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CONCEPTS AND PHARMACEUTICAL APPLICATIONS OF SUPERCRITICAL FLUID TECHNOLOGY

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A B S T R A C T

In light of environmental apprehension, supercritical fluid technology (SFT) exhibits excellent opportunities to accomplish key objectives in the drug delivery sector. Supercritical fluid extraction using carbon dioxide (CO2) has been recognized as a green technology. It is a clean and versatile solvent with gas-like diffusivity and liquid-like density in the supercritical phase, which has provided an excellent alternative to the use of chemical solvents. The present commentary provides an overview of different techniques using supercritical fluids and their future opportunity for the drug delivery industry. Some of the emerging applications of SFT in pharmaceuticals, such as particle design, drug solubilization, inclusion complex, polymer impregnation, polymorphism, drug extraction process, and analysis, are also covered in this review. The data collection methods are based on the recent literature related to drug delivery systems using SFT platforms. SFT has become a much more versatile and environmentally attractive technology that can handle a variety of complicated problems in pharmaceuticals. This cutting-edge technology is growing predominantly to surrogate conventional unit operations in relevance to the pharmaceutical production process. Supercritical fluid technology has recently drawn attention in the field of pharmaceuticals. It is a distinct conception that utilizes the solvent properties of supercritical fluids above their critical temperature and pressure, where they exhibit both liquid-like and gas-like properties, which can enable many pharmaceutical applications. For example, the liquid-like properties provide benefits in extraction processes of organic solvents or impurities, drug solubilization, and polymer plasticization, and the gas-like features facilitate mass transfer processes. It has become a much more versatile and environmentally attractive technology that can handle a variety of complicated problems in pharmaceuticals

Keywords:supercritical fluid technology, applications of SFT, SCF in Liposomal Formulations,

1. Introduction:

By keeping up with the rapid growth of research, technology, and the global economy, the pharmaceutical industry has become one of the largest and most fascinating sectors. The creation and implementation of robust, scaleable, and cost-effective manufacturing processes are critical to the success of drug products. Many of the traditional unit activities in pharmaceutical production rely heavily on high-temperature processing and/or the careless use of organic solvents, which is a barrier to the medicinal products' final safety and correct activity. The industry is looking for quick techniques of medication development and purification, as well as a predictable scale-up, that will affect the product's success by enhancing quality and safety while lowering environmental hazards on a financial and strategic level. A considerable industrial development is expected in the near future as a result of this. According to research, the exponential trend in the number of scientific publications relevant to

the use of supercritical fluids (SCFs) in the pharmaceutical area has nearly reached maturity in the previous two decades. [1]

SFT is a unique concept that makes use of the solvent properties of supercritical fluids (SCFs) above their critical temperature and pressure limits. A SCF is a phenomenon in which the temperature and pressure readings rise above the critical point at the same time. SCFs have dense and extremely compressible characteristics outside of the supercritical zone. The greatest temperature at which a gas can be turned into a liquid by increasing pressure is known as the critical temperature (TC). The greatest pressure at which a liquid may be turned into a conventional gas by raising the liquid temperature is known as critical pressure (PC). When both critical pressure and temperature parameters have been attained or exceeded, very distinct traits can be noticed. To put it another way, a SCF can act like a liquid or a gas, but it is neither. The supercritical state refers to a state in which the substance's physicochemical properties, such as density, viscosity, diffusion coefficient, and thermal conductivity, are halfway between those of a liquid and a gas. Because the substance has a liquid-like density and gas-like viscosity and diffusivity in the critical area, the liquid and gaseous phases are similar and homogeneous, allowing for good mixing and mass transfer. SCFs display both liquid-like and gas-like characteristics above such critical thresholds, allowing for a wide range of medicinal uses. The liquid-like qualities, for example, help with organic solvent or impurity extraction, medication solubilization, and polymer plasticization, while the gas-like properties help with mass transfer and reaction selectivity. [2,3,4,5,6]

Under the impact of SCF conditions, certain organic solvents exhibit favourable properties such as low viscosity and high solvent power. Above their respective critical pressure and temperature parameters, almost all gases can be converted into SCFs. In some situations, reaching the SCF state necessitates extremely high temperatures and pressures. With increasing molecule weight, intermolecular hydrogen bonding, or polarity, the critical pressure and temperature values rise. [7,10]

Carbon dioxide (CO2) is, without a doubt, the most often used SCF in pharmacological applications. CO2 as the SCF has been used in about 98 percent of applications due to its low critical temperature (31.2 °C) and pressure (7.4 MPa), which is advantageous in relatively simple processing and production for medicinal items. Due to increasingly stringent environmental rules, supercritical CO2 (SC-CO2) has acquired widespread popularity in recent years as a non-toxic, noncombustible, recyclable, readily available, environmentally friendly, and cost-effective replacement to harmful solvents. [7,8,9,10]

Other SCFs with low critical temperature and pressure values, such as xenon (Xe) and sulphur

hexafluoride (SF6), are restricted for commercial usage due to their high manufacturing costs. Even though gases like nitrous oxide (N2O) and ethane have low critical values, safety concerns limit their use. Overall, safety and commercial viability must be addressed in order to qualify as a SCF for pharmaceutical manufacturing. Table 01 shows the critical temperature and pressure values of several SCFs. [6,11]

Table No. 1: Critical Temperature and Pressure Values of Supercritical Fluids

Supercritical Fluid	Chemical Formula	Critical Temperature (T_C) °C	Critical Pressure (P_C) MPa
Water	H_2O	374	22
Xenon	Xe	16.6	5.9
Sulpha hexafluoride	SF_6	45.5	3.8
Nitrous oxide	N_2O	36.5	4.1
Ethylene	C_2H_4	9.1	5.1
Trifluormethane	CHF_3	25.9	4.7
Carbon dioxide	CO2	31.2	7.4
Propene	$CH_{2=}CHCH_3$	36.5	4.6
Methane	CH_4	19.0	4.6

2. Supercritical fluid technology (SFT) techniques:

Based on the expansion of a supercritical solution containing a solute, SFT is divided into two categories:

1. The SCF is used as a good solvent, and
2. The precipitation of a solute dissolved in an organic solvent by means of SCF used, that is, the SCF is used as an antisolvent.

