Supercritical fluids for the treatment of bioactive components

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Abstract. *Supercritical fluids are marked as green solvents able to substitute toxic organic solvents. Therefore, processes involving supercritical fluids are seen as, besides others, green chemical processes with a huge potential for the food and pharmaceutical industry. Research on supercritical fluids evolved with time, spreading the possibilities and opportunities. The following paper gives a brief overview of the process involving supercritical fluids. Supercritical extraction, supercritical drying and impregnation of aerogels and particles from gas saturated solution are explained through the research performed and published by our research group. The most significant achievements are thoroughly explained and compared with the results available in the literature. The most important conclusions are underlined, and future remarks are discussed.*

Keywords. Supercritical fluids, supercritical extraction, aerogels, PGSS™, supercritical impregnation

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1 Introduction

The principles of green chemistry [1] and the principles of green engineering [2] raised concerns about environmental issues. They resulted in the development of "green chemical processes" that are safe and environmentally friendly. The possible solution for making chemical processes greener was to substitute hazardous solvents with aqueous solutions, supercritical fluids, ionic liquids, low-toxicity organic solvents. Therefore, the development of alternative technologies is essential. Reducing energy consumption, lowering toxic residues and byproducts, increasing conversion efficiency (reactants to products), and increasing final products' quality and safety are crucial requirements for future processes [3]. One of the ways to fulfill these demands is to apply high-pressure technologies.

Among high-pressure technologies, subcritical and supercritical fluids offer a high range of excellent technologies due to their physicochemical properties. The idea of supercritical fluids goes back to the 1820s, but an interest in them really began in the 1960s and 1970s, with research focused on extraction techniques. Later on, attention was drawn to the unique solvent properties of supercritical fluids. As presented by Fig. 1, three regions correspond to the pure compound's solid, liquid or gas states, separated by curves that meet at the triple point (TP). The vaporization/liquefaction curve has an end point called a critical point (CP), beyond which only one phase exists, the supercritical fluid (SCF).

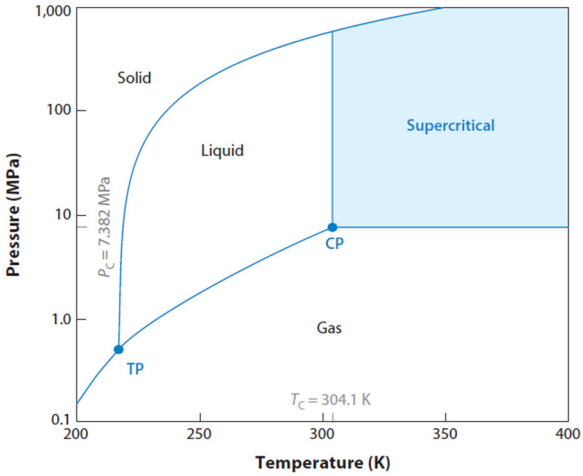

Figure 1. Phase diagram for the pure compound (CO2) [4]

Supercritical fluids have temperatures and pressures above their critical values ($T>T_c$, $p>p_c$). At a critical point, liquids and gases coexist, and supercritical fluids possess properties that are different from either liquids or gases under standard conditions. Their physical and chemical properties are between those of liquids and gases. Their main characteristics are densities similar to liquids, viscosities similar to gases, and diffusivities between those of gases and liquids, as shown in Table 1.

Table 1. Physical properties of gases, supercritical fluids and liquids [5].

Mobile Phase	Density (kg/m^3)	Viscosity (kg/m/s)	Diffusivity (m^2/s)
Gas	1	10^{-5}	10^{-4}
SCF	$0.3*10^{-3}$	10^{-5}	10^{-7}
Liquid	10^{3}	10^{-3}	$5*10^{-10}$

At the critical point, the diffusion coefficients are more than ten times higher than in liquids, making the mass transfer with supercritical fluids generally fast. Density is also highly sensitive to small changes in temperature and pressure. Even though the density values are similar to liquids, the solubilities can be much higher.

SCFs have the ability of liquids to dissolve materials and the ability of gases to penetrate porous solid materials. Furthermore, there are no surface tensions, thus no capillary forces during processing [6]. The solvent power of SCF is directly related to its density. By regulating the pressure, the solvent power can be manipulated to imitate the properties of organic solvents and eventually replace them. The separation of substances from solvents is easy to achieve.

Furthermore, it is possible to add modifiers to SCFs to change their polarities. For example, the nonpolar nature of $scCO_2$ can be overcome by adding modifiers in the form of polar organic co-solvents. This means that they have the potential to replace numerous chemicals in industries [6].

Table 2 gives insight into the most used sub and supercritical fluids and their critical values [7].

Table 2. Critical temperatures and pressures of the most used supercritical fluids

Supercritical fluid	T_c ($^\circ$C)	p_c (bar)
Argon (Ar)	-122.5	48.6
Carbon dioxide (CO_2)	31.1	73.8
Water (H_2O)	374.0	220.6
Sulphur hexafluoride (SF_6)	45.5	37.6
Xenon (Xe)	16.6	58.3
Fluoroform (CHF_3)	25.9	48.2
Difluoromethane (CH_2F_2)	78.1	57.8
Dimethyl ether (C_2H_6O)	126.9	54.0
Propane (C_3H_8)	96.7	42.5

The most exploited supercritical fluid is carbon dioxide since it is chemically inert, nonflammable, nontoxic, recyclable, and naturally abundant. Additionally, the critical point of CO_2 occurs at mild temperature and pressure conditions of 31.1 $^\circ$C and 73.8 bar. Therefore, it is a commonly used solvent in the food and pharmaceutical industries. Since it is a gas in ambient conditions, it can easily be removed from products by simply reducing pressure. Its critical temperature is close to the ambient, making it ideal for natural products thermolabile. It can be recovered for recycling and is miscible with organic solvents but can also replace them [8]. Carbon dioxide (O=C=O) is generally a nonpolar molecule, with the presence of a

small polarity, owing to a quadrupole moment, and, therefore, is categorized as a hydrophobic solvent. It dissolves lipids that are water-insoluble compounds, such as vegetable oil, butter, fats, hydrocarbons, etc. However, it does not dissolve hydrophilic compounds, such as sugar, proteins, salts, metals, etc. In industry, $scCO_2$ has mostly been used for coffee decaffeination, tea decaffeination, and the extraction of fatty acids from spent barley, pyrethrum, hops, spices, flavors, fragrances, and corn oil, as well as the extraction of color from red peppers. Some other applications include polymerization, polymer fractionation, particle formation for pharmaceutical and military use, textile dyeing, and the cleaning of machine and electronic parts [9].

