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Recent advances in the preparation of drug delivery systems using supercritical fluid technologies

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Alla mia famiglia e a Irene

SOMMARIO

Introduzione

Una delle maggiori cause che condizionano negativamente l'immissione sul mercato dei farmaci è la loro scarsa biodisponibilità, dovuta per lo più alla loro bassa solubilità in acqua. La scarsa biodisponibilità dei farmaci fa sì che essi debbano essere somministrati in quantità maggiori del dovuto, aumentandone così gli effetti collaterali. Una delle migliori soluzioni che l'industria farmaceutica ha adottato negli anni è la formazione di sistemi a rilascio controllato dai farmaci che, oltre a migliorare la biodisponibilità dei farmaci stessi, fornisce loro protezione da alcune condizioni sfavorevoli come alte temperature, presenza di ossigeno e pH acidi.

La tecnologia a fluidi supercritici si è contraddistinta, negli ultimi anni, nella produzione di questi sistemi di rilascio di farmaci, in quanto supera molti degli ostacoli che caratterizzano i metodi convenzionali. Un fluido supercritico può essere definito come qualsiasi sostanza che viene riscaldata e compressa al di sopra del suo punto critico. La caratteristica che più rende interessante la tecnologia a fluidi supercritici è la possibilità di modificare le proprietà di questi fluidi semplicemente variando la pressione e/o temperatura del sistema, permettendo di ottenere dei prodotti finali con le caratteristiche desiderate. I bassi valori di temperatura e pressione critiche, l'economicità, la stabilità chimica, la non infiammabilità e tossicità, rendono la CO_2 la sostanza più utilizzata come fluido supercritico.

Lo scopo di questa tesi è quello di fornire una panoramica generale dei principali sistemi di rilascio di farmaci preparati con la tecnologia a fluidi supercritici, con una particolare attenzione dedicata agli studi pubblicati negli ultimi cinque anni (2016-2020). I sistemi di rilascio di farmaci trattati in questo lavoro sono (i) micro- e nanoparticelle polimeriche, (ii) aerogel, (iii) schiume microporose, (iv) liposomi, (v) nanoparticelle lipidiche solide e (vi) supporti impregnati con farmaci tramite l'utilizzo dell'impregnazione con solvente supercritico.

La tesi è divisa in tre capitoli. Nel primo capitolo vengono fornite le principali informazioni sui fluidi supercritici e sulla biodisponibilità dei farmaci, utili per una buona comprensione del contenuto degli altri capitoli. Nel secondo capitolo vengono spiegate le principali caratteristiche dei sistemi di rilascio di farmaci trattati. Nel terzo ed ultimo capitolo, invece, vengono discussi gli articoli che, negli ultimi cinque anni, hanno utilizzato i sistemi di cui sopra. Dall'analisi di tali recenti progressi vengono fornite alcune conclusioni e delineate le prospettive future.

1. I fluidi supercritici

Qualsiasi sostanza che viene riscaldata e contemporaneamente compressa a valori di temperatura e pressione più alti dei due valori critici, è conosciuta come *fluido supercritico* [1].

Ciò che rende i fluidi supercritici interessanti da un punto di vista tecnologico, è la possibilità di variare le loro proprietà fisiche da valori tipici della fase gassosa a valori tipici di quella liquida, agendo sulle condizioni termodinamiche del sistema. Per esempio, è possibile aumentare la densità di un fluido supercritico (aumentandone quindi il potere solvente) semplicemente aumentando la pressione del sistema [1].

L'anidride carbonica (CO_2) è la sostanza più utilizzata, in quanto ha delle condizioni critiche molto blande (31 °C e 74 bar), è economica, chimicamente stabile, non infiammabile e non tossica.

Proprietà della CO_2 supercritica

Le tre proprietà più importanti che caratterizzano l'applicazione tecnologica di un fluido supercritico, in generale, e della CO_2 supercritica, in particolare, sono densità, viscosità e diffusività.

La densità della CO_2 supercritica è più vicina a quella di un liquido piuttosto che a quella di un gas [3]. Ciò le conferisce un buon potere solvente. Riguardo le proprietà di trasporto come viscosità e diffusività, i loro valori sono più vicini a quelli tipici di un gas e ciò permette, ad esempio, di realizzare processi di estrazione più veloci e con minor perdite di carico.

Un'altra proprietà dei fluidi supercritici da tenere in considerazione è la tensione superficiale. In particolare, essendo questi fluidi in una fase omogenea, la loro tensione superficiale è nulla. Ciò li rende ideali nell'impregnazione di un farmaco in matrici solide [1].

Solubilità dei composti organici in fluidi supercritici

Come già sottolineato, le proprietà di un fluido supercritico, e quindi anche il suo potere solvente nei confronti di specifici soluti, possono essere modificate variando pressione e/o temperatura del sistema. Quando la pressione sale (a temperatura costante) la solubilità di un soluto in un fluido supercritico cresce [4]. A pressioni più elevate, infatti, la densità del fluido cresce, aumentando di conseguenza il suo potere solvente. Il modo in cui una variazione di temperatura (a pressione costante) influisce sul potere solvente di un fluido supercritico è invece dipendente dalla posizione della pressione rispetto alla cosiddetta *pressione di crossover*, una caratteristica comune dei

dati di solubilità per un singolo soluto in un fluido supercritico [4]. In entrambi i casi (a valori di pressione sia inferiori che superiori a quella di crossover) un incremento isobaro della temperatura porta ad un aumento della tensione di vapore del soluto solido e ad una diminuzione della densità del fluido supercritico. L'aumento di tensione di vapore del soluto causa un aumento della solubilità, mentre l'effetto opposto è causato dalla diminuzione della densità del fluido. Ne consegue che il risultato finale dipende da quale dei due fenomeni prevale. In particolare, al di sotto del punto di crossover si registra un a diminuzione della solubilità con la temperatura, mentre l'effetto opposto avviene al di sopra di esso [4].

Aspetti generali dei farmaci e della loro biodisponibilità

La scarsa solubilità in acqua, e di conseguenza in fluidi fisiologici, della maggior parte dei farmaci, ne condiziona il tasso di dissoluzione e la biodisponibilità. Ne risulta una somministrazione del farmaco in dosi maggiori di quanto necessario, accentuando i suoi effetti collaterali. Tra i vari metodi utilizzati per migliorare la biodisponibilità dei farmaci, i più comuni sono (i) la riduzione della loro dimensione e (ii) l'utilizzo di sistemi di rilascio di farmaci [9].

La riduzione della dimensione di un farmaco aumenta la sua area superficiale specifica, migliorandone il tasso di dissoluzione nei fluidi fisiologici.

La preparazione di sistemi di rilascio di farmaci, invece, consente loro di essere rilasciati in maniera controllata. A seconda delle applicazioni, infatti, potrebbe essere utile un rilascio veloce del farmaco, così come un suo rilascio prolungato. Inoltre, il sistema di rilascio di farmaci protegge il farmaco dalla degradazione dovuta a condizioni particolari come alte temperature di servizio, presenza di ossigeno e pH acidi [10].

La *biodisponibilità* di un farmaco è definita come la porzione inalterata di farmaco che raggiunge la circolazione sistemica [11]. La valutazione della biodisponibilità di un farmaco è molto complessa e richiede dei test molto costosi. Tra i vari tentativi fatti per la stima della biodisponibilità di un farmaco, la cosiddetta *rule of five* è risultata essere la migliore [12]. Secondo questa regola, un farmaco possiede un'accettabile biodisponibilità se ha le seguenti caratteristiche: (i) massa molecolare < 500 Dalton, (ii) $\log(p) \leq 5$, (iii) numero di donatori di legami a idrogeno < 5, (iv) numero di accettori di legami a idrogeno < 10, dove p è il coefficiente di permeazione del farmaco attraverso le membrane cellulari della barriera gastrointestinale [12].

Le due caratteristiche biofarmaceutiche più importanti sono solubilità e permeabilità del farmaco. Il sistema BCS (Biopharmaceutical Classification System) classifica i farmaci in quattro classi diverse, sulla base del valore di queste due caratteristiche [13]. I composti appartenenti alla *classe I* hanno elevati valori di solubilità e

permeabilità. Per questo, sono assorbiti rapidamente dal corpo umano e la loro biodisponibilità è determinata dal loro tasso di dissoluzione nel sito di assorbimento [8]. La bassa solubilità e l'elevata permeabilità dei composti appartenenti alla *classe II* fanno sì che la loro biodisponibilità sia limitata dal loro basso tasso di dissoluzione. Al contrario, la biodisponibilità dei farmaci appartenenti alla *classe III* è limitata dal loro basso valore di permeabilità. Infine, i farmaci appartenenti alla *classe IV*, hanno valori bassi sia di solubilità che di permeabilità [8,12].

Somministrazione dei farmaci

Un farmaco può essere somministrato in molti modi diversi. Le vie più comuni sono [8]: (i) enterali, (ii) parenterali, (iii) per inalazione, e (iv) transcutanee. Le vie enterali si possono dividere a loro volta in orali, rettali e buccali. Quando un farmaco viene somministrato per via orale (la più utilizzata), le fasi di raggiungimento della circolazione sistemica possono essere raggruppate in due step principali, che sono la dissoluzione del farmaco in liquidi intestinali ed il suo assorbimento attraverso le membrane cellulari [12]. Entrambi i fenomeni possono essere sfruttati per migliorare la biodisponibilità di un farmaco. Lo step di dissoluzione, in particolare, è governato dall'equazione di Noyes-Whitney (si consulti il capitolo 2). L'equazione che descrive invece l'assorbimento deriva dalla seconda legge di Fick, secondo la quale il flusso del farmaco attraverso le membrane è favorito da elevati valori di coefficiente di permeabilità del farmaco e da una sua elevata concentrazione nel bulk di liquido [12].

2. Sistemi di rilascio di farmaci

Micro- e nanoparticelle polimeriche

Per micro- e nanoparticelle polimeriche si intendono quei sistemi in cui un farmaco viene rivestito con un polimero. I processi a fluidi supercritici utilizzati per la produzione di micro- e nanoparticelle polimeriche vengono spesso suddivisi sulla base del ruolo del fluido supercritico. RESS, SAS e PGSS sono le tecniche più conosciute, in cui la CO_2 supercritica viene usata rispettivamente come solvente, antisolvente e soluto. [14].

L'equazione che governa la velocità di dissoluzione massica di una particella solida è l'equazione di Noyes-Whitney [12]:

$$\frac{dM}{dt} = \frac{DS}{h}(C_S - C_b) \tag{2.1}$$

dove M è la massa di farmaco, D il suo coefficiente di diffusione attraverso uno strato di diffusione di spessore h, S la sua area superficiale e (C_s-C_b) la forza spingente del processo di dissoluzione, cioè la differenza tra la solubilità del farmaco nel mezzo acquoso e la sua concentrazione nel mezzo stesso. Riducendo le dimensioni delle particelle, l'area superficiale S aumenta, migliorando così il tasso di dissoluzione del farmaco e, di conseguenza, la sua biodisponibilità [15,16].

Le tecniche convenzionali usate per ridurre la dimensione delle particelle tendono a generare particelle con un'ampia distribuzione del diametro, forme irregolari e con presenza di solventi organici [14,19,20]. Per superare queste difficoltà, la tecnologia a fluidi supercritici è una valida alternativa.

Nel processo *RESS (Rapid Expansion of Supercritical Solution)*, il fluido supercritico viene utilizzato nel ruolo di solvente.

La configurazione del processo RESS prevede principalmente due unità: l'unità di estrazione e quella di precipitazione. La CO_2, dopo essere riscaldata e compressa fino al raggiungimento delle sue condizioni supercritiche, viene mandata in un serbatoio (unità di estrazione) in cui sono presenti il farmaco e il polimero di interesse, che vengono disciolti nel fluido supercritico. Successivamente, la soluzione formata viene fatta espandere attraverso un ugello micrometrico fino a pressione atmosferica, in un altro serbatoio (unità di precipitazione). La rapida perdita di carico induce il fenomeno di super saturazione, dovuto alla rapida diminuzione della solubilità dei soluti nel fluido supercritico, che porta alla precipitazione di micro- o nanoparticelle [24].

Il processo RESS ha una buona efficacia solamente se usato per incapsulare farmaci che hanno un'ottima solubilità nel fluido supercritico [23]. Il più importante vantaggio che la tecnica offre è l'assenza di utilizzo di un solvente organico, che richiederebbe ulteriori step di purificazione del prodotto [24].

Sono state proposte, durante gli anni, alcune modifiche del processo RESS, al fine di espanderne il campo di utilizzo e migliorare le qualità del prodotto finale. Tra questi, il più utilizzato è il processo *RESOLV (Rapid Expansion of Supercritical Solutions into a Liquid Solvent)*. In questa tecnica, l'unità di espansione viene riempita con un solvente liquido, piuttosto che con semplice aria. Nel solvente liquido vengono disciolti dei tensioattivi e agenti stabilizzanti che prevengono la coalescenza delle particelle prodotte [24].

Nel processo *SAS (Supercritical Antisolvent)*, il fluido supercritico viene utilizzato nel ruolo di antisolvente. In questo modo, il campo di utilizzo della tecnologia a fluidi supercritici viene ampliato, aprendo alla possibilità di processare soluti con più bassa solubilità in CO_2 supercritica di quelli processabili con la tecnica RESS.

Il processo SAS consiste nell'inviare una corrente di farmaco e polimero disciolti in un solvente organico in un serbatoio di precipitazione riempito con CO_2

supercritica. Quando la soluzione organica si miscela con il fluido supercritico, la solubilità del farmaco nella nuova miscela diminuisce rapidamente, inducendo lo stesso fenomeno di super saturazione coinvolto nel processo RESS, e portando quindi alla precipitazione dei soluti in forma di micro- o nanoparticelle [14,37].

Il processo SAS produce buoni risultati se i soluti coinvolti hanno una buona solubilità nel solvente organico e una solubilità molto inferiore nel fluido supercritico. Rispetto al processo RESS, la tecnica è maggiormente utilizzata per l'incapsulamento di farmaci in polimeri, piuttosto che per la sola micronizzazione del farmaco stesso [24]. Questo porta ad alcune difficoltà legate al fatto che i due soluti (farmaco e polimero) possono precipitare in tempi diversi [37].

Tra i vari altri processi in cui il fluido supercritico è coinvolto con il ruolo di antisolvente, il più utilizzato è il processo *SFEE (Supercritical Fluid Extraction of Emulsions)* [50]. In questo processo, la CO_2 supercritica viene utilizzata per estrarre il solvente organico contenuto nelle goccioline di un'emulsione formata da solvente organico e acqua. Il farmaco è disciolto nel solvente organico e, dopo il contatto con la CO_2 supercritica, precipita.

Nel processo *PGSS (Particles from Gas-Saturated Solutions)*, il fluido supercritico viene utilizzato come soluto. In particolare, il suo ruolo è quello di ridurre la temperatura di fusione del polimero coinvolto, in modo tale da poter lavorare con temperature operative inferiori [24,52].

La configurazione del processo PGSS prevede la presenza di un'autoclave ad alta pressione e di un serbatoio di precipitazione. Inizialmente, il farmaco ed il polimero vengono posizionati nell'autoclave, la quale viene riscaldata fino alla temperatura operativa e saturata con CO_2 supercritica. Di conseguenza, i composti solidi vengono fusi, formando una miscela liquida satura di CO_2. Questa miscela viene fatta passare attraverso un ugello micrometrico, espandendosi nel serbatoio di precipitazione. L'improvvisa perdita di pressione porta ad un rapido raffreddamento della miscela (dovuto all'effetto Joule-Thomson) con la conseguente atomizzazione delle sostanze in microparticelle [54,55].

Il processo PGSS, a differenza del SAS, non prevede l'uso di alcun solvente organico. Sebbene la CO_2 supercritica abbassi la temperatura di fusione del polimero, la temperatura operativa richiesta è comunque maggiore rispetto a quella di altri processi, e potrebbe risultare in una degradazione termica del farmaco [24].

I fluidi supercritici sono usati come soluti anche in altri processi. Tra questi, il processo SAA è il più utilizzato per gli scopi di questa tesi. Nel processo *SAA (Supercritical-Assisted Atomization)*, viene formata una soluzione gas-liquida miscelando una soluzione acquosa (contenente il farmaco e il polimero) con la CO_2 supercritica. Per raggiungere la saturazione, la miscelazione viene fatta in una colonna impaccata.

In questa tecnica, l'atomizzazione viene migliorata soprattutto grazie al rilascio del fluido supercritico dalle goccioline liquide sature [62].

In tutti e tre i processi descritti (RESS, SAS e PGSS) è possibile modificare le proprietà del prodotto finale variando alcuni parametri caratteristici, tra cui variabili termodinamiche come temperatura e pressione operative, nonché geometriche come lunghezza e diametro degli ugelli micrometrici utilizzati.

Aerogel

Si definisce *aerogel* qualsiasi solido poroso ultraleggero ottenuto rimpiazzando la componente liquida di un gel con aria. Gli aerogel hanno elevate porosità e area superficiale, nonché una bassissima densità. Queste caratteristiche li rendono dei materiali ideali nel loro impiego come sistemi di rilascio di farmaci, soprattutto per l'elevata quantità di farmaco che è possibile includere nella loro matrice [14,66]. Gli aerogel possono essere inorganici (per lo più aerogel di silice), organici e "speciali" (cioè, possono essere introdotte alcune sostanze al fine di migliorarne alcune caratteristiche) [14,74,76].

Il processo di preparazione di un aerogel prevede principalmente tre step: (i) formazione del "sol", (ii) gelificazione ed (iii) essiccamento [66]. Nel primo step, degli opportuni precursori vengono miscelati con una soluzione acquosa (dove è contenuto anche una certa quantità di un solvente organico come un alcol o acetone) e reagiscono per formare il "sol", una soluzione colloidale dove la sospensione è formata da particelle solide molto piccole. Nel processo di gelificazione, le particelle reticolano o polimerizzano ulteriormente per formare un gel ad elevata porosità, dove i pori sono riempiti con la soluzione. Dopodiché, l'idrogel formato viene coinvolto in uno scambio di solventi, in cui la soluzione acquosa viene rimpiazzata con un solvente organico puro (generalmente acetone o etanolo), portando alla formazione del cosiddetto *alcogel*. Infine, l'essiccamento consiste nella rimozione del liquido che riempie i pori dell'alcogel, risultando nella formazione dell'aerogel [66]. L'essiccamento è il processo più critico, in quanto potrebbe portare al danneggiamento della struttura del solido. Quando l'essiccamento dell'alcogel prevede la semplice evaporazione dell'alcol, la struttura del gel viene compromessa. Quando il liquido evapora, infatti, la riduzione del raggio di curvatura dell'interfaccia liquido-vapore porta ad una tensione superficiale che esercita una pressione sulla superficie del gel [66]. L'utilizzo del cosiddetto *essiccamento supercritico* permette di superare questo problema. Tra i vari modi in cui l'essiccamento supercritico può essere condotto, il più utilizzato consiste nell'estrarre il solvente che riempie i pori dell'alcogel con CO_2 supercritica, seguito da uno step di depressurizzazione con cui viene rimossa la CO_2 in fase gassosa [14]. Ciò che rende fondamentale l'essiccamento supercritico nella formazione degli

aerogel è la mancanza di un'interfaccia liquido-vapore, che fa in modo che la CO_2 supercritica abbia una tensione superficiale nulla, evitando quindi stress meccanici alla struttura solida del prodotto finale [66].

Le modalità con cui il farmaco può essere introdotto nella struttura dell'aerogel sono molteplici. Il farmaco, infatti, può essere introdotto prima della formazione del "sol", in concomitanza con lo step di gelificazione, nonché dopo la formazione dell'aerogel stesso, con l'utilizzo della tecnica di impregnazione a solvente supercritico [71,72,73].

Schiume microporose

Le *schiume microporose* sono materiali multifase con un'elevata porosità e una bassa densità, in cui una delle fasi è un gas. Tra le possibili applicazioni, le schiume microporose sono utilizzate come sistemi di rilascio di farmaci, impalcature nel campo dell'ingegneria tissutale e per isolamenti termici ed acustici [78]. Riguardo il loro utilizzo come sistemi di rilascio di farmaci, la loro struttura microporosa è vantaggiosa in termini di velocità di rilascio del farmaco stesso, grazie al percorso più breve che esso deve attraversare nel polimero [81].

La tecnologia a fluidi supercritici con cui può essere prodotta una schiuma microporosa è conosciuta come *schiumatura supercritica*, rappresentata in figura 2.5.

Il primo step di formazione della schiuma microporosa prevede che il fluido supercritico venga disciolto in un polimero fuso in pressione, formando una soluzione polimero/gas. Dopodiché, la solubilità del fluido supercritico nel polimero viene ridotta attraverso una depressurizzazione del sistema, causando nucleazione e crescita di bolle. Quando la temperatura di transizione vetrosa della soluzione polimero-fluido supercritico supera la temperatura operativa, la vetrificazione del polimero interrompe il processo di schiumatura. Un ritardo nella vetrificazione del polimero potrebbe portare ad una indesiderata deformazione della struttura della schiuma microporosa [14,78].

L'incapsulamento di un farmaco all'interno della struttura di una schiuma microporosa può essere fatta sia con la tecnica di impregnazione a solvente supercritico, condotta contemporaneamente alla schiumatura, che a monte della schiumatura supercritica, con altri tipi di processi.

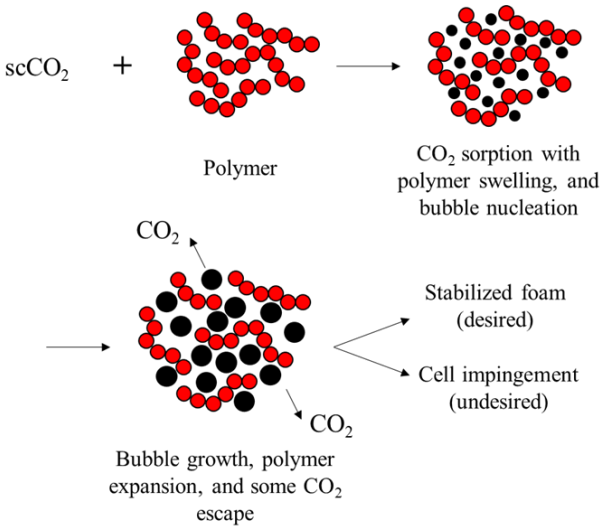

Fig. 2.5 Rappresentazione schematica del processo di schiumatura supercritica.

La schiumatura supercritica può essere condotta (i) in modalità batch, (ii) con un estrusore e (iii) con stampaggio ad iniezione [78]. Tra queste modalità, la schiumatura con estrusione è quella che si presta maggiormente ad un possibile scale-up industriale. Durante il processo di schiumatura con estrusore la CO_2 viene miscelata con il polimero fuso; questa miscela è pressurizzata come conseguenza al suo scorrimento lungo il cilindro dell'estrusore e, infine, la rapida caduta di pressione che causa la nucleazione delle bolle di CO_2 avviene all'uscita dell'estrusore stesso [78].

Liposomi

I *liposomi* sono vescicole artificiali in cui uno o più strati lipidici bimolecolari circondano un nucleo acquoso, come è possibile apprezzare dalla figura 2.6 [88]. Essi sono costituiti principalmente da fosfolipidi, molecole caratterizzate da una coda alifatica apolare e una più piccola testa polare. Le proprietà idrofobiche della coda dei fosfolipidi e le proprietà idrofile della loro testa, rende i liposomi sostanze anfifiliche, in cui possono essere incorporati farmaci sia idrofili che lipofili [89].

Ciò che rende i liposomi un sistema ideale per trasportare farmaci è la non tossicità, la biodegradabilità e la biocompatibilità dei fosfolipidi che li compongono [90].

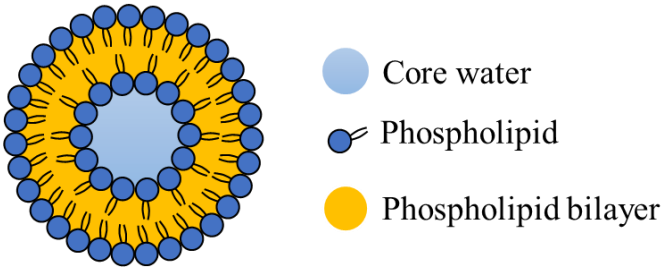

Fig. 2.6 Struttura di un generico liposoma.

Le tecniche a fluido supercritico proposte per superare gli svantaggi delle tecniche convenzionali sono principalmente tre: scRPE, DESAM e SuperLip. Nel processo *scRPE (Supercritical Reverse Phase Evaporation)* i fosfolipidi sono miscelati con CO_2 ed etanolo a valori costanti di pressione e temperatura. Dopodiché, una lenta introduzione di acqua ed una rapida riduzione di pressione portano alla formazione dei liposomi [88]. Nel processo *DESAM (Depressurization of an Expanded Solution into Aqueous Media)*, invece, i fosfolipidi vengono sciolti in un solvente organico e la CO_2 viene aggiunta per ottenere una soluzione lipidica espansa. Infine, la soluzione viene fatta passare attraverso un ugello in un mezzo acquoso caldo, a pressione atmosferica, portando alla struttura dei liposomi [94].