2.1. Supercritical Solution as a Solvent:

2.1.1. Rapid Expansion of Supercritical Solution (RESS):

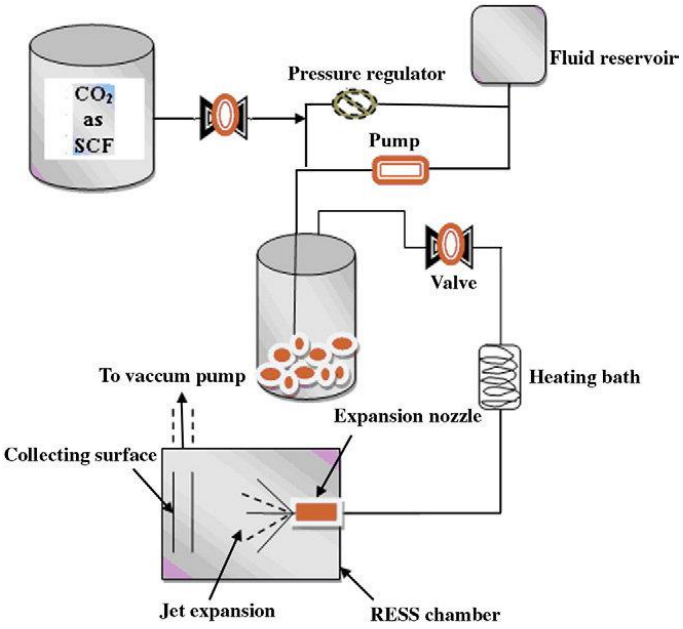


Figure No.1: Rapid Expansion of Supercritical Solution

One of the simplest and earlier approaches in SFT to manufacture drug-encapsulated particles is rapid expansion of supercritical solution (RESS). [10] This approach involves saturating the SCF with a solid substrate, quick depressurization, and then expansion of a supercritical solution into a low-pressure chamber via a heated capillary or laser-drilled nozzle. When substrate-loaded SCFs are passed from supercritical to ambient settings, pressure declines quickly and uniformly, reducing solvent power. This causes the solution to decompress, resulting in supersaturation, superfluid nucleation, and uniform particle formation. SC-CO2 is the most often utilised solvent in the RESS technique. Propane, chlorodifluoromethane, pentane, ethanol, acetone, diethylether, and nitrousoxide are among the other solvents employed. **[4,10,12,13]**

Solute solubility in SC-CO2, temperature, pressure, capillary design angle, and impact of the capillary jet against the surface are the governing parameters in this process. The particles obtained are entirely dry and solvent-free, and no further processing is required. The method is already in use in pharmaceutical applications such as microparticle and film fabrication. The RESS method has the advantage of being a reasonably straightforward procedure to implement as a lab scale component due to its control parameters in the absence of a solvent system. Low solubility of many medicinal chemicals in SCF, poor prediction control of particle size and morphology, tendency toward particle aggregation, and difficulties in exploring at production scale are the most significant RESS constraints. **[4,10,12,13]**

2.1.2. Rapid Expansion of a Supercritical Solution into a Liquid Solvent (RESOLV):

RESOLV (rapid expansion of a supercritical solution into a liquid solvent) is a modified version of

RESS that intends to reduce aggregation during particle formation. This approach entails depressurizing or inflating solid substrate-containing SCFs through a laser-drilled aperture into a collection container containing a room-temperature aqueous solution. As a stabiliser, different water-soluble polymers or surfactants are added to the aqueous medium. [4,14,15]

2.2. Supercritical Solution as an Antisolvent

In most cases, antisolvent-based approaches were used to treat poorly soluble chemicals in SCFs. In this case, compressed CO2 behaves as an antisolvent, causing a solute to precipitate from an organic solvent. This procedure allows for better control of particle agglomeration in the precipitator, resulting in improved RESS process performance. Because supercritical antisolvent approaches offer more versatility, they've been examined more fully for drug delivery system preparation. [16]

2.2.1. Gas Antisolvent Recrystallization (GAS):

The gas antisolvent recrystallization (GAS) method was created to reduce the particle size of hydrophobic materials that are unsuitable for RESS processing due to their poor solubility in SCF. A SCF is used as an antisolvent in the GAS method. It entails injecting an antisolvent SCF to precipitate a solute existing in an organic solvent. CO2 diffuses into the organic solvent, resulting in its evaporation into the gaseous phase, a volume expansion, and a decrease in solvent density. The organic solvent's solvent power is reduced, and it is no longer a good solvent for the solute. The whole process favours the commencement of nucleation, causing the solute to precipitate. When the SCF is entirely miscible with the solvent and the solute is insoluble in the SCF, the GAS approach works well. [4,15,17-19]

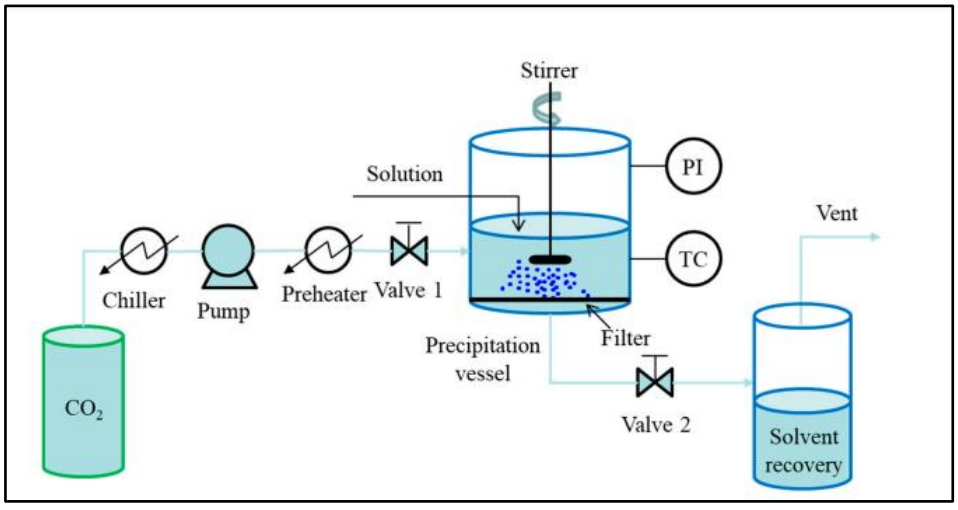


Figure No.2 : Schematic diagram of batch SAS process: GAS process

2.2.2. Supercritical Antisolvent Recrystallization (SAS) and Precipitation with Compressed Antisolvent (PCA):

By spraying an organic solution comprising medication and polymer via an opening into a compressed gas or SCF, the antisolvent property of SCFs can be used. CO2 is pumped into the high-pressure vessel in both procedures until the system reaches the desired pressure and temperature. The organic solution, which has a proper drug and polymer concentration, is sprayed into the vessel containing the SCF using a nozzle. Particles are formed by managing important parameters such as pressure and temperature, and they are collected on a filter attached to the bottom of a precipitation vessel. The supercritical antisolvent recrystallization (SAS) uses SCFs as an antisolvent, whereas the precipitation with compressed antisolvent (PCA) uses either a liquid or supercritical antisolvent. In SAS, high-pressure vapor-liquid equilibrium and mass transfer between the liquid and the SCF are also important. [3,6]