The application of SCF is increased, especially in the field of chemical and biochemical reaction [10], for the synthesis of new materials and new catalyst support such as aerogels [11], for special separation techniques such as chromatography [12], extraction processes [13], and particle formation and product formulation [14].

SCFs are considered green solvents for the future due to their ecological benefits, particularly low energy consumption. Nowadays, they have been used in several processes operating on a large scale in the pharmaceutical, food, textile, and chemical industries [15].

2 Supercritical Extraction

Supercritical fluid extraction (SFE) is a separation process in which solids or liquids from the matrix are separated using SCF as the solvent. The specific properties of SCFs allow the solvent characteristic to be changed during the extraction process simply by changing the temperature and pressure. Investigation of SFE technology started a few decades ago. Hundreds of supercritical extraction plants operating at excessively high pressures (up to 2000 bar) have been designed worldwide. The most common high-pressure processes on a large industrial scale are the decaffeination of tea and coffee, the extraction of hop components, and the separation of lecithin from oil. Several industrial plants use different dense gases to isolate or fractionate various components. For example, the extracts obtained from the extraction of oils from seeds, fruits, leaves, and flowers, are further used in the food, pharmaceutical, and cosmetic industries [16, 17].

The use of supercritical fluids in extraction processes allows the production of environmentally friendly and safer extracts compared to conventional techniques. Using supercritical fluids in industrial extraction can replace much more harmful traditional solvents. One of the most important advantages of using SCFs as an extraction media is the possibility of selective extraction of components or fractionation of total extracts. One of the most important influencing factors is the mass transfer of the solute in the supercritical solvent, which depends on the solubility of the solute in each solvent. The mass transfer also significantly impacts the economics of the extraction process itself [18, 19].

Different compounds have different solubilities at different processing conditions. Temperature and pressure have the most significant influence on solubility in SCFs. For example, an increase in temperature at constant pressure decreases the density of the solvent.

As a consequence, the solubility of the solute decreases. Similarly, an increase in temperature at constant density increases the vapor pressure of the solute. Therefore, the substance is more soluble in the supercritical fluid. Depending on the system, one of the effects will prevail. The effect of pressure is more direct. A higher density of supercritical fluid will be achieved by increasing the pressure. Furthermore, the greater the density of the medium, the greater the solute's solubility [16].

The most common solvent used for supercritical extraction is $scCO_2$, especially for the extraction of nonpolar compounds. For the extraction of more polar components, a polar modifier or co-solvent is added to the $scCO_2$. Consequently, the solubility of the polar components in the supercritical solvent will increase. Examples of such modifiers are methanol, ethanol, etc. By adding a modifier, the density, and the viscosity increase. It results in a decrease in the mobile phase's diffusivity, thereby reducing the mass transfer [16].

For obtaining the highest possible yields, the process has to be optimized. Firstly, the influence of the process parameters on the extraction has to be investigated. One way to define the optimal process parameters is response surface methodology (RSM). This method uses a multiple regression model with the second-order polynomial equation from which the optimum parameters are chosen [20]. Extraction rates also depend on the morphology of the material and the location of the desired compounds in the plant material. Extraction rates will be high if desired compounds are located on the surface of a material. Conversely, if the desired components are located deeper inside the material, they will require more time to be extracted. Mass transfer depends on the particles' shape and size and the porosity of the solid material. If the material's structure is more complex and the desired compounds are deeper in the material, higher diffusion resistance is expected [21]. For this reason, sample preparation is crucial. The material needs to be mechanically pre-treated (grinding, milling, cutting, etc.) to reduce the average particle size. Smaller particles provide faster extraction due to smaller diffusion pathways and lower diffusion resistance. It is necessary to find a suitable particle size, as these particles should not be too fine despite mechanical processing.

2.1 Extraction of solids and liquids using supercritical fluids

Using SCFs as an extraction processing media enables processes at lower operating temperatures without organic solvents residuals and lower energy consumption. Final products are solvent-free. The basic scheme of the extraction procedure is shown in Fig. 2. The extractor is loaded with a certain amount of material. The gas is introduced from the solvent tank into the extractor by the high-pressure pump into the extractor at P_{ext} and T_{ext}. The material (extract) is collected at T_{S1} and P_{S1} in the separator.

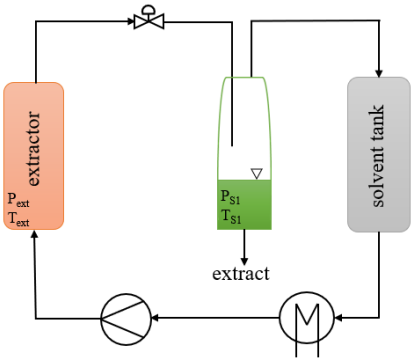

Figure 2. Basic flow sheet of SCF extraction apparatus [22]

Very important is selective extraction of components or fractionation of total extracts. This can be achieved by using different gases to isolate/fractionate the components and/or by changing the process parameters. The solubility of each component in the SCF is a fundamental input for the design of supercritical extraction processes for solid and liquid mixtures. The requirement for the extraction is the solubility of the components or mixtures of compounds in the SCF. In the extraction step solubility of the substance has to be at its highest level. While in the separation step, the solubility should be the lowest. That being the case, information on the phase equilibria of the systems is crucial for designing the operating pressures and temperatures of the SCF in the extraction unit. The theoretical mass of the SCF required to separate the compound from the solid or liquid mixture should be determined. The solute's solubility data in SCF mass transfer also has a major influence on the economy of the extraction process. Mass transfer models usually describe the extraction efficiency as a function of extraction time. For the design of extraction plants, it is preferable to present the efficiency as a function of the solvent to feed ratio (S/F). Typical extraction curves are shown in Fig. 3, describing the efficiency of chia seeds extraction as a function of S/F [23]. It is shown that pressure has a strong influence on the extraction yield. Higher operating pressures contributed to higher extraction yields at a constant temperature.