La bassa riproducibilità della tecnica scRPE e la bassa efficienza di incapsulamento della tecnica DESAM hanno spinto alcuni ricercatori alla proposta di un nuovo processo continuo basato sulla tecnologia a fluidi supercritici, il processo *SuperLip (Supercritical Assisted Liposome formation)* [92]. In questo processo, i liposomi sono formati attorno a delle goccioline nanometriche di acqua prodotte dall'atomizzazione di una corrente di acqua in una miscela liquida espansa di lipidi, etanolo e CO_2. Gli autori che hanno proposto questa tecnica, ne hanno anche testato le potenzialità studiando gli effetti dei principali parametri di processo sul prodotto finale. I risultati sono stati incoraggianti, soprattutto nel miglioramento dell'efficienza di incapsulamento di farmaci.

Nanoparticelle lipidiche solide

Le *nanoparticelle lipidiche solide* (fig. 2.9) sono sistemi colloidali su scala nanometrica composti da una matrice lipidica contenente il farmaco di interesse, generalmente dispersi in una fase acquosa contenente un tensioattivo, utile a rendere il prodotto fisicamente stabile, impedendone la coalescenza [14,96]. La matrice lipidica di queste nanoparticelle è solida a temperatura ambiente.

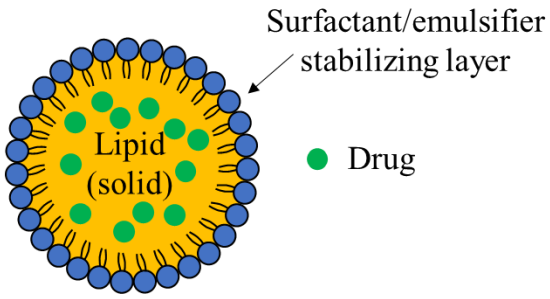

Fig. 2.9 Struttura di una generica nanoparticella lipidica solida.

Alcuni studi hanno dimostrato che la struttura interna di queste nanoparticelle può essere rappresentata con diversi modelli [95]. Per esempio, tra gli altri, alcuni autori [99] hanno osservato un arricchimento di farmaco nel guscio esterno delle particelle, che ha portato ad un suo veloce rilascio nella parte iniziale dello step di dissoluzione, seguito da un rilascio più prolungato. Sebbene la vasta possibilità di modelli complichi la produzione delle nanoparticelle lipidiche solide, questa alta flessibilità permette una maggior ingegnerizzazione del profilo di rilascio [99].

Il più grande svantaggio di questo tipo di sistemi è la limitata quantità di farmaco che è possibile incorporare nella loro struttura [100]. Dato che i farmaci vengono incorporati nelle imperfezioni della struttura cristallina delle nanoparticelle lipidiche, una soluzione alla scarsa quantità di farmaco incorporata è quella di creare quante più imperfezioni possibili, per esempio usando lipidi sia solidi che liquidi nel nucleo della nanoparticella. Questi tipi di sistemi sono conosciuti come *trasportatori lipidici nanostrutturati* [14].

Riguardo all'impiego della tecnologia a fluidi supercritici nella produzione delle nanoparticelle lipidiche solide, le tecniche che si usano sono le già descritte RESS, SFEE e PGSS. Tra queste, la più utilizzata è la PGSS.

Impregnazione con solvente supercritico

Lo stato amorfo di un farmaco è stato spesso associato alla sua instabilità, vista la tendenza di un solido amorfo di cristallizzare [106]. Tuttavia, la forma amorfa di un farmaco risulta avere maggiore biodisponibilità della sua forma cristallina, per via della maggiore mobilità delle sue molecole [107].

Al fine di evitare la spontanea cristallizzazione di un farmaco, esso potrebbe per esempio essere adsorbito in un solido poroso. Lo stato amorfo del farmaco, in questo modo, verrebbe garantito dallo stato energetico inferiore che il farmaco stesso ha quando è adsorbito sulla superficie porosa dell'adsorbente [116]. Inoltre, se i pori

della matrice sono abbastanza piccoli, il numero di molecole dentro un poro potrebbe non essere sufficientemente alto da indurre la nucleazione e crescita dei cristalli [118].

Per impregnare un farmaco in uno specifico materiale, si può ricorrere alla tecnica di impregnazione con solvente supercritico.

L'impregnazione con solvente supercritico è una tecnica in cui un farmaco viene disciolto in CO_2 supercritica e messo in contatto con il supporto da impregnare per un certo periodo di tempo. Una depressurizzazione del sistema, infine, consente alla CO_2 di lasciare il supporto in fase gassosa [107].

I materiali usati come supporto possono essere solidi porosi (come aerogel e materiali mesoporosi di silice o ossido di zinco), polimeri rigonfiabili e ciclodestrine.

Quando viene usato un solido poroso, il soluto viene adsorbito sulla superficie dell'adsorbente e precipita a seguito della depressurizzazione del sistema. In questo processo, la scelta di temperatura e pressione viene fatta in modo tale da evitare la formazione di una fase liquida, che potrebbe causare il collasso della struttura solida [107].

Quando viene usato un polimero rigonfiabile, l'amorfizzazione del farmaco è favorita dall'abilità della CO_2 supercritica di rigonfiare il polimero, consentendo una migliore diffusione del farmaco [114]. La scelta della temperatura può basarsi sull'aumento della solubilità del farmaco nella CO_2, migliorandone la quantità caricata, così come sul rigonfiamento del polimero, che dipende da diffusività e assorbimento della CO_2 nel polimero stesso [120].

Le ciclodestrine sono oligosaccaridi ciclici composti da un certo numero di unità glucopiranosio. Le conformazioni a sedia di queste unità permettono di formare ciclodestrine di forma troncoconica o toroidale, in cui la superficie esterna e la cavità interna sono rispettivamente idrofile e lipofile. Le ciclodestrine hanno l'abilità di formare complessi di inclusione con farmaci poco solubili in acqua [115]. Di solito, un ambiente ad alte temperatura e pressione è favorevole per l'efficienza di inclusione [127,129]. Inoltre, l'efficienta del processo di inclusione può essere migliorata dall'aggiunta di alcuni agenti ausiliari, come lisina e PVP [115].

3. I progressi più recenti (2016-2020)

In questo capitolo vengono discussi gli articoli che, negli ultimi cinque anni, riportano studi sui sistemi di rilascio di farmaci descritti nel capitolo 2. Per ogni sistema di rilascio di farmaci descritto viene inoltre riportata una tabella bibliografica (qui omessa per motivi di sintesi) che riporta le caratteristiche principali di prodotti ottenuti e processi utilizzati nei vari articoli.

Micro- e nanoparticelle polimeriche (2016-2020)

Il 63 % degli articoli analizzati, riguardanti l'incapsulamento di composti attivi in particelle polimeriche, ha impiegato la CO_2 supercritica come antisolvente. Di questi, il 54 % ha utilizzato la tecnica SAS. Il 34 % dei lavori ha impiegato la CO_2 supercritica come soluto (PGSS) mentre soltanto il 3 % l'ha impiegata nel ruolo di solvente (RESS). Seguono il ruolo di soluto con il 34 % e quello di solvente con il 3 %. SAS e PGSS sono quindi le tecniche più utilizzate. Il contributo della tecnica RESS è, invece, trascurabile, probabilmente per il fatto che il suo utilizzo richiede che i soluti abbiano una elevata solubilità nel fluido supercritico.

Il risultato più importante derivante dall'analisi degli articoli che hanno usato la tecnica SAS è l'impiego del polimero polivinilpirrolidone (PVP). Infatti, è stato riscontrato che esso ha l'abilità di ritardare la cristallizzazione dei soluti in gioco, risolvendo il problema (introdotto nel capitolo 2) del mancato successo della co-cristallizzazione di farmaco e polimero [44,46,131,132]. Inoltre, è stato ampliato il numero di polimeri utilizzabili con successo per il processo SAS, incapsulando con ottimi risultati alcuni composti attivi nella zeina, un biopolimero naturale [133]. Gli altri processi, che coinvolgono il fluido supercritico come antisolvente, sono SFEE e SEDS. È stato riscontrato che questi due processi sono stati utilizzati perlopiù per incapsulare composti bioattivi (come vitamina E, antiossidanti e oli essenziali), piuttosto che farmaci commerciali (come ibuprofene e paracetamolo).

Riguardo l'utilizzo del fluido supercritico come soluto, i due processi più utilizzati sono PGSS e SAA. Il processo PGSS, sebbene usato in maniera meno frequente rispetto al processo SAS, ha il grande vantaggio di non utilizzare alcun solvente organico. È ritenuta quindi una valida alternativa al processo SAS. Infine, il processo SAILA ha prodotto buoni risultati, ma, vista la sua giovane età (2012), ulteriori studi dovranno essere fatti in futuro per testarne le reali potenzialità.

Aerogel (2016-2020)

In questa sezione vengono discussi gli articoli che hanno previsto il caricamento di composti attivi in aerogel prima della formazione dell'aerogel stesso.

Dall'analisi dei risultati di questi lavori è emerso che sono stati usati tanti modi diversi di caricare il farmaco nella struttura dell'aerogel, dimostrando quindi la mancanza di un processo ben consolidato. Come mostrato in tab. 3.2, i composti attivi possono essere caricati: (i) prima della formazione del "sol", (ii) simultaneamente all'essiccamento supercritico, o (iii) in concomitanza con il processo di gelificazione [71,72,186].

Tab. 3.2 The different routes used to load the active compound in the aerogel matrix.

Ref., authors, year	In what stage of the aerogel production the drug was loaded
[71], Tkalec et al., 2016	In the first part of the sol-gel process, when the "sol" is not already formed.
[186], Salgado et al., 2017	Simultaneously with the supercritical drying process.
[72], Horvat et al., 2018	Concurrently to the gelation step, after the formation of the "sol".
[187], Iglesias et al., 2019	Concurrently to the gelation step, after the formation of the "sol".
[73], Buratto et al., 2019	Direct wet impregnation in the first part of the sol-gel process, as [71]. Indirect wet impregnation after the aging process.
[191], Santos et al., 2020	Indirect wet impregnation after the aging process.
[189], Ulker et al, 2017	After the aging process, GAS-like method.
[190], Falahati et al., 2019	After the aging process, GAS-like method.

Inoltre, la stampa termica a getto d'inchiostro è stata proposta come una tecnica promettente per la realizzazione di dispositivi farmaceutici destinati alla somministrazione polmonare di farmaci.

Schiume microporose (2016-2020)

Così come per gli aerogel, in questa sezione vengono discussi gli articoli che hanno caricato il composto attivo di interesse prima della formazione della schiuma.

Numerosi autori [194,196,197], negli ultimi cinque anni, hanno focalizzato la loro attenzione nella preparazione di schiume microporose per applicazioni nel campo dell'ingegneria tissutale, confermando la tendenza del passato. Il policaprolattone è stato il polimero più utilizzato a questo scopo. La sua bassa velocità di degradazione, infatti, lo rende ideale per essere impiantato nel sito di interesse.

Due nuove tecniche sono state proposte per la preparazione di schiume microporose. La prima [197] consiste nella combinazione dei processi *freeze-drying* e schiumatura supercritica, in cui dei *pellet* di policaprolattone vengono impregnati con una soluzione del farmaco in un solvente organico, asciugati tramite *freeze-drying* e soggetti a schiumatura supercritica. I risultati hanno dimostrato che è possibile ottenere dei prodotti con rilascio prolungato del farmaco. La seconda tecnica [194] consente di ottenere schiume a temperatura inferiori della temperatura di fusione del polimero. In questo processo, viene usato un solvente per sciogliere sia il policaprolattone che il farmaco, mentre, la successiva schiumatura supercritica, avviene all'interno di uno stampo cilindrico.

Liposomi (2016-2020)

La tecnica più utilizzata, negli ultimi cinque anni, per la produzione di liposomi con fluidi supercritici è stata la SuperLip. Tutte le altre tecniche come RESS, SEDS, SAS e scRPE hanno trovato scarso impiego [203,204,207,208,209].

La tecnica SuperLip è stata introdotta solamente nel 2014 [92]. Da allora, il suo potenziale è stato ampiamente testato. Di seguito verranno descritti i passi più importanti che sono stati fatti nell'esplorazione di questa tecnica.

Nel 2016 [210], è stata per la prima volta esplorata la possibilità di usare la tecnica SuperLip per produrre liposomi con una diversa composizione del doppio strato lipidico, impiegando una miscela di fosfatidilcolina e fosfatidilglicerolo, piuttosto che solamente fosfatidilcolina, ottenendo ottimi risultati in termini di efficienza di incapsulamento di un farmaco. Successivamente [211], il doppio strato lipidico è stato ottimizzato con l'aggiunta di due lipidi naturali (colesterolo e fosfatidiletilanolammina), i quali hanno avuto effetti rilevanti sul prolungamento del rilascio di un farmaco impiegato in soluzione acquosa. Nel 2018 è stato per la prima volta incapsulato un farmaco lipofilo in un liposoma tramite la tecnica SuperLip [212]. Alcuni passi verso un possibile scale-up del processo SuperLip sono stati fatti studiando il tempo necessario al raggiungimento delle condizioni stazionare [216] e sviluppando un processo di produzione continua con una produttività fino ad 1 g/h [218].

La tecnica SuperLip ha consentito di ottenere efficienze di incapsulamento dei farmaci spesso più alte del 90 %.

Infine, tantissime tipologie di principi attivi sono state incapsulate in liposomi con questa tecnica, come, per esempio, proteine, oli essenziali, antibiotici, anticorpi e integratori alimentari [216].

Nanoparticelle lipidiche solide (2016-2020)

Il processo a fluidi supercritici più utilizzato recentemente per la produzione di nanoparticelle lipidiche solide contenente farmaci è stato il processo PGSS. Gli altri processi utilizzati sono stati i processi RESSAS e SAS [227,228]. È stato inoltre proposto un nuovo processo, denominato *atomizzazione del lipide espanso con anidride carbonica* [229].

La sfida di produrre particelle con un diametro medio inferiore al micron non è stata ancora superata. Infatti, solamente in uno degli articoli che ha impiegato il processo PGSS i risultati hanno portato alla produzione di particelle lipidiche con diametro inferiore al micron [226]. Questo risultato è stato ottenuto grazie all'espansione della miscela satura di CO_2 in un bagno di acqua, anziché in aria. Lo stesso fenomeno ha consentito di ottenere, con la tecnica RESSAS, particelle di diametro inferiore al micron [227].

Yang et al. [229] hanno proposto un nuovo processo a fluidi supercritici, l'*atomizzazione del lipide espanso con anidride carbonica*, per produrre, per la prima volta, particelle lipidiche solide "bucate". Questo processo differisce dalla tecnica PGSS per il fatto che il lipide viene fuso prima di entrare nel serbatoio ad alta pressione. In questo modo, quando la CO_2 viene insufflata all'interno del serbatoio miscelandosi con il lipide fuso e si espande a seguito della depressurizzazione del sistema, ne risulta la formazione di un guscio lipidico vuoto che si solidifica poi per effetto Joule Thomson.

Impregnazione con solvente supercritico (2016-2020)

Tra la grande varietà di materiali utilizzabili come supporto per l'impregnazione dei composti attivi con solvente supercritico, gli aerogel sono stati quelli di gran lunga impiegati, probabilmente per via delle loro elevate porosità e aree superficiali, che consentono di incorporare grandi quantitativi di composto. Tenendo in considerazione anche gli articoli analizzati nella sezione "Aerogel (2016-2020)", il 67 % dei lavori ha incorporato il composto attivo in un aerogel già pronto. Considerando che nel rimanente 33 % sono stati usati metodi di caricamento del farmaco diversi fra loro, il ruolo dell'impregnazione a solvente supercritico si conferma essere quello più significativo.

De Marco et al. [235,236] hanno condotto due analisi LCA (Life cycle assessment) per studiare l'impatto ambientale del processo di produzione di aerogel a base di amido incorporati con vitamine E e K_3, ed hanno stabilito che il processo di essiccamento supercritico è quello che più influisce sui quasi tutte le categorie di impatto esaminate (come cambiamenti climatici, riduzione dell'ozonosfera e altri). Segue il

processo di impregnazione con solvente supercritico, con un impatto di almeno il 5 % in ogni categoria. Gli autori hanno spiegato che i consumi delle due tecniche sono dovuti principalmente all'elevata richiesta di energia termica dei processi di condensazione e riciclo della CO_2.

Riguardo invece alle schiume microporose, solamente due articoli [256,257] hanno ottenuti dei prodotti con schiumatura e impregnazione supercritiche.

Nel 2018, Gurikov e Smirnova [107] hanno sottolineato il fatto che la bassa solubilità in CO_2 supercritica di alcuni farmaci non è necessariamente legata al basso contenuto di farmaco incorporabile in un supporto poroso. L'analisi degli articoli più recenti che hanno usato polimeri rigonfiabili come supporti, porta ad estendere la conclusione di Gurikov e Smirnova anche a questo tipo di supporti.

Infine, l'ossido di zinco ZnO è stato usato, per la prima volta, per incorporare con successo l'ibuprofene e il clotrimazolo [234,238,239]. Maggiori studi andranno fatti per ampliare l'utilizzo di questo tipo di supporti e testarne le potenzialità.

Conclusioni

Il potenziale della tecnologia a fluidi supercritici nella produzione di sistemi di rilascio di farmaci è stato ampiamente esplorato durante gli anni. L'analisi degli studi pubblicati nel periodo 2016-2020 ha mostrato che molti sforzi sono stati compiuti in questo campo. La grande varietà di caratteristiche dei sistemi di rilascio di farmaci trattati non consente di generalizzare una conclusione in termini di scelta del miglior sistema di rilascio. Questa scelta, infatti, dovrebbe essere fatta in funzione dell'applicazione desiderata. In ogni caso, il contenuto di questa tesi consente di avere una panoramica generale circa i sistemi di rilascio trattati, nonché dei più recenti progressi, che potrebbe aiutare i ricercatori a decidere su quale di queste tecnologie investire le loro risorse. La mia personale opinione è qui sotto riportata, con il tentativo di dettare alcune linee guida.

Tra i possibili parametri che potrebbero essere usati per discriminare un processo dall'altro, io credo che i più importanti siano (i) la natura "solvent-free" del processo e (ii) la sua scalabilità. Quest'ultima può essere favorita da una configurazione continua e/o da una maggior semplicità di processo.

I candidati migliori per la produzione di micro- e nanoparticelle polimeriche sono i processi SAS e PGSS. Basando il ragionamento sui due punti di cui sopra, il processo SAS, oltre ad essere stato il più utilizzato negli ultimi anni, ha una configurazione continua. Comunque, esso richiede l'uso di un solvente organico. Il processo PGSS, che invece è "solvent-free", ha una configurazione batch. È importante anche ricordare che la solubilità del farmaco di interesse nel fluido supercritico influisce sulla

scelta tra questi due processi. Infatti, il processo SAS è limitato ai soluti che non sono solubili in CO_2 supercritica e che sono idrofobi.

Per quanto riguarda gli aerogel, la discussione degli articoli più recenti ha mostrato che i due processi supercritici, essiccamento ed impregnazione, sono fondamentali per il loro sviluppo. L'essiccamento supercritico è infatti la sola tecnica in grado di prevenire il collasso meccanico della struttura del solido. L'impregnazione a solvente supercritico, invece, sembra essere l'unico processo consolidato per incorporare farmaci in aerogel. Comunque, le analisi LCA condotte da De Marco et al. [235,236] hanno evidenziato l'enorme impatto ambientale che i due processi hanno nella produzione degli aerogel. Data la loro grande potenzialità, e data l'importanza che i due processi supercritici hanno nella loro produzione, è fondamentale, nel futuro, provare a ridurne l'impatto ambientale.

Quando l'applicazione farmaceutica desiderata ricade nel campo dell'ingegneria tissutale, non ci sono dubbi sullo scegliere le schiume polimeriche di policaprolattone. Da un punto di vista industriale, la schiumatura con estrusore offre le migliori opportunità, grazie alla sua configurazione continua e alle elevate produttività.

I sistemi di rilascio lipidici, come i liposomi e le nanoparticelle lipidiche solide, sono dei sistemi innovativi ed emergenti. L'uso di lipidi fisiologici li rende molto più biocompatibili, biodegradabili e non tossici, se confrontati con i polimeri di solito impiegati o con i supporti inorganici come quelli a base di silice, che sono biocompatibili ma non biodegradabili. Se si focalizza l'attenzione nella struttura del sistema di rilascio di farmaci, le nanoparticelle lipidiche solide sembrano superare alcuni dei problemi dei liposomi, come la perdita di farmaco e l'instabilità durante la conservazione. Se, invece, ci si focalizza sull'applicabilità industriale, la tecnica SuperLip (per produrre liposomi), nonostante la sua giovane età, sembra avere un grande potenziale in un futuro più prossimo.

Sebbene l'impregnazione con solvente supercritico sia un metodo consolidato per l'incorporazione di farmaci in un'ampia varietà di materiali, è importante considerare che il suo utilizzo richiede l'utilizzo di un materiale già pronto. Per questo motivo, non dovrebbe essere considerato necessariamente come un'alternativa agli altri sistemi discussi, bensì una loro possibile integrazione. Da un punto di vista di processo, potrebbe essere integrato (come già provato a fare con gli aerogel) agli altri processi a fluidi supercritici, includendo un ricircolo della CO_2 supercritica usata.

Table of contents

INTRODUCTION

The poor water-solubility of many drugs is universally recognized to be one of the major causes of their low bioavailability. The low bioavailability of the drugs negatively affects their introduction in the global market, since it leads to their administration in higher dosages than necessary, so increasing the adverse effects. Many efforts have been done, over the years, to enhance the bioavailability of the drugs. Generally, two main approaches are followed: (i) *particle engineering*, in which the reduction of the drug particle size allows their dissolution rates to be improved, and (ii) *drug delivery systems*. Among the advantages that loading a compound in a drug delivery system provides, the controlled release of the active compound is the most important. In fact, based on the desired applications, either a fast or prolonged release of the drug could be obtained. Moreover, the carrier protects them from hard conditions such as temperature, oxygen, and pH, so enhancing their stability.

Many types of conventional techniques are currently used to produce a wide variety of drug delivery systems. However, they suffer from several drawbacks, such as the high temperature conditions, which may cause the degradation of some active compounds, as well as the use of organic solvents, which require downstream purification processes. To overcome these drawbacks, the *supercritical fluid (SCF) technology* has been proposed as a good alternative. A generic substance becomes a supercritical fluid when temperature and pressure of the system exceed its critical temperature (T_c) and pressure (P_c). The properties of a SCF are intermediate between those of a liquid and a gas, and they can be tuned by varying the thermodynamic conditions of the system. The tunable properties of the supercritical fluids allow the SCF technologies to produce modified final products, based on the desired application. The supercritical CO_2 is the most used SCF, due to its mild supercritical conditions, inexpensiveness, chemical stability, non-flammability, and non-toxicity.

The aim of this work is to provide a general overview of the main drug delivery systems prepared by SCF technology, with a special focus on those prepared in the last five years. The drug delivery systems discussed in this thesis are polymer micro- and nanoparticles, aerogels, microporous foams, liposomes, solid lipid nanoparticles, as well as those prepared with the supercritical impregnation technique.

This work is divided into three chapters. In the first one, the general aspects of the supercritical fluids and the drugs' world are reported, in order to provide the reader the preliminary information needed for a good comprehension of the content of the other chapters. Moreover, the main properties of the supercritical fluids are discussed, highlighting the possibility to control them by tuning the thermodynamic conditions

of the system. Finally, some basic information about the bioavailability of the drugs are included.

The second chapter is devoted at providing a general overview of those drug delivery systems produced with the SCF technology treated in this thesis. In addition to the explanation of their general structure, the main functioning of the SCF-based processes used for their production is reported. Moreover, the reason why they are useful to improve the bioavailability of the drugs is provided.

The third chapter is focused on the discussion of those studies that, in the period 2016-2020, have prepared the active compounds-loaded drug delivery systems discussed in the second chapter, by employing the SCF technology. Moreover, for each drug delivery system treated, a bibliographic table shows the most important characteristics of each collected work. The database of the discussed recent works has been formed by using the subscripted international bibliographic databases available the *Politecnico di Torino*, like Scopus, as well as its electronic subscripted periodicals, such as Science Direct, Taylor & Francis Online, ACS Journals, Wiley Online Library, and Springer Link. The database includes papers that have been published from January 2016 until October/November 2020.