3. Pharmaceutical Applications of SCF Technology

Figure No.3: Supercritical fluid technology

3.1. Particle Design in Drug Delivery Applications

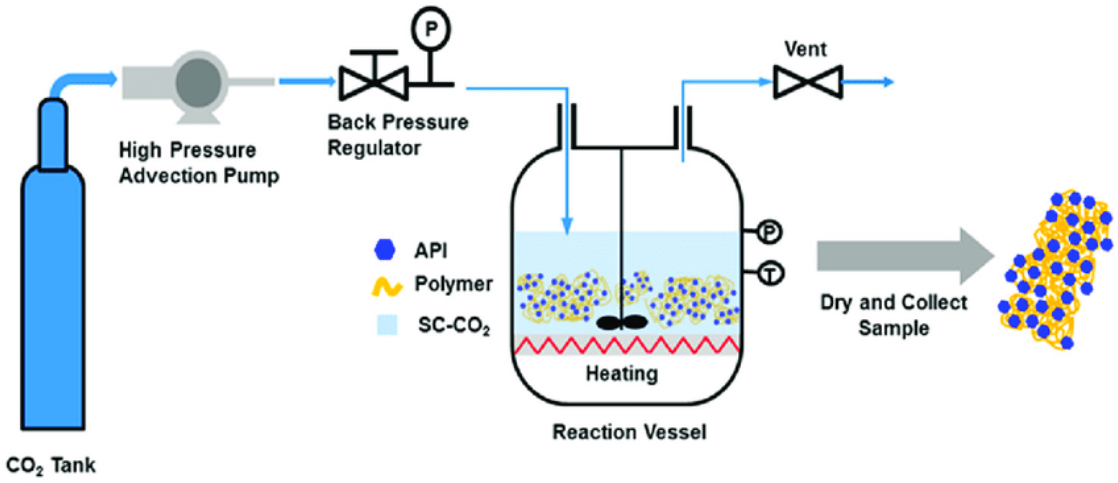


Figure No.4: Particle Design in Drug Delivery Applications

The majority of present pharmaceutical particle creation and pretreatment procedures are still somewhat crude, inefficient, and constrained. The classic high-energy milling approach for particle size reduction is ineffective and susceptible to morphological and crystallographic changes. The physicochemical stability of the shrunk material may be affected by such modifications. In this domain, the alternative technique of using SFT for crystal and particle engineering of pharmaceutical materials and drug delivery systems has a lot of potential. Traditional methods' limitations can be overcome by making efficient use of supercritical technology to generate micron- and submicron-sized particles. The SAS technique has showed considerable promise in producing micron-sized particles for a variety of chemicals, including insulin, lysozyme, trypsin, methylprednisolone, and hydrocortisone, in recent years. [21]

Budesonide and polylactic acid (PLA) microparticles were synthesised in one application using a compressed antisolvent (PCA) approach and incremental changes in temperature and pressure. The drug solution and antisolvent were injected by a capillary tube, and the budesonide-PLA microparticles had mean sizes of 1–2 micrometres. [22]

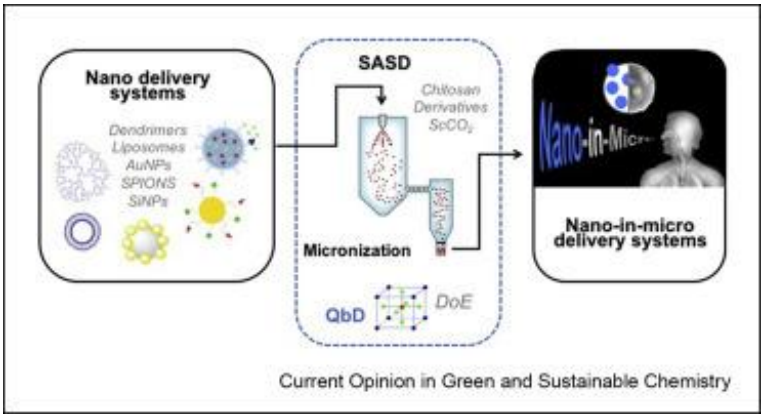
The controlled-release matrices for paclitaxel were successfully manufactured using a modified SAS method. The findings indicate that the method can produce sub-micron particles without the use of specialised nozzles or high temperatures. The use of ultrasonication in the SAS process dramatically improves the mixing of the organic solvent and CO2 phases, resulting in significantly smaller particles. [2]

The supercritical-assisted atomization (SAA) technique has proved to be promising for micronization of hydrophilic substances. SAA successfully synthesised cefadroxilmicroparticles from

a mixture of water and ethanol solvent. [23] Using a high-pressure CO2 or nitrogen (N2) atomization technique similar to the SAA technology, lysozyme particles in the range of 0.1–5 micro mater were produced from aqueous ethanol solutions. [24]Padrela et al. showed that SFT has the potential to be used as a screening tool for cocrystals with particle engineering applications. SFT was used to create particulate indomethacin-saccharin cocrystals with various morphologies and sizes ranging from micron to nano. [25] Using the gas-saturated solution approach, the poorly water-soluble medication nifedipine was micronized down to the size range of 15–30 micrometres. [2,26] The SAA approach was used to micronize griseofulvin particles, and mean diameters of spherical particles in the range of 0.5–3 micrometre were obtained. [27] SFT has proven considerable improvements over conventional approaches in the preparation of naproxen-loaded microspheres for controlled-release applications. In instance, as compared to traditional methods, the SAS methodology has demonstrated to be more beneficial. The particle size distribution of the naproxen-loaded microparticles obtained is smaller and homogeneous. [28]Reverchon et al. employed the SAA process to make hydroxypropyl methylcellulose (HPMC)–based microparticles using ampicillin trihydrate as a model drug in their study. Co-precipitation of HPMC with ampicillin was performed using a buffer solution as a solvent for micronization of HPMC alone. A uniform particle size distribution was created with a diameter range of 0.05–5.20 micrometres. The particles that were collected have a spherical or doughnut-like shape. [29]

Pathak et al. used RESOLV-based approaches to nanosize ibuprofen molecules in a different way. The nanoparticles were allowed to expand in an aqueous media containing various hydrophilic polymers after they were first created. The RESOLV approach was also used to investigate the effects of various stabilising agents on the characteristics of nanoparticles. [30] Using the SAS method, Kim et al. were able to make amorphous atorvastatin calcium nanoparticles. Mean particle sizes ranging from 152 to 863 nm were produced by optimising several process parameters such as drug solution concentration, CO2 and drug solution feeding rates, and the pressure and temperature conditions of the precipitation vessel. The particles had a spherical shape and a uniform dispersion. The generated amorphous atorvastatin calcium nanoparticles demonstrated increased solubility and thus intrinsic dissolving rates as compared to the crystalline state of the unprocessed medication. The reduction in particle size, which resulted in an increase in specific surface area, was credited with these results. [31] For the production of controlled-release products, Falk et al. used a PCA-based method. To make gentamicin, naltrexone, and rifampicin nanoparticles, the researchers used PLA as a polymer. These nanoparticles were spherical in shape and ranged in size from 200 to 1000 nanometers. [32]