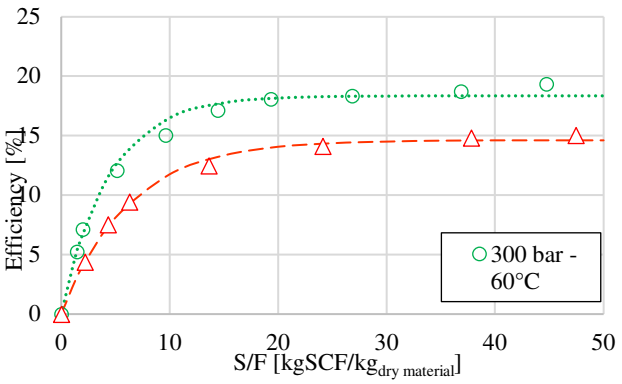

Figure 3. The extraction efficiency of chia seed extraction as a function solid to feed ratio

2.2 Published research

Our research group has performed multiple extractions with SCFs, published in scientific papers. Table 3 summarizes the natural materials, extracted compounds, fluid, and conditions used for the extraction processes. Most commonly, extraction of phenolic compounds was performed at different pressures and temperatures from various natural sources such as elderberry, pomegranate, oregano, etc. The most frequently used solvent was $scCO_2$. The extraction of phenolic compounds from grape marc and elderberry has been investigated by Vatai et al. [24]. Extracts with high anthocyanin content, showing stability during storage, would be potentially interesting for commercial applications as natural colorants. Furthermore, saw palmetto berries, chia seeds, and sunflower seeds were extracted to obtain fatty acids by unconventional techniques [23, 25, 26].

Using water as a sub- or supercritical medium has become very interesting. Subcritical water is a good substitute for organic solvents and an environmentally friendly medium for treating various materials without adding a catalyst. Large amounts of residues are produced in the food industries. The treatment or extraction of natural waste matter with different SCFs for extracting more valuable active substances is attractive. For instance, Gagić et al. [27] extracted the chestnut bulk using subcritical water. The influence of process parameters, such as extraction time, temperature, and solvent-solid ratio on extraction yield was examined. Moreover, the yield of the main compounds, total phenol content, total tannin content, and antioxidant activity have been studied. Bark extract was highly rich in ellagic acid and with acceptable total phenols and total tannins levels. In a separate study, the separation of valuable compounds from cocoa shells by applying a sustainable green separation process of subcritical water extraction (SWE) was investigated. Used temperatures were in the range between 120 and 220˚C. Different concentrations of theobromine, caffeine, theophylline, epicatechin, catechin, chlorogenic acid, and gallic acid were determined [28]. Phenolic compounds from large wood waste were extracted using SWE in the range of 100 to 300˚C [29].

The industrial processing of tobacco generates a significant amount of waste. It consists of residues, pith, and dust. A majority is released directly into the environment and causing pollution. Therefore, the use of waste raw materials is preferable. Subcritical water, as an environmentally friendly and cheap technology, was used for the extraction of active compounds from different types of industrial tobacco waste. Valuable active compounds like nicotine, rutin, and phenolic acids (3,4-DHBA and chlorogenic acid) and other specific compounds, such as nicotinamide and nicotinic acid, were characterized [30].

Table 3. Published results from our research facility

Natural material	**Extracted compound**	**Type of fluid***	**Conditions**	**Ref****
Black yeasts	proteins	CO_2	100, 300 bar, 35 ° C	[31]
Chamomile	matricine	CO_2	100, 250 bar, 30, 40 ° C	[32]
Chestnut bark	ellagic and gallic acids, ellagitannins, sugars and sugar derivatives, furfural and levulinic acid	H_2O	150, 250 ° C	[27]
Chia seed	fatty acids	n-propane	up to 300 bar, 40, 60 ° C	[23]
Chilli pepper	capsaocinoid	CO_2	100-400 bar 40-80 ° C	[33]
Cocoa shell	theobromine, caffeine, theophylline, epicatechin, catechin, chlorogenic acid and gallic acid	H_2O	120-220 ° C	[28]
Common juniper	terpenes	CO_2	80-100 bar, 40 ° C	[34]
Curcuma longa L.	curcuminoids	H_2O	100-200 ° C	[35]
Elder berry	phenolic compounds	CO_2	150, 300 bar, 40 ° C	[24]
Feverfew flower	parthenolide	CO_2	200-800 bar 40-80 ° C	[36]
Ganoderma lucidum	phenolic compounds	CO_2	250, 300 bar 40, 50 ° C	[37]
Grape marc	phenolic compounds	CO_2		
Green tea	caffeine, major catechins, flavonols	CO_2	225-350 bar 50-80 ° C	[38]
Hop	α- and β-acids	CO_2	50-150 bar, 20-80 ° C	[39]
Horse chestnut	escins, esculin, fraxin, phenolics	H_2O	100-250 ° C	[40]
Larch wood waste	phenolic compounds	H_2O	100-300 ° C	[29]
Origano	phenolic compounds	CO_2	150, 250 bar 40, 60 ° C	[41]
Pomegranate	phenolic compounds	H_2O	100-220 ° C	[42]
Rosemary	carnosic acid	CO_2	100, 200 bar, 35, 60 ° C	[43]
Saw Palmetto berries	fatty acids and β-sitosterol	CO_2	300, 450 bar, 40, 60 ° C	[25]
Silybum marinum	vitamin E rich oil	CO_2	100-300 bar 35-80 ° C	[44]
Sunflower seeds	fatty acids	H_2O	60-160 ° C	[26]
Tagetes erecta	lutein	CO_2 n - propane	300 bar, 40-60 ° C 100-200 bar, 40, 60 ° C	[45]

