furthermore, the separation of the anti-solvent from the particles after precipitation was readily accomplished. This allows for the avoidance of substantial solvent by-products and the potential establishment of an advantageous solvent and anti-solvent flow.

As the substance *Retama Raetam* is not soluble in supercritical CO_2, it is first solubilized in a supercritical organic solvent (itself soluble in CO_2). Then, the solution is confined in an atomization tank through a coaxial spray nozzle where it is brought into contact with CO_2 which will separate the molecule from the organic solvent, leading to precipitation. Simultaneously, the solvent evaporates in the supercritical phase, elevating the solute's concentration. This dual-directional mass transfer is the key factor behind the swift supersaturation of the solute, resulting in its nucleation.

The application of this process to pharmaceutical compounds involves specific constraints. Particular attention must be paid to the choice of the solvent. The choice of the solvent is based on three requirements. The first is its good miscibility with the anti-solvent (CO_2). The usual solvents such as ethanol, toluene and acetone show complete mutual miscibility with SC-CO_2. The second is the solubility of the solid to be crystallized and the third is its human safety. Indeed, the solvent must generally belong to class 3 (non-toxic) of the pharmaceutical guidelines. SAS precipitation results in the production of solvent-free materials, affording precise control over the morphology, size, and polymorphic phase of the precipitated material.

Figure 2 illustrates a schematic representation of the apparatus employed in the Supercritical Anti-Solvent (SAS) process. From literature, we can underline potentialities of the kind of crystallization as the tiny during the process which occur a very fine droplet and a high specific surface area for mass transfer. The freshly precipitated aggregates are retained within the system, while the supercritical fluid and organic solvent are continuously drained from the system. As the carbon dioxide is used in this process, it can be removed easily from the system by depressurizing. We mention that precipitations were obtained at the inner of the vessel. The experiments conducted in this study are mainly focused on the variation of carbon dioxide pressure, temperature on porous film dimensions and distribution.

The SAS process commences by pressurizing CO_2 to the desired level within the precipitator, followed by precise temperature control. CO_2 is methodically transitioned into a supercritical state, ensuring meticulous attainment of the designated pressure (10 and 12 MPa) within a high-pressure vessel. This prepares the groundwork for executing Supercritical Anti-Solvent (SAS) experiments, as per established protocols. The selection of a 2 kg/h CO_2 flow rate is a judicious decision, informed by prior research, particularly the findings of Rejab and Ksibi (17) which demonstrated its effectiveness in achieving solution atomization.

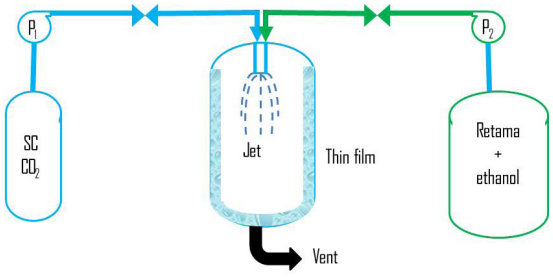

Figure 2- A schematic representation of the SAS process

Within this meticulously controlled environment, a liquid solution is introduced, comprising a carefully crafted mixture of powdered *Retama Raetam* and ethanol. This solution is expertly sprayed into the reactor using a purpose-designed nozzle, where the solvent undergoes rapid diffusion. This diffusion process causes the solution droplets to disperse into the bulk supercritical fluid, initiating the intricate sequence leading to the solute's precipitation. The culmination of these precisely engineered conditions results in the formation of a porous film, as detailed in Table 1.

Subsequently, we undertake a washing step, during which the precipitator is depressurized to atmospheric levels, and the film that has precipitated on the inner wall of the precipitator is meticulously collected for further analysis and examination. Our experimental runs were conducted to assess the process's efficiency in generating thin *Retama Raetam* films with controlled pore structures. Such films hold promise for addressing challenges associated with the incorporation of additives that are difficult to swallow, ensuring precise dosing, and maintaining consistent particle sizes. During each run, we systematically varied the operating conditions to explore the effects of different combinations. The results derived from each operational run are derived from observations made during a stabilized operating period under a specific set of operating conditions.

Table 1- Operating Run conditions

Run	T °C	P MPa	Volume of solution /ml
1	30	10	40
2	40	10	25
3	40	10	40
4	50	12	40

Results and Discussion

The application of film coating or deposition in general, to achieve customized surface properties holds significant relevance in the fields of pharmaceuticals, cosmetics, and food manufacturing. Through the engineering of plant materials, it becomes possible to impart specific physical, chemical, biochemical, and pharmaceutical characteristics by creating a thin, porous film over active principle particles. This versatility allows for the fine-tuning of critical parameters such as operating pressure, temperature, dissolution rate, chemical reactivity, and flow rate, thereby accommodating a wide spectrum of applications, Souiy et al. (18). Several studies show that SAS is not merely an effective process for nucleated material morphology control and scalability. Conducting processing under mild conditions can additionally enhance the stability of biopharmaceuticals without compromising their conformational structure.