3.2. Preparation of Pharmaceutical Powders



FigureNo.5: Building dry powder formulations using supercritical CO_2 spray drying

SC-CO2 in SFT has inherent features that can be exploited to process macromolecules like proteins, peptides, and nucleic acids. Compared to traditional lyophilization procedures, this process provides a number of advantages. The inclusion of sensitive biologicals without losing biological activity, as well as control over the morphology of the powder particles, are both advantages. Several studies have shown that SCFs can produce a variety of particles when processing aqueous protein solutions into powders. Tservistas et al. used SEDS technology to create plasmid DNA-loaded medicinal powders. **[33]**

To make microparticles of insulin and catalase, Debenedetti et al. used an antisolvent technique, in which the SCF expanded and nucleated with the liquid solvent, allowing for the creation of submicron protein particles. **[34]**

3.3. SCF in Drug Solubilization Applications

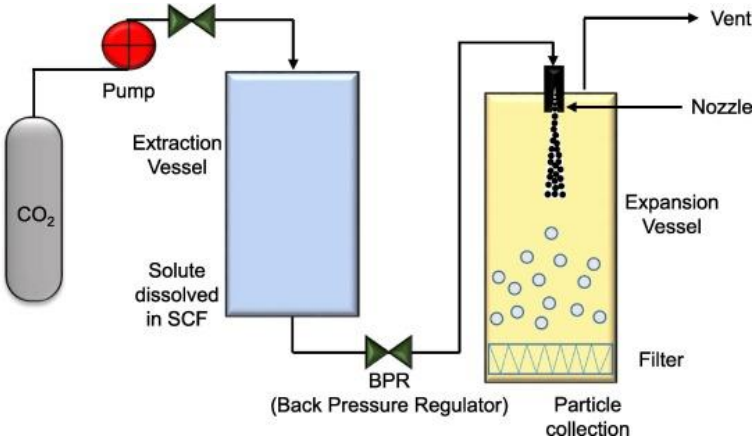


Figure No.6: Application of supercritical fluid technology for solid dispersion to enhance solubility and bioavailability of poorly water

One of the regulating criteria of medication delivery design is drug solubility. A variety of

pharmacological compounds are frequently insoluble in aqueous media, and their applications are frequently limited due to their low bioavailability. SFT is a promising approach for increasing the solubility and thus bioavailability of medicinal drugs. A number of researchers looked into the use of SFT to improve solubility. Turk et al. demonstrated that RESS processing of griseofulvin improves the drug's solubility rate, resulting in increased bioavailability. [35]

By adsorbing a poorly water-soluble fenofibrate onto silica, Sanganwar et al. enhanced its dissolution rate. The fenofibrate is first dissolved in SC-CO2 and then depressurized onto silica for adsorption. In vitro dissolving tests were used to evaluate the finished product. When compared to micronized fenofibrate, the dissolving rates of silica-adsorbed drug product increased significantly. This is due to an increase in the surface area of the medication and a decrease in its crystallinity following adsorption onto silica. The resulting crystalline fenofibrate has also been shown to be more stable than the amorphous form of the chemical in terms of stability. Solvents were not employed in the drug loading procedure in this method, hence the finished product was free of residual solvents. [36] Badens et al. used excipients such poloxamer, polyethylene glycol, and polyvinylpyrrolidone (PVP) to manufacture oxeglitazar, a poorly water-soluble molecule, and processed it using the SAS approach. SC-CO2 was used to pass the drug-and-polymer-blended feed solutions through a capillary nozzle. Particle shape, particle size, crystallinity, polymorphic purity, precipitation yield, and specific surface area were all superior in the formulations. The study's authors were successful in improving the dissolution kinetics of the study chemical oxeglitazar. [37]

In addition to these studies, various experimental results show that SFT is promising for the creation of submicron particles and that particle size, surface area, and wettability of the treated powders affect enhanced dissolving performance. [36]

3.4. SCF in Inclusion Complexes

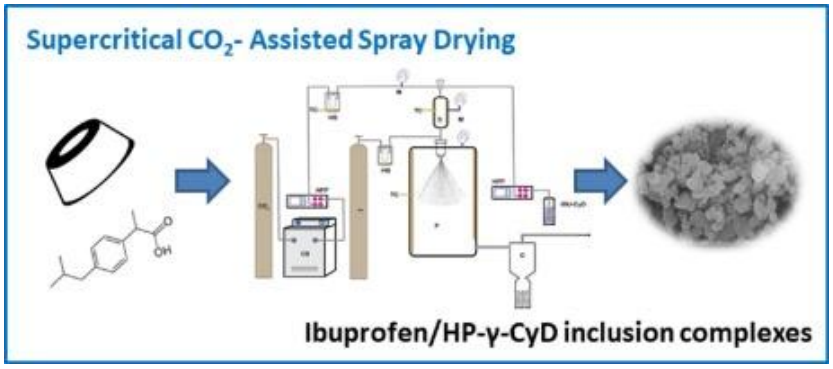


Figure No.7: Preparation of ibuprofen/hydroxypropyl-γ-cyclodextrin inclusion complexes using upercritical CO_2

Another way to increase drug dissolving qualities with SFT is to load or combine poorly aqueous soluble pharmaceuticals into a solid carrier, which improves the drug's water solubility. [38]

SC-CO2 was used to make piroxicam/Beta-cyclodextrin complexes in a solid form, for example. The impact of a number of operating variables was investigated. Physical combinations of piroxicam/Beta-cyclodextrin/l-lysine in the molar ratio of 1:2:1.5 in contact with CO2 at 150 °C and 15 Mpa produced the best results. [39]

The intrinsic dissolving rate of carbamazepine solid dispersions in PVP K30 was determined using either a traditional solvent evaporation or a SCF technique. Supercritically processed carbamazepine/PVP K30 had the best intrinsic dissolution rate, which was 4-fold higher than pure carbamazepine. Solid dispersions with intrinsic dissolving rates higher than ordinary solid dispersions were generated using a supercritical-based technique. [40]Bounaceura et al. used SC-CO2 to create a complex between ketoprofen (KP) and a Beta-cyclodextrin (CD). They also went over the process controls involved in SFT in great detail. The findings point to possible explanations for the occurrences that occur during inclusion formation. In all cases, increasing process-related parameters such as pressure, temperature, maturation period, agitation, and SC-CO2 density resulted in a rise in the KP with CD association rate. The SC-CO2 mixture's mass ratio had a crucial role in the complex's development. It was discovered that using more CO2 led in dilution of KP, which was detrimental to the complexation rate. The procedure resulted in a larger percentage of complexation without the use of organic solvents by controlling the operating conditions of SC-CO2 (pressure, temperature, maturation duration, agitation, and density). [41]