Natural material	Extracted compound	Type of fluid*	Conditions	Ref**
Tobacco waste	Nicotine phenolic compounds	CO_2, H_2O	100-300 bar, 40-80 ˚C	[46]
Tobacco waste	nicotine, DHBA, chlorogenic acid, rutin	H_2O	150-250 ˚C	[30]

*Type of sub/supercritical fluid, **Reference

Few laboratory-scale studies are available for liquid – SCF extraction [47]. Several data on binary systems between liquid and SCF were found, but there is a lack of data for liquid/liquid/SCF systems. Extraction of liquid mixtures with supercritical fluids is similar to liquid-liquid extraction, except that compressed gas is used instead of an organic solvent. Pressure plays a vital role in the extraction of liquids with SCF. Selective extraction of the components or fractionation of the total extracts can also be carried out using different gases and by varying the parameters themselves. Also, in liquid-SCF extraction, one of the most important advantages of using SCF is the easy regeneration of the solvent. In conventional liquid-liquid extraction, solvent regeneration in most cases involves the necessary re-extraction or distillation, which is energy-intensive and therefore expensive. Another advantage is that liquid phase can be introduced into and continuously removed from the high-pressure unit.

To summarize, green extraction technologies such as supercritical fluid extraction can satisfy all current and likely future regulations relating to health, safety, and the environment. In addition to the most used carbon dioxide, other sub- or supercritical solvents are also used for sub- or supercritical extraction. The use of sub- and supercritical water has become particularly interesting. Currently, water is the cheapest solvent, and many substances are highly soluble in water. Industrial waste is a major problem in developed countries, with economic, environmental, and social impacts. For this reason, waste treatment has received a lot of attention in the last few years.

The main drawback of the SFE relates to high investments. The price of products obtained by SFE can be relatively high compared to those obtained by conventional methods. However, this disadvantage is balanced by the avoidance of legal costs, restrictions on solvents, and solvent residues in products used for animal or human utilization. In addition, there is the possibility of isolating and fractionating specific compounds from whole extracts. It is also possible to formulate them immediately and sterilize them without the use or high temperatures. All this promotes the use of dense gases for extraction purposes.

3 Aerogels: a method for impregnation of bioactive components

Nowadays, the term "aerogels" may refer to a wide range of different materials. At the beginning of their production in the 1930s [48], aerogels were materials obtained by applying supercritical drying technique, a novel and promising method in synthesizing dry without collapsing the solid network. Their main component is air, surrounded by a solid network. Back then, the interest in this field was low. In the 1980s, aerogels science was revived, leading to a huge interest in academic and industry research. As a result, the estimated market volume of silica aerogels was around 300-400 M euros in 2019 [49, 50]. Up to date, aerogels are recognized as materials having unique properties such as high specific surface areas, low densities, and high porosities. These properties lead to others, such as low thermal conductivity, low sound velocity, and high optical transparency. These properties are mainly obtained by applying the supercritical drying technique. However, the literature nowadays presents materials with all of the mentioned properties obtained without the supercritical drying technique [51]. This is why the definition and the term "aerogel" became controversial and prone to changes over time. In the more narrow context, aerogels are solid materials with open-celled, highly porous, and predominantly mesoporous structures (2-50 nm pores) derived from wet gels [52].

Most commonly, aerogels are prepared from molecular precursors through sol-gel processing. Firstly, sol is formed from the solution of precursors. Once the sol particles are condensed, gelation takes place, and gel is formed. A three-dimensional network of gel is usually filled with water or alcohol, forming hydrogels or alcogels. Obtaining dry gels from wet gels while maintaining the solid network may be challenging. At ambient pressure drying, the gel shrinks strongly during the evaporation of the solvent from the pores. The presence of liquid/gas interface in the pores during evaporation will cause the formation of capillary forces and pressure gradient on the pore walls. This leads to the collapse of the gel structure and the formation of the so-called xerogels [53]. The supercritical drying process overcomes the disadvantages of ambient pressure drying. If the solvent is put in a supercritical state, the presence of two phases is avoided. Both temperature and pressure are raised above the critical point of the corresponding solvent and kept there for a certain period of time. In a modified version mostly applied nowadays, the original solvent from the pores is exchanged with carbon dioxide (CO_2) already in a supercritical state [54]. The use of CO_2 is convenient due to mild temperature and pressure conditions for the critical point. By simply reducing the pressure, it can be removed from the products since it is a gas at ambient conditions. It is convenient for thermolabile products due to low critical temperature. Lastly, supercritical CO_2 can sorb into many polymers causing their swelling and making them appropriate for the impregnation [54].

Aerogels are materials with various chemical and physical properties, leading to many applications [55]. To name just a few, they can be used in cosmetics [56], for diverse medical applications, especially as drug delivery systems [57], in microelectronics [58], as catalysts

[59], insulators [59], capacitors [60] or carriers for drugs in food and pharmaceutical industry [61].

Different types of aerogels are recognized in the literature: inorganic, inorganic-organic hybrid, and organic. Silica (SiO_2) gels are the best-investigated materials in inorganic chemistry [50]. SiO_2 gels were used in the first attempt to produce SiO_2 aerogels by Kistler. The production process of SiO_2 aerogels was optimized over time and simplified. The network formation begins with the aqueous solution of salts or molecular precursors, usual alkoxysilanes in organic solvents. The prepared solution can be employed through sol-gel processing and, eventually, drying. In the case of non-silicate inorganic gels (titanium, zirconium, tin, aluminum), the network is formed by the same principles [50]. Organic molecules can be combined with the structural elements of inorganic materials. Organic molecules or groups are integrated during sol-gel synthesis. By modifying oxide aerogels with organic groups, the properties of aerogels can be improved. For example, the hydrophobicity and the elastic properties of SiO_2 aerogels can be improved by incorporating organic groups [62, 63]. As an alternative to inorganic aerogels, purely organic resorcinol-formaldehyde aerogels were synthesized [64]. Despite the structural differences coming from different precursors, organic aerogels had common properties, such as high surface areas and high porosities.