Nevertheless, it is crucial to highlight a notable observation from our experiments. Instead of adhering to the intended surfaces, the film coating unexpectedly occurred within the chamber, specifically on the inner wall of the precipitator and its bottom. We subsequently scraped the film coating from these surfaces and subjected it to examination under a scanning electron microscope (SEM).

Biopharmaceuticals processed with SAS process are also submitted to atomization spray formation. However, several studies have shown that RESS, PGSS and other supercritical processes conduct only particle (spheres and sticks) after nucleation and separation. The tunable properties of $scCO_2$ mean that material engineering can speed up the process. Habulin and Knez (19) found that an increase of supercritical carbon dioxide flow rate led to a loss of relative activity and therefore, a narrow distribution of particle size with a quasi-uniform morphology. It is important to call attention to the fact that no excipients were used during drying and the product (thin film or particles) is free of solvent.

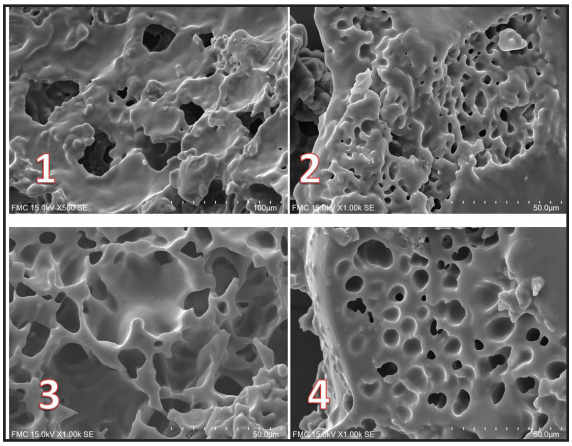

Figure 2- formed films on the inner wall vessel for several run cases

The present study delves into the analysis of various film deposits within a defined study area using ImageJ software. Our primary objective is to determine the average pore area within the blackened regions and establish a corresponding distribution function for each experimental condition. With reported porosity levels ranging from 40-50%, as per Rejab and Ksibi (16), we noted differences in the average pore class distribution across various study locations.

Figure 3 presents the outcomes from multiple experimental runs, revealing the consistent formation of spongy thin films in each case. The figures depicting *Retama Raetam* deposits at a microscopic scale offer insights into the internal structure of the atomization slot and the resulting film. These variances in pore size can be attributed to working temperature, pressure conditions, and the volume to be dissolved, highlighting the intricate relationship between these factors in film deposition. The examination of regenerated Retama Raetam film surfaces was carried out using a *Zeiss Auriga Compact* focused ion beam scanning electron microscope (FIB-SEM) with an accelerating voltage set at 10 kV. The scale was chosen at 50 µm to get a clear idea about pores morphology, area, distribution and depth. Thin films obtained by the SAS process have exhibited the highest organic porous film efficiency offering a best stability to incorporate active principles. With SAS process, it would be interesting to study optimal conditions *Retama Raetam* extract deposition from spray-drying as thin film.

Discussion

Utilizing digital processing techniques on these microphotographs, which involved the use of image analysis software and *Gnuplot* surface tracing, we were able to gain valuable insights into the cumulative intensity within the porous regions, both along and across the deposited material as shown in figure 3.

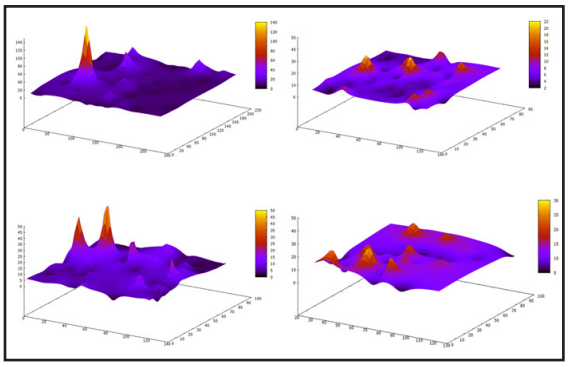

Figure 3- Cumulative Porous Area Intensity (µm²) of the Film from Bidirectional PSD Compilation in Multiple Run Cases

Notably, in the cases of runs 1 and 3, we observed a significant accumulation of porous zones. This accumulation suggests that the *Retama Raetam* -based matrix created in these runs has a substantial capacity for incorporating additional pharmaceutical additives. The various peaks evident in these cases reflect the varying degrees of incorporation within the matrix, shedding light on the potential for controlled release and distribution of these additives.

