Superkritični fluidi za izolacijo in formulacijo bioaktivnih snovi

Supercritical fluids for the isolation and formulation of bioactive substances

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Izvleček

Tehnologije, ki vključujejo pod- in nadkritične tekočine omogočajo pridobivanje novih izdelkov s posebnimi lastnostmi, ki so še posebej potrebni v farmacevtski industriji. Z uporabo pod- ali nadkritičnih tekočin kot procesnega orodja se lahko izognemo tudi zakonskim omejitvam glede ostankov topil in omejitvam rabe konvencionalnih topil, hkrati pa omogočimo nove postopke izdelave oz. procese, ki so okolju neškodljivi in so trajnostni. Ekstrakcija snovi iz rastlinskih materialov, njihovo oblikovanje in situ v izdelke s posebnimi lastnostmi, oblikovanje delcev, penjenje, nanašanje in sterilizacija so med aplikacijami superkritičnih tekočin deležni intenzivnih raziskav znanstvene javnosti. V tem preglednem prispe-

Abstract

Technologies involving sub- and supercritical fluids offer the possibility to obtain new products with special characteristics, which are especially needed in the pharmaceutical industry. The use of sub- or supercritical fluids for processing can also address legal limits for solvent residues and restrictions on the use of conventional solvents, while enabling new processes that are environmentally friendly and sustainable. Extraction of substances from plant materials, their in situ formulation in products with specific properties, particle formation, foaming, deposition, and sterilization have attracted much attention among the applications of supercritical fluids. This short

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vku podajamo trenutno stanje raziskav in aplikacij ter pričakovan prihodnji razvoj raziskav tehnologij pod- in nadkritičnih tekočin.

overview reports the current status and expected future developments of research into sub- and supercritical fluid technologies.

INTRODUCTION

It has been known since prehistoric times that some plant-derived substances have therapeutic properties, while others can be potent toxins. Herbs have been used to treat illnesses for thousands of years, and even in modern pharmacy, many remedies are still prepared directly from natural materials (1,2).

The isolation of active compounds has changed over time. Moreover, they have been utilized in different formulations; in teas and tinctures, or as extracts obtained using nontoxic or even toxic organic solvents. Mixtures of biologically active compounds from natural materials, their extracts, or essential oils have been shown to have antioxidant, antimicrobial, anti-inflammatory or other beneficial properties in the human body (3–6). To overcome the limitations of conventional extraction, novel techniques have been implemented to increase separation efficiency, reduce the use of raw materials, solvents, and energy, and to have a positive environmental impact (8,9). In addition, the use of "green" solvents, which are completely biodegradable, recyclable, non-corrosive, non-carcinogenic, and non-ozone-depleting, enables preparation of products that are recognized as safe and are preferred by consumers.

Processes using supercritical CO_2 have many advantages over other methods, because CO_2 is nontoxic and non-flammable. Consequently, the use of CO_2 as a solvent under supercritical conditions has been increasingly exploited. Supercritical CO_2 can also be used to load isolated active compounds into tailored pharmaceutical formulations that are most suitable for the patient.

The aim of this manuscript is to illustrate some recent advances of these relatively new processes.

WHAT IS THE SUPERCRITICAL STATE OF A SUBSTANCE?

In high pressure industrial processes, pressures range from approximately 50 bar (extraction or particle formation processes) to over 200 kbar (conversion of graphite to diamond). The use of supercritical fluids (SCFs) as solvents in chemical synthesis has advantages that include environmental, health, and safety benefits (7).

One of the primary process benefits stems from the thermo-physical properties of SCFs: high diffusivity and low viscosity, density, and dielectric constant. These properties can be finely tuned by varying the operating pressure and/or temperature.