Polysaccharide aerogels are among the best-investigated materials in the aerogels science community nowadays. Polysaccharides are natural materials with high stability, renewability, availability, and low toxicity [65]. Moreover, they are bioavailable, biodegradable, and prone to chemical modifications [66]. As such, polysaccharides are key ingredients for producing bio-based materials in life sciences (food, cosmetics, medical devices, and pharmaceutics). The wide range of polysaccharides will allow incorporation into pharmaceutical products with different routes of delivery, target organs and/or drug release profiles. Furthermore, they can be applied as solid matrices in different forms, such as monoliths, beads, micro-, or nanoparticles [67].

3.1 Methods of impregnation

Due to their outstanding properties, aerogels are proposed as possible carriers for bioactive components. The impregnation process is convenient since aerogels are highly porous materials with high specific surfaces areas and open porous structures. The impregnation process is defined as imbuing or saturating a material or substance with something. Bioactive components, e.g., drugs, can be impregnated into aerogels following two different routes, as presented by Fig. 4. The first method implies the addition during the sol-gel process. It is a flexible and straightforward method in which the drug is added to the sol solution before gelation. However, it is essential to investigate if and how the added component may influence the gelation process. It must be able to withstand all the processing steps during the aerogel's synthesis. During supercritical drying, the added component may be washed out by CO_2. The second possibility is to impregnate bioactive components through the post-treatment of dried aerogels, e.g., supercritical impregnation, vapor deposition, reactive gas treatment. The

method implies the penetration of the drug into the aerogel's pores from the supercritical, liquid, or gaseous phase. The method is, however, limited by diffusion [50]. Supercritical impregnation of aerogels is a method mainly applied for poorly water-soluble components. The technique implies the incorporation of desired bioactive components, e.g., drugs, into aerogels under supercritical conditions. It is of the most promising methods of improving the dissolution and adsorption of poorly soluble drugs. Furthermore, supercritical impregnation has been proven to be more effective and tunable compared to the traditional sol-gel incorporation [68]. When it comes to poorly water-soluble drugs, the way to enhance their dissolution is by impregnating them into water-soluble carriers [69].

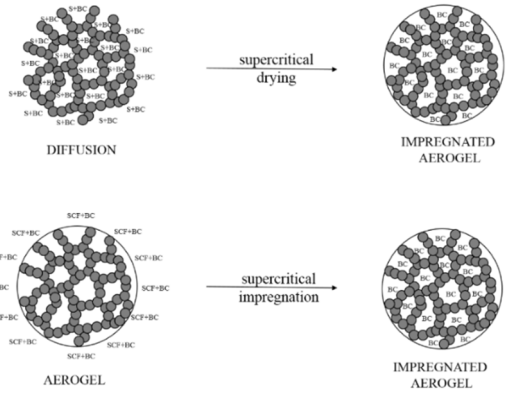

Figure 4. Impregnation processes of aerogels: diffusion via sol-gel versus supercritical impregnation process

3.2 Published results

Over the years, the research on aerogels in our laboratory evolved from silica-based to organic, polysaccharide-based aerogels. Table 4 summarizes the types of aerogels used, their shapes, impregnated bioactive components, methods for their impregnation as well as achieved loadings. The first progress was achieved with alginate aerogels, prepared as both beads and monoliths. The drug nicotinic acid was used as a bioactive component for the impregnation experiments. The drug was added via sol-gel synthesis and achieved loadings varied from 2.2 to 21% [70]. The drug dissolution profiles were improved by simply adding multi membranes to the core beads. Aerogels with more membranes resulted in higher loadings [23, 24]. The research was spread from alginate to pectin aerogels investigating the influence of ionic and non-ionic drugs on the drug release. Loadings as high as 37% were achieved for nicotinic acid and 33% for theophylline [24, 25]. In another attempt, both alginate, pectin, and their mixtures aerogels were used as carriers for diclofenac sodium achieving loadings as high as 80% depending on the used cross-linker for obtaining gels [74].

Since more than 40% of newly discovered drugs are hydrophobic [75], the focus shifted from soluble to poorly water-soluble drugs and finding ways to improve their bioavailability. Pectin aerogels were used as carriers for nifedipine, employing both techniques diffusion via sol-gel synthesis and supercritical impregnation [76]. Higher loadings were achieved in the

case of diffusion via sol-gel synthesis, namely 22%, compared to 13% in the case of supercritical impregnation. In separate research, the study on nifedipine was spread. Other polysaccharides, such as alginate, guar, and xanthan, were used as well. Diffusion via ethanol was used for the impregnation technique. Obtained loading varied between 21 to 37% [77]. Fat-soluble vitamins, cholecalciferol (vitamin D_3), and menadione (vitamin K_3) were supercritically impregnated into alginate aerogel beads as a way to improve their dissolution and bioavailability [78]. The highest achieved loadings for vitamin D_3 were 20 % and 16 % for vitamin K_3. The dissolution of vitamin D_3 was significantly improved, reaching 20 times higher concentration than its crystalline form. Since vitamin D_3 showed high sensitivity to elevated temperatures, among others (oxygen, light), the subcritical impregnation technique was employed to preserve its stability and prevent degradation of the vitamin. The method implied the usage of subcritical CO_2 and temperatures below 31.1˚C [79]. The temperatures from 5˚C to 25˚C were used, and loadings as high as 16% were obtained. The research focus shifted to producing coated aerogels.