Conversely, when we examine the diagrams for runs 2 and 4, we notice that the peaks are much lower. This implies a more consistent and evenly distributed porous structure throughout the film. The quasi-regularized distribution in these cases may have its own advantages, de-

pending on the intended use and desired characteristics of the pharmaceutical coating or film.

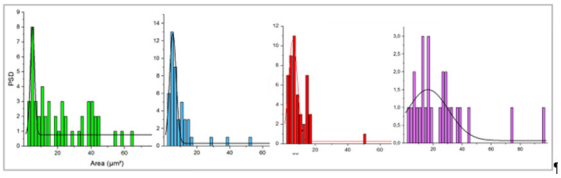

Figure 4- Pore Size Distribution in Various Deposition Films

Figure 4 illustrates the distribution of porous area intensity in the Retama Raetam film deposits, corresponding to the images in Figure 2. Various pore shapes and sizes were achieved by adjusting key parameters. By referencing Table 1 and the pore area distributions depicted in Figure 4, it becomes evident that the operational conditions of the SAS process had a substantial impact on both thin film morphology and pore size distribution.

In the first experiment, Run 1, we conducted operations at a pressure of 10 MPa, maintaining the temperature in close proximity to the critical point of CO2. Our objective was to fabricate a thin film characterized by micron-scale porosity. In this instance, we observed that the medium pore area averaged around 5µm², albeit with the presence of some larger pores.

Moving on to Manipulation Run 3, we maintained the same pressure as the previous run while elevating the temperature beyond the critical point (T=40°C). This adjustment resulted in a subtle increase in pore size, with an average exceeding 10µm but displaying a narrower distribution. A careful examination of the Particle Size Distributions (PSDs) presented in Figure 5 clearly indicated the absence of large pores in this scenario.

In Run 2, we replicated the thermodynamic conditions of Run 3 but introduced a modification by reducing the volume of the solution (comprising *Retama Raetam* and ethanol) from 40ml to 25ml. Under these altered conditions, the film collected within the precipitation chamber exhibited an average pore size of 5µm, distinguished by a considerably narrower distribution. Consequently, the reduction in solution volume not only led to a shortened deposition time but also restricted the growth time of film porosity.

Lastly, in Run 4, we conducted experiments under high-pressure and high-temperature settings, yielding a deposited film characterized by a diverse range of pore sizes. Consequently, the pore area exhibited variations spanning tens of microns, resulting in a distribution that extended widely across the spectrum.

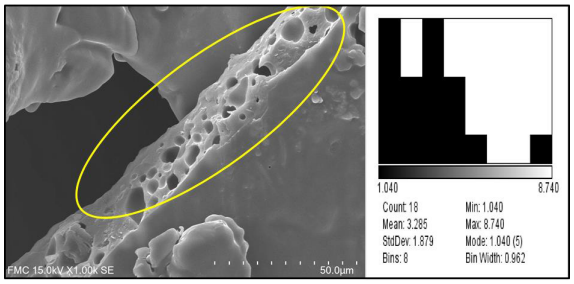

Figure 5- Run 2 Film thickness and Corresponding Pore Area Distribution

During the second experimental run (Run 2), we succeeded in producing a film with an impressive thickness of approximately 18 µm. This film displayed distinct, visually observable pores, as illustrated in Figure (b). What sets this particular material apart is its classification as a truly uniform porous medium. The average pore area within this medium measures approximately 3.5 µm², serving as compelling evidence of the remarkable uniformity and consistent distribution of pores throughout the entire film.

This level of uniformity and controlled porosity is of significant interest in various applications, such as drug delivery systems or materials with specific filtration requirements. The findings from Run 2 highlight the potential for creating materials with precisely engineered porosity, which can be instrumental in achieving desired characteristics and performance in various fields of science and technology.

Conclusion

The SAS coating technique holds significant potential as an environmentally mindful approach for potentially entrapping microparticles through pore creation. This is achieved by generating porous, thin films using vegetable matrices like Retama Raetam. This method finds application in the pharmaceutical industry and other sectors where preventing chemical interactions is of paramount importance.

The figures depicting *Retama Raetam* deposits via SAS process at a microscopic scale offer insights into the internal structure of the atomization slot and the resulting film. These variances in pore size can be attributed to working temperature, pressure conditions, and the volume to be dissolved, highlighting the intricate relationship between these factors in film deposition. In fact increasing pressure led to very close pore distribution and a mean area pore about 7µm² whereas increasing temperature favorites the formation of film with large pores.

List of abbreviations

SAS: Supercritical AntiSolvent process

SC-CO_2: Supercritical carbon dioxide

FIB-SEM: focused ion beam scanning electron microscope.

CPA : Cumulative Pore Area

PSD : Particle Size Distribution

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