Simvastatin (SV), another hydrophobic medication, was investigated utilising the SAS technique in an inclusion complex with hydroxypropyl -cyclodextrin (HP-Beta-CD). The findings confirmed that SV's water solubility, dissolving rates, and bioavailability were improved. The SAS procedure was found to be useful in the creation of inclusion complexes, according to the authors. Thus, the SCF process appears to be efficient and is a good alternative for the synthesis of inclusion complexes for weakly water-soluble pharmacological active chemicals, based on the findings of numerous research groups. [42]

3.5. Polymer Impregnation

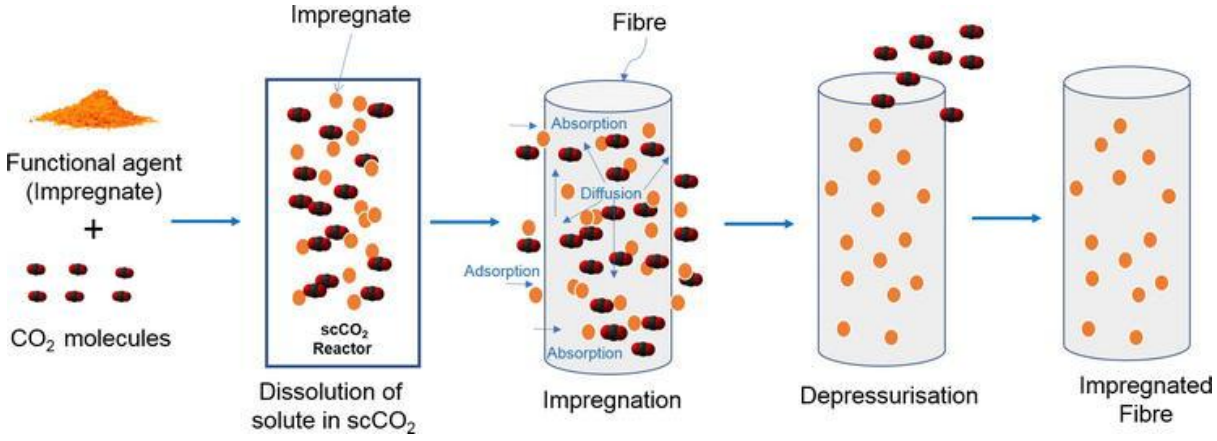


Figure No.8: Impregnation of Materials in Supercritical CO_2

Placing a polymeric substrate in a pressure vessel at atmospheric pressure while simultaneously mixing it with a mixture of a carrier liquid and an impregnation additive, where the impregnation compound is substantially insoluble in a SCF, is known as polymer impregnation. All of these components (polymeric substrate, carrier liquid mixture, and impregnation additive) were simultaneously exposed to a SCF in a closed pressure vessel for a significant amount of time, allowing the polymeric substrate to expand. The pressure in the vessel is released after closed pressure vessel exposure, allowing the carrier liquid to seep out of the swollen polymeric substrate, trapping a quantity of the impregnation additive within the polymeric substrate. [43]

Lactic acid, glycolic acid, polyolefin, polyamide, polyurethane, silicone, and protein derivatives are some of the polymeric substrates that can be employed. A suitable combination of polymeric substrates in an appropriate ratio can also be used in some of the procedures. This is also true with carrier liquids, which can be used alone or in combination with water, methanol, ethanol, isopropanol, or hexane. Impregnation additives can be dye, protein, polypeptide, nucleotide, medication, or monomer. [44]

The use of polymer impregnation in drug delivery applications has been the subject of numerous research studies. Uzer et al. presented a polymer impregnation process for controlled-release delivery devices based on SC-CO2. Polymeric component, polymethylmethacrylate (PMMA), and compound naphthalene were utilised in the impregnation procedure. Temperatures of 35–45 °C and pressures of 80–150 bar were used to produce impregnation and swelling phases. Impregnated samples expanded 2- to 4-fold under these conditions, with impregnation doses ranging from 103.7 to 289 mg naphthalene/g of PMMA. [44]Banchero et al. used the impregnation approach to acquire the required amorphous form

of piroxicam. PVP K-15 and piroxicam samples were impregnated at a pressure of 300 bar and a temperature of 100 °C. In comparison to simple physical mixes, the amorphous structure of piroxicam resulted in a considerable increase in dissolving rates. [45]

A hydrogel-type ophthalmic medication delivery was created employing a supercritical solvent impregnation (SSI) approach in another application. The anti-inflammatory medicine flurbiprofen and the anti-glaucoma drug timolol maleate were impregnated into several chitosan derivatives [N-carboxymethyl chitosan (CMC), N-carboxybutyl chitosan (CBC), and N-succinyl chitosan (SCC). Impregnation tests were carried out at 303.0, 313.0, and 323.0 K at pressures ranging from 9.0 to 14.0 MPa. The results showed that the impregnation procedure can successfully prepare drug delivery devices for eye treatment. [46]

Dias et al. used an SSI approach to load two separate natural-origin bioactive chemicals, quercetin (anti-inflammatory) and thymol (anaesthetic characteristics), onto polymeric biomaterials like N-carboxybutylchitosan (CBC) and agarose (AGA). SC-CO2 impregnation experiments were performed at 10 and 20 MPa and 303 and 323 K. When higher pressures and temperatures were used, higher amounts of quercetin and/or thymol were loaded, according to release kinetics data. The authors also found that the SSI procedure helped to reduce the size of the loaded quercetin particles, which helped to increase solubility metrics significantly. As a result, the use of impregnation technology in the production of polymer-drug composites employing SC-CO2-assisted infusion for a variety of medications can help to improve drug solubility and dissolution kinetics. [47]

3.6. SCF in Liposomal Formulations

The use of significant volumes of organic solvents in liposomal manufacture, as well as limited encapsulation efficiency, are two of liposomal manufacturing's major drawbacks. SC-CO2 can be used as a co-solvent for liposomal formulations to remedy these difficulties. [6]Otake et al. produced one of the most efficient supercritical procedures.