By analyzing the recent advances of the above-mentioned drug delivery systems, some conclusions are reported, and some future perspectives are proposed.

CHAPTER ONE

Supercritical Fluids

This chapter aims at explaining some general aspects of the supercritical fluids and the drugs' world, to provide the reader the preliminary information needed for a good comprehension of the content of the other chapters. The first part of the chapter is focused on providing a general overview of supercritical fluids, by reporting their definition and main properties, and highlighting the possibility to control them by tuning the thermodynamic conditions of the system. Finally, in the last paragraph (the paragraph 1.4), some basic information on the drugs and their bioavailability is reported, and one of the major challenges of the pharmaceutical industry (which is the improvement of the bioavailability of the drugs) is introduced.

1.1 Introduction to supercritical fluids

In the 1822, Charles Cagniard de la Tour, through his experiments with cannons, showed the existence of a critical temperature above which a single substance can exist only as a single fluid, with intermediate properties between those of a liquid and a gas, known as *supercritical fluid* (SCF) [1]. To observe this phenomenon, it is useful to refer to figure 1.1.

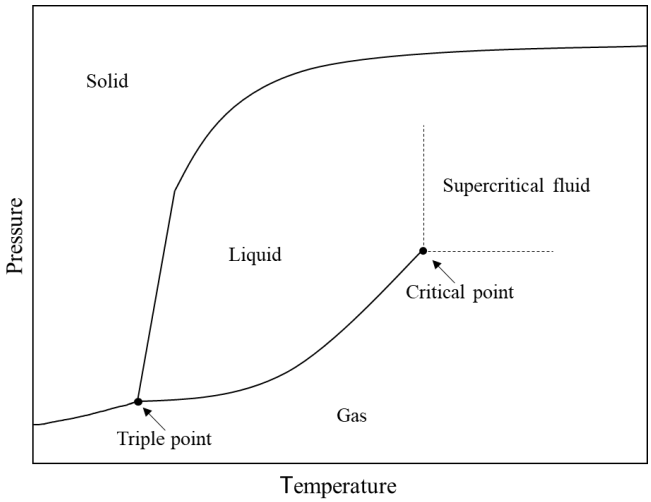

Fig. 1.1 Phase diagram of a single substance.

The main purpose of the figure is to illustrate, qualitatively, the phase diagram of a single substance. As known, in a generic phase diagram like the one described in figure 1.1, the triple point represents a pair of values of pressure and temperature in which three phases coexist, while each line represents those pressure and temperature values at which the two bordering phases coexist.

Moving from left to right along the liquid-gas coexistence line, both temperature and pressure increase. The liquid phase becomes less dense because of the thermal expansion, while the gas phase becomes more and more dense due to the increase in pressure. When certain values of pressure and temperature are reached, the densities of the two phases are equal and the liquid phase cannot be distinguished from the gaseous one any longer. The temperature and pressure of this point are named as the *critical temperature* (T_c) and *critical pressure* (P_c), respectively. Together, they identify what is called the *critical point*, which is different for each substance [1]. To get an idea, some values of the critical temperature and critical pressure are reported in table 1.1.

Tab. 1.1 Useful substances as supercritical fluids and their supercritical conditions. The table was reproduced with modifications from [1].

Substance	Critical temperature, K	Critical pressure, bar
Carbon Dioxide	304	74
Water	647	221
Ethane	305	49
Ethene	282	50
Propane	370	43
Xenon	290	58
Ammonia	406	114
Nitrous oxide	310	72
Fluoroform	299	49

Moving down or left inside the supercritical fluid zone, due to the absence of coexistence lines between two phases, the fluid properties do not change suddenly. Moreover, the liquid phase in the liquid region just to the left of the supercritical zone has characteristics very similar to those of the supercritical fluids, and for this reason it is studied in a similar way (in the literature [1] the substances in this liquid region are named as *subcritical fluids* or *near-critical fluids*). It must be pointed out that the best benefits of operating with a supercritical fluid come out when they are used not too much above their supercritical temperature.

Supercritical fluids have important characteristics, such as high compressibility and homogeneity as well as a continuous variation of all properties from those typical of the liquid phase to those typical of the gas phase. As it can be observed from the phase diagram in fig. 1.1, a supercritical fluid cannot be liquefied by increasing the pressure.

Although they are often used for environmental reasons, the major cause of interest in supercritical fluids is the fact that their properties are intermediate between those typical of gases and liquids. In fact, compared to liquids, they have lower density and viscosity, and higher diffusivity. Furthermore, it is possible to change their properties by simply acting on both pressure and temperature, so giving the SCF technology the ability to change the characteristics of the final product, as it can better be appreciated in the second chapter of this work. As an example, the pressure could be increased to approach the typical densities of liquids. Near the critical temperature, the change of properties versus pressure becomes more sudden [1].

In many industrial applications, supercritical fluids are ready to substitute the conventional solvents, mainly for environmental benefits and the quality of the final products. Among the substances reported in tab. 1.1, supercritical carbon dioxide ($scCO_2$) is the most largely used. Almost all the articles cited in work used CO_2 as the supercritical fluid. For this reason, often the term “supercritical fluid” will be used in place of “CO_2” or “$scCO_2$”, and vice versa.

In this paragraph, a general overview about supercritical fluids has been done, while in the next one, the attention is focused on $scCO_2$, in order to deepen how its main properties change with pressure and temperature.

1.2 Supercritical CO_2

As reported in tab. 1.1, carbon dioxide becomes a supercritical fluid when it is heated and simultaneously compressed to temperature and pressure values greater than about 31 °C and 74 bar, respectively. Carbon dioxide is the most used substance as a supercritical fluid because of its mild supercritical conditions, inexpensiveness, chemical stability, non-flammability, and non-toxicity. The mild conditions allow, for example, thermolabile compounds to be processed. Moreover, $scCO_2$ is an eco-friendly alternative to organic solvents. It is obtained in large quantities as a by-product of the processes of fermentation, combustion and ammonia synthesis and would be directly released into the atmosphere, if it were not used as a supercritical fluid [1]. The molecule of CO_2 is a linear symmetric molecule with no net dipole moment, therefore it can be classified as a non-polar solvent. Its solvent power is intermediate between that of a completely non-polar solvent, such as hexane, and that of a weakly polar solvent. Despite this, it shows some limited affinities towards polar solutes due

to its quadrupole moment. Anyhow, to enhance its affinity towards polar solutes, a little amount of polar co-solvents can be added [2].

1.2.1 Properties of scCO₂

Density is one of the most important parameters for a supercritical fluid. In general, the density of a supercritical fluid is closer to that typical of liquids [3]. The higher density of these fluids compared to that of gases provides them a higher solvent power. The behavior of density versus temperature and pressure is illustrated in fig. 1.2, which shows the variation of CO_2 density with pressure, at three different temperatures.

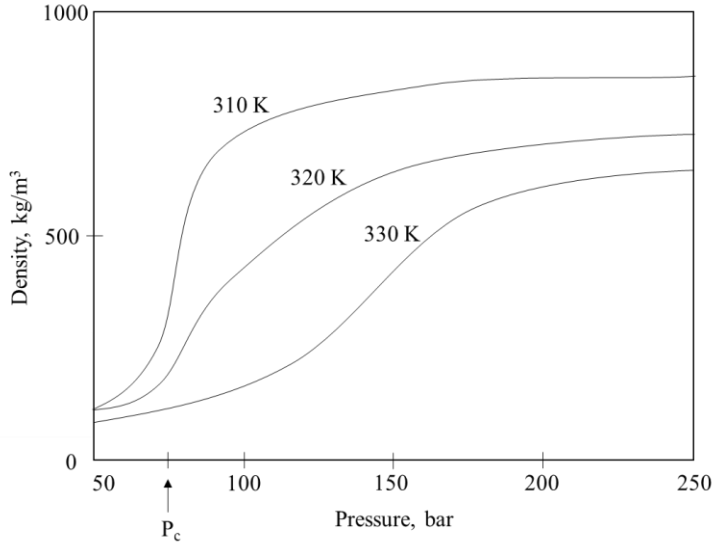

Fig. 1.2 Density vs Pressure isotherms for CO_2. The figure was reproduced with modification from [3].

As already mentioned in paragraph 1.1, if the pressure rises, the properties of a supercritical fluid go from those typical of gases to those typical of liquids. Fig. 1.2 confirms this last statement, as well as that at higher temperature values density is lower. Fig. 1.2 also points out that a more rapid change of properties occurs near the critical point. In fact, looking at the isotherm that is closer to the critical temperature (the isotherm at 310 K), the increase in density versus pressure is more accentuated with respect to that occurring at higher temperatures. The greater the distance of an isotherm with respect to the critical temperature, the less abrupt the change in density versus pressure will be. Consequently, since density and solvent power are strongly

connected, near the critical point the control of the experimental conditions of any process involving a supercritical fluid can be very difficult and should be avoided.

Many other properties, like *enthalpy*, show a similar behavior at temperatures near the critical one [1].

As well as the transport properties, like the *dynamic viscosity* (μ) and *diffusivity* (D) are concerned, their values are much closer to those typical of gases with the latter often being one order of magnitude greater than the typical values of liquids. Therefore, due to the almost gas-like dynamic viscosity, when supercritical fluids are involved pressure drops through chromatographic columns, extractors and other equipments are much lower than the equivalent processes involving liquids. Regarding the diffusivity values, the extra order of magnitude with respect to the liquids implies the advantage of faster extraction processes [1]. Some typical values of the properties discussed in this paragraph, are reported in Table 1.2.

Tab. 1.2 Typical values of density, viscosity, and diffusivity of CO_2. The table was reproduced with modification from [1].

Phase	CO_2		Naphtalene in CO_2
	Density, kg · m^{-3}	**Viscosity, μPa · s**	**Diffusion Coeff., m^2 · s^{-1}**
Gas (313 K, 1 bar)	2	16	$5.1 \cdot 10^{-6}$
Supercritical (313 K, 100 bar)	632	17	$1.4 \cdot 10^{-8}$
Liquid (300 K, 500 bar)	1029	133	$8.7 \cdot 10^{-9}$

One last interesting property, which deserves to be analyzed, is the *surface tension*. A generic supercritical fluid, being a homogeneous phase (liquid and gas phases are indistinguishable), has zero surface tension. Therefore, supercritical fluids, and in particular $scCO_2$, are suitable vehicles to achieve the impregnation of organic compounds, i.e. drugs, into solid matrices [1].

1.3 The solubility of organic compounds in supercritical fluids

The *solubility* of organic compounds in a supercritical fluid is a property that has a great impact in the set-up of a process. It can, for example, directly affect the velocity, the yield, and affordability of a process [1]. Depending on the type of process, higher or lower values of solubility of specific compounds are desired. As an example, for the correct functioning of the RESS process (see paragraph 2.1.1), a high solubility of the solute in $scCO_2$ is required, while when the $scCO_2$ is used as an antisolvent (for

example in SAS, see paragraph 2.1.2) it is important to have a low solubility of the solute in the $scCO_2$/organic solvent mixture.

One of the most important advantages of the SCF technology is the ability to tune the solubility of solutes in supercritical fluids in a continuous way in a wide range, which can be obtained by manipulating (i) pressure at constant temperature, or (ii) temperature at constant pressure. To understand how the solubility can change with pressure and temperature, figure 1.3 shows the solubility of chlorpropamide in $scCO_2$ versus the operative pressure at three different temperatures. The solubility is defined here as the molar fraction of solute in the solution solute + solvent. From the figure 1.3 it can be observed that when the pressure rises (with constant temperature) the solubility increases. This happens because when the pressure rises, the density rises too, with the consequent increase of the solvent power. About the role of the temper-

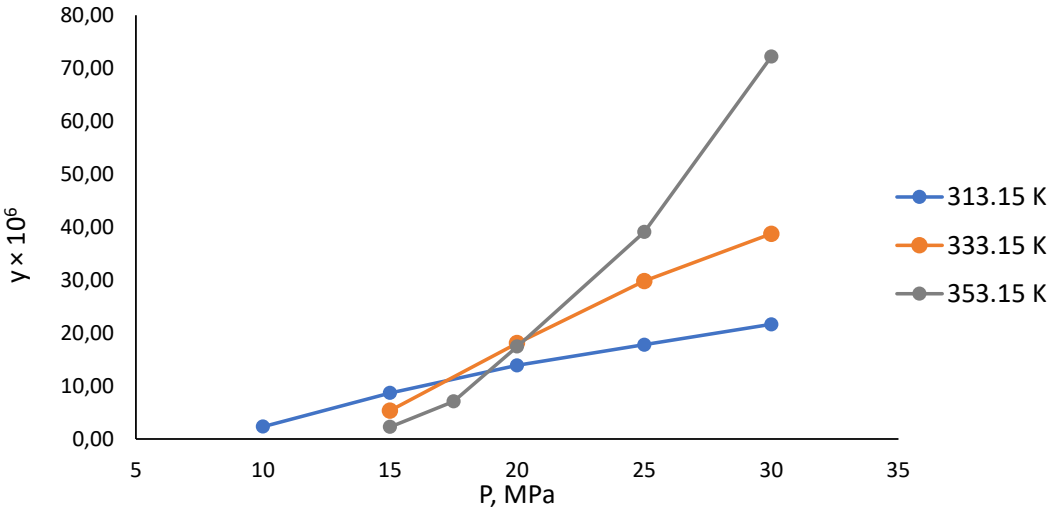

Fig. 1.3 Solubility of chlorpropamide in CO_2 at different temperatures and pressures. The figure was reproduced with modifications from [4].

ature, a more complicated reasoning needs to be done. In fact, the trend of the solubility with temperature at constant pressure depends on the pressure value, if this is placed above or below the *crossover pressure*, which is a common characteristic of solubility data for single solutes dissolved in a SCF, as it can be observed in the figure in the range 17-20 MPa [4].

The crossover pressure is a pressure value around which the isotherms tend to converge [5]. Below the crossover pressure, an isobaric increase of temperature causes a decrease of solubility, while the opposite trend is observed above. To understand this behavior, two conflicting effects must be considered. In both cases (above and

below the crossover pressure) when the temperature increases, the vapor pressure of the solid solute rises, while the density (therefore also the solvent power) of $scCO_2$ decreases. The trend of solubility vs temperature depends on which of the two phenomena prevails. Below the crossover pressure, where the compressibility is higher, the density effect dominates, so determining a decreasing trend of the solubility when temperature rises. Above the crossover pressure, instead, the effect of vapor pressure prevails, with the consequent increasing trend of the solubility versus temperature [4].

As mentioned above, the solubility is a very important parameter for processes involving SCFs. In the second chapter of this work it is explained how the knowledge of the solubility in $scCO_2$ is crucial to decide which process is more suitable for the solute involved. Therefore, many studies [4, 6, 7] are focused on the determination of the solubility of solutes in $scCO_2$, aiming at to enriching the database of solubility data. Because of the wide range of temperatures and pressures in which the solubility of a solute can be experimentally determined, before performing these experiments it is useful to have the phase diagrams of the mixtures of interest [8], which helps the researchers to choose the most appropriate range of temperatures and pressures, so drastically reducing the amount of work to be done.

After providing a general overview of supercritical fluids and their properties, the last paragraph of the first chapter provides some preliminary information about drugs and some general aspects of their bioavailability, since the main subject of this thesis is to show how the bioavailability of a drug can be improved through the use of SCF technology in the production of drug delivery systems.

1.4 General aspects about drugs and their bioavailability

Most of the *Active Pharmaceutical Ingredients* (APIs) are poorly soluble or completely insoluble in aqueous media and, then, in body fluids, because of their hydrophobic nature. This negatively affects the drug dissolution rate and bioavailability, whose improvement is, currently, one of the major challenges of the pharmaceutical field. In fact, these low-soluble drugs are generally administrated in a much higher amount than they should be to achieve the desired therapeutic effects, which leads to an increase in side effects of the drug and its toxicity for the human body.

To overcome the issue of the poor bioavailability of the APIs, many solutions have been studied for many years. Among these, two different approaches are often followed, (i) *particle engineering* and (ii) *drug delivery systems*. As far as particle engineering is concerned, as it can be better appreciated in the first part of the second chapter, the dissolution rate of a drug is enhanced by the reduction of its particle size. The micro- or nanonization of the particles allows them to permeate more effectively through biological barriers in the body due to their vast specific surface area [9]. Drug

delivery systems, instead, refer to the preparation of solid dispersions of the drug in a solid carrier, generally an inert biocompatible polymer. The encapsulation of the drug in the carrier allows to protect the core component from rapid degradation, and can also be useful to release the involved drug in the targeted site in the human body or to prolong the duration of bioactive agents [9]. Moreover, the carrier can be suitable to control the drug release rate, so allowing the release of the drug to occur only when some fundamental conditions such as optimum pH and temperature are reached [10]. In addition to solubility, also the *permeability* is a key factor for the improvement of the bioavailability of the drugs.

1.4.1 The Bioavailability of an API

The *bioavailability* is defined in the first volume (in 1973) of the Journal of Pharmacokinetics and Biopharmaceutics [11], which recites:

"Bioavailability is a term used to indicate the rate and relative amount of the administered drug which reaches the general circulation intact. In the context of this definition, general circulation refers primarily to venous blood (excepting the hepatic portal blood during the absorptive phase) and arterial blood which carries the drug to the tissues."

From the above definition, it is clear that the bioavailable drug is the portion of unaltered API that reaches the systemic blood circulation. The bioavailability can be classified as *absolute bioavailability* and *relative bioavailability*.

The *absolute bioavailability* (*in vivo*) is defined by the equation:

$$F = \frac{AUC_{ev}}{AUC_{iv}} \tag{1.1}$$

where AUC represents (mathematically) the integral of the curve C(t) obtained during the drug administration (where C is the concentration of the drug in the biological fluid involved and t is the time), and the subscripts "ev" and "iv" refer to extra-vasal and intra-vasal, respectively [12]. Therefore:

$$AUC = \int_0^\infty C(t)dt \tag{1.2}$$

Of course, from an experimental point of view, the highest extremity of the integral will be a limited level of time [12].

The *relative bioavailability*, instead, has a definition similar to that of the absolute one, but now, in the ratio, the bioavailability of two drug dosage forms (containing similar API and administered in a similar way) are compared [12]:

$$F_{rel} = \frac{AUC_{test}}{AUC_{referent}} \tag{1.3}$$

The study of the bioavailability of a drug is very complex and needs very expensive tests. The API concentration in some biological fluids must be measured. Unfortunately, there is not a common method for the different drugs, but each method is unique.

The general approach for the prediction of the bioavailability of a drug aims at simplifying the procedures, and to do this, the physiological characteristics of the human gastrointestinal tract, and physico-chemical properties of API need to be known. Among the parameters affecting API absorption after peroral administration, bile acid level, gastric juice acidity and gastrointestinal tract peristalsis are important. As far as physico-chemical properties of the drug are concerned, some important parameters are, for example, the melting point, solubility, hydrogen bonds, charge, molecular mass, lipophilicity and amphiphilicity [12]. According to Golovenko and Borisyuk [Dd], the knowledge of the physico-chemical properties of a drug is the most important tool to predict its bioavailability. The authors stated that, among the numerous attempts to find important correlation between the bioavailability of drugs and their physico-chemical properties, the most used is the so-called “rule of five”, according to which, an API has an acceptable bioavailability if it has the following characteristics: (i) molecular mass < 500, (ii) log(p) ≤ 5, (iii) the number of H-bond donors < 5, (iv) the number of H-bond acceptors < 10, where p is the permeation coefficient.

1.4.2 The Biopharmaceutical Classification System (BCS)

In the various steps of the feasibility study of a drug-preparation process, which includes screening, selection and introduction of innovative processes, it is recommended to predict the bioavailability of the candidate drug. The prediction of the bioavailability of a drug can be performed according to the *Biopharmaceutical Classification System* (BCS), as Golovenko and Borisyuk explain in their study [12]. The BCS is a system proposed by Amidon et al. in 1995 [13], which classifies the drugs on the basis of their solubility and permeability, two important biopharmaceutical characteristics. API solubility refers to aqueous media while the permeability coefficient quantifies its mass transfer through the lipophilic part of the bio-membrane of

the gastrointestinal wall. The subdivision of drugs in classes, according to the BCS, is showed in figure 1.4.

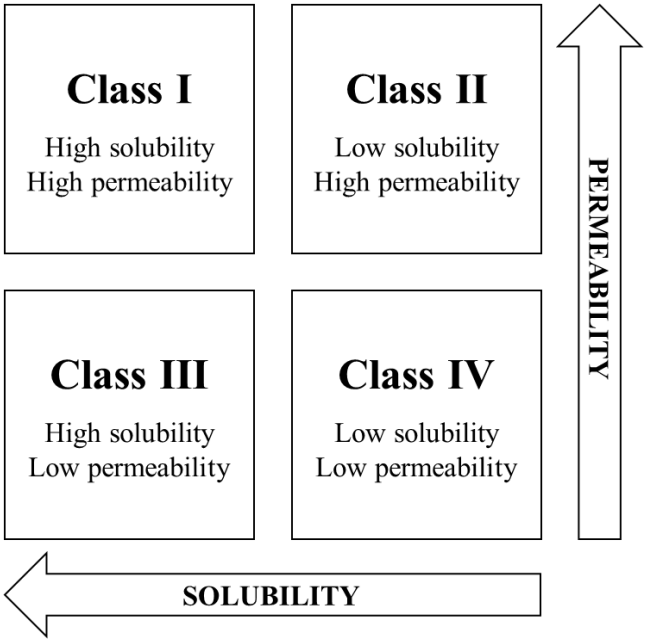

Fig. 1.4 Scheme of the classification of the drugs in classes, according to the BCS.

As it can be appreciated from the figure, the solubility and permeability values are the discriminants to understand at which class a drug belongs.

Compounds belonging to *class I* are both very soluble and very permeable, and then they are rapidly absorbed by the body [8]. The bioavailability of the API is determined by the rate of its delivery to an absorption site. Drugs belonging to this class display high dissolution rates (about 85 % of drug dose is dissolved in 15 minutes) [12]. Moreover, class I drugs have a molecular mass below 500, water solubility higher than 1 mg/ml and lipophilic character.

The low solubility and the high permeability of the drugs belonging to *class II* of the BCS imply that what limits the bioavailability of the API is its low dissolution rate. Because of this, the bioavailability of class II drugs can be improved trying to increase their dissolution rate [8].

Conversely, the bioavailability of the drugs belonging to *class III* is limited by the very scarce penetration through bio-membranes.

The drugs belonging to the *class IV* of the BCS are very limited for peroral administration, due to the low values of both solubility and permeability [12].

1.4.3 Administration of drugs

The administration of a drug can take place in different ways. Among them, the most common are [8]: (i) enteral routes, (ii) parenteral routes, (iii) by inhalation, (iv) transcutaneous routes. Among the enteral routes, the oral, rectal, and buccal ones can be distinguished. According to Golovenko and Borisyuk [12] the 84 % of drugs available in American and European pharmaceutical markets is in solid oral dosage form. When the drug is in solid oral dosage form, the following interrelated processes occur in the gastrointestinal tract [12]: (i) solid carrier degradation with the release of the API, (ii) API solubilization in the liquid medium contained inside the gastrointestinal tract, (iii) penetration of the API from the liquid medium to the gastrointestinal mucosal surface, (iv) transfer of the API into the mucosal layer, and (v) penetration of the API from the mucosal layer into systemic blood circulation.

As far as the *dissolution step* is concerned, a diffusion border layer surrounding the surface of the dissolving API is formed. In this border layer, the concentration of the dissolving drug changes from the concentration on the solid surface of the API to the concentration in the bulk of the solution, where the thickness of the layer depends on hydrodynamic parameters. Some other information (like the Noyes-Whitney model) about the dissolution rate of drugs can be found in the first part of the second chapter, when the improvement of the drug bioavailability connected with particle size reduction is discussed.

The *absorption step* is the transfer of the drug through bio-membranes, which involves intracellular transport (also known as *passive transport*) [12]. The equation that describes the transport of drug through bio-membrane comes from the Fick's first law, and it is showed here:

$$J = pC_b \tag{1.4}$$

where J is defined as the mass of API passing through the bio-membrane in the unit of area and time, p is the permeability coefficient, and C_b the API concentration in the bulk of the aqueous medium. The permeability coefficient is evaluated as:

$$p = \frac{DK}{h} \tag{1.5}$$

where D is the diffusion coefficient of the API in the membrane, K is the mass transfer coefficient, and h is the thickness of the membrane. From equation (1.4) it can be observed that the penetration of the API through the bio-membrane rises when C_b increases. In addition to the hydrophilicity required to achieve a high solubility in

water, it is also important that the API has a reasonable level of lipophilicity in order to pass from the aqueous medium into the lipophilic part of the membrane. In fact, when lipophilicity increases, the permeability coefficient increases too and, from equation (1.4), the flux of API is enhanced. Moreover, by calculating the diffusion coefficient with the Stocks-Einstein equation (equation (1.6)), it is easy to observe that drugs with smaller radius r have a bigger value of D and, then, can penetrate through mucosa more effectively than larger drugs.

$$D = \frac{RT}{6\pi\mu r N_A} \tag{1.6}$$

In this first chapter, a general overview of the supercritical fluids and the bioavailability of the drugs have been reported. The second chapter is focused on the general explanation of those drug delivery systems produced with the SCF technology treated in this work, which are polymeric micro- and nanoparticles, aerogels, microporous foams, liposomes, and solid lipid nanoparticles. A specific section is also devoted to the supercritical solvent impregnation technique, which is used to load drugs in supports such as polymers, aerogels, mesoporous silica and ZnO carriers, and cyclodextrins.