The research focus shifted to the preparation of coatings. Firstly, xanthan-pectin coatings were prepared for orthopedic applications. Bioactive components, diclofenac sodium, and indomethacin were impregnated via sol-gel synthesis. Loadings as high as 4.5 and 4.2 % were achieved [80]. Furthermore, pectin aerogels coated with chitosan improved the dissolution and bioavailability of curcumin compared to its crystalline form. On the other side, coated pectin aerogels, unlike neat pectin aerogels, showed controlled curcumin release [81]. In the extended study, chitosan coatings were used for both pectin and alginate aerogels. The idea was to prepare neat polysaccharide aerogels (alginate, pectin, and chitosan) and compare them with coated pectin and alginate aerogels. The tested bioactive component was esomeprazole impregnated using both sol-gel synthesis and supercritical impregnation, and release profiles were optimized. Achieved loadings were similar, slightly higher in the case of sol-gel synthesis. The values varied from 4% to 22% for sol-gel synthesis and 2% to 17% for supercritical impregnation [82].

It is essential to distinguish between these two techniques. As practice showed that higher loading could be achieved using impregnation via diffusion through sol-gel synthesis (either water for soluble or ethanol for poorly water-soluble), the quantity of used bioactive components is huge (depending on their solubility). On the contrary, a much lower quantity of bioactive components is required using supercritical fluids and supercritical impregnation. At the same time, the loadings achieved are comparable to those obtained by sol-gel synthesis.

As mentioned, extensive research work has been done over the years.

The primary focus was on silica aerogels. Silica aerogels possess extremely high porosities (up to 99.8%), high inner surface areas (100-1600 m^2/g), and low densities (0.003-0.5 g/cm^3) [49]. Due to these outstanding properties, they have been proposed for biomedical applications. Silica aerogels with different modifications, hydrophilic, hydrophobic, amino-functionalized silica, etc., were used as carriers for different bioactive components [83]. The impregnation process and final loading showed to be dependent on the type of the drug, the solubility of the drug, type and characteristics of aerogel, impregnation conditions, etc. [83]. One of the most important revelations was that above a certain concentration, a solute of

bioactive component crystallizes, causing the collapse of the aerogel's structure. Using concentrations below the saturation concentrations is crucial to obtain amorphous products. The amorphous form of drugs is a highly desirable product, stable over a more extended period [84–87]. When it comes to supercritical impregnation of aerogels, it was discovered that the density of CO_2 under certain conditions is high, so the adsorption of CO_2 molecules takes place along with the bioactive components. After the depressurization, CO_2 molecules desorb, and only the traces of CO_2 stay in the aerogels at ambient pressures [88]. Ketoprofen and ibuprofen are the best investigated bioactive components for impregnation into aerogels. Loading as high as 16-30% [89–91] in the case of ketoprofen and 24-29% [88, 92] in the case of ibuprofen were achieved.

In the last decade, the world has been moving towards natural materials over synthetic materials. Many natural materials and polysaccharides among them are gaining increasing attention. Polysaccharide-based aerogels, like silica aerogels, are highly porous (up to 99%), lightweight (0.07-0.46 g/cm^3) with high inner surface areas (70-680 m^2/g). They proved to enhance drug bioavailability and drug loading capacity [67]. Besides polysaccharides, other organic materials such as proteins have also been proposed for the preparation of natural organic aerogels. Aerogels based on natural proteins are a new opportunity for life science and food applications due to their biocompatibility and biodegradability [93, 94]. Using natural organic aerogels, loadings for ketoprofen varied from 9 to 22% and for ibuprofen from 20-30%. Ketoprofen and ibuprofen loading showed to be dependent on the type of the polysaccharide or protein used. Alginate, alginate/pectin, alginate/κ-carrageenan, corn starch, starch, pectin, whey protein were used for the impregnation of ketoprofen [67, 91, 94–96], while alginate, starch, and silk fibroin protein were used for the impregnation of ibuprofen [92, 93, 97].

Besides mentioned bioactive components, many others have been used. To name just a few, active components such as miconazole, flurbiprofen, griseofulvin, dithranol [98], terfenadine, niclosamide [89], domperidone [99], artemisinin, rifabutin, loratadine, dihydroquercetin [92], α-tocopherol [100] have been used for the impregnation into a variety of aerogels.

As presented, aerogels are an inexhaustible source for impregnation for many bioactive components. Throughout extensive research, aerogels proved to be extraordinary carriers for bioactive components for a variety of biomedical applications. In the area where the concern for environmental issues is on its maximal level, we are able to produce products using green energy, green processes, and completely natural materials. Even though aerogels haven't yet found their way into the pharmaceutical and food industry, they must not be excluded or underestimated. They have a remarkable potential to grow, develop and stand out.

Table 4. Published results from our research facility

Type	**Bioactive component**	**Method**	**Loading, %**	**Reference**
Alginate, beads	Nicotic acid	Sol-gel synthesis	11.6-21	[70]
Alginate, monoliths			2.2-4.1	
Alginate, multi membrane beads	Nicotic acid	Sol-gel synthesis	6.4-179.3*	[71]
Alginate, multi membrane beads	Nicotic acid Theophylline	Sol-gel synthesis	53.5-179.3* 35.1-644.8	[72]
Pectin, multi membrane beads	Nicotic acid Theophylline	Sol-gel synthesis	25-37 21-33	[73]
Alginate beads	Diclofenac sodium	Sol-gel synthesis	40.5-79.6	[74]
Pectin beads			44.2-79.0	
Alginate-pectin beads			35.9-56.9	
Pectin, tablets	Nifedipine	Sol-gel synthesis Supercritical impregnation	22 13	[76]
Guar, tablets	Nifedipine	Sol-gel synthesis	25.7	[77]
Pectin, tablets			37.4	
Xanthan, tablets			34.9	
Alginate, tablets			21.7	
Alginate beads	Vitamin D_3 Vitamin K_3	Supercritical impregnation	5.2-20.1 6.1-16.0	[78]
Alginate beads	Vitamin D_3	Supercritical impregnation Subcritical impregnation	9-20 2-16	[79]
Xanthan-pectin coatings	Diclofenac sodium Indomethacin	Sol-gel synthesis	4.5 4.2	[80]
Pectin, tablets Pectin coated chitosan, tablets	Curcumin	Sol-gel synthesis	 **	[81]
Pectin, tablets	Esomeprazole		19.5 ± 2.0	[82]
Alginate, tablets		Sol-gel synthesis	11.5 ± 0.5	
Chitosan, tablets			22 ± 1.5	
Pectin coated chitosan, tablets			4 ± 0.5	
Alginate coated chitosan, tablets			8.5 ± 0.5	
Pectin, tablets	Esomeprazole	Supercritical impregnation	16.5 ± 1.0	[82]
Alginate, tablets			10 ± 0.5	
Chitosan, tablets			15.5 ± 1.5	
Pectin coated chitosan, tablets			2.5 ± 0.5	
Alginate coated chitosan, tablets			9 ± 0.5	