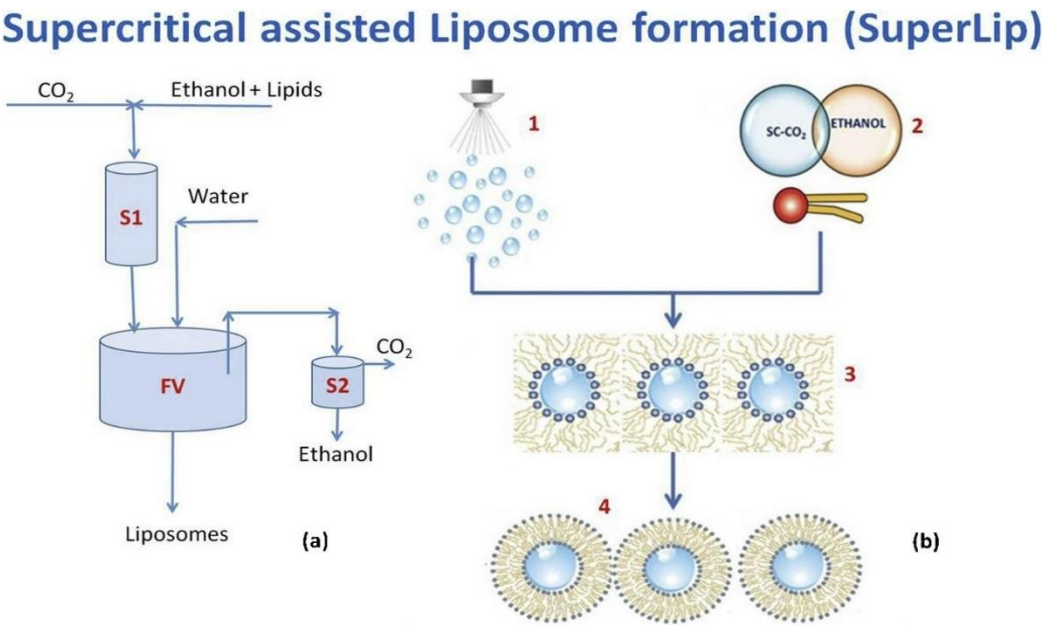


Figure No.9: SCF in Liposomal Formulations

The study group used a reverse-phase evaporation approach with SC-CO2 to manufacture liposomes without utilising any organic solvents. The researchers made aqueous liposome dispersions by emulsifying a predetermined volume of water into a homogenous mixture of SCCO2/l-Alpha-dipalmitoylphosphatidylcholine/ethanol with enough stirring and subsequent pressure reduction. According to the findings, the supercritical reversephase evaporation method is the preferred way for manufacturing large unilamellar liposomes with excellent entrapment efficiency in a single step. [48]

Amphotericin B–intercalated liposomes were made by dissolving amphotericin B and purified phosphatidyl choline in a suitable solvent and using the antisolvent approach to precipitate microsized particles in SC-CO2. Using the laser approach, the particle size distribution was investigated, revealing nanosize particles with a narrow size distribution and a greater intercalation efficiency of generated liposomes. [49] The ASES procedure can also be utilised to make dry and reconstitutable liposomes. An optimised ASES procedure was used to make dry liposomes containing miconazole as a model medicine, using varied compositions of spraying solution containing phosphatidylcholine, cholesterol, and poloxamer 407. Partially crystalline, spherical, and nonporous microparticles ranging in size from a few to 40 micrometres were produced using this method. The microparticles are also within a certain range of residual solvents. To overcome the disadvantages associated with routine production methods, several approaches utilising SCFs can be successfully modified to make liposomal preparations. [50]

3.7. Purification and Polymorphism

The absence of contaminants in the production of active pharmaceutical ingredients (APIs) is critical. Aside from the physical instability of compounds, the presence of even amounts of impurities can

cause chemical characteristics to change. SFT has recently emerged as an excellent method for isolating certain process contaminants in APIs. SFT is especially important in the API sector because of its use in polymorph isolation and chiral control. Several publications on the use of the gas anti-solvent recrystallization (GAS) process for separation and purification can be found in the literature. Purification of Beta-carotene, cholesterol, anthracene, bilirubin, citric acid, and proteins, as well as fractional crystallisation of anthracene and anthraquinone mixes and lecithin from egg yolk, phenanthrene and naphthalene, natural products, hydroxybenzoic acid isomers, and racemic combinations SC-CO2 crystallisation has also been used to explore polystyrene fractionalization by chain length and to separate semi-crystalline and amorphous polymers (l-lactic acid). [51]

Polymorphic forms of a crystalline material can have distinctly different physical and chemical properties in solid state pharmaceuticals, such as melting point, solubility, and even bioavailability. The separation of polymorphs of paracetamol, carbamazepine, terbutalin sulphate, deoxychloric acid, sulfathiazole, and salmeterolxinafoate by processing in SC-CO2 has been reported by many research groups. During pharmaceutical production, critical procedures such as granulation, drying, spray drying, homogenization, lyophilization, and compression are unavoidable. The impact of these processes on solid states is very susceptible to unwanted polymorph conversion, solvate desolvation, solvate creation, and other phenomena that can be avoided by using SFT methods. [51]

3.8. Drug Extraction Process and Analysis

Drug extraction techniques and analysis have gotten a lot of attention as a result of the considerable development of the field of SFT. The use of supercritical fluids for natural chemical extraction and fractionation has already gained traction. Studies on the extraction of traditional compounds like essential and seed oils from diverse sources—seeds, fruits, leaves, flowers, rhizomes, and so on—with or without the addition of a co-solvent have been published throughout the previous decade. [52]

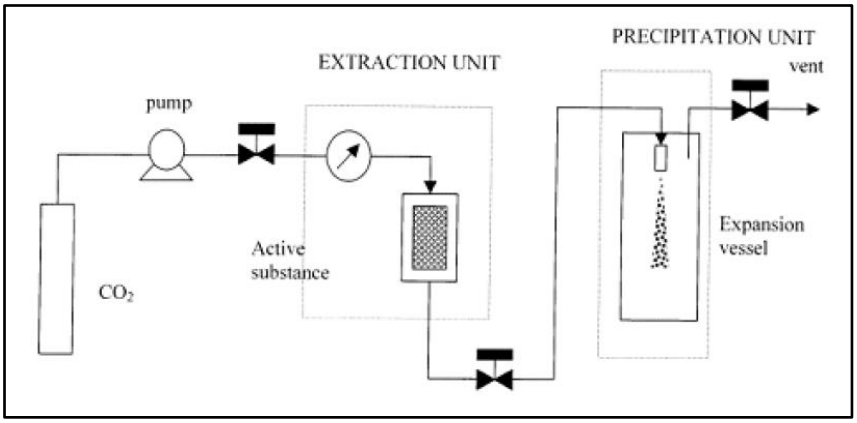


Figure No.10: Drug Extraction Process

Klime et al. used supercritical fluid extraction (SFE) to test pharmaceuticals in plasma. The high-performance liquid chromatography (HPLC) approach in combination with SFE was used to assess anti-inflammatory medicines such as ibuprofen, indomethacin, and flufenamic acid. The procedure was carried out at a CO2 pressure that was acceptable for drug extraction. This research established the best method for extracting ibuprofen, indomethacin, and flufenamic acid from plasma. [53]