CHAPTER TWO

Drug Delivery Systems

This chapter aims at explaining the general structure of the drug delivery systems treated in this thesis, as well as the main functioning of the SCF processes used for their production. Although the polymeric micro- and nanoparticles are referred to the system in which an active compound is coated by a polymer, also the micro- and nanonization of the particles alone is treated to further understand the operating principles of the SCF-based processes involved. For each drug delivery system, the explanation of their structure and of the reasons why they are useful to improve the bioavailability of the active compounds are first provided. Second, the SCF-based processes used for their production are illustrated, highlighting the advantages and disadvantages that each process provides.

2.1 Polymeric micro- and nanoparticles

The first pharmaceutical drug carriers discussed in this work are polymeric micro- and nanoparticles produced with SCF technology. The processes are usually classified on the basis of the role of the supercritical fluid. This may occur in three different ways: CO_2 can act (i) as a solvent, (ii) as an antisolvent and (iii) as a solute. For each of the cited roles, many SCF processes can be found in the literature. The main techniques used to produce polymeric micro- and nanoparticles are RESS, SAS and PGSS, which use the SCF as a solvent, an antisolvent and a solute, respectively [14]. In this section, these three techniques are explored, by explaining their main functioning and how the process and/or technological parameters can affect the quality of the final product. Although the aim of this work is to talk about the drug delivery systems, to better understand these three techniques, the studies performing the micro- or nanonization of drugs without coating material are analyzed too.

As it has been explained in the paragraph 1.4.3, during the dissolution step, a diffusion border layer appears on the API surface, whose thickness depends on hydrodynamic parameters. The equation governing the mass dissolution rate is the Noyes-Whitney equation [12]:

$$\frac{dM}{dt} = \frac{DS}{h}(C_S - C_b) \tag{2.1}$$

where M is the dissolving API mass, D is the diffusion coefficient of the API through the diffusion layer of thickness h, S is the surface area of API particles, and (C_S-C_b) is the *driving force* of the dissolution process, i.e., the difference between the saturation solubility of the drug in the aqueous medium and its concentration in the same medium.

It is easy to observe from equation (2.1) that the dissolution rate dM/dt is enhanced by higher values of the API diffusion coefficient, the surface of the solid particles and the driving force, as well as a finer diffusion layer. In order to increase the driving force of the equation (2.1), it is important to have a good solubility of the drug in the body fluid involved (C_S), also considering that it depends on the composition of the aqueous medium, including its pH. Besides, to increase S and consequently dM/dt, a *reduction of particle size* can be performed, and this is how the micro- or nanonization of the APIs improves the bioavailability of the drugs themselves [15,16]. A very strong assumption of the Noyes-Whitney equation is to consider S as a constant in the whole duration of the dissolution step. However, it should be noted that in the dissolution process, S gradually decreases. Moreover, it must also be highlighted that many parameters involved in the dissolution process depend on the physiological conditions of the gastrointestinal tract, which also change over time. These are the reasons why the Noyes-Whitney equation fails to predict the experimental trend of *in vivo* dissolution tests when the influence of the different factors on API solubility is not known in depth. Instead, the equation describes very well what happens during *in vitro* release tests [12]. As the International Union of Pure and Applied Chemistry (IUPAC) recommends, the locutions *in vitro* and *in vivo* refer to the study in the laboratory and the study performed on a living organism, respectively. It should be accentuated that, in the pharmaceutical field, usually the *in vitro* release tests involve the dissolution of the drug in fluids which simulate the biological ones [17,18].

The above discussion has pointed out that a high surface-to-volume ratio of the particles guarantees a higher mass dissolution rate. Conventional techniques used for particle micronization, such as spray-drying, liquid antisolvent precipitation, jet milling and spray freeze drying have several drawbacks like the tendency to generate particles with high surface energy, which causes static charging and agglomeration, so resulting in poor flow properties. Moreover, during conventional micronization, irregular particles with broad size distribution are produced, which may also be potentially degraded by mechanical and thermal stresses as well as polluted with organic solvents, so requiring downstream purification processes [14,19,20]. To overcome the main difficulties of conventional micronization techniques, supercritical fluid technology has been proposed as a good alternative, since it exploits the SCFs characteristics as liquid-like density and tunable solvent power, as well as gas-like transport properties.

The quality of the final product and the performances of SCF technology in micro- and nanonization processes (in this case RESS, SAS and PGSS) are evaluated in terms of some parameters such as (i) particle size distribution (PSD), (ii) mean particle size, (iii) drug loading, and (iv) precipitation yield, as well as other characteristics like the amorphous form obtained and the dissolution rate.

The *span* of size distribution is defined with the following relation [16]:

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \tag{2.2}$$

where, as an example, d_{10} is the diameter below which 10 % of the particles is obtained. Based on this, the equation (2.2) provides an idea, normalized to the central point d_{50}, about how far apart d_{90} and d_{10} are. Small values of span mean a narrower PSD. The *mean particle size* is simply the value of d_{50}.

The *drug loading* can be calculated by the following equation [9]:

$$Drug\ loading\ (\%) = \frac{M_d}{M_T} \cdot 100 \tag{2.3}$$

where M_d is the weight of the drug in the drug-polymer system and M_T is the total weight of the same system.

Finally, the *precipitation yield* is defined as the amount of drug in the processed powder compared with the total amount of the processed drug, eventually expressed as a percentage.

Following the classification proposed by Franco et al. [21], *nanoparticles* have a mean diameter smaller than 0.2 μm, *sub-microparticles* have a mean diameter between 0.2 and 0.4 μm, and *microparticles* have a mean diameter bigger than 0.4 μm.

After this general introduction, the next paragraphs are focused on the description of the RESS, SAS and PGSS techniques, used to achieve the micro- and nanonization of the particles, both with and without a coating agent.

2.1.1 RESS (Rapid Expansion of Supercritical Solution)

The *Rapid Expansion of Supercritical Solution* (RESS) is a process that involves the use of a supercritical fluid as a solvent, to overcome the previously mentioned drawbacks of conventional methods. The RESS process is not only used for micro- and nanonization of drug particles but, among other uses, for the preparation of liposomes, solid lipid nanoparticles, and microporous foams. The main purpose of this paragraph is to explore this technique in its use for the micro- and nanonization of

drugs and polymer-coated drugs, focusing on how this technique works and how the main parameters of the system affect the quality of the final product. Just like in any other process involving supercritical fluids, CO_2 is the most used solvent for the RESS process, for the reasons already discussed.

Sharma et al. [22] underline that the administration of nanoparticle carriers, which are unnecessary foreign bodies, into the biological system, could produce long term adverse side effects in the body, and this would explain why the most experiments are focused on micro- and nanonization of the drugs themselves.

In the RESS process, the supercritical fluid plays the role of the solvent and this process can be used only for drugs that have good solubility in it (usually, to have a good efficiency, the solubility of the drug, expressed as mole fraction, must be in the range of 10^{-5}-10^{-4}) [23]. The knowledge of the solubility of the drugs allows the designer to make the right choice on which SCF process provides the best results. For this reason, it is an important challenge to have a very large database of drug solubilities, and, therefore, many authors begin their works by measuring the solubility of the investigated drug in the employed supercritical fluid.

Among the main advantages, the RESS is an environmentally friendly process with mild operating conditions, which allows micro- and nanoparticles to be obtained in a single step without solvent residues. Moreover, it allows high purity products to be obtained, with a narrower particle size distribution whose dimensions can be controlled by tuning the process parameters. Again, mild working conditions permit thermo-sensitive compounds to be processed [24]. The main disadvantages of the RESS process are its limited use with solutes having a relatively high solubility, and an undesirable aggregation of particles, with the consequent difficulty to collect the final product [24]. To overcome these limits, some RESS-modified processes, and other different techniques (like SAS) can cover other ranges of solubility, so expanding the field of application of the SCF technology.

Operating principles

Figure 2.1 shows a typical schematic flowsheet used to perform the RESS process. The process consists of two main units: the extraction (or dissolution) and precipitation units. First, the SCF is pumped in the extraction unit, in which the drug and coating compound (or only the drug) are dissolved. Before entering the extraction unit, the pump and a heat exchanger allow the solvent to reach its supercritical conditions. Then, the supercritical fluid solution flows through a fine device, such a capillary or orifice nozzle, to expand from high pressure to atmospheric one in the precipitation unit (usually a glass vial). This rapid pressure drop leads to supersaturation phenomena to occur (due to the decrease in solubility of solute/s in SCF), so allowing the

precipitation of micro- or nanoparticles [24]. The expansion of the supercritical solution via the nozzle increases the speed of the fluid at values higher than the speed of sound [25]. The solubility reduction, which leads to supersaturation, is very high, since, as already highlighted, a pressure reduction leads the SCF to change its properties from liquid-like to gas-like, and the solubility of a solute in a gas is much lower compared to that in a liquid. Micro- or nanoparticles are collected in the bottom of the precipitation unit, while SCF goes out from the top.

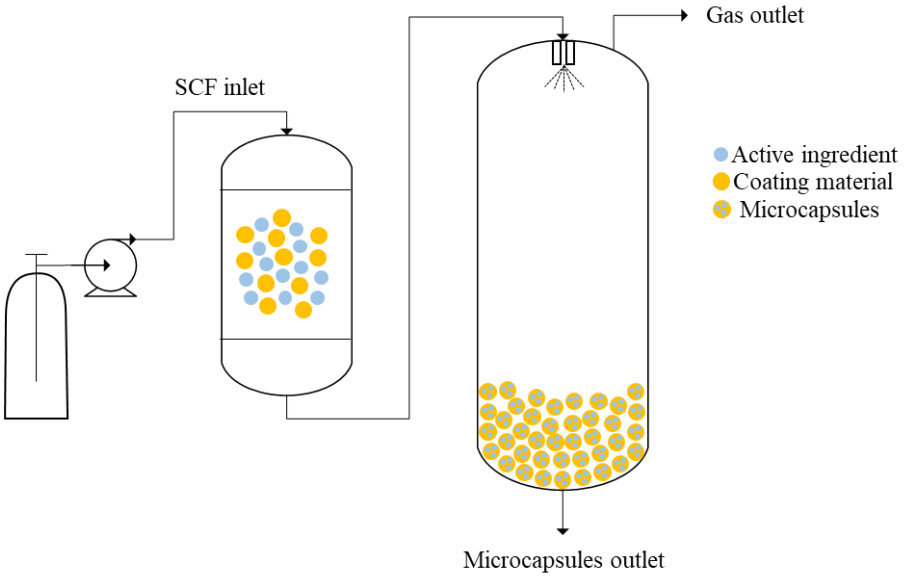

Fig. 2.1 Schematic representation of RESS process.

The driving force of this process is the reduction of the density of the solvent, that, leading to the supersaturation phenomenon, allows the nucleation and crystallization of particles to occur. The higher the density drops, the higher the solvating power drops, and the higher the nucleation rate will be, which leads to smaller particles to be obtained [24]. Typical nozzles have internal diameters in the range of 25-150 μm with short lengths to prevent the precipitation inside the nozzle due to the rapid pressure drop. The length of typical capillary nozzles is in the range of 50-500 μm [24].

As discussed above, in these types of processes there is the possibility to control the characteristics of the final products by tuning some parameter of the process. The relationships between the RESS parameters (including the geometry of the nozzle) and the characteristics of the final products are the next step of the discussion.

Effects of the RESS parameters on the final product

Size, morphology, and particle size distribution (PSD) are very important characteristics of the final product for its potential use in the pharmaceutical industry. The RESS process allows their control by varying the geometric and process parameters involved, like the temperature and pressure of different sections of the process, the nozzle length and diameter as well as the spray distance (which is the distance from the tip of the nozzle to the bottom of the precipitation vessel). The relationship between these parameters and the characteristics of the final product are listed below.

The *extraction temperature* affects the particle size of the final product with two phenomena, which have an opposite effect on supersaturation. On one hand, by increasing the extraction temperature, the solute vapor pressure rises, which results in a solubility and, consequently, a supersaturation increase. Moreover, viscosity and surface tension go down, favoring the atomization. On the other hand, the density of SCF decreases when the extraction temperature increases, which translates into a decrease in solvent strength with the consequent decrease of solubility and supersaturation. To better understand how the change of supersaturation affects the particle size, it is useful to cite the classic *theory of nucleation*. According to the latter, when supersaturation rises, the nucleation rate in the expansion step increases, which leads to the generation of a higher number of particles; the critical size of the nucleus decreases, which results in smaller particles [26]. Depending on which of the two previously-cited phenomena prevails, the relationship between particle size and extraction temperature changes. In particular, the first phenomenon (increase of the solute vapor pressure) leads to a decrease of the particle size (by increasing temperature), while the opposite trend is the result of the second phenomenon (decrease of the solvent strength). This dual behavior makes it very difficult to generalize the results when the extraction temperature is varied. Consequently, the general approach is to evaluate each RESS-processed drug case-by-case. Fattahi et al. [15], for example, performed RESS for simvastatin nanoparticles formation, and, studying the effect of extraction temperature on particle size, found that increasing the temperature from 30 °C to 50 °C, the mean particle size decreased from 74 to 47 nm. This means that, in their experiment, the first phenomenon prevailed. The same trend was found by Ghoreishi et al. [26]. However, the authors claimed that the reduction of the mean particle size with temperature was decelerated at higher temperature values, due to the increased contribution of the second phenomenon. Ciou et al. [27], instead, by processing diuron particles at a fixed extraction pressure of 15 MPa, obtained a mean particle size ranging from 6.1 to 32.4 μm, when temperature was increased from 273 to 313 K, i.e., the second phenomenon prevailed. However, when the pressure was fixed at 20 MPa, the behavior was reversed. It should be noted that, as explained in chapter 1, the dependence of solubility

of a drug in SCF with temperature can be different depending on whether pressure is higher or lower than the crossover point. This implies that when experiments are performed with an extraction pressure higher than the crossover one, by increasing the temperature, the solubility increases too, so resulting in a decrease of the mean particle size.

The effect of *extraction pressure*, when temperature and volume are fixed, has also been investigated. When the pressure increases, the velocity and, therefore, the turbulence and mass flow rate in the nozzle increase too, which leads to a decrease in the residence time inside the nozzle. A low residence time inside the nozzle means that the particles have less time to grow, which results into smaller particle size. Moreover, an increase of pressure leads to an increase of density of the SCF, which means higher solvent power, so leading to higher supersaturation and smaller particles. By applying a RSM (Response Surface Methodology), Ghoreishi et al. [26] found that there is a quadratic positive effect in the relationship between particle size and pressure. Huang et al. [28] suggested that, an extraction pressure increase can decouple the processes of nucleation and growth. When extraction pressure rises, the concentration of the drug is higher, and the growth process is favoured, which results in bigger particles. In the experiments of Chen et al. [23], who processed pyridine-4-amine, when the extraction pressure increased from 15 to 20 MPa, the particle size went down from 0.7 μm to 0.39, while an opposite trend was reported when the pressure rised from 20 to 25 MPa, since the particle size increased from 0.39 to 0.53 μm. This turnaround confirms that at higher pressures the growth phenomenon can prevail. Instead, Fattahi et al. [15] have not found statistically significant differences in mean particle size of simvastatin, by varying the extraction pressure. Probably, the two phenomena previously described had similar intensity, so balancing their opposite effects.

To understand the effect of the *spraying distance*, it should be noted that the coagulation of particles continues when they leave the nozzle. Consequently, a longer residence time implies more time to grow, so resulting in the formation of bigger particles. On the other hand, after a certain distance, the breakage of agglomerated particles occurs, so leading to smaller particles. The first trend here described was observed, for example, by Hezave et al. [29] for creatine monohydrate, since particles ranging from 6.30 to 7.85 μm were obtained, when the spraying distance increased from 1 to 7 cm. Other authors had similar results [16,30]. Fattahi et al. [15], instead, obtained the second trend for nanoparticles of simvastatin with a mean particle size of 54 nm when the spraying distance was equal to 3 cm, which decreased down to 47 nm when the spraying distance was set to 7 cm. Other authors found a similar trend [31].

An increase of the *pre-expansion temperature* leads to a decrease of the mass flow rate, with the consequent increase of the residence time in expansion vessel, which

involves longer time for the particles to grow. On the other hand, always depending on the system pressure (if below or above the crossover point) the density of the SCF is related with temperature with two different behaviors. Ciou et al. [27] obtained smaller particles of diuron at higher temperatures. Türk et al. [32], instead, obtained the opposite effect for paracetamol.

Also the *pre-expansion pressure* affects the system by varying the mass flow rate of the supercritical solution. In fact, there is an increase of the mass flow rate when the pre-expansion pressure increase; this decreases the residence time in the expansion chamber, which translates into shorter time to grow and smaller particles. Türk et al. [32] obtained this trend for paracetamol particles. However, the same authors did not find a consistent change in the particle size by varying the pre-expansion conditions when nabumetone and tolbutamide were investigated.

After the expansion in the collector, the *post-expansion temperature* also affects the kinetics of nucleation and growth. By increasing the post-expansion temperature, the increase of growth rate leads to bigger particles. Ciou et al. [27] confirm this trend, since they obtained an increase in the particle size of diuron from 6.1 to 32.4 μm, when the post-expansion temperature raised from 273 to 313 K. The same trend was observed from other authors [16].

As far as the effect of the *nozzle length* is concerned, Wang et al. [33] claim that by increasing it, the growth time of the particles inside the nozzle is prolonged, leading to bigger sizes. The same trend was obtained by Hezave et al. [29] for creatine monohydrate. Karyak et al. [20], instead, found, for ibuprofen, that by increasing the nozzle length, the particle size decreased. This was explained by the fact that when the nozzle is shorter, the pressure reduction starts earlier in the expansion device, leading to a more gradual decrease of the pressure that results into higher supersaturation and nucleation rates.

Finally, when the *nozzle diameter* decreases, the supersaturation and nucleation rate at the tip of the nozzle increase, which leads to smaller particles. This was obtained by Hezave et al. [29], while other authors did not find a consistent variation of the particle size with the nozzle diameter [23,26].

Solid characterization and dissolution rate

Many drugs can crystallize in more than one crystalline form (polymorphism). Different crystalline forms can change the chemical and physical properties of the final product, including solubility, melting point, and density. When micro- and nanoparticles are prepared, it is important to obtain the most stable crystalline form, i.e. the one with the lowest Gibbs free energy [34]. Many experiments [20,25,32] pointed out that RESS-processed micro- and nanoparticles have (in addition to smaller mean

particle size) (i) narrower particle size distribution (PSD), (ii) the possibility to obtain the desired polymorphic form by changing the pre-expansion conditions, (iii) enhanced *in vitro* and *in vivo* dissolution rates (iv) a decrease of the degree of crystallinity, which leads to a thermodynamic instability that improves the aqueous solubility of drugs. As far as this last point is concerned, Chen et al. [35] explain that varying the physical form of the particles from crystalline to amorphous state usually results in an enhancement of the free energy and entropy. This internal thermodynamic instability improves the dissolution rate of the drug powder [36]. Fattahi et al. [15] enhanced up to four times the dissolution rate of simvastatin, by reducing the mean particle size from 26 μm to 47 nm, without chemical degradation and with a reduction of the melting point from 142 to 126 °C, which was explained by a decrease of degree of crystallinity. Ghoreishi et al. [26] started from chitosan particles with irregular shape and wide particle size distribution and, after applying the RESS process, obtained more uniform and smaller particles with narrower PSD. In the experiments of Ciou et al. [27] with diuron particles, an irregular crystal habit became a rod-like one without any chemical degradation. Türk et al. [32], by processing tolbutamide and other drugs, proved that by changing the operating conditions, the favourable tolbutamide polymorphic form II could be obtained.

RESS-derived processes

In order to overcome the disadvantages of the RESS process, some alternatives have been proposed, such as the Rapid Expansion of Supercritical Solutions into a Liquid Solvent (RESOLV) and the Rapid Expansion of Supercritical Solutions into an Aqueous Solution (RESSAS). In both cases, the only modification compared to the original process, is that the expansion of the supercritical solution is performed into a liquid solvent (RESOLV) or in an aqueous solution (RESSAS), instead of air. The receiving liquid is usually a solution with surfactants or stabilization agents, with the aim to avoid the collision of the particles, so preventing the coalescence phenomenon and resulting in particles smaller than those obtained with the original RESS [24]. As expected, the introduction of a solution with surfactant/stabilizer implies the knowledge of other parameters, which complicates the system.

In addition to the reduced size of the particles and a narrower PSD, the Rapid Expansion of Supercritical Solutions with a Solid Co-solvent method (RESS-SC) provides another advantage. The addition of a solid co-solvent, in fact, may enhance the solubility of the drug in the SCF. Moreover, the precipitation of the co-solvent around the API may help avoid the aggregation of the drug particles [24].

Another RESS-derived process is the Rapid Expansion of Supercritical Solution with a Non-Solvent method (RESS-N). Actually, this method is not used for micro-

and nanonization of particles, but it is used to coat, usually with a polymer, some already produced particles that are not soluble in $scCO_2$. A liquid co-solvent is present in the $scCO_2$-based fluid, which also contains the coating polymer, to increase the solubility of the polymer in $scCO_2$. The $scCO_2$-based fluid is mixed with the API particles and expanded in the precipitation unit at room pressure. It is important that the co-solvent, at ambient conditions, is a non-solvent for both the API and the polymer, to assure the formation of the coated particles. At the end of the process, in fact, the non-solvent is removed with the SCF, and the polymer precipitates around the drug, so resulting in the encapsulation [24].

2.1.2 SAS (Supercritical Antisolvent)

The S*upercritical Antisolvent* (SAS) technique is a process in which the supercritical fluid is used as an antisolvent. As for the previous technique, this part of the work provides a general description of the technique and, although it is used to obtain many kinds of products, here its application on the production of micro- and nanoparticles of drugs and polymer-coated drugs is highlighted. Unlike the RESS process, the SAS process is often used to obtain coated drug systems [24]. This generally increases the complexity of the process, especially due to the fact that when a co-crystallization step is performed, the solubilities of the drug and coating compound both in the organic solvent and in the SCF antisolvent are different, which leads to different supersaturation levels and the consequent separate precipitation of the solutes (i.e. in two different moments of time). This results in irregular and coalescing particles, with broad particle size distribution. It has been shown, for example, that the use of PVP (Polyvinylpyrrolidone) as a coating material can overcome this problem, by retarding the crystal growth [37].

By using the SCF as an antisolvent, the operative range of the SCF technology is expanded to the solutes that are not soluble in the supercritical fluid.

Despite the SAS technique requires the use of an organic solvent, the final product could be (almost) solvent-free. As an example, Cuadra et al. [38] obtained co-crystals of diflunisal into nicotinamide with a purity higher than 99 %. Moreover, just like many other processes involving the use of CO_2, working at mild temperature conditions, makes it possible to handle heat sensitive biomolecules like enzymes and proteins [39]. Besides, the final product is easily recovered, because SCF is removed as a gas at ambient conditions, leaving the precipitate in the collector [10]. Besides the already cited risk of obtaining co-crystals, the SAS process introduces a third component, which makes the management of the whole process more complicated. Moreover, due to the necessity of a good miscibility between the solvent and supercritical CO_2, the SAS process is limited to hydrophobic compounds [40,41].

Operating principles

As shown in the figure 2.2, the SAS process is similar to the RESS one, with the difference that here the SCF is not used as a solvent, but as an antisolvent. First, the solute is dissolved in an organic solvent and then, the solution flows through a nozzle and expands in the precipitation chamber that is filled with a fluid (generally CO_2) in its supercritical state, which acts as an antisolvent. When the organic solution mixes with the antisolvent (SCF) the solubility of the drug in the new mixture decreases rapidly (the new mixture has less solvent power), so resulting in supersaturation, which drives nucleation, so leading to the precipitation of the drug particles. At the end of the precipitation, an excess of CO_2 is sent to wash the expansion chamber, so removing the solvent residual content. Finally, the precipitator is gradually depressurized, so collecting the precipitated powder [14,37]. The configuration for coated-drug systems is the same, where also the coating compound is dissolved in the organic solution before the expansion step.