In the literature (7), the SC state of a substance is defined as a state of a compound, mixture, or element above its critical pressure (*pc*) and critical temperature (*Tc*) but below the pressure required to condense into a solid. In the pressure-temperature phase diagram, shown in Figure 1, three regions correspond to solid, liquid, and gaseous states of a pure compound, separated by

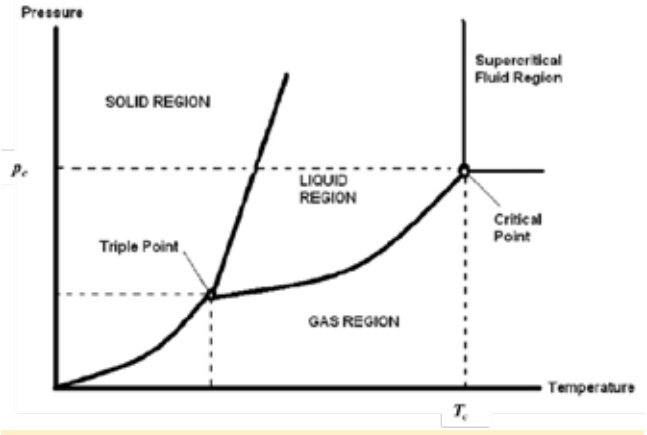

Figure 1. Pressure-temperature phase diagram for a pure compound (8).

equilibrium curves that meet at the triple point. The evaporation/liquefaction curve ends at the so-called critical point, beyond which only one phase occurs, which is the supercritical phase.
The most important properties of SCFs are their liquid-like density, their gas-like viscosity, and their diffusivity, which lies between those of gases and liquids, as shown in Table 1.

Table 1: *Physical properties of gases, liquids, and SCFs (9)*

Phase	Density [kg m^{-3}]	Diffusivity [m^2 s]	Viscosity [kg s m^{-1}]
Gas	1	10^{-4}	10^{-5}
Liquid	10^{3}	$5 \cdot 10^{-10}$	$5 \cdot 10^{-10}$
SCFs	$0.3 \cdot 10^{-3}$	10^{-7}	10^{-7}

liquids, for dissolution of materials, and to gases, for permeation of porous solid materials. Knowledge of the high pressure behaviour of liquids in the supercritical state has been used to develop new separation processes in numerous fields, such as in the pharmaceutical, food, and fine chemical industries (10).

EXTRACTION OF HERBAL SUBSTANCES USING SCFS

The choice of appropriate extraction method and solvent is crucial to ensure purity of the isolated substances and to enable isolation of specific compounds without interference. Extraction of hop constituents and decaffeination of tea and coffee are among the most performed extraction processes on an industrial scale. Several industrial plants are also dedicated to the extraction of bioactives for pharmaceutical applications. The advantages of SCFs for isolation of natural products are well described in the literature (11), with the most important being selective extraction of components and fractionation of total extracts.
Various compounds and their mixtures can be isolated with SCFs by using different gases for isolation/fractionation of components and/or adapting the process parameters, i.e., extraction pressure and/or temperature. The regulatory constraints surrounding organic solvent residues in applications involving humans, coupled with the need for isolation/fractionation of special components from total extracts and alternative formulation and sterilization processes (e.g., in drug release studies), will increase the use of SCFs for extraction purposes.

PARTICLE FORMATION USING SCFS

Particle size is an extremely important parameter affecting the dissolution rate of bioactive compounds. SCF-based micronization to form solid particles has been studied intensively to overcome the drawbacks of conventional processes, and several types of SCF-based micronization process have been developed. The type of micronization process employed depends on the physicochemical properties of the active compound as well as on the final application.