Apart from that, SFE was used to remove various benzodiazepines from the matrices of their usual dosage forms. [54]

For the separation and/or estimation of medicinal substances, several chromatographic methods in conjunction with SFE are used. SFE liquid chromatography procedures are designed to separate analytes with high polarity and molecular weight that can't be separated using either method alone. Because of the great sensitivity, versatility, and compatibility of the detectors, SFE gas chromatography is an extensively used combination approach. [6]

3.9. Scale-up and GMP Processing

SFT has several appealing qualities from the standpoint of GMP, particularly in terms of providing a completely enclosed, single-step process for controlled particle creation. The efficient scale-up of laboratory procedures will be based on a mechanistic knowledge of SCF particle formation processes and rigorous descriptions of mass transfer and nucleation processes. SFT can be used to create a variety of pharmaceutical products, including polymeric microparticles and nanoparticles, drug powders, drug polymorphs, and liposomes. A demonstration of techniques that can be scaled to produce considerable quantities of practical yield in production batches is necessary for major commercial feasibility. For antisolvent-based systems, the optimum single-stage processing equipment is completely enclosed, has no moving parts, and is made of high-grade stainless steel, with clean-in-place capabilities available for larger-scale equipment. In comparison to traditional technologies such as spray-drying and freeze-drying, SC-CO2 technology has unique qualities that make it an excellent choice. As a result, GMP compliance is not a significant barrier to commercialising SCF processing. In fact, numerous pharmaceutical companies have already constructed particle-design factories on a pilot and industrial scale, all while maintaining strict quality control and adhering to GMP guidelines. An interdisciplinary strategy combining engineering, physicochemical, pharmaceutical technology, and biopharmaceutical skills is required in this regard. [6,21]

3.10. Supercritical fluids technology in bioprocess industries

In recent years, supercritical fluids (SCFs) have been of interest in the biotechnological processes (Williams et al 2002). They provide solutions to drastic problems related to bacterial (Dillow et al.

1999; Enomoto et al. 1997; Spilimbergo et al. 2003), enzyme (Hong and Pyun 2001), viral (Fages et al. 1998) and yeast inactivation (Hashizume et al. 1995), as well as permeabilization (Aaltonen and Rantakyla 1991; Mesiano et al. 1999) and extraction of fermentation products (Bruno et al. 1993; Cocks et al. 1995; CygnarowiczProvost et al. 1999; Hampson and Ashby 1999; Isenschmid et al. [55]

The most often used fluid is supercritical carbon dioxide (SC-CO2), which has a low critical temperature (31.1°C) and pressure (73 bar), making it an appropriate medium for processing volatile products (Goodarznia and Eikani 1998) and other innovative developments (Marr and Gamse 2000). The most essential properties are non-toxicity, non-flammability, selectivity of the procedure, and ease of recovery (Wells and DeSimone 2001). SC-CO2 was also used as anantisolvent for making PHB microspheres for use as drug delivery systems (Bleich and Mueller 1996; Breitenbach et al. 2000; Bustami et al 2000). The use of SCF in polymer processing has been carefully examined (Kazerian 2000). At temperatures ranging from 30 to 70°C and pressures ranging from 122 to 355 bar, Khosravi et al. reported the equilibrium solubility of poly(hydroxyl butyrate) (ultra-molecular weight) in SC-CO2 (Khosravi-Darani et al. 2003). Khosravi et al. have also evaluated several elements of SCF extraction in bioscience downstream processing (Khosravi-Darani and Vasheghani-Farahani 2005). [56]

3.10.1. Product recovery

Due to product concentration, broth viscosity, and biomaterial characteristics, product recovery from a fermentation broth is more complex than chemical procedures. SCE may be able to address some unique constraints, such as low product concentration. SCE with CO2 can be done at fermentation temperature, preventing any heat damage to the product and saving energy. Table I presents a list of fermented bioproducts that have been isolated. SCFs have been used to extract biomaterials from biomass in a variety of ways (Khosravi-Darani and Vasheghani-Farahani 2005), with the following classifications: post fermentation extraction of products; in situ extraction from microbial fermentation biomass; fractionation of cellular biomass; and removal of biostatic agent from fermentation broth. Furfural, a growth inhibiting byproduct of Clostridium fermentation on sugars, was successfully eliminated by adding liquefied CO2 into the aqueous solution at ambient temperature and 5.9 MPa. Several SCF were used in the fermentations of Propionibacteriumfreudenreichii and E. coli, including ethane, CO2, Xe, and halogenated refrigerants (Tomasko and Chon 1997). The growth of broth P. freudenrechii and E. coli was unaffected by refrigerants 13 (CClF3) or R116 (CF3CF3). The use of reverse micelle production as a new technique for increasing polar species extraction with SC-CO2 is discussed. Prior to SFE, adding a reversemicelle forming reagent speeds up the quantitative extraction

of the analyte. (Jimenez-Carmona and colleagues, 1998). [55,56]

Table No.2: Application of SCF in downstream of biotechnological processes

Product	Microorganism	Process Condition	Reference
Ethanol	Saccharomyces cerevisiae	Pilot scale, continuous countercur rent	Van-eijs 1983
Acetic/Propanoic/Buta-noic acid	Clostridium thermoaceticum	Laboratory scale, batch	Van-eijs 1983
Aceton/Butanol/Ethanol	Clostridium acetobutylicum	Laboratory scale, batch	Van-eijs 1983

3.10.2. Detecting the presence of a microorganism

SCF can also be used to detect the presence of a bacterium in a sample, which is a fascinating application in biotechnology. The nucleic acid of the pathogen will be isolated following exposure to SCF in this method. Then, using the hybridization and PCR methods, contamination to a specific sequence will be detected (Nivens and Applegate 1996). This approach, in particular, can be used to detect harmful microbes in water supplies. This approach has the benefit of being applicable to all microbes (preferably, a bacterium; alternatively, a protozoan, parasite, or virus.). Even if partial or selective lysis occurs, the recovered DNA can quickly be typical of sample contamination. Hydrostatic pressure appears to have no effect on DNA. When pressures of up to 10000 at were applied for 60 minutes at 25-40°C, the structural integrity of calf thymus or salmon sperm DNA remained unchanged. [57]