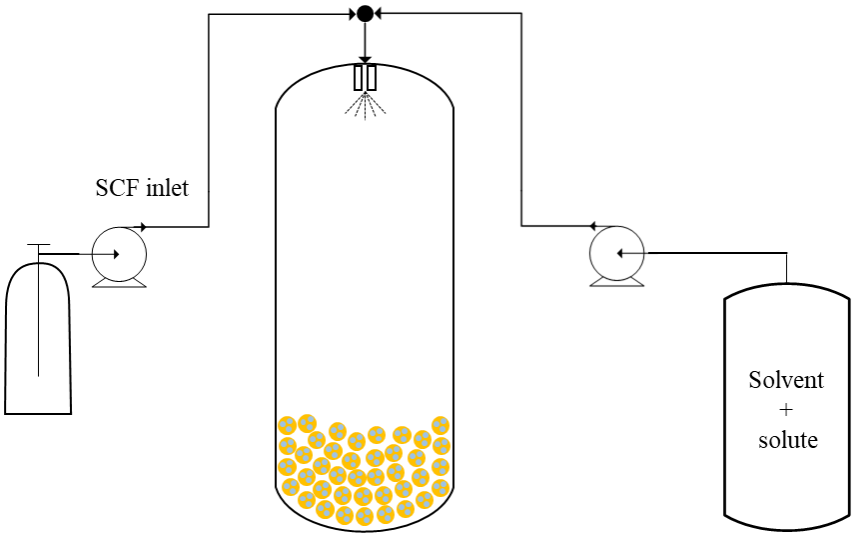

Fig. 2.2 Schematic representation of SAS process.

To appropriately perform the SAS process, the solutes must have good solubility in the organic solvent and a much lower one in the SCF antisolvent, to allow the driving force for supersaturation to be achieved. Moreover, solvent and antisolvent must be highly miscible with each other, at the process conditions.

As already mentioned for the RESS process, the most important characteristics of the final product are size, morphology and particle size distribution. Also, for the SAS

process these can be controlled by manipulating the parameters involved in the process.

Effects of the SAS parameters on the final product

As well as for the RESS process, the SAS process parameters can affect the quality of the final product.

As far as the effect of the *coating-material/drug mass ratio* is concerned, Liu et al. [42], by varying this ratio from 1:5 to 1:25, obtained particles of 10-hydroxycamptothecin (HCPT) incorporated into zein with mean size ranging from 3.7 to 0.84 μm. Moreover, the lower values of mass ratio resulted in uniform microspheres, while irregular microspheres were produced with the higher mass ratio values. Besides, some drug particles on the zein surface were observed when a low mass ratio was employed due to the increase of the amount of drug used. Therefore, decreasing the coating-material/drug mass ratio allowed smaller particles to be obtained and a relationship between mass ratio and morphology of particles was also observed. Prosapio et al. [37] studied the effect of the coating-material/drug mass ratio for the systems dexamethasone/PVP, prednisolone/PVP and budesonide/PVP. With the first two systems, by operating with a mass ratio of 1:2, crystals and coalescing sub-microparticles were obtained (unsuccessful coprecipitation). In the budesonide/PVP case, with the same ratio of 1:2, the experiments led to successful precipitation. Since these results differ with each other, the budesonide/PVP system was also investigated at a ratio of 1:1 with the aim to see if a transition from microparticles to separated crystals could occur. The hypothesis was confirmed, and coalescing particles were obtained. As a further confirmation a similar transition was observed by studying the first two systems with mass ratio ranging from 1:3 to 1:20: well-separated spherical microparticles were obtained. As far as the mean particle size is concerned, Prosapio et al. found an opposite trend with respect to Liu et al. [42] (i.e., the mean diameter rose when the coating material/drug mass ratio was decreased).

In general, particle size increases slightly when the *drug loading* rises up to a limit point. After, the particle size starts to increase dramatically. When the incorporation of HCPT drug into zein was investigated, Liu et al. [42] found this trend. The phenomenon was explained according to Chen et al. [43] for whom higher values of drug loading meant that less molecules of the coating compound would surround the drug so favouring its flocculation and agglomeration and causing the dramatic increase of particle size.

As far as the effect of the *overall concentration* (which refers to the concentration of drug and coating compound in the organic solvent) is concerned, Prosapio et al. [37], by changing the overall concentration of prednisolone/PVP (with a mass ratio of

1:10) in ethanol from 10 to 30 mg/ml, obtained microparticles from 2 to 3.5 μm, with a wider particle size distribution. The same authors, in another study [44], did not find a significant change in the mean diameter by varying the overall concentration of the system nimesulide/PVP in dimethylsulfoxide (DMSO). Miao et al. [45] obtained the same trend as Prosapio et al. [37] operating at concentrations higher than 15 mg/ml of naringenin in a acetone/dichloromethane solution, which was explained by supposing that, due to the high amount of drug, this would crystallize earlier during the droplet expansion, so leading to multi-core formation. The collision of these nuclei would cause the aggregation of particles. Moreover, Paola Franco et al. [46] observed that a high concentration of solutes may also has a not negligible influence on the vapor-liquid equilibria (VLE). By varying the overall concentration from 10 to 50 mg/ml, they obtained well separated microparticles with a mean diameter ranging from 3.09 to 3.81 μm, while, when the overall concentration was set to 100 mg/ml, irregular crystals were obtained. The experiments were performed at 90 bar, just above the mixture critical point (MCP), which is equal to about 86 bar. The authors supposed that at higher overall concentration the solutes could influence the equilibrium, leading to a shift of the MCP at higher values, so that an operating pressure of 90 bar would not fall in the biphasic region.

When the *operating pressure* rises, the diffusion coefficient of the drug in the organic solution increases, resulting in a higher degree of supersaturation and smaller particles. This trend was observed by several authors [44,46]. On the other hand, when the pressure rises (with constant temperature), density and viscosity of $scCO_2$ increase and the diffusivity of the drug in the supercritical fluid decreases. In general, when the diffusion coefficient diminishes and viscosity increases, the mass transfer between the droplets and carbon dioxide is enhanced, so resulting in larger particles [45]. Miao et al. [45] prepared naringenin microparticles and observed the first phenomenon, obtaining a mean particle size ranging from 717 to 660 nm when the pressure was changed from 8 to 12 MPa; however, the second phenomenon was also observed and particles with a mean size of 947 nm were obtained with a further increase of pressure up to 20 MPa.

When the *operating temperature* rises, the density of $scCO_2$ decreases. Moreover, the solubility of the drug in the organic solvent can decrease, which leads to lower supersaturation and, consequently, larger particles. This trend was obtained, for example, by some authors [44,45]. In analogy to other physical properties, the morphology is also influenced by the temperature. As observed by Cuadra et al. [47], a variation from the plate-like to the needle-like agglomerate can be obtained in the co-crystallization of carbamazepine in saccharin with an increase of temperature.

As far as the effect of the *flow rates of CO_2 and organic solution* are concerned, by changing the two flow rates, the CO_2 mole fraction in the precipitator can be tuned.

When the mole fraction of CO_2 is high, this results in a more significant antisolvent effect that favours the production of smaller particles. Ciou et al. [48] obtained this trend, i.e., smaller particles at higher CO_2 flow rate and lower organic solution flow rate.

Among the other influences, also the *choice of the organic solvent* affects the quality of the result. For example, Ciou et al. [48] performed the crystallization of warfarin with five different solvents, acetone, methanol, methyl ethyl ketone (MEK), dichloromethane (DCM), and acetonitrile (ACN), obtaining different results in terms of yield recovery, crystal habit and mean particle size.

Mechanisms affecting the production of drug micro- or nanoparticles

It has been demonstrated that obtaining microparticles rather than nanoparticles depends on the competition between two different characteristic times of the process, the time of jet break-up (t_{JB}) and the time of surface tension vanishing (t_{STV}) [21]. When the *time of jet break-up* is the process controlling time, the formation of liquid droplets is the result of the break of the liquid jet with the subsequent drying with $scCO_2$, which results in the production of *microparticles*. This is optimal when co-precipitation of different compounds is desired since each droplet works as a confined drying system [40]. When, instead, the *time of surface tension vanishing* is the process controlling time, *nanoparticles* are obtained. In this case the liquid jet disappears before the break-up and the particles are obtained by a gas-to-particle formation mechanism (nucleation and growth); in this case the coprecipitation does not occur, because the two compounds have different nucleation and growth times, which leads to the formation of a sort of physical mixture [40]. As mentioned in paragraph 2.1.2, by choosing the right carrier (for example PVP), the conditions to produce coprecipitates can be improved, since the carrier may inhibit the crystal growth. The controlling mechanism between the two above cited characteristic times depends on the distance of the operative point from the MCP of the binary solvent/antisolvent mixture. When the operative point is near the MCP, $t_{JB} > t_{STV}$ and microparticles are formed. Instead, when the SAS operative point is far above the MCP (fully developed supercritical conditions), $t_{STV} < t_{JB}$ and nanoparticles are obtained [40].

Other processes involving SCF as an antisolvent

The first antisolvent process using $scCO_2$ was proposed in the literature in 1989 [49] and was denoted as *Gas Antisolvent Crystallization* (GAS). With respect to the SAS, which is now preferred, in the GAS process the expansion is made without the presence of a capillary device.

Another $scCO_2$ antisolvent process in which the atomization is not performed is the *Supercritical Fluid Extraction of Emulsions* (SFEE). The SFEE differs from the GAS process because the $scCO_2$ is used to extract the organic solvent contained in the droplets of previously formed organic solvent-in water emulsions (usually an oil-in-water or water-in-oil emulsions). In this process, the drug is dissolved in the dispersed phase (the organic solvent contained in the emulsions) and after contacting the $scCO_2$, which is able to dissolve the organic solvent, is precipitated in water to form a micro- or nanosuspension. By using an oil-in-water emulsion or a water-in-oil emulsion, both hydrophilic and lipophilic compounds can be encapsulated by using the SFEE process, respectively [50].

On the other hand, considering the processes in which atomization is performed, *Supercritical Antisolvent with Enhanced Mass Transfer* (SAS-EM) is a SAS-derived process in which the spray jet is equipped with a horn vibrating at ultrasonic frequencies able to deflect the solvent droplets in order to break them and obtain potentially smaller particles [24].

Another variation of the SAS process is the *Solution Enhanced Dispersion by Supercritical Fluids* (SEDS) technique. In this process, the use of a coaxial nozzle with a mixing length at the end of it allows the drug solution and the $scCO_2$ to be premixed, so enhancing the mass transfer of the process [51].

Other less used processes that employ the $scCO_2$ as an antisolvent are, for example, the *Supercritical Antisolvent with Drug-Excipient Mixing* (SAS-DEM) and the *Aerosol Solvent Extraction System* (ASES). A brief description can be found elsewhere [24].

2.1.3 PGSS (Particles from Gas-Saturated Solutions)

The *Particles from Gas-Saturated Solutions* (PGSS) is a SCF technology in which the supercritical fluid is used as a *solute*. The role of $scCO_2$ as a solute is to act as a high mobility additive to reduce the melting temperature of the involved polymer (see next section, operating principles) [24,52]. As highlighted above, the RESS process is inefficient for solutes with too low solubility in CO_2, which results in the use of a huge amount of supercritical fluid to surmount this obstacle. The SAS process overcomes this trouble but introduces the use of an organic solvent, which complicates its market launch and makes it less environmentally friendly. These two drawbacks are overcome by the PGSS process. In fact, with respect to the RESS and SAS processes, the PGSS needs a much lower amount of $scCO_2$ and is completely solvent-free [24]. In addition, other advantages are that the involved substances must not be necessarily soluble in $scCO_2$, and very high yields (frequently up to 100% [53]) are often obtained. However, many disadvantages can also be cited: (i) particle size and morphology are

difficult to control, (ii) spray nozzles can easily clog, (iii) high processing temperatures are required, and (iv) the production of nano- and sub-microparticles is very difficult [24].

Operating principles

Figure 2.3 shows a classic PGSS scheme. The first step of the PGSS process is to load a high-pressure autoclave with the pre-grounded compounds of interest, for example the drug, carrier, and excipients. The autoclave is heated up to the operative temperature and saturated with $scCO_2$. As a consequence, the solid compounds melt, and a CO_2-saturated liquid mixture is formed. Finally, the mixture is forced to pass through a micro-nozzle in a collection chamber where a pressure reduction leads to a sudden cooling of the mixture (due to the Joule-Thomson effect) with the consequent atomization of the substances in microparticles, while the depressurized CO_2 is discharged from the top in gaseous phase [24,54,55].

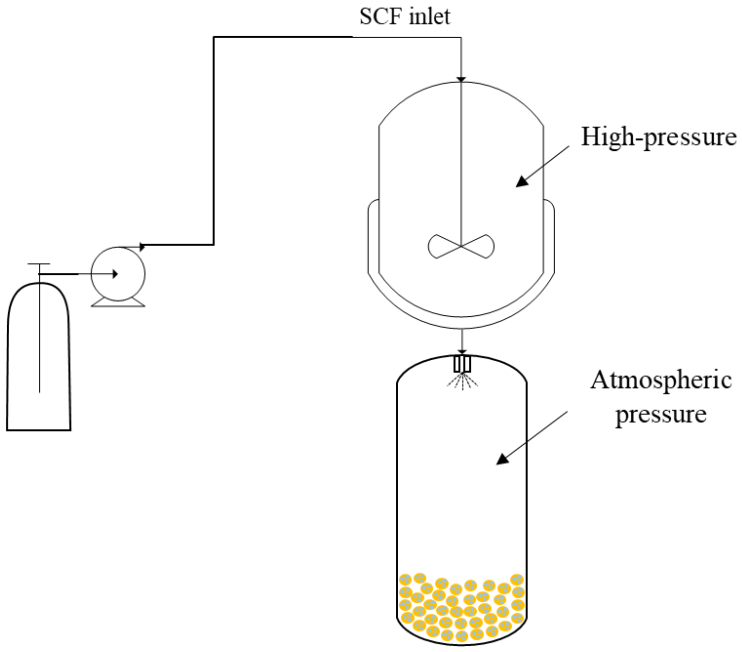

Fig. 2.3 Schematic representation of PGSS process.

To achieve the melting of the polymer, the system should be heated (at the pre-expansion temperature) above its melting point (T_m) or its glass transition temperature (T_g), which could lead to the thermal degradation of the polymer and/or the drug [54].

However, in this process, the low viscosity and high diffusivity of $scCO_2$ allow its penetration in the polymeric matrix to act as a plasticizer, so resulting in a reduction of the polymer T_g, which allow the atomization process to be conducted at lower temperatures [55]. Moreover, the viscosity and interfacial tension of the melted mixture is decreased, allowing it to be better sprayed [54]. The reduction of the glass transition temperature is the main feature of PGSS, the reason why $scCO_2$ is used as a solute.

The appropriate use of this process requires that the CO_2 is absorbed in the polymeric carrier. In order to have a good CO_2 sorption in the polymer, it is important that this has CO_2-philic groups, i.e. electron-donating groups like ether, fluorine, ketone, or carbonyl [52].

Often, a PGSS-modified process is used, where a high-pressure liquid pump for the gas-saturated solution allows the control of the solution flow rate [56].

Effects of PGSS parameters on final product

The role of the *operating/pre-expansion temperature* on the PGSS process was clearly explained by Labuschagne et al. [52], which processed shellac and obtained particles with the smallest average diameter when the lowest temperature was set. They attributed this trend to the lower solubility of CO_2 in shellac at higher temperatures, which led to poorer atomization efficiency. Moreover, the authors obtained significantly lower PSD at higher temperatures, finding a much higher $d_{0.1}$ and attributing this to the phenomenon of agglomeration that may occur when the temperature increases. Chen et al. [56] observed the same trend of the mean particle size by encapsulating ibuprofen into PEG6000, and they attributed the trend to the fact that at higher temperature values more time is necessary to solidify the droplets generated after passing through the nozzle, so resulting in larger particles. On the other hand, the viscosity of the melting mixture diminishes at higher temperatures, and this can lead to the formation of finer particles during the expansion through the nozzle. As far as the influence of temperature on the encapsulation efficiency (EE) is concerned, Vo et al. [55] found that this increased after heating from 45 to 55 °C, while a decrease was observed when the temperature was further rised up to 65 °C. This was explained by the occurrence of two contrasting phenomena. On one side, the melting of the polymer could be improved by a temperature increase, which resulted in a greater sorption of CO_2, with the consequent increase of the EE. On the other hand, at higher temperature values the degradation rate of the drug could increase during the equilibration time and reduce the overall drug content as well as the EE.

Hu et al. [57] explained that when the *operating/pre-expansion pressure* increases, finer particles are obtained. In fact, by increasing the pressure drops between the pre-expansion chamber and the nozzle, the Joule-Thomson cooling effect is accentuated,

which results in higher speed of solidification and smaller particles. Other authors observed the same trend [56]. Regarding the influence of pressure on the encapsulation efficiency, Vo et al. [55], who encapsulated fucoxanthin-rich oil in PEG by PGSS, found that this gradually increased when pressure was raised from 100 to 250 bar and slightly decreased when pressure varied from 250 to 300 bar. They declared that the influence of pressure on the encapsulation efficiency is very complex, because it depends on the core material and PEG molar mass. Varona et al. [58], instead, experimentally observed a decrease of the EE with a rise of pressure. In their opinion, the increase in solubility of the compound of interest in CO_2 with pressure led the drug to be completely miscible with the CO_2 at pressures above the mixture critical point.

Labuschagne et al. [52] showed how the CO_2 sorption and polymer swelling can be affected by pressure and temperature (shellac was the polymer used in this research). In an isothermal experiment, by increasing the pressure, an initial increase of CO_2 sorption was found, due to the increase of CO_2 solubility, and this was followed by a region in which no significant sorption of CO_2 was observed with a further pressure increase. Besides, in an isobaric experiment, at higher temperatures, since the CO_2 solubility is lower (because of its lower density), a low degree of absorption was observed. When CO_2 was absorbed in the polymer matrix, a swelling phenomenon occurred. Initially the polymer swelling increased when pressure rised, until it reached a plateau. At a certain pressure, the swelling phenomenon was found to be greater at the lowest temperature, while fixing the pressure at a higher value, the effect of temperature on swelling became less clear. It was explained that this is due to two conflicting effects. First, a greater temperature allowed the improvement of polymer chains mobility to be achieved, which resulted in more CO_2 molecules partitioned in shellac matrix. On the other hand, a greater temperature reduced the CO_2 density, with the already seen consequence. The meeting point between these two phenomena determined the swelling intensity of the polymer.

Even the *drug concentration* in drug/polymer system affects the mean particle size. As an example, Hu et al. [57] found that up to a drug concentration (CoQ_{10} in CoQ_{10}/PEG6000) of 40 % almost nothing changes. Then, suddenly, the mean particle size rose, passing from 220 nm to 2.36 μm when the mass fraction of the drug was increased from 40 to 50 %. They attributed this to the difficult dispersion of the CoQ_{10} in the mixture at lower PEG6000 mass fraction.

As already mentioned, with the configuration of the PGSS-modified process, the *flow rate of the gas-saturated solution* can be controlled. By exploiting this feature, it can be observed [57] that, by increasing the flow rate, the mean particle size rises too. Lower flowrates, in fact, guarantee higher gas-liquid ratio so resulting in stronger atomization and decreasing the mean diameter.

Other processes involving SCF as a solute

In addition to the PGSS technique, many other processes use the supercritical fluid as a solute. For example, the *Gas-Assisted Melting Atomization* (GAMA) method was proposed [59] to enhance the atomization features of the PGSS process. The GAMA process will be explained in the paragraph 2.5.1 since it was born to enhance the production of the solid lipid nanoparticles.

Another modification of the PGSS process was proposed by Sievers [60], to allow the PGSS method to be used with any water-soluble compound. They named it as the *Carbon Dioxide Assisted Nebulization with a Bubble Dryer* (CAN-BD) method. In this technique, a gas-liquid solution is formed by mixing a drug aqueous solution, the excipient/s and the $scCO_2$. Then, this solution is atomized by a flow restrictor, resulting in an aerosol of microbubbles and micro-droplets, which is then dried by, for example, a hot stream of air or nitrogen.

To enhance the atomization efficiency of the PGSS process, and (to) explore lower ranges of mean particle sizes, the *Supercritical-Enhanced Atomization* (SEA) can also be used. This can be obtained by using a coaxial nozzle into a micrometric tee, where an aqueous solution containing the drug passes through the inner tube while the SCF flows through the outer one; the sudden depressurization of the two flows causes an intense shear stress, which enhances the break-up of the liquid, so obtaining sub micro-sized droplets [61].

The *Supercritical-Assisted Atomization* (SAA) method was proposed by Reverchon et al. [62], who, by trying to perform their experiments with an apparatus similar to the one of Sievers [60], failed to work at reproducible conditions. Therefore, they introduced a thermostated packed tower to obtain the equilibrium solubilization of CO_2 in the liquid (saturation), so exploiting both the advantages of the solution proposed by Sievers [60] (the atomization enhancement by the mixing) and the release of the SCF from the saturated liquid droplets (which the authors labeled as "effervescent atomization"), to enhance the atomization. The so-called "effervescent atomization" can be potentiated by the *Supercritical-Assisted Atomization with a Hydrodynamic Cavitation Mixer* (SAA-HCM) process, proposed by Cai et al. [63], who introduced an hydrodynamic cavitation mixer to the SAA process to enhance the mass transfer between the liquid solution and the SCF.

All the above-mentioned processes must deal with a drawback that is usually present when nanoparticles are involved, which is the recovery of the particles, which is particularly critical especially when they are dispersed in a high velocity gas [24]. The cyclones do not assure good efficiencies for this purpose, and the commonly used filters are not easily scalable [24]. So, other techniques were proposed to avoid the involvement of a gas phase to capture the particles. The *Depressurization of an*

Expanded-Liquid Organic Solution (DELOS) method is an example [24]. First, the $scCO_2$ is dissolved in an organic solvent (which already contains the solute) at "quasi-saturation" conditions of both the solute and the $scCO_2$. Then, the system is depressurized to atmospheric conditions and the consequent Joule-Thomson effect allows a high temperature drop to occur, so resulting in the consequent supersaturation and precipitation of the solute into very small particles.

In 2012, Campardelli et al. [64] proposed a novel technique where the SCF is used as a solute, the *Supercritical Assisted Injection in a Liquid Antisolvent* (SAILA). In this process, an expanded liquid, composed by an organic solvent, a solid solute and the SCF, is continuously injected in an appropriate liquid antisolvent. SAILA is very similar to SAS, but, here, the antisolvent is not the SCF, which, instead, act as a co-solute to decrease the solution viscosity and surface tension, which favours the atomization process in the liquid antisolvent. To proper perform this process, the solid solute must be soluble in the solvent and insoluble in the antisolvent. Moreover, the solvent and antisolvent must be completely miscible with each other. Although the introduction of an organic solvent is undesired, if water is used as an antisolvent, non-toxic solvents like acetone and ethanol may generally be used, since they are water miscible [65].

2.2 Aerogels

An *aerogel* is an ultra-light and porous solid that can be obtained from a gel by replacing its liquid component with air. The high porosity and surface area and the low density make the aerogels ideal for their use as drug delivery systems, since, these properties, allow them to achieve high levels of drug loading, improve the physical stability of the impregnated APIs and influence their *in vivo* release kinetics [14,66].

Generally, the aerogels are prepared via a procedure that involves three main steps, (i) sol formation, (ii) gelation (also called gel formation) and (iii) drying [66]. In the *sol formation*, some precursors (generally silicon alkoxides [67]) are placed in an appropriate solvent/water solution (where, for example, the solvent is an alcohol or acetone [67]) and react to form the "sol", which is a colloidal solution where the suspension is made of very small solid particles [66]. In the second step, the *gelation*, the small particles crosslink or further polymerize to form a gel that has a very high porosity and where the pores are filled with the solution, whose volume counts for the 95 % of the total volume of the gel. Sometimes, the gel structure can be fortified by the use of an aging process, which consists in the introduction of the gel in an aging solution (water/alcohol solution, in which the alcohol is generally ethanol), which makes the gel structure more compact, so preventing the shrinkage and damage. Moreover, the formed hydrogel is involved in a solvent exchange, in which the aqueous

solution is replaced by a pure organic solvent (generally acetone or ethanol). This allows the hydrogel's pores to be cleaned from the impurities and to become an *alcogel*, which can be more easily subjected to the last step, the drying process. Due to the relevant role that the pores and matrix structure of the final product have in its technological application, the *drying step* is the most critical one, since it should guarantee the removal of the pore-filling liquid while preserving the integrity of the matrix structure. Moreover, this is the step in which the supercritical fluid may be involved to overcome the drawbacks connected with the use of more conventional drying techniques [66].