Rapid expansion of supercritical solutions (RESS)

Crystallization from supercritical solutions (CSS) is a process in which fine particles are formed by substances dissolved in supercritical solvents. When the solvent is an SCF, supersaturation may be induced by varying not only the temperature but also the pressure.
Micronization using the RESS process is an alternative to CSS. Here, a solid is dissolved in a pressurized SCF, followed by rapid expansion of the solution to lower pressure, resulting in precipitation of the solid. A diverse range of materials, including polymers, pharmaceuticals, and inorganic substances, have been successfully micronized using the RESS process (12,13). The RESS process produces very fine particles, even down to the nanometre scale. The particle size and size distribution can be controlled, and the process is free of organic solvents. Conversely, there are drawbacks related to the need for high ratios of gas/substance due to low substance solubility. This results in difficulty separating (very) small particles from large volumes of expanded gas, and necessitates the use of large-volume pressurized equipment.

Gas anti-solvent processes

The application of SCFs as anti-solvents is an alternative technique for processing solids that are insoluble in SCFs. This method exploits the ability of gases to dissolve in organic liquids and to lower their "solvent power", inducing precipitation of compounds from solution.

The benefits of GASR (Gas Antisolvent Recrystallization) include the ability to generate extremely fine particles, control of particle size and size distribution, as well as previously demonstrated suitability for a diverse range of active substances (14–16). Disadvantages, on the other hand, are related to the use of organic solvents, batch processing, uncertainties surrounding scale-up for particle formation despite recent advances, and the need to remove residual organic solvent. In addition, highly diluted product streams and the need to add gas to solvent can be problematic in industrial processes.

Particles from gas-saturated solutions (PGSS™)

This process allows the formation of particles from substances that, while insoluble in SCFs, absorb a large amount of CO_2 that either swells the substance or decreases its melting temperature. This process can also be used for micronization of suspensions and emulsions (17).

In PGSS™, the compressible medium is solubilized in the substance to be micronized (17). Subsequently, the gas-containing solution is rapidly expanded in an expansion unit and the gas is evaporated. As a consequence of the Joule-Thomson effect and/or evaporation, as well as the volume-expansion of the gas, the solution cools below the solidification temperature of the solute, inducing formation of fine particles. The solute is separated and fractionated from the gas stream using a cyclone and electrofilter. The PGSS™ process has been tested at pilot and technical scales for various classes of substances, including pharmaceuticals, polymers, resins, waxes, and surface-active components. The obtained powders exhibit narrow particle-size distributions, and have improved properties compared to conventionally produced powders (18–20). Figures 2a and 2b show the shapes of particles obtained by PGSS™.

IMPREGNATION AND DEPOSITION USING SCFS

In the last decade there has been a tremendous effort to develop tissue engineering strategies for repairing or replacing damaged tissues. In most cases, these involve the creation of scaffolds that can fulfil the requirements, such as control of porosity and pore size, and maintenance of mechanical properties. Additionally, a variety of material requirements must be satisfied, including biocompatibility, biodegradability