* not standardized method, ** not reported

4 Particles from gas saturated solutions (PGSS™)

In PGSS™ process, compressible media is solubilized in the substance or mixture of substances that should be micronized [101]. Several substances which are practically insoluble in sub-critical or supercritical fluids dissolve a considerable mass of gasses in the liquid phase. For several solid substances in the presence of gas, the melting point decreases with increasing gas pressure due to the solubilization of gas in the solid. After solubilizing SCF into emulsion suspended liquid or molten material, SCF containing liquid is rapidly expanded (through a heated nozzle) into a pressurized vessel leading to precipitation and formation of fine particles [102]. Gas saturated solution in an expansion with the compressible media is evaporated, and due to the Joule-Thomson effect, the solution is cooled below the melting point [103].

Equipment required for the process includes a compressed $scCO_2$ cylinder, two high-pressure liquid pumps for $scCO_2$, and the other solvents, high-pressure chambers, product separation units, liquefying units, recirculating pumps, manometers, in-line filters, thermocouples, and a host of others. Parameters that need to be optimized for each application include temperature, pressure, and feed emulsion rate. Technically for the batch-wise operated plant presented in Fig. 5, the substance to be micronized is filled in an autoclave, later, the gas is loaded and the system is equilibrated. Gas is solubilized in the substances or mixture of substances. The gas saturated system is expanded via a nozzle [104]. In a continuously operated plant, the substance to be powdered (molten or liquid, emulsion or suspension) is fed to a static mixer, mixed with sub-critical or supercritical fluid. After mixing, the multicomponent system is expanded via a nozzle. Produced particles of micron size are easily separated from gas streams in a spray tower cyclone. The PGSS™ process could be used to produce particles of pure substances, but the main advantage of the process is the production of composites of miscible or even immiscible substances. Frozen emulsions or liquid-filled particles could be produced [105]. As mentioned before, the PGSS™ process could operate batch-wise or continuously, and today several plants from the lab up to industrial-scale are in use in different industries [106].

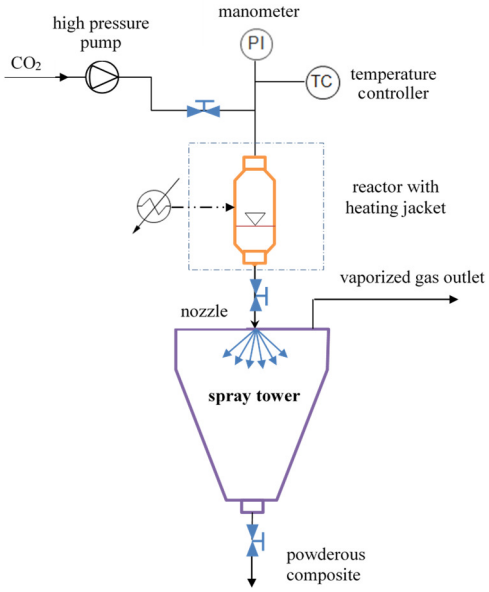

Figure 5. Scheme of a batch PGSS^{TM} process [107]

The technique known as supercritical melt micronization (scMM) is more efficient in atomizing and drying at low temperatures when compared with spray drying [108]. It has found application in powdering food and food-derived products like soy lecithin, chocolate, cocoa butter, citric acid, β-carotene, menthol, ricinus fat, shea butter, phytosterols, mono, and diglycerides [109]. Edible water in oil emulsion wrapper product has been prepared using supercritical melt micronization. Prepared products had a firm texture and were temperature stable. Smaller water droplet size emulsions were produced, leading to an increase in microbiological stability. Wrapper products have applications mainly in baking, frying, and cooking without leaving any sticky/waxy mouthfeel. Milk fat micronized by PGSS^{TM} proved stable β´ polymorphism. As milk fat is temperature-sensitive, this technique was well suited, and the properties of powder formed were well preserved during storage. Lavandin essential oil was micronized using PGSS drying [110]. Several carriers and pharmaceutical substances have also been micronized using a PGSS^{TM} process [111, 112].

PGSS^{TM} process was applied for powder formation of monostearate and tristearate. It allowed the formation of particles below 50 μm from substances that are non-soluble in supercritical fluid but absorb a large amount of gas that either swells the substance or decrease its melting point. Micronized samples had narrow particle-size distributions and improved properties compared to the conventionally produced powders [113]. The influence of process parameters like pre-expansion pressure and pre-expansion temperature on the degree of crystallinity, crystal form, particle size, particle-size distribution, and morphology of micronized monostearate and tristearate was investigated. Micronization was performed in a pressure range from 60 to 210 bar and at two different temperatures. The degree of crystallinity and crystal form of micronized samples were determined after micronization and

after three months of storage under controlled conditions. The average sizes of obtained particles from starting tristearate of approximately 4 mm, and monostearate of approximately 150 μm, were reduced to 10–40 μm depending on pre-expansion pressure and temperature. Agglomeration of samples during storage occurred. The polymorphic form remained the same after micronization, but the degree of crystallinity for both substances was lower compared to the samples before micronization. The samples during storage were re-crystallized. Re-crystallization process for monostearate was almost the same for all pressures and temperatures. The results for tristearate showed faster re-crystallization for pressures higher than 115 bar at a higher temperature. During storage, polymorphic form remained the same for monostearate (β form), and in the case of tristearate, the slow transformation from β′ to β form occurred. SEM analysis indicated particles with irregular and porous shapes after micronization, where the surface changed with storage in both cases.