The most used conventional drying technologies are (i) ambient pressure drying and (ii) freeze drying [66].

The first technique allows the wet gel to be dried by the evaporation of the pore-filling liquid at temperatures ranging from the room one to 200 °C, and ambient pressure. The main limitation of this technique is that it makes the gel crack or shrink. In fact, as the liquid evaporates from the gel's pores, a reduction of the radius of curvature of the vapor-liquid interface occurs, so allowing the surface tension to exert a pressure on the gel surface, which is inversely proportional to the diameter of the gel's pores [66]. Due to the nanoscale of the gel's pores, the capillary pressure may reach very high values, up to one thousand bars, which is so high that the fragile porous structure of the gels collapses. In order to overcome this drawback, a solvent with low interfacial surface tension could be used, but, due to the nano-size of the pores, this approach does not guarantee to prevent the damage of the gel structure. One of the technological solutions that can be adopted is to begin the process with a reinforced matrix, so performing the *ambient pressure drying with matrix strengthening* [66].

The *freeze-drying* technique, instead, allows the gel structure to be dried without the formation of a liquid-vapor interface, so preventing the above cited problems. First, the solvent inside the gel's pores is frozen, and then, it is sublimated by reducing the pressure. Although this process solves some of the previously cited drawbacks (like the shrinkage), it introduces other limitations. For example, the freezing of the solvent might damage the gel structure, due to the mechanical stresses caused by the crystal growth inside the pores [66].

The use of the *supercritical drying* technique avoids the drawbacks of the above-cited conventional ones. The figure 2.4 shows the three main routes that can be used to dry the alcogel by using the SCF technology [14].

The first route is the most used one and consists in the extraction of the pore-filling liquid (i.e. the alcohol) by the $scCO_2$. This is followed by a depressurization step, which frees the gel's pores from the SCF, so resulting in the dried aerogel (where the air fills the pores of the gel).

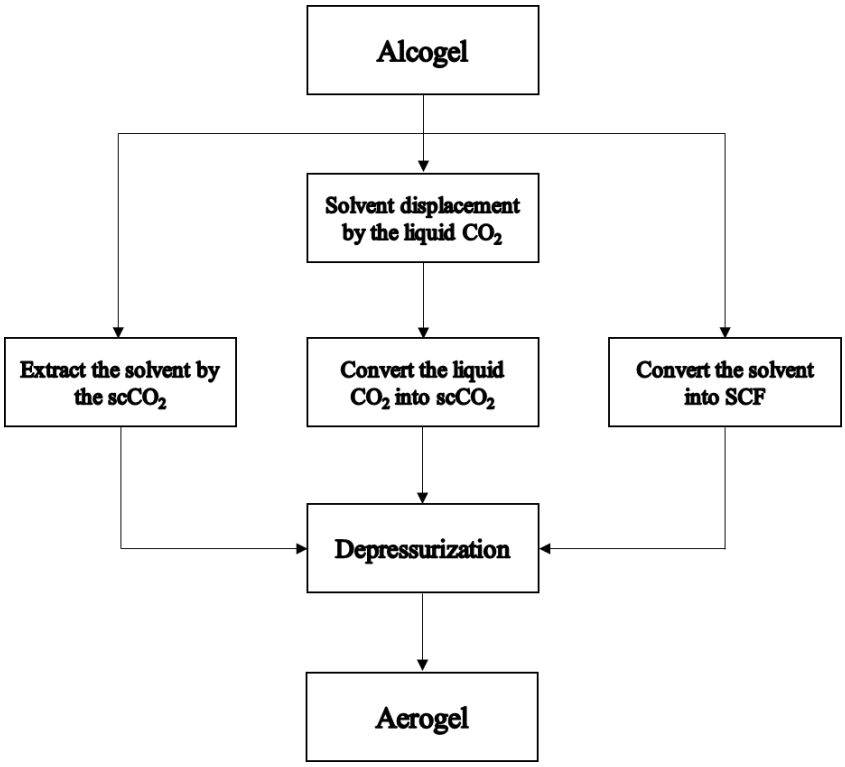

Fig. 2.4 The main routes to perform the supercritical drying of an alcogel. The figure was reproduced with modifications from [14].

The second route, instead, involves the displacement of the pore-filling liquid by liquid CO_2, which is followed by the pressurization and heating of the system, to allow the supercritical state of the liquid CO_2 to be reached. The final depressurization step, again, is able to free the pores from the $scCO_2$ to obtain the aerogel.

The last route reported in fig. 2.4 does not involve the use of an external supercritical fluid. The thermodynamic conditions of the system are directly varied until the supercritical conditions of the pore-filling liquid are reached, which is followed by the usual depressurization step. Since, in this route, the SCF is not the carbon dioxide, the advantage of the mild thermal conditions generally provided by the SCF technology is not exploited. For example, if the pore-filling liquid is ethanol, the system must be heated up a temperature higher than 243 °C, which could degrade the produced aerogel [14].

The use of a supercritical drying process does not involve the formation of a vapor-liquid interface and its consequent capillary pressure, so preserving the integrity of the matrix and high porosity of the gel [14]. Moreover, as it can be expected from any

SCF technology, the aerogel structure features (such as the density, porosity, pore size and surface area) can be modified in a controlled manner. Besides, the supercritical drying allows aerogels with higher pore volumes, porosities, and surface areas to be obtained [68].

2.2.1 Supercritical drying model and kinetics

Generally, in the laboratory scale experiments, the researchers tend to overestimate the drying time to make it sure that the fully removal of the liquid occurs [69]. This approach can be considered a good practice in the laboratory scale, where the time and energy consumptions have not a huge impact. However, in the industrial production, they play a more important role, and their minimization, without affecting the quality of the final product, should be achieved. Moreover, García-Gonzàlez et al. [69] showed that for silica aerogels (the most used inorganic aerogels), no improvements in their textural properties are observed after 2-3 h of drying, and, besides, a worsening of the textural properties of starch aerogels occurs after a certain period of time. Thus, the study of the supercritical drying kinetics is a key aspect for the production process of the aerogels. Mukhopadhyay et al. [70] modelled the supercritical drying technique of silica aerogels with parallel cylindrical mesopores through the first route shown in fig. 2.4, and found out that a two-way mass transfer of the $scCO_2$ and ethanol (the pore-filling liquid) to and from the cylindrical pores occurred. The mechanism of the supercritical drying process proposed by the authors in their single pore model, includes three steps. In the first one a flow of the $scCO_2$ surrounds the wet gel at the required temperature and above the MCP of the CO_2-ethanol mixture. The $scCO_2$, then, spreads in the pores filled with ethanol and the ethanol vapours are dragged by the convective flow of the CO_2 before a spillage phenomenon occurs, due to the accumulation of the liquid mixture in the solid, which is caused by the high dissolution of the CO_2 in the ethanol and the relatively low convective transport of the ethanol itself. Eventually, as time goes by, the liquid mixture inside the pores is increasingly enriched with the CO_2, until the conditions at which the mixture critical mole fraction (MCMF) at the MCP is reached and surpassed. The extraction of the ethanol continues and the amount of the ethanol at the end of the process is so small that its condensation in the pores does not occur when the expansion to atmospheric pressure is performed.

García-Gonzàlez et al. [69], by studying the *kinetics* of the supercritical drying process in the formation of silica aerogels, showed a time-dependent profile of the pore-filling liquid removal. After only ten minutes, more than 60 % of the liquid in the gel was removed. After, the drying rate slowed down, especially for the removal of the last 5 % of ethanol. This trend suggested that different mass transfer resistances

may affect the various steps of the supercritical drying process. Based on the above described model, in the first part of the process, only convective mass transfer resistances are expected. When the amount of the ethanol decreases, instead, the mass transport resistances in the solid (diffusion) would start to be more and more important. The results of the experiments of García-Gonzàlez et al. totally agreed with the proposed model. In fact, by fitting a mathematical solution of the Fick's second law to describe the amount of ethanol outgoing from an isotropic cylindrical material in the first step of the process, the authors found a very bad prediction of the experimental results. On the other hand, the same equation predicted very well the experiment related to the last part of the solvent removal step (< 5-8 %). These results confirm that the first part of the supercritical drying process is controlled by convective mass transfer, while the last part is controlled by diffusion mass transfer. In the range between the two above cited regimes of solvent removal (from 60 to 92 % of the ethanol removal), both the diffusion and convection mass transfer controlled the drying kinetics. To further confirm the mass transfer mechanisms, the geometry of the aerogels from a cylindrical shape to a spherical one was changed, and the drying profile was observed. The results, again, totally agreed with the model. With respect to the cylindrical aerogels, the only difference consisted in an acceleration of the drying rate after the 60 % of the solvent removal for the microspheres. The authors suggested that, since the Fick's second law implies that the drying rate must be influenced by the minimum diffusion path length, the higher drying rate in microspheres further confirms the predominance of the diffusion mass transfer in the last part of the drying process.

2.2.2 The use of the aerogels as drug delivery systems

Once the aerogel is produced, it can be loaded with an API, to exploit its structural properties for its use as a drug delivery system. The active compound can be loaded in many different ways, based on the stage in which the loading is performed. It can be, for example, loaded before the formation of the "sol" [71], concurrently to the gelation step [72] or after the aging process [73], as well as after the formation of the aerogel.

The drug delivery aerogels developed in the literature are both inorganic and bio-based (organic) aerogels, as well as specialized aerogels. In order to become familiar with these systems, some studies are discussed here. A more detailed discussion of the use of the aerogels as drug delivery systems will be done in the third chapter, by analysing each study available in literature of the last five years.

As far as the *inorganic aerogels* are concerned, the silica-based materials are the most used. Belhadj-Ahmed et al. [74] impregnated the drug α-tocopheryl acetate into

a silica-based aerogel, by using $scCO_2$. The obtained drug loading was similar to that reached for the impregnation in a liquid media, the hexane, but with a reduction of the process time up to four times and with the advantage of obtaining a solvent-free final product. Caputo et al. [75], instead, loaded the nimesulide in a silica aerogel and, by studying the release profile of the drug from the support, found a higher release rate of the adsorbed drug compared to the release rate of the same drug in its crystalline form. They attributed this to two features of the aerogels: (i) a higher surface area of the adsorbed drug, (ii) and the non-crystalline structure of the drug adsorbed in the aerogel.

Betz et al. [76] prepared a *bio-based aerogel*, the whey protein aerogel, and studied its use as a drug carrier, by employing ketoprofen as the active compound. Their results showed a drug loading capacity up to 9.5 % of the drug in the aerogel obtained through SCF drying, which was much higher than that obtained for the drug loaded in a cryogel (i.e., the aerogel obtained with the conventional freeze-drying technique) where it ranged from 0.4 to 0.8 %. Generally, the inorganic aerogels have a higher surface area with respect to that of the organic ones. However, the better biocompatibility and biodegradability of the organic materials has made them more and more interesting during the last years [14].

Finally, some *specialized aerogels* can be prepared, such as the composite aerogels, surface functionalized aerogels, and layered aerogels. In the composite aerogels, a second material is introduced in the aerogel matrix, to improve some properties. For example, if a polymer is introduced in the solid structure of the aerogel, the mechanical strength of the aerogel matrix can be improved so that the use of the supercritical drying technique is not needed anymore to avoid the matrix collapse [14]. In the surface functionalized aerogels, for example, the erosion of the hydrophilic surfaces in aqueous media can be avoided by including some hydrophobic groups. With this surface modification, the intrusion of the dissolution medium in the solid matrix is avoided and the drug release profile can be regulated by tuning the surface hydrophobicity of the aerogel [77]. As far as the layered aerogels are concerned, they have the features to (i) protect the hydrophilic layer of the aerogels from its possible collapse in the aqueous media, (ii) regulate the drug release from the core of the matrix, (iii) release different drugs, sequentially, depending on the hydrophobicity of the different layers and (iv) release the drugs under specific physiological conditions [14].

2.3 Microporous Foams

The *microporous foams* are multiphase materials, which have high porosity (at the micro-scale) and low density, where one of the phases is a gas. Among the possible technological applications, the microporous foams are used for tissue engineering

scaffolds, drug delivery systems, and heat/sound insulation [78]. The main characteristics provided by the cellular structure of the microporous foams that make them ideal for *in vivo* structural materials are the low density, biodegradability, high porosity, and biocompatibility. Particularly, when the foams are produced from natural polymers, they have an attractive biodegradability and biocompatibility for their use in tissue cultures [79]. In fact, a current technological trend is to substitute petroleum-based foams with biodegradable ones, also considering that natural-polymers need an upstream thermo-plasticization process, whose conditions are relevant for the foamability of the polymer [80].

As far as the use of microporous foams as drug delivery systems is concerned, their microporous structure is ideal for an enhanced release rate, because of the shorter diffusion path that the loaded drug must cross in the polymer. As an example, Wang et al. [81] obtained a very low release rate for the hydrophobic paclitaxel encapsulated in compressed disks of PLGA, due, in part, to the low diffusivity of the drug in the polymer matrix, so resulting in a sub-therapeutic dose. Lee et al. [82], instead, by using the same substances (paclitaxel in PLGA), but by performing a two-stage fabrication process (spray drying + supercritical gas foaming), obtained a microporous foam which showed a nearly constant release rate (*in vitro*) for up to 8 weeks. This was a very good result in the perspective of using the final product as a surgical implant for a sustained release of the anti-cancer drug.

Some conventional methods were used to produce foams, such as the solvent casting-salt technique, freeze-drying, microwave heating and gas foaming [79]. Among these, one of the most used, which also includes the encapsulation of the drugs, is the salt leaching technique. This consists in the following steps: first, the polymer is dissolved in an organic solvent (for example methylene chloride) and cast upon a salt particles bed; then, the solvent is removed under vacuum conditions, and, by immersing the polymer in a bath of distilled water, the salt particles are leached, so resulting in the production of the porous matrix [83]. The main drawbacks of this technique are (i) the dispersion of the drug in the organic solvent phase, (ii) the possibility of a drug leakage during the leaching process, and (iii) the use of a large amount of organic solvent, which may require a downstream purification process.

The use of the SCF technology, by performing *supercritical foaming*, allows these drawbacks to be overcome [83]. The supercritical foaming process is described in the next paragraph.

2.3.1 Basic principles of the supercritical foaming technique

The microporous foams can be produced by the supercritical foaming technique. A schematic representation of this process is showed in figure 2.5. This is a very complex process, which involves mainly two different steps. First, the blowing agent (in this case the SCF) is dissolved in a molten polymer under pressure, forming a polymer/gas solution. Then, a depressurization of the system reduces the solubility of the SCF in the polymer matrix, so causing the bubble nucleation and growth. When the glass transition temperature of the polymer-SCF mixture is higher than the operative one, the polymer vitrification stops the foaming process, so preventing the cell coalescence and stabilizing the cellular structure [14,78].

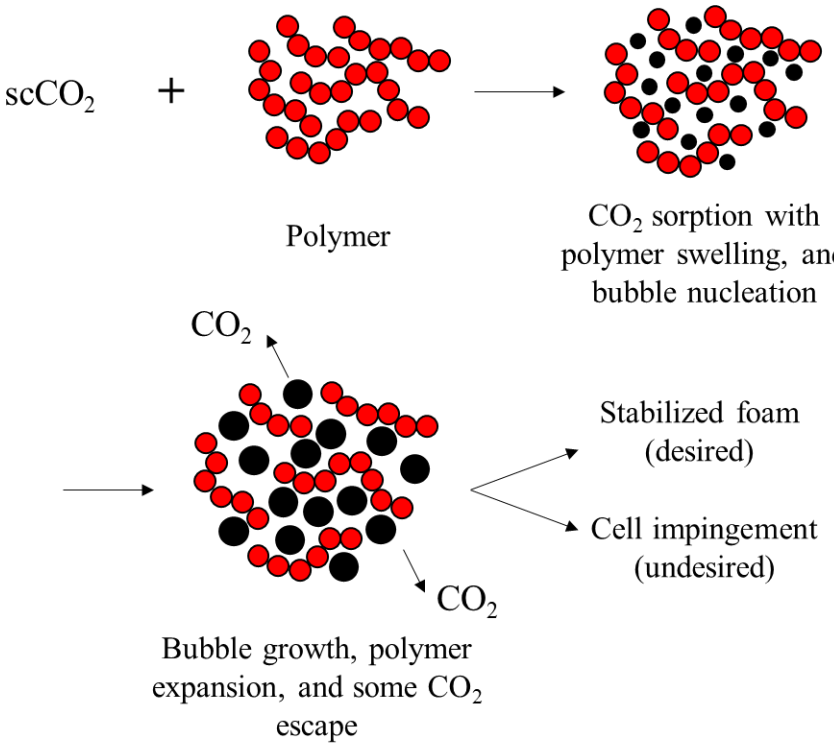

Fig. 2.5 Schematic representation of the supercritical foaming process.

Besides the depressurization of the system, the bubble nucleation and growth can also be obtained by an increase of temperature. If the temperature of the system is quite low, the depressurization step could be replaced by an increase of temperature, without affecting the thermal stability of the involved drugs [78].

As it can be observed from the figure, after the growth of the bubbles, an undesired product can be obtained. In fact, the deformation of the cell wall could cause the impingement of the neighbouring cells, resulting in the cell wall rupture and coalescence, and in the possible collapse of the foamed structure, if multiple coalescence occurs [78]. This undesired deformation of the cell wall is the result of delayed vitrification, which depends on the nature of the polymer and growth dynamics.

As far as the encapsulation of the drugs in the foam is concerned, two types of processes can be employed: (i) the single-step impregnation and foaming process and (ii) the two-step encapsulation and foaming process. In the single-step process, the $scCO_2$ has two different roles. It is a solvent for the drug and a solute due to its sorption in the polymer matrix. The procedure is the same already described for the supercritical foaming, but, here, the drug is impregnated in the foamed structure by its dissolution in the $scCO_2$, which is finally vented off [2]. The main limitation of this technique is that the drug loading in the foam depends on the solubility of the drug in the SCF. This problem can be overcome by the two-step process. The idea is to process an already prepared drug-encapsulated polymer matrix, and then, the supercritical foaming is performed as a second step. In this way, the drug loading is not limited by its dissolution in the SCF anymore. A disadvantage of this process is the involvement of an organic solvent in the first step. However, the amount of residual solvent is generally quite low, due to its removal by the CO_2 during the foaming process [2].

The most used types of supercritical foaming are (i) batch foaming, (ii) extrusion foaming, and (iii) foam injection molding [78].

The *batch foaming* is a process in which a polymer is placed in a high-pressure vessel and exposed to a flux of carbon dioxide for the time necessary for its absorption. This time depends on the mutual diffusivity between polymer and carbon dioxide. Then, the system is decompressed at ambient pressure, and, depending on the operating temperature in the vessel, two different situations can occur. In a first case, if the operating temperature is lower than the T_g of the polymer, after the decompression, this can be too rigid to foam. Consequently, the solid matrix, which contains the entrapped CO_2, must be removed from the vessel and heated to soften the polymer, so inducing the bubble nucleation and foaming. This latter type of batch foaming is named as *solid-state foaming* [78]. In a second case, the sorption of the CO_2 is performed above the T_g of the polymer, so achieving nucleation and foaming only by means of the depressurization step, without involving the heating one. This other type of batch foaming is named as *pressure-induced foaming* [78].

The *extrusion foaming* is a process in which the carbon dioxide is sent in an extrusion barrel and mixed with a molten polymer. As in the previous case, the process is conducted at high pressure, but now, the pressure increase is the result of the polymer flow along the barrel, instead of being imposed by the external gas stream. Here, the

rapid pressure drop that causes the de-mixing of CO_2 from the polymer/CO_2 mixture and the subsequent bubble nucleation occurs at the die (of the extruder). The plasticising properties of the CO_2 allow the operating temperature of the system to be lowered. Moreover, larger volume expansions of the polymer at the exit of the extruder are obtained, which means that the CO_2 is also used as an expansion agent of the polymer [84].

Finally, the *foam injection molding* is a good method to produce foams with complex three-dimensional geometries. In this process, care should be taken to be sure that foaming occurs in the mould and not during the injection step. First, the mould cavity is filled with the polymer. Then, after a certain residence time, a part of mould is sharply moved along the axial direction. The expansion of the cavity allows the gas dissolved in the polymer to experience a sudden depressurization, so resulting in a uniform foaming step in the whole moulded product [78,85].

As far as the advantages, disadvantages and the comparison of these three described foaming methods are concerned, some conclusions can be highlighted:

- the batch foaming is a very common technique used in this research field, because it is a relatively simple technique to carry out and few grams of samples can be used.
- From an industrial point of view, the extrusion foaming is better than the batch process because of its continuous production with higher productivity and easier scale-up.
- Despite the productivity advantages of extrusion foaming, this technique suffers from many drawbacks, such as the low time available for the CO_2 sorption (which is linked to the path of the polymer before its exit from the extruder) and the foam collapse after exiting the extruder.
- To avoid the CO_2 leakage (which limits the expansion of the polymer), a new generation of extruders needs to be designed, to increase the sorption level in the extruder without the technological issues of the traditional ones [78].

2.3.2 The energy barrier to achieve homogeneous bubble nucleation

Just like for the crystallization phenomenon, the foaming process occurs through the mechanisms of nucleation and growth. Therefore, the growth mechanism of a spherical gas bubble in a polymer is relevant. Using the classic theory of nucleation, Goel and Beckman [86] described how the density of the pores changes with the pressure. According to the authors, for a homogeneous nucleation of bubbles in the

polymer solution, a gas bubble must overcome the energy barrier described by the following relation:

$$\Delta G_{homo} = \frac{16\pi\gamma^3}{3\Delta P^2} \tag{2.4}$$

where ΔG_{homo} is the energy barrier, ΔP represents the difference between the pressure in the gas nucleus and the pressure in the polymer/gas solution, while γ is the polymer/gas interface surface tension. The equation (2.4) shows an inverse relationship between the energy barrier and the pressure drop. Therefore, the higher the pressure drops between the gas bubble and solution, the lower will be the energy barrier, so resulting in the increase of the nucleation rate and cell density. According to this relationship, Lee et al. [82], obtained an inverse relation between the mean pore size and the operating pressure. Other authors obtained the same trend [87].

2.4 Liposomes

The *liposomes* are artificial microscopic vesicles in which one or more bimolecular lipid layers surround an aqueous core, as it can be appreciated in the figure 2.6 [88]. They are mainly composed by phospholipids, which are molecules characterized by a voluminous apolar aliphatic tail and a smaller polar head. Therefore, they show hydrophobic properties in the tail and hydrophilic properties in the head, and for this reason the phospholipids are amphiphilic substances [89]. In the liposomes, the hydrophobic phospholipidic chains are incorporated in the bimolecular lipid layer and the hydrophilic heads are exposed to the extravascular solution [14].

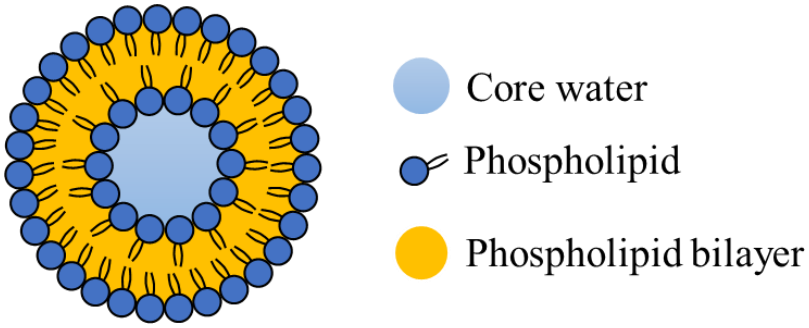

Fig. 2.6 The basilar structure of the liposomes.

The non-toxicity, biodegradability and biocompatibility of the phospholipids make the liposomes ideal to be used as drug delivery systems [90], especially for external applications, since they remain on the skin or in the stratum corneum after their

application [88]. Liposomes have characteristics similar to mammal membranes, which further enhance their non-toxicity and biodegradability [91]. The diameter range of liposomes varies from about 100 nm to many microns. [92].

An important feature that make the liposomes very interesting is the possibility to incorporate both the hydrophilic and lipophilic drugs, in the inner aqueous core and in the lipid bilayers (or between the bilayers, if more than one lipid bilayer is present), respectively.

A common classification of the liposomes is based on the number and dimension of the phospholipid bilayers. When the liposomes have only a single bilayer, they are named as unilamellar vesicles (UVs), and, depending on whether they are extremely small or large they can be labeled as SUVs or LUVs, respectively. When, instead, the liposomes have more than one phospholipidic bilayer, they are named as multilamellar vesicles (MLVs). These last ones are ideal for time-release drugs since the multiple layers allow the release of the drug at each step of the membrane's degradation to be obtained [91].