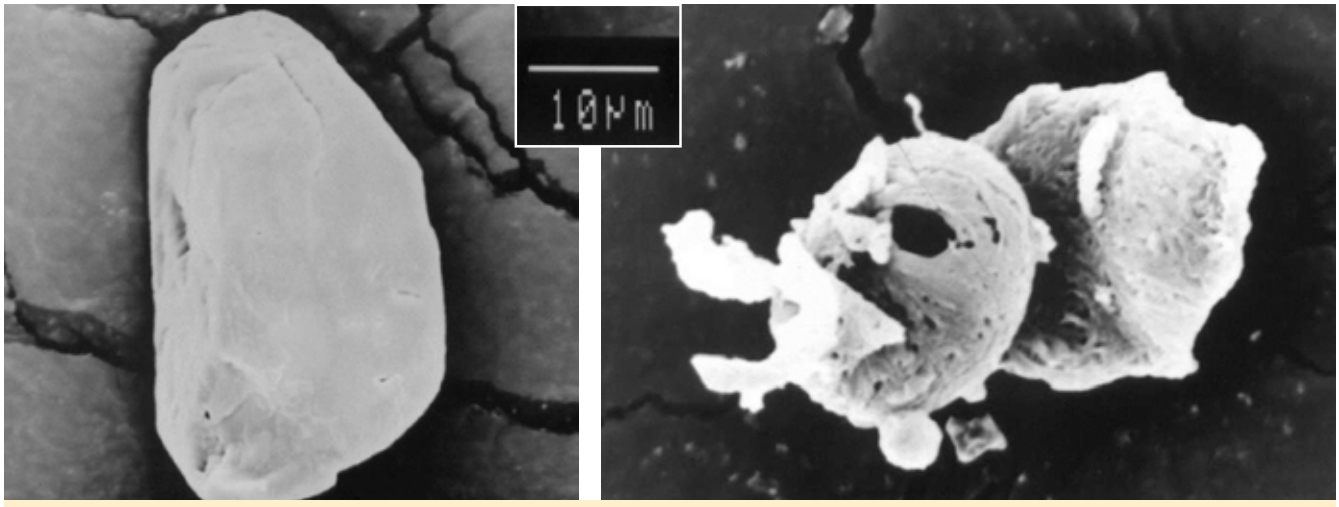

Figure 2. Particles of nifedipine; a) before and b) after PGSS™ micronization.

(with a corresponding rate of new matrix production), and a porous structure with interconnected pores of adequate size to allow cell adhesion, cell proliferation, and subsequent tissue growth. In short, the scaffolds must be degradable in the human organism and be able to bond with the surrounding tissue (21–27).

Processing methods that use SCFs have many advantages over standard methods for the preparation of tissue engineering scaffolds. These include avoidance of toxic organic solvents, the ability to control the morphology of an internal pore structure, and the potential to incorporate active compounds under mild conditions, thus preserving their properties (28). These methods overcome problems around incorporation of thermolabile compounds (synthetic drugs or natural-origin active components) during processing. Supercritical impregnation is used to diffuse active compounds into porous materials, offering straightforward optimization of loading and distribution by adjusting pressure and temperature. Higher pressure generally allows for better solubility, while higher temperature improves diffusion rates (29,30). Supercritical impregnation is also promising in the field of wound healing, where conventional drug delivery is often insufficient to ensure sustained release of active compounds at the wound site. This approach allows active compounds to be distributed homogeneously in wound dressings and facilitates precise control of release kinetics.

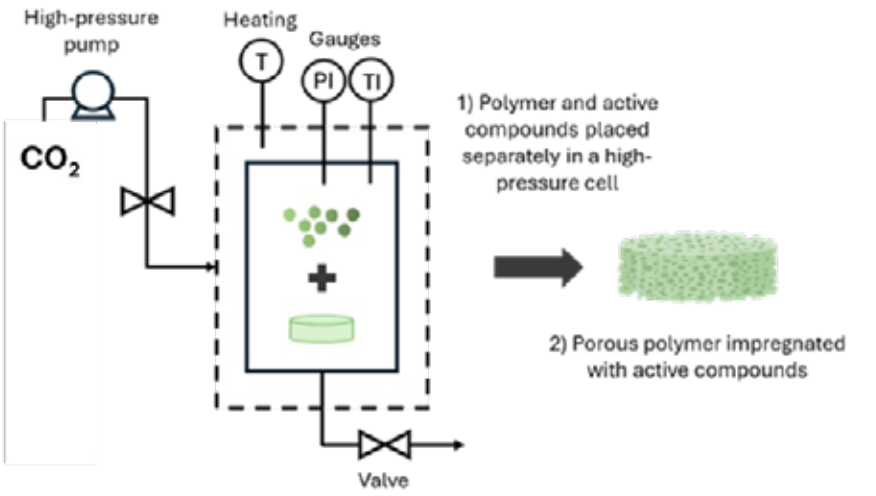

Figure 3. Schematic representation of one-step supercritical CO_2-assisted foaming and impregnation for production of porous polymers loaded with active compounds.