For the detection of earth-based microorganisms, a SCF extraction approach and a chromatographic separation/detection method were devised. Several redox chemicals could be recovered from bacteria after microorganisms in a sand or soil sample were hydrolyzed in a diluted NH4OH/acetone solution (trimethylamine- SC-CO2 at 35°C and 300 at). The findings showed that the approaches outlined might be used to detect the chemical signature of life in desolate desert sand samples (Lang et al. 2002). [56]

3.10.3. Inactivation of microorganisms

Pasteurization and sterilisation of foodstuffs (especially in the liquid phase), as well as chemically, thermally, and hydrolytically sensitive materials in biomedical applications, using supercritical is a viable alternative technique. Khosravi has studied the creation of this successful alternative strategy as

well as the fatal effect of high hydrostatic pressure CO2 on microorganisms (2007). [57]

3.10.4. Cell disruption

The potential utilisation of intracellular storage products like poly(-hydroxybutyrate) (PHB) and recombinant metabolites has sparked interest in efficient and cost-effective cell disruption to enable the recovery of intact intracellular microbial products (Lee et al. 1979). Because of the cost savings, mechanical disruption methods are preferred. Although only relatively dilute biomass slurries can be adequately treated, homogenization with a sufficient pretreatment is a proven recovery strategy (Tamer et al. 1998). Harrison has demonstrated that chemical (adding an anionic detergent and a monovalent cation) or lysozyme pretreatment can weaken the cell wall enough to reduce energy consumption and allow for lower pressures or fewer homogenizer passes (Harrison et al. 1990). Because of its low power consumption and resilience, bead mill disruption is also recommended for PHB recovery (Heremans and Smeller 1998). The quantity of energy consumed in this approach is affected by the agitation speed, although when rotation was increased to a critical level, only a small increase was observed, with the extra energy being converted to heat (Schutte et al. 1983). [58]

The physical parameters of the cell slurry are affected by the cell disruption operation, such as viscosity (Mosqueira et al. 1981), density, particle size, and suspension settle ability (Lee et al. 1979). The necessity of harvesting the generating cells in order to extract an internal element is a significant economic disadvantage, and it contributes to the current obsession with manufacturing high-value items (Chisti and Moo-Young 1986). Few research on this topic have been published due to the high capital and operating costs of large-scale intracellular product separation, as well as the need for large teams of scientists and technical employees to acquire important biochemical engineering data (Chisti and Moo-Young 1986). [58]

SCF has only been used in yeasts (Castor and Hong 1995, Lin et al. 1991; Nakamura et al. 1994) and a few bacteria for cell disruption (SC disruption) (Foster et al. 1962; Fraser 1951; Juhasz et al. 2003). The procedure entails a rapid release of the imposed SC-CO2 pressure, allowing it to penetrate the cells. Following the expansion of gas within the cells, a pressure flash discharge drives the cell wall apart, causing cell disruption. The method is straightforward and may be simply scaled up (Juhasz et al. 2003). Furthermore, the cells are subjected to minimum shear stresses and no heat is generated, which would otherwise have a negative impact on the yield of temperature sensitive and labile material recovery. [57]

Fraser (Fraser 1951) was the first to disclose the disruption of microbial cells by consecutive pressurisation and explosive decompression, which was later developed by other researchers to

retrieve intracellular enzymes and recombinant-DNA proteins (Castor et al. 1996; Foster et al. 1962; Lin and Chen 1994; Lin et al. 1991; 1992; 1993; Nakamura et al. 1994). SCF has been shown to inactivate cells de several investigations. [58]

3.10.5. Biochemical reactions in SCF

The solubility of substrate in SCF, protein stability (Athes et al. 1998), and enzyme stability in SCF have all been extensively researched (Adams and Kelly 1998; Jimenez-Carmona and Luque de Castro 1998; Mozhaev et al. 1994; 1996; Nakamura 1990; Noel Marie et al. 2000). The usage of lipase, among the enzymatic processes in SCF, has the largest commercial potential. A mixture of SC-CO2/H2O can be employed as a reaction media for hydrolytic or synthetic reactions catalysed by lipase and other hydrolases (Giebauf et al. 1999). The constants of the reaction and mass transfer, such as rate constant, solubility, effective diffusivity, mixing diffusivity, and mass transfer coefficient, are affected by temperature, pressure, and flow velocity in a continuous acidolysis of triolein with stearic acid reaction (Nakamura 1990). [56,57,58]

In SC-CO2), immobilised Candida antarctica lipase B was utilised as a catalyst to successfully synthesis butyl butyrate from butyl vinyl ester and 1- butanol. For both liquids and SC-CO2 media, a drop in fluid density resulted in a clear increase in synthetic activity and selectivity. The synthesis activity of the lipase-membrane derivative was increased up to 84-fold in all SC conditions compared to the organic solvents. The enzymatic membrane was tested under the best SC conditions (60°C, 8 MPa) using 6 h/day operational cycles, revealing a 360 cycle half-life time in their synthesis activity (Lozano et al. 2004). [59,60]

Adsorption of a commercial solution of free Candida Antarctica lipase B (Novozyme 525L) onto 12 distinct silica supports modified with unique side chains also immobilised a commercial solution of free Candida Antarctica lipase B (Novozyme 525L) (e.g. alkyl, amino, carboxylic, nitrile, etc.). In both ionic liquid/hexane and ionic liquid/SC-CO2 biphasic media, the immobilised derivatives were tested for rac-1-phenylethanol kinetic resolution. The best results were obtained when the in water activity was increased from 0.33 to 0.90 (e.g., the CALB/butyl-silica activity was increased up to five times). The "philicity" between alkyl chain lengths of both the silica support and the cation of ionic liquids improved the synthetic activity of immobilised derivatives coated with ionic liquids by up to six times in SC-CO2 compared to the hexane medium, which agrees with the "philicity" between alkyl chain lengths of both the silica support and the cation of ionic liquid (Lozano et al. 2007). [5]

4. Conclusion

SFT is no longer in its infancy; it is rapidly expanding, but one of the biggest problems now is ensuring that meaningful business opportunities in the pharmaceutical industry are generated. SFT's unique qualities, such as easier and more flexible processes and lower environmental impact, make it perfect for developing techniques for extracting, purifying, and recrystallizing fine chemicals and medicines, as well as improving particle-size engineering for drug delivery. SFT can also be used to improve the dissolution rate of drugs, particularly those with poorly soluble active ingredients, as well as for a variety of other purposes, including the formation of microparticles and nanoparticles, polymer impregnation, drug extraction, co-crystallization, and complexes with host molecules. Importantly, several academic research papers have documented promising SFT applications, but they have also noted a dearth of or extremely modest commercial production to date. To deepen the current understanding of SFT and, eventually, to employ it properly in industrial applications, more research and development of SCF processing for medicines is required.

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