The most common used conventional methods to produce liposomes are the Bangham method, reversed-phase evaporation technique, rehydration-dehydration technique, and the organic solvent injection method. The main drawbacks of these methods are (i) the large amount of solvent involved, (ii) the multiple preparation step required (iii) the severe thermodynamic conditions, which can lead to a denaturation of the phospholipids and encapsulated drugs and, (iv) the low encapsulation efficiencies [14,91,92].

In the next paragraph, the use of the SCF technology to overcome these drawbacks is reported.

2.4.1 $scCO_2$-based processes for liposomes formation: a focus on SuperLip

In order to overcome the above cited drawbacks of the conventional methods, some $scCO_2$-based processes were proposed. These can be approximately divided into two different families: (i) two-step processes and (ii) one-step processes [92]. In the first family, an organic solution (usually ethanol) that contains the phospholipids is sprayed in a vessel containing $scCO_2$. The contact between the organic solution and the SCF leads to the extraction of the organic solvent by means of the $scCO_2$ (this is the main reason why the SCF is used) and to the supersaturation of the solution, so resulting in fast nucleation with the formation of dried phospholipidic particles. A second step, the rehydration of phospholipidic particles, allows the formation of the liposomes to occur. The difficulties related to these types of processes are (i) the control of the particles diameter and size distribution and (ii) the low encapsulation

efficiencies [92]. An example of a one-step SCF-based process is the *Supercritical Reverse Phase Evaporation* (scRPE), proposed, in 2001, by Otake et al. [88]. In this process, the phospholipids are mixed with the $scCO_2$ and ethanol in a constant stirring variable volume cell at constant temperature and pressure. Then, the last steps are the slow introduction of water and a rapid reduction of the pressure, which results in the formation of liposomes. Although this process has the advantage to produce the liposomes in just one step, the batch configuration and the low reproducibility of the particle size distribution cannot be avoided. The low reproducibility of this process is a consequence of the rapid decompression step from the supercritical conditions to room ones to mix the lipids and water, without the use of any device (like a nozzle) which generate different size characteristics. A solvent-free variation of the scRPE was proposed by the same authors in another study [93]. In this method, an inhomogeneous mixture of phospholipids and water solution was prepared using a stirrer. Then, the system was pressurized by CO_2 and, after 40 min, depressurized to form the liposomes.

Another one-step process that can be found in literature is the *Depressurization of an Expanded Solution into Aqueous Media* (DESAM) process [94]. In this process, the phospholipids are dissolved in an organic solvent, and then, the CO_2 is added to obtain an expanded lipid solution. Finally, the expanded solution is passed through a nozzle into a hot aqueous medium, at ambient pressure, so resulting in the production of liposomes. The introduction of the nozzle during the expansion step allow a more reproducibility of the particle size distribution to be achieved. An important limitation related to this process is the low encapsulation efficiency, since the drug is dissolved in the water phase, and only a part of water is entrapped in the lipidic layers.

With the purpose to overcome this last drawback, Santo et al. [92], in 2014, proposed a new continuous $scCO_2$-based process, the *Supercritical Assisted Liposome formation* (SuperLip) process. The main feature of this technique is that the liposomes are formed around water-based micro- and nanodroplets, which are produced by water atomization into a lipid + ethanol + CO_2 expanded liquid mixture. The figure 2.7 shows a schematic representation of the SuperLip process. First, the CO_2 and an ethanolic solution containing the phospholipids are pumped in a saturator (which is a heated high-pressure static mixer), forming an expanded liquid, which is, then, sent inside the high-pressure vessel. The water, instead, is continuously sprayed through a nozzle, which cause its atomization in the high-pressure vessel, so producing very small water droplets, which, then, contact the lipids contained in the expanded liquid. The mechanism proposed by the authors for the liposome's formation is illustrated in the figure 2.8. The water droplets are surrounded by a lipid layer formed by the spontaneous and rapid assemble of the lipids contained in the expanded liquid; a water-in-CO_2 emulsion is initially generated and, after, the final formation of water-in-water

emulsion (the liposomes) occurs, when the droplets fall down in the water bath, as it can be observed from the figure.

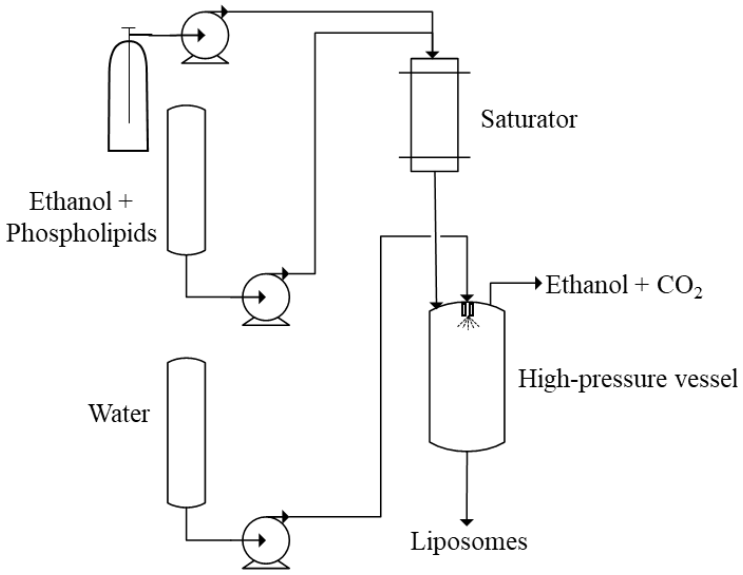

Fig. 2.7 Schematic flowsheet of the SuperLip process. The figure was reproduced with modifications from [92].

The authors reported [92] that the above described technique would assure a better size control and a higher encapsulation efficiency, which was demonstrated by performing the encapsulation of a water-soluble model protein (BSA, bovine serum albumin).

Since SuperLip can be considered the most promising approach to produce liposomes through a supercritical technique, Santo et al. [92] performed a series of experiments by varying the pressure, temperature, nozzle diameter, and the tube length to investigate how these parameters affect the results of the process. Moreover, they studied the effect of the depressurization rate on the encapsulation efficiency.

It was found that, among all parameters, the diameter of the water droplets formed by the atomization through the nozzle mostly affected the dimensions of the final product. In general, the smaller the diameter of the water droplets, the smaller the diameter of the final liposomes. This phenomenon is connected with the role of temperature, pressure, and nozzle diameter. By increasing the *pressure*, the density of the CO_2 and, consequently, that of the expanded liquid mixture increase. If the density of the mixture is higher, the jet disruptive forces, which are produced when the water jet impacts on the mixture, rise, so resulting in smaller water droplets. The same trend of the density was observed by decreasing the *temperature*. Finally, a smaller *nozzle*

diameter allowed smaller water droplets to be obtained. It can be concluded that the smallest liposomes can be obtained operating at high pressure, low temperature, and with small nozzle diameters. As far as the tube length is concerned, the authors tested two different lengths. In the first solution, the tube was not submerged in the bulk of the water phase set at the bottom of the vessel. In the second case the tube was longer and was immersed, injecting directly the expanded liquid in the bulk of water, with the purpose to achieve a further production of liposomes thanks to a more intimate interaction between the expanded liquid and the bulk of water set at the bottom of the vessel. The liposomes formed with the longest tube presented a bimodal distribution, with larger dimensions with respect to the results obtained when the shortest tube was used. This supported the thesis that the formation mechanism of the liposomes occurred according to the above described mechanism, showed in fig. 2.8. As far as the effect of the depressurization rate on the final product is concerned, the authors, by knowing that the lipids can rearrange during depressurization (to recover the suspension), performed two experiments, one at slow and the other one at very fast decompression rate. The obtained results only slightly differed from each other, so they claimed the advantage of the SuperLip process to remove the dependence of the quality of the final product by the depressurization rate. Finally, they demonstrated the improvement of the encapsulation efficiency achieved by the SuperLip technique, by loading the hydro-soluble drug BSA with an encapsulation efficiency ranging from 85 to 90 %.

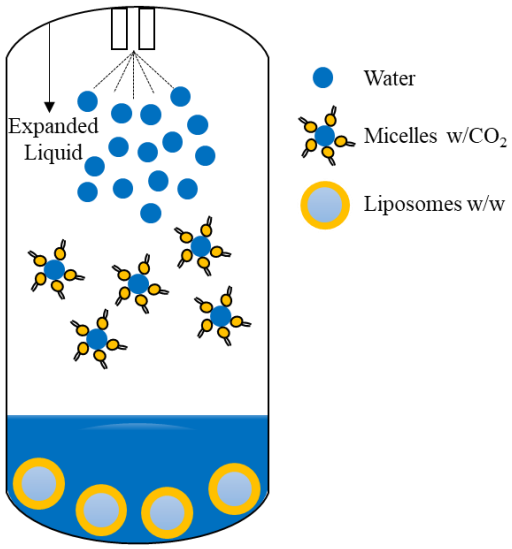

Fig. 2.8 An illustration of the mechanism of the liposomes formation in the Superlip. The figure was reproduced with modifications from [92].

2.5 Solid Lipid Nanoparticles

The *Solid Lipid Nanoparticles* (SLNs, showed in fig. 2.9) are an alternative drug carrier developed to overcome the scarcity of the safe biocompatible polymers, which limit the widespread use of the nanoparticles in the pharmaceutical field [95]. They are nano-sized colloidal drug carriers made of a lipid matrix with the encapsulated drug, which are generally dispersed in an aqueous phase that can contain a surfactant, useful to make the SLNs physically stable by preventing their coalescence [14,96]. The lipid matrix of the SLNs is a solid at room temperature. Ram et al. [95] stated that the SLNs can be considered the most effective lipid-based colloidal carriers, since, according to the authors, they overcome the disadvantages of lipid emulsions and liposomes (including drug leakage and instability during storage). With respect to the polymers, the biocompatibility of the solid lipid nanoparticles is higher since physio-

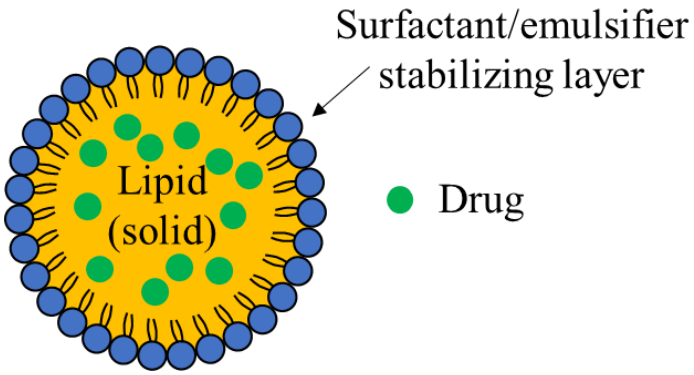

Fig. 2.9 The basilar structure of the solid lipid nanoparticles.

logical lipids are used. Due to the lipidic core matrix, the encapsulated drugs are generally lipophilic, but example of hydrophilic drugs encapsulation can be found in the literature [97]. The lipophilic drugs are very difficult to deliver via the classical routes of administration, such as the oral route and the intravenous one. However, the internal lipidic core of the solid lipid nanoparticles offers a good place to encapsulate the lipophilic drugs. The solid lipid core matrix of the SLNs allows the encapsulated drugs to be released in a controlled manner, which can be also extended to bio-macromolecules as proteins and peptides, by appropriately designing the SLNs [97]. As an example, Xu et al. [98] showed that, by loading an endosomal escaping agent in the lipidic core of a SLN, the incorporated drug (insulin) preserved its biological activity, after an oral administration, during the intracellular transport. It should be pointed out that proteins like insulin suffer from a chemical degradation during the transport through the gastrointestinal tract, after oral administration [97].

During the last years, some different models of the internal structure of the SLNs have been proposed [95]. As an example, Mühlen et al. [99], by observing a burst drug release (100 % of the drug release in less than a minute) for tretracaine and etomidate loaded in SLNs, stated that a drug enrichment in the outer shell of the particles occurred. It was also observed, by investigating another drug, the prednisolone, that a prolonged release could be obtained, which would open the possibility of using the SLNs as prolonged release formulations. In the same study, the authors investigated the effects of some parameters, like the temperature and surfactant concentration. They found that the burst release of the drug increased by increasing both parameters. At higher temperatures and surfactant concentration, in fact, the solubility of the drug in the outer aqueous phase is higher, so resulting in the above-described model (i.e. a drug enrichment in the shell of the solid lipid nanoparticle). By applying the hot homogenization technique (explained below), instead, when the final cooling step was performed, the precipitation of the drug-containing lipid core occurred and, simultaneously, the appropriate modulation of the drug solubility (which can be tuned by varying the temperature and surfactant concentration) allowed a drug enrichment in the shell of the SLN to be obtained. This is a great potential of the SLNs, because of the tunable initial burst release of the drug, which is followed by the prolonged release, so resulting in the possibility to perform a specific design of the drug release profile.

Although the vast different models for drug incorporation into SLNs complicates their production, the high flexibility helps control the SLN structure to design the desired drug release profile [99]. Other advantages of the SLNs led to claim that they combine the advantages of the liposomes and of the polymeric systems, like the above cited biocompatibility and non-toxicity for the human body, the excellent physical stability, and the protection of the sensitive drugs. Moreover, they offer an easy scale up of the production techniques [95,97]. Among the disadvantages, the relatively high-water content of the dispersions and the poor drug loading capacity can be cited [95,100].

In order to overcome the drug loading limitation, the structure of the lipid matrix can be engineered to create more space for the incorporation of the drugs. These types of modified SLNs are named as *nanostructured lipid carriers* (NLCs) [14,97]. The idea is to create as many imperfections as possible, since the drugs are incorporated in the imperfections of the crystal structure of the lipid nanoparticles. This is also the reason why the drug leakage occurs, i.e., the number of imperfections decreases, during the storage of the SLNs, due to a structure modification towards the configuration with the lowest energy possible. Unlike SLNs, the NLCs have both solid and liquid lipids in the core of the nanostructure, so resulting in the formation of more structural imperfections. Moreover, the liquid lipids solubilize the drugs in a better way than the solid ones [100].

2.5.1 Production of the SLNs

Some of the conventional techniques that are used to produce the solid lipid nanoparticles are (i) hot homogenization and (ii) emulsion precipitation [14].

As far as the *hot homogenization* technique is concerned, a melt, formed by the lipid and the drug to be incorporated, is dispersed in an aqueous solution, in which a surfactant is present, at a temperature higher than the melting temperature of the investigated lipid. Then, a pressure increase step makes the pre-emulsion to be homogenized, so forming a nano-emulsion. Finally, a cooling step to room temperature allows the lipids to be recrystallized, so resulting in the formation of the solid lipid nanoparticles [95].

In the *emulsion precipitation* technique, instead, a solution of the lipid and drug in a water-immiscible organic solvent is emulsified in an aqueous solution containing a surfactant, which is followed by an evaporation, extraction or dilution step to remove the solvent, and achieve the subsequent precipitation of the lipid-drug mixture. The mild temperature conditions make this technique suitable for thermolabile drugs. However, possible limitations in the solubility of the lipid in the organic solvent may affect the success of the technique [95].

The main advantage in the use of the *SCF technology* to prepare solid lipid nanoparticles, is the possibility to tune the supersaturation that provides the precipitation of the SLNs, by controlling the depressurization of the supercritical fluid. Some of the SCF-based processes that can be used for this purpose are the already-discussed RESS, SFEE and PGSS processes [14]. Here, a brief description of these processes is again reported, in order to clarify the role of the lipid, which was not discussed in the previous sections.

In the RESS process, the SCF is used as a solvent to dissolve the lipid and drug, and, the formed solution, is then depressurized through a nozzle, so resulting in the precipitation of the lipid-drug matrix. As an example, Akbari et al. [101] loaded the ibuprofen in SLNs by using the RESS process. They obtained ultrafine spherical particles of SLNs with a high drug loading capacity and an increased drug solubility in a gastric fluid.

In the SFEE process, the $scCO_2$ extracts the organic solvent present in the droplets of an already-formed emulsion, in which the lipid and drug form the dispersed phase and the water the continuous one, allowing the formation of a nanosuspension due to the precipitation of the lipid and drug in water. As an example, Chattopadhyay et al. [102] used the drugs ketoprofen and indomethacin and the lipids tripalmitin, tristearin and Gelucire 50/13, to produce some solid lipid nanoparticles suspensions by using

the SFEE technique. The authors obtained suspensions with a narrow size distribution and a low mean diameter.

As far as the PGSS process is concerned, the lipid and drug are melted, saturated with $scCO_2$. Finally, the saturated solution passes through a nozzle, so resulting in the precipitation of the lipid-drug matrix. Many studies focused on the production of SLNs by using the PGSS technique [103,104,105]. The interest in the production of solid lipid nanoparticles with the PGSS technique is significant, due to the solvent-free nature of the process, the direct production of dry SLN powders and its simplicity. However, the SLNs produced with the PGSS process have, usually, a mean particle size higher than 1000 nm.

In order to exploit the advantages of the PGSS process and to solve the just mentioned limitation, in 2009, Salmaso et al. [59] proposed a PGSS-modified process where the atomization step has been improved, which was named as the *Gas-Assisted Melting Atomization* (GAMA) technique. This process differs from the PGSS one for the use of a second gas stream (air) for two different purposes: (i) assisting the nozzle atomization through a tangential air flux, and (ii) preventing the adhesion of the particles on the precipitation chamber walls.

2.6 Supercritical Solvent Impregnation (SSI)

The amorphous state of a drug has often been associated to its instability, due to the tendency of an amorphous solid to crystallize [106]. However, this has gained an increasing attention over the years, due to the higher bioavailability of the amorphized drugs (see paragraph 2.6.1 for further information) [107]. Among the possible ways to transform a crystallin solid into an amorphous one, the preparation of an intrinsically non-crystalline form (such as a solution), followed by a high-interaction intensity process (such as adsorption or precipitation on a solid support) is a possibility [107].

In the so-called *solvent impregnation process* a drug-containing solution is kept in contact with an adsorbent material, until the adsorption equilibrium is reached, which is then followed by the removal of the solvent. As it has already been stressed, the use of conventional solvents should be avoided in the pharmaceutical field. By replacing the conventional solvent with $scCO_2$, the solvent impregnation process is referred as the *supercritical solvent impregnation* (SSI) technique. Besides the good solvent power, the SCFs can also plasticize and swell some types of adsorbents, so enhancing the penetration of drugs in the solid matrix [108]. Although the supercritical process is solvent-free, the introduction of a solvent is needed when a poorly $scCO_2$-soluble active compound is employed. However, the analysis of Gurikov and Smirnova [107]

showed that the poor $scCO_2$-solubility of the drugs does not necessarily affect the drug loading into porous supports.

Many types of supports are loaded with active compounds by means of $scCO_2$, such as polymers [109], aerogels [110], mesoporous silica carriers [111], mesoporous ZnO carriers [112] and cyclodextrins [113].

When a swellable matrix (like many polymers) is used as the adsorbent, the matrix swells and the active compound is impregnated into the bulk of the solid [114].

When a non-swellable carrier matrix (such as aerogel, silica and ZnO) has a large surface area and is very porous, the solute is first adsorbed on the surface of the adsorbent, which is then followed by its precipitation upon depressurization. To better describe the above-cited mechanism, Gurikov and Smirnova [107] recommended the scientific community to refer it as *adsorptive precipitation*.

Finally, the cyclodextrins are cyclic oligosaccharides composed by 6,7 or 8 α-D-glucopyranose units, and are referred as α-, β- and γ-cyclodextrins, respectively [115]. The chair conformation of these units allows the cyclodextrins to form truncated cone or torous-like shapes, in which the outer surface and inner cavity are hydrophilic and lipophilic, respectively [115]. Due to the ability of cyclodextrins to form inclusion complexes with poorly water-soluble drugs, their interest in the pharmaceutical field is increasing over the years [115]. When a SCF is used to include an active compound in cyclodextrins the process referred as *supercritical fluid inclusion complexation*.

2.6.1 Stabilization of amorphous drugs in porous and non-swellable adsorbents

Although the analysis of Qian et al. [116] showed many gaps in understanding the thermodynamic behavior of the amorphous drug molecules confined in the pores of a solid matrix, it is generally recognized that they have an enhanced mobility with respect to those present in the crystal lattice. The high mobility of the molecules has important implications in pharmaceutical applications, since it influences the solubility and dissolution rate in physiological fluids, and, consequently, the bioavailability of the involved drugs [116]. However, the higher free energy state of the amorphous state drives the spontaneous recrystallization of the drugs and increases their chemical reactivity, so resulting in a loss of the benefits provided by the amorphous state itself [117].

Among the ways to reduce the driving force for crystallization, the use of a porous adsorbent is an option. In fact, the interaction with the large surface area of a porous adsorbent, which corresponds to a high surface free energy, allows the drug to be adsorbed in a lower free energy state [116]. The adsorbed drug can then be kept in its

amorphous state, and, due to the lower Gibbs free energy, the system is physically stable.

Besides the thermodynamic considerations, the physical stability of the amorphous state can be enhanced by geometric ones. According to the classical theory of nucleation, upon reaching a critical size, the crystal growth proceeds spontaneously. Therefore, the nucleation and growth of the crystals can be prevented by imposing a size constraint, so preserving the amorphous state of the adsorbate. For example, if the matrix pores are small enough, the number of molecules inside a pore could not be sufficiently high to reach the critical size [118]. According to Gurikov and Smirnova [107], in order to minimize the risk of the recrystallization of the amorphized solutes, a 10-20 factor between the diameter of the adsorbent pores and the solute should be used. As an example, for small-molecule drugs (with a 0.3-1 nm range diameter), it would be ideal to work with adsorbent mesoporous materials having pores between 2 and 50 nanometers.

Finally, as a rule of thumb, a stronger drug-solid matrix interaction increases (i) the probability of the drug to be confined in amorphous form, and (ii) the amount of the drug loading [107].

2.6.2 Operating principles

The supercritical solvent impregnation (SSI) technique mainly involves three steps. First, the drug is dissolved in the supercritical fluid. Then, the mixture is kept in contact with the material to be loaded and, finally, the depressurization of the system allows the $scCO_2$ to be removed [119].

To perform the SSI process two different approaches are mainly used in laboratory, which are the batch and semi-continuous SSI [107,119]. In the batch operation, the drug and the adsorbent are placed inside two different parcels and introduced inside a high-pressure autoclave, which is then pressurized with $scCO_2$ until the desired pressure is reached. After a fixed period of time the autoclave is depressurized [107,119]. In the semi-continuous operation, the solubilization and sorption processes are performed in two different vessels. The solubilization occurs in a high-pressure saturator, which is connected with a second high-pressure vessel that contains the adsorbent material [119].

Porous and non-swellable adsorbents

As far as the thermodynamic considerations are concerned, the pressure and temperature conditions should be accurately chosen to avoid the presence of a liquid phase for the entire duration of the process, which could be originated because of the melting

point depression of the drug provided by the pressurized CO_2. The liquified drug, in fact, can hinder the mass transfer of the drug within the adsorbent and cause the collapse of its solid structure. However, usually the melting point of the drugs are much higher than the CO_2 critical temperature [107]. As Gurikov and Smirnova [107] reported in their review, the 303-333 K and 12-24 MPa ranges are the mostly used for temperature and pressure, respectively.

As far as the depressurization step is concerned, when highly CO_2-soluble drugs are used, both a slow and a fast depressurization rate may lead to their crystallization. Therefore, it would be useful to work with drug concentrations below their solubility in $scCO_2$. Although the scarce information in the literature does not allow us to state general conclusions, for poorly CO_2-soluble drugs a fast depressurization rate should generally increase the amount of precipitated drug [107].

Swellable polymers

The amount of drug amorphized into a swellable polymer is favoured by the swelling effect of the $scCO_2$ [114]. The SSI process is generally affected by the sorption and diffusivity of the $scCO_2$ in the polymers, as well as the solubility of the drug into the SCF [120,121].

In general, as already stressed, the solubility of a drug in $scCO_2$ increases with *temperature* above the crossover point (while the opposite trend is observed below), so enhancing the drug loading. Moreover, an increase of temperature leads to a decrease of the sorption of $scCO_2$ into the polymer, and to an increase of its diffusivity. These two phenomena could be exploited to tailor the swelling of the polymer [120]. Furthermore, Ameri et al. [121], by investigating the impregnation of lansoprazole into PVP and HPMC, stated that the lower CO_2-solubility of the drug, which resulted from an increase of temperature below the crossover point, led to promote the partition coefficient of the drug between the CO_2 and the polymer in favour of the polymer itself, so enhancing the drug loading.

As far as the role of the *pressure* is concerned, the scientific community is divided into two different beliefs. On one hand [122,123], the solute-$scCO_2$ interactions would be intensified at higher pressures, so lowering the drug loading. On the other hand [124,125,126], the increase in pressure would lead to a more intense swelling of the polymer, so increasing the drug loading.