PGSS™ is a favorable technique for polymeric encapsulation of drugs, proteins, and peptides as fine particles without employing organic solvents. In this process, carriers such as polymers are melted with the dissolved or suspended bioactive components contained within them. Ibuprofen has been successfully micronized with different carrier materials such as polyethylene glycol (PEG) 6000 [114], poloxamers, Gelucire1, and glyceryl monostearate [115]. PEG 4000 has also been used as a carrier for the micronization of poorly water-soluble drugs [116]. Sievers *et al.* [117] described a process to obtain fine particles usable in a dry powder inhaler form. The procedure consists of the production of a dense, fine droplet aerosol plume followed by a drying step. This patented process has been performed with lactose for developing a dry powder inhaler of anti-asthmatic drugs: albuterol sulfate and cromolyn sodium. The fine spherical particles in the range of 0.1– 3 μm made the product suitable for inhalation. The particle size of nifedipine was reduced from 50 to 15 μm in a pre-expansion pressure range from 100-200 bar. The influence of pressure on particle size has been evaluated. At higher pressures, smaller particles were formed [118]. Fenofibrate lipid-based solid dispersion formulation containing fenofibrate and Gelucire1 50/13 has been described by Pestieau *et al.* [119].

Fenofibrate solid dispersions were also investigated by Krananja *et al.* [107]. PGSS™ process was applied to the carrier materials Brij S100 and polyethylene glycol PEG 4000 to incorporate the insoluble drugs nimodipine, fenofibrate, and o-vanillin to improve their bioavailability and dissolution rate. With increasing pre-expansion pressure, the mean particle size of nimodipine/Brij S100, vanillin/Brij S100, and vanillin/PEG 4000 decreased. In a mixture of fenofibrate/Brij S100, anticipated effective surface areas were probably slightly reduced with pressure due to agglomeration and resulted in increased mean particle size of precipitated particles. The influence of drug/carrier ratio on particle size distribution was investigated in a nimodipine/Brij S100 system. The mean particle size at pressures higher than 150 bar increased with increasing drug/carrier ratio. For example, at a pressure of 200 bar, the mean particle size decreased from 61.28 μm at 0.10 drug/carrier ratio and up to 47.92 μm at 0.20 drug/carrier ratio. The effect of temperature was investigated in the o-vanillin/PEG 4000 system. After the temperature increased from 45˚C to 60˚C at 150 bar, particle size increased from 41.45 to 59.5 μm.

The research has also been performed for the formulation of the anthocyanin-based extracts and concentrates into powder form by using different supercritical micronization processes and different carriers to provide higher stability of the product during storage. Using the PGSS™ method, encapsulation of different anthocyanin-based extracts and concentrates with palm fat as carrier material was tested. Different types of anthocyanin concentrate, their concentrations, as well as different mass ratios of the carrier and the liquid to be encapsulated, were investigated. Anthocyanins, used in research, were extracted from grape marc or elderberry. The batch micronization experiments were carried out at pre-expansion temperatures of 70˚C and in the pressure range between 120 and 150 bar. The melted palm fat was mixed with emulsifier and with anthocyanin-concentrate using an electrical homogenizer. The formulated products were analyzed for their color properties. In the study, homogeneous, free-flowing anthocyanin-based powders were obtained. According to the different types of extract and the different concentrations of the extract in the solution, the visually observed color of the products varied from light pink to darker red-brownish. The average particle size of the obtained products varied from 8 to 18 μm, and the particle size distribution was relatively narrow. With increasing the liquid content on the carrier, the particle size of the powders decreased. Particles were very porous and mostly amorphous [120]. The obtained powderous anthocyanin-palm fat products showed good color stability, which gives suitable bases for potential applications in the future. Table 5 presents the examples of compounds and carriers formulated by PGSS™.

Table 5. Published results

Bioactive component	**Carrier**	**Conditions**	**Reference**
Monostearate Tristearate	without carrier	115-215 bar, 54, 60, 70 ˚ C	[113]
Green tee extracts	without carrier	73-100 bar, 33-79 ˚ C	[121]
Mixtures of ceramide 3A and cholesterol	without carrier	60-210 bar, 60, 70, 80 ˚ C	[122]
Nimodipine Fenofibrate O-vanillin	Brij S100 PEG 4000	100-250 bar, 45, 60 ˚ C	[107]
Banana puree Strawberry puree Blueberry concentrate	Maltodextrin	112-152 ˚ C	[123]
Curcuminoids	PEG 1500	160 bar, 50 ˚ C	[124]
Anthocyanin concentrates from grape residues	Palm fat	100 bar, 60 ˚ C	[120]

5 Conclusion

Technologies based on supercritical fluids offer various advantages over technologies based on organic solvents. The significant benefits are the thermophysical properties of SCFs, which can be easily tuned by adjusting the operating pressures and temperatures. Subcritical and supercritical CO_2 and subcritical water are solvents with great potential for extraction processes. They are non-carcinogenic, nontoxic, not mutagenic, nonflammable, and thermodynamically stable. CO_2 allows operations at low temperatures, while water is the cheapest available solvent. The demand for new products with special characteristics, high purity, and lower energy consumption for applications in different fields are increasing. With the use of SCFs, such products can be produced.

The processing of natural products with new technologies has been an extensive area of research in the past few decades. The main advantages of using SCFs are solvent-free products, no co-products, and already mentioned low processing temperatures. In addition, selective extraction of components or fractionation of total extract is a huge advantage. The possible limitations could be the prices of prepared products, which are relatively high compared to conventionally prepared.

The main advantage of using sub or SCFs to produce fine particles is the tunability of solvent properties. Micronisation processes can be easily connected to supercritical extraction process processes. On the other side, the impregnation of bioactive components (extracts as well) using aerogels can increase the bioavailability of poorly soluble drugs, improve their stability and release kinetics. A large number of research articles concerning aerogels in drug delivery application is proof of the aerogel's great potential.

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