Coatings play a crucial role in enhancing material properties, protecting surfaces, and enabling specific functionalities. Various deposition methods have been employed to create coatings, each with advantages and limitations regarding control of film thickness, composition, and morphology (31). Traditional techniques include evaporation, 3D printing, electrospinning, electrophoretic deposition, dip coating, drop casting, sol-gel deposition, biomimetic deposition, spray coating, physical or vapor deposition, layer-by-layer assembly, and anodization (32,33).

SCF-based technologies have great potential for creating multifunctional coatings for biomedical applications through aerogel and foam synthesis. Aerogels are derived from gels in which the liquid is replaced by gas during supercritical drying, resulting in a solid assembly with a highly porous structure (34,35). Supercritical CO_2-assisted foaming, on the other hand, utilizes supercritical CO_2 as a foaming agent to dissolve a polymer, which, similarly to aerogels, results in a porous structure upon depressurization.

Supercritical foaming and impregnation can be achieved simultaneously in a simple one-step procedure for fabrication of highly porous bone scaffolds from thermoplastic polyesters uniformly loaded with active compounds (36). Moreover, the gas is removed rapidly, eliminating the need for subsequent treatments (drying or cleaning). Both aerogels and foams can be used for impregnation with active compounds for localized drug release, as presented in Figure 3 (13).

STERILIZATION USING SUBCRITICAL FLUIDS AND SCFS

Early work showed that gaseous CO_2 and N_2O, even at low pressure (below critical pressure), inhibited the growth of microorganisms (37,38), including spores during irradiation (39) or thermal treatment. Heat treatment at temperatures of 50–55 °C in the presence of CO_2 at a pressure of 6 bar has the same lethal effect on several bacteria, fungi, and yeasts as heat treatment at 60–65 °C in air at ambient pressure. It has been proven that high pressure can reduce pasteurization time at a given temperature by 50% (40).

Sterilization using supercritical CO_2, as for other processes, has many advantages compared with conventional methods, because CO_2 is non-toxic, non-flammable, abundant, and its use as an SCF constitutes an additional means of carbon reuse. Furthermore, supercritical CO_2 can be used to treat thermosensitive materials due to its low *Tc* of 31.06 °C. At the end of the process, CO_2 is spontaneously separated from the material during depressurization and can be recycled. In addition, the United States Food and Drug Administration (FDA) considers supercritical CO_2 as being a "Generally Recognized as Safe" (GRAS) solvent. As a consequence of the specific properties of SCFs, supercritical CO_2 easily penetrates various solid materials, including cell walls and polymeric matrices. The latter is especially important for materials in medical applications when they are entirely or partly made of polymers. Sterilization must often be carried out in pouches or blisters made of polymers. Moreover, because of the high-pressure conditions, there are no dead volumes in the treated materials. Finally, the temperature and pressure can be conveniently modulated to achieve targeted lowering of the bioburden; the number of contaminated microorganisms that are found in material before a sterilisation procedure (41).

CONCLUSIONS

This paper provides a limited overview of high pressure (supercritical) technologies for pharmaceutical and medical applications. Initial studies have shown promising results, but further research is needed to gain a deeper mechanistic understanding of the processes, assess their safety profiles, and determine optimal processing conditions for specific applications. While modern medicine might rely on phytomedicinal preparations with numerous health benefits (e.g., taxol, etoposide from Podophyllum peltatum, etc. (42)), it remains crucial to prioritize patient safety. The procedures for regulating plant-derived medicinal products with proven effectiveness are very strictly defined; they are carried out by authorized bodies, and their availability is strictly controlled. It must be emphasized that only pharmaceutically active ingredients have verified quality and can be guaranteed to be safe and effective. Most dietary supplements do not meet the same standards, and their labelling and use are not as precisely regulated. When incorporating individual plant-derived substances, it is imperative to conduct additional research in accordance with relevant standards to ensure the production of safe, high quality products that contribute to overall patient health.

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