Cyclodextrins

As already mentioned, when the $scCO_2$ is used to include an active compound in cyclodextrins, the process is referred as supercritical fluid inclusion complexation. In

this process, the $scCO_2$/drug solution is kept in contact with the cyclodextrin, until the drug inclusion complexation occurs.

Many authors [115,127,128] observed that the temperature has a positive effect on the inclusion efficiency. At higher temperatures, in fact, the equilibrium of complexation is shifted towards the inclusion complex.

As far as the effect of pressure on the inclusion efficiency is concerned, many authors found that it is not as important as the effect of the temperature [115,127]. When the pressure had a not negligible influence, the complexation was favoured by higher pressures, and this was attributed to the higher solubility of the involved drugs in $scCO_2$ [113,129].

It was demonstrated that the addition of some auxiliary agents could improve the efficiency of the inclusion process. As an example, Banchero et al. [115] showed that the addition of PVP and lysine in the process was able to improve the inclusion yield of the piroxicam/hydroxypropyl-β-cyclodextrin system. Moreover, the auxiliary agents allowed the amount of cyclodextrin used to be lowered.

CHAPTER THREE

Recent Advances (2016-2020)

In this chapter, the studies that prepared active compound-loaded drug delivery systems in the last five years are discussed. As well as for the second chapter, each drug delivery system is reported in a different paragraph. For each of them, after the discussion, the main conclusions are reported. Moreover, for each drug delivery system treated, a bibliographic table shows the most important characteristics of each work collected. The database of the discussed recent works has been formed by using the subscripted international bibliographic databases available the *Politecnico di Torino*, like Scopus, as well as its electronic subscripted periodicals, such as Science Direct, Taylor & Francis Online, ACS Journals, Wiley Online Library, and Springer Link.

As Guaadaoui et al. [130] reported, there is not a unique definition of "bioactive compounds". To avoid any type of misunderstanding, the following clarification is necessary. In this thesis, the terms *APIs* and *drugs* refer to the commercial drugs (such as the ibuprofen and paracetamol) that chemically synthesized. All other compounds that have any biological activity for the human beings are classified as *bioactive compounds*. These are generally naturally-derived compounds that are extracted from plants or other organic substrates. Among the bioactive compounds encapsulated in polymeric carriers, the polyphenols (such as flavonols) have a strong antioxidant activity, as well as the carotenoids (like astaxanthin) and, among others, the vitamin E. Besides, the carotenoids are also immunostimulants. Many plant extracts can have interesting biological activities, such as curcumin (anticancer, antioxidant, antimicrobial and anti-inflammatory activities) or andrographolide (immunostimulatory and anti-inflammatory ones), as well as phytosterols (hypocholesterolemic activity). Finally, vegetable matrix oils are widely explored since they can contain many molecules like vitamins, carotenoids, and polyphenols.

3.1 Polymeric micro- and nanoparticles (2016-2020)

In the last five years, many studies (showed in table 3.1) focused on the incorporation of the drugs or bioactive compounds into a polymeric carrier, by using the SCF technology. The figure 3.1 (a) shows the distribution of these articles based on the role that the supercritical fluid has in the process.

As it can be observed from the figure, the use of the SCF as an antisolvent was the preferred choice in the last years. Among the processes that involve the SCF as an antisolvent, the figure 3.1 (b) shows that the SAS process was the most used.

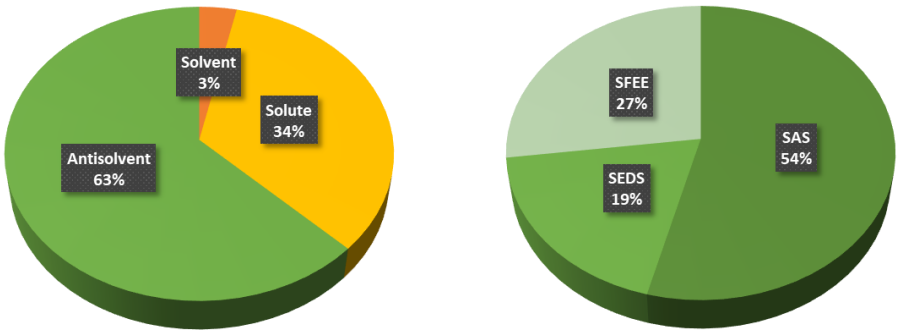

Fig. 3.1 Distribution of the articles which, in the last five years, performed the encapsulation of a drug/bioactive compound into a polymeric carrier. Data are referred to: (a) the role of the SCF in the process; (b) the type of process in which the SCF acts as an antisolvent.

It is evident, according to the explanations reported in the second chapter, that the use of the SCF as a solvent to achieve the microencapsulation of APIs/bioactive compounds in a polymer is very limited by the low solubility of the solutes in the SCF itself, especially for the high-molecular-weight solutes such as the polymers. Although the solubility of the compounds in the SCF can be enhanced by the introduction of a cosolvent in the process, this complicates the system, and the consequent gain in solubility is not enough to make the RESS and derived-RESS processes more advantageous than the antisolvent ones. As far as the SAS and PGSS processes are concerned, their percentages of use in the last five years are comparable, although the antisolvent role of the SCF was preferred. They are both good processes to encapsulate active compounds in a polymeric carrier. The SAS processes must deal with the use of an organic solvent, while the PGSS and SAA processes are completely solvent-free, but they must deal with a higher operative temperature that can degrade the active compounds and polymers.

The discussions in the next paragraphs provide some other information about the use of these two SCF-based processes.

The SAS process was abundantly employed for the encapsulation of both the APIs and bioactive compounds. Because of this, they are treated in two different sections.

Although the SFEE process can be used also to process the drugs, the table 3.1 shows that only bioactive compounds were encapsulated by SFEE in the last years. This could be a consequence of the fact that the SFEE process requires the preparation of an emulsion, which is typically an oil-in water emulsion. Most of the studies showed in the table, in fact, treated the oils extracted from a vegetable matrix. However, the same consideration cannot be stated for the SEDS process, where (again) only bioactive compounds were processed.

As Soh et al. [2] reported in their review, the PGSS process is often used to deal with essential oils because of the combination of the mild operating conditions and the absence of the solvent. The same consideration should be extended to the SAA process.

3.1.1 The SAS-processed drugs

As discussed in the second chapter, the failure of the SAS process for the coprecipitation of two different compounds is a common drawback, since this generally results in their separated precipitation. However, Prosapio et al. [44], by exploiting the ability of the PVP to retard the crystal growth of the particles, obtained well-separated spherical microparticles of nimesulide/PVP, with negligible residual solvent, and with a dissolution rate in a phosphate buffered saline solution 2.5 times faster with respect to that of the original drug. The use of the PVP for its above-mentioned feature is an increasing trend in the last years. The same authors, in fact, performed, for the first time, a successful encapsulation of three corticosteroids (dexamethasone, prednisolone and budesonide) into PVP [37], so resulting in almost-free solvent products with a dissolution rate of all the coprecipitates 4-5 times faster than that of the unprocessed drugs. Other studies exploited this PVP unique feature [46,131,132]. It must be pointed out that the use of the PVP for successful drug encapsulation contributed to the large number of articles that used the SAS process in the last years. In fact, from the table 3.1 is easy to evaluate that, for the SAS process, 40 % of the compounds were encapsulated into PVP carriers.

Franco et al. [21] tried to encapsulate a different drug, the diclofenac, into zein, aiming at expanding the still limited number of polymers suitable for the SAS process. However, a successful coprecipitation with a sustained release of the drug was only found with a zein/diclofenac ratio of 30/1, so pointing out that the polymer/drug ratio needs to be accurately selected. In a subsequent work, the same authors [133] obtained a successful incorporation of two antibiotics, the amoxicillin and ampicillin, into zein particles, and claimed that two different applications could be proposed: (i) a zein/amoxicillin system for long-term antibiotic therapy and (ii) a zein/ampicillin system for short-term antibiotic therapy.

Finally, Djerafi et al. [134] highlighted that the solvent nature can affect the success of the SAS process for the encapsulation of the rifampicin into ethyl cellulose. In fact, an unsuccessful coprecipitation was obtained by using ethyl acetate as the solvent, while, by using a mixture of ethyl acetate and dimethyl sulfoxide, successful results were obtained, so resulting in a device able to provide sustained drug release.

3.1.2 The SAS-processed bioactive compounds

The antioxidant activity is an important feature of some bioactive compounds (like the polyphenols) that needs to be preserved. This can be obtained by encapsulating these bioactive compounds into a polymeric carrier that can protect them from degradation. Ozkan et al. [132], for example, encapsulated two flavonoids, quercetin and rutin, into PVP. They claimed that the coprecipitation of these two flavonoids had never been performed with satisfying results. Therefore, they decided to exploit the above-mentioned crystallization-inhibition ability of PVP to achieve the coprecipitation, and, for the first time, obtained successful products with dissolution rates in a phosphate saline solution (PBS) up to 10 times faster than those of the unprocessed compounds. The passion fruit seed oil [135], astaxanthin [136], quercetin [132,137] and rutin [132] are examples of other antioxidants that have successfully been encapsulated in polymer carriers by SAS.

Many authors failed to encapsulate curcumin in PVP [138,139,140]. Therefore, the aim of the work by Matos et al. [141] was to deeply investigate the curcumin/PVP system. They showed that the composition of the solvent mixture of acetone and ethanol plays the major role in the improvement of the results. They stated that the SAS process is effective for the preparation of curcumin/PVP formulations, since they obtained a final product with a very increased apparent solubility in water, compared to that of the physical mixture of the same compounds (more than 600 times higher).

3.1.3 Discussion of the SFEE-processed bioactive compounds

By investigating the encapsulation of vitamin E in polycaprolactone at lab scale, Prieto et al. [142] obtained particles with a nanoscale mean particles size in the 8-276 nm range, with a narrow size distribution, an encapsulation efficiency of about 90 %, and a good morphology (spherical nanocapsules). Moreover, the size and morphology of the obtained nanoparticles remained unchanged at storage-times up to 12 months. This very interesting results allowed the same authors to perform a scale-up of the process, by realizing a high-pressure packing column in a counter-current configuration, whose conditions were optimized with help of the commercial process simulator Aspen Plus [50]. The results showed a lower encapsulation efficiency with respect to

the lab-scale operation and a lower solvent content residual (the solvent used was acetone), which was lowered down to the legal specifications only with a packing height of 3.5 meters. Some different options for the scale-up of the above-mentioned encapsulation were compared in a further study by Prieto et al. [143], in which the SFEE technique was used with four different installations: (i) a bubble column, (ii) a bubble column with a gas redistributor, (iii) a spray column and (iv) a packed column. Briefly, the last two configurations produced the smallest particles and lowest encapsulation efficiencies, while the smallest solvent removal and highest encapsulation efficiencies were obtained with the first two configurations, but with a bigger mean particles size. Other successful encapsulations were obtained by other authors [144,145,146,147].

3.1.4 Discussion of the SEDS-processed bioactive compounds

Machado Jr. et al. [148] successfully employed the SEDS technique to recover the carotenoid astaxanthin (an antioxidant) after the disruption of the cell wall of *Haematococcus pluvialis* and encapsulate it into the co-polymer poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV). The authors claimed that the combination of the enzymatic lysis (used for the disruption of the cell wall) and the SEDS technique is successful for the development of the new bioproduct from microalgae. Other works focused on the extraction and encapsulation of antioxidants, like resveratrol [149] and, again, astaxanthin [51], both with successful coprecipitation into PHBV and PVP, respectively, and with improved antioxidant activity of the bioactive compounds. Moreover, Lee et al. [150] successfully prepared microcapsules of red palm oil into maltodextrin, with a good retention of two bioactive compounds with antioxidant properties, carotene and vitamin E, so resulting in the preservation of their oxidative stability. The main challenge of the work was to reach good results starting from an oil-in water emulsion, without the use of any organic solvent. Generally, the main difficulty is that the solubility of the water in $scCO_2$ is low, and consequently its extraction power is low too. However, the authors demonstrated that, by changing the operating pressure, temperature and the $scCO_2$/emulsion flow rate ratio, successfully results could be obtained.

Scapinello et al. [151] demonstrated the possibility to encapsulate phytosterol-containing extracts from the plant *Philodendron bipinnatifidum*, into PHBV. They obtained an increase up to 35 % in the concentration of β-sitosterol in the extract, which is the most important compound. The phytosterols are a group of plant sterols able to lower the cholesterol level in human blood.

Lee et al. [152] studied the role of the polymer in the inhibition of the crystal growth in a SEDS coprecipitation process. They encapsulated the diterpenoid andrographolide (which has many biological activities such as the immunostimulatory and

anti-inflammatory ones) into three different polymers, Pluronic F127, Eudragit EPO, and Eudragit L100-55. The coprecipitation was successful only with the polymer Eudragit L100-55, with a large improvement of the dissolution rate of the bioactive compound in a PBS solution, which they attributed to the restriction of the crystal growth during the precipitation provided by the polymer.

Among the other studies that employed the SEDS technique, Andrade et al. [153] prepared a polymer-active compound system that had never been proposed until then. They succeed in encapsulating, for the first time, pink pepper oil into PHBV. The biological activities of the pink pepper oil, which need to be preserved by its encapsulation for pharmaceutical applications, are the antioxidant and anti-inflammatory ones.

3.1.5 Discussion of PGSS-processed drugs/bioactive compounds

Getachew et al. [154] successfully encapsulated the coffee oil into the polymer PEG, so overcoming the usual loss and oxidation of the flavor during its storage. By performing a Response Surface Methodology (RSM), they optimized the parameters of the PGSS process to maximize the encapsulation efficiency (up to 79.78 %), and, at these optimum conditions, the results showed a good retention of the fatty acids and flavor compounds. Other authors [55] performed a RSM in the PGSS process to encapsulate fucoxanthin-rich oil into PEG, with an encapsulation efficiency up to 82 %, which resulted in a much higher antioxidant activity of the bioactive compound, but without significant improvement of the storage stability. An improvement of the oxidative stability was found for the encapsulation of another essential oil (citrus oil) into PEG [155]. Akolade et al. [156] successfully prepared capsules of citrus aurantifolia essential oil into PEG and studied the effects of the introduction of the lauric acid (LA) in the system. They concluded that the specific interactions between PEG and LA allowed the loss of the volatile oil during the thermal treatment to be reduced, and the oil loading capacity to be improved. Moreover, the inclusion of the LA extended the mean release time of the oil in the physiological solution investigated. The advantage of using the PGSS technology for the encapsulation of thermo-sensitive materials, volatile compounds, and essential oils, was claimed also for the encapsulation of the limonene [157], ω-3 PUFAs and astaxanthin-rich salmon oil [158], n-3 PUFAs [159], and rice bran oil [160].

According to the investigated literature works, only Kravanja et al. [54] have recently used the PGSS process to encapsulate two commercial drugs, nimodipine and fenofibrate. They studied the system Brij S100/CO_2 (where Brij S100 is the commercial name of the polyoxyethylene stearyl ether) and found that, in similar thermodynamic conditions, the sorption of the CO_2 in Brij S100 is higher (of about 25 %) than

in PEG 4000. This higher concentration of CO_2 in the molten polymer led to a reduction of both the melting point and interfacial tension of the molten system, so improving the atomization step.

3.1.6 Discussion of the SAA and SAA-HCM-processed bioactive compounds

According to the authors, Adami et al. [138] solved for the first time the problem of the dissolution rate of the curcumin in physiological solutions, by incorporating this bioactive compound into PVP with the use of the SAA process. The investigated dissolution test showed a dissolution rate in PBS of the formed capsule up to 4.5 times faster than that of the PVP/curcumin physical mixture.

Many authors used the SAA process to encapsulate bioactive compounds with antioxidant activity in polymers, like phenolic compounds from olive pomace [161], propolis [162] and β-carotene [163], by exploiting the low temperatures that the SAA process allows to deal with. The coprecipitation of β-carotene with PVP, performed by Di Capua et al. [163], resulted in a very high improvement of the dissolution rate in PBS of the coprecipitates, up to 22 times faster than that of the physical mixture. Before the above cited research was conducted, the best results for the system β-carotene/PVP had been obtained by using the SAS technique, which resulted in slower enhancement of the dissolution rate of the coprecipitates (10 times) with respect to the physical mixture [164].

Some studies [165,166] focused on the SAA-HCM process. Shen et al [165] fabricated inhalable dry powders of insulin loaded into N-trimethyl chitosan (TMC) with a preserved structure of the insulin, and claimed that the novel SAA-HCM process offers a good alternative for the production of polymer/protein dry powders, for pulmonary administration.

3.1.7 Discussion of the SAILA-processed drugs/bioactive compounds

Ever since it was proposed for the first time, in 2012, the SAILA technique has been used only by the researchers from the University of Salerno. The studies published from 2012 to date are 9, three of them are focused on the encapsulation of active compounds in a polymer matrix (and are here discussed), while the others are focused on simple micronization [64,167,168], the production of solid lipid nanoparticles [169], and nanosuspensions [170,171]. To better understand the potential of this technique, a much higher number of experiments should be performed also from other research groups to provide a different point of view.

Campardelli et al. [65] employed the SAILA process for the encapsulation of three non-steroidal anti-inflammatory drugs (diclofenac, piroxicam and indomethacin) in the polymer PLGA. For all the drug/polymer systems investigated, well-defined spherical microparticles were obtained. By choosing the PLGA/piroxicam system as a model, the authors optimized the operative parameters for this system, and the experiments at optimal conditions showed a prolonged release in water, much slower than the release from the physical mixture of the same compounds. The other two works obtained similar successful results for the encapsulation of luteolin in zein [172], and α-lipoic acid and eugenol in polycaprolactone [173].

Summary of the conclusions

In this section, the main conclusions that come out from the analysis of the articles of the last five years that performed an encapsulation of drugs/bioactive compounds in a polymeric carrier are listed:

1) the use of the SCF as an antisolvent for the encapsulation of the drugs or bioactive compounds in a polymeric carrier was the preferred choice in the 2016-2020 period. However, the PGSS and SAA processes were also abundantly used. SAS must deal with the use of an organic solvent, while the PGSS and SAA processes are completely solvent-free, but they must deal with a higher operative temperature that can degrade the active compounds and polymers.

2) Among the processes that involve the SCF as an antisolvent, the SAS process was the most used.

3) The use of the SCF as a solvent is negligible, due to the low solubility of the solutes involved in the SCF.

4) The SFEE, SEDS, PGSS, and SAA processes were mostly used for the encapsulation of bioactive compounds in biocompatible polymers, while the SAS process was indifferently used both for the encapsulation of drugs and of bioactive compounds. In particular, the PGSS process was abundantly used for the essential oils.

5) The PVP was the most used polymer in the SAS process, due to its important feature to retard the crystal growth of the particles, so overcoming the main challenge of the use of the SAS process, which is the non-simultaneous precipitation of the active compound and polymer. This had a strong impact on the number of articles that used the SAS process in the last years.

6) The zein polymer is a good alternative to the usual polymers used for the SAS process, if appropriate process conditions are accurately selected.

7) The SAILA process showed good results. However, many other experiments should be performed to understand if it can be considered a valid alternative to the other processes.

8) Some compounds have successfully been coupled with specific polymeric carriers for the first time, so creating new drug/polymer matches. Dexamethasone, prednisolone and budesonide have been encapsulated into PVP, by using the SAS process; similarly, also quercetin and rutin have been incorporated into PVP with satisfying results, while PHBV has been loaded with pink pepper oil.

Tab. 3.1 List of the main properties and operative conditions of the preparation of polymeric micro- and nanoparticles in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the abbreviations.

Process	Active compound	Polymer	Solvent	P, bar	T, °C	D, µm	Ref.
RESOLV	Tetrahydrocurcumin	PLLA	EtOH/CO_2	210-330	55-100	0.08-0.11	[174]
RESOLV	Disulfiram	PVP	Water	250	55	0.62-0.71	[9]
	Disulfiram	mPLLA/PEG	Water	250	55	0.62-0.82	
	Disulfiram-Cu c.[a]	PVP	Water	250	55	0.27-0.68	
	Disulfiram-Cu c.[a]	mPLLA/PEG	Water	250	55	0.69-0.92	
SAS	Quercetin	PVP	DMSO/EtOH	90-130	40	0.47-9.52	[132]
	Rutin	PVP	DMSO/EtOH	90-130	40	0.84-8.17	
SAS	Gefitinib	PLLA	DCM/EtOH	90-120	33-48	1.08-3.75	[175]
SAS	Lycopene	Lechitin/α-Toc.[b]	DMF	80-120	35	0.8-3	[176]
SAS	α-Methyltestosterone	PLA/PLC	DCM	80	40	23-54	[177]
SAS	P.F. seed oil[c]	PLGA	DCM	90-110	35-45	0.72-1.50	[135]
SAS	C. mangga extract[d]	PVP	Acetone/EtOH	150-210	35-45	0.11-0.21	[178]
SAS	Astaxanthin	PLLA	DCM/Acetone	80	42	0.955	[136]
SAS	Tamoxifen	PLLA	DCM	130	38	n.r.	[10]
SAS	Irbesartan	Pluronic® F-127	EtOH	100-200	40-73	0.097	[39]
SAS	10-HCPT	Zein	DMSO/EtOH	80-140	30-45	0.84-3.70	[42]
SAS	Quercetin	CAP	Acetone	90	40	0.145	[37]
SAS	5-Fluorouracil	PLLA	DMSO/DCM	120-250	35-50	0.6-1.2	[179]
SAS	Ivermectin	PMMA	Acetone	90-110	40-60	0.06-0.17	[180]
SAS	Diclofenac sodium	Zein	DMSO	85-150	35-60	0.105-2.953	[21]
SAS	AMOXI	Zein	DMSO	90	40-50	0.26-12	[133]
	AMPI	Zein	DMSO	90	40-50	0.23-19	
SAS	Rifampicin	EC	EA & EA/DMSO	100	35-60	0.19-0.23	[134]
SAS	Curcumin	PVP	Acetone/EtOH	90-120	35-50	0.051-0.327	[141]
SAS	α-Tocopherol	PVP	DMSO	90	35-50	1.69-4.08	[131]
	Menadione	PVP	DMSO	90	35-50	2.64-5.09	
SAS	Nimesulide	PVP	DMSO	90	35-45	1.7-4	[44]
SAS	Dexamethasone	PVP	EtOH	90	40	1.8-2.5	[37]
	Prednisolone	PVP	EtOH	90	40	2-3.1	
	Budesonide	PVP	EtOH	90	40	3-3.6	

SFEE	Quercetin	Polysorbate 80	EA	40-120	25-50	0.026-0.03	[181]
SFEE	Pepper oleoresin	Hi-Cap 100	EA	90-110	40	0.137-0.167	[182]
SFEE	Vitamin E	PCL	Acetone	80	40	0.009-0.084	[50]
SFEE	Vitamin E	PCL	Acetone	80	40	0.008-0.276	[142]
SFEE	Vitamin E	PCL	Acetone	*	*	0.06-0.153	[143]
SFEE	Fish oil	PCL	Acetone	80	40	0.006-0.073	[144]
SFEE	L.L.[e] essential oil	Hi-Cap 100	DCM	100	40	0.24-0.318	[145]
SFEE	Neem seed oil	PHBV	Chloroform	100	40	0.153-0.307	[146]
SFEE	Yacon leaf extract	Hi-Cap 100	EA	90-130	40	0.093-0.450	[147]
SFEE	L.L.[e] essential oil	Hi-Cap 100	DCM	90-110	35-45	0.2-1	[183]
SEDS	Astaxanthin	PHBV	DCM	80-100	35	0.224-0.396	[148]
SEDS	Resveratrol	PHBV	DCM	80-150	35	0.39-0.57	[149]
SEDS	Astaxanthin	PVP	Acetone/EtOH	80-150	40-60	0.1-0.203	[51]
SEDS	*Bipinnatifidum* extract	PHBV	EA	80	35-40	0.622-0.971	[151]
SEDS	Andrographolide	Pluronic® F-127	Acetone	150	40	n.r.	[152]
	Andrographolide	Eudragit EPO	Acetone	150	40	n.r.	
	Andrographolide	Eudragit L100-55	Acetone	150	40	n.r.	
SEDS	Red palm oil	Maltodextrin	Water	100-150	40-60	4.5-9.03	[150]
SEDS	Pink pepper extract	PHBV	DCM	80-125	35-55	0.39-25.4	[153]
PGSS	Coffee oil	PEG	-	200-300	40-50	78	[154]
PGSS	FO	PEG	-	100-300	45-65	0.38-234	[55]
PGSS	Nimodipine	Brij S100	-	100-250	60	45-61	[54]
	Fenofibrate	Brij S100	-	100-250	60	47-70	
	o-Vanillin	Brij S100	-	100-250	60	36-62	
	o-Vanillin	PEG-4000	-	100-250	60	46-70	
PGSS	Lime essential oil	PEG	-	120	45	2	[156]
PGSS	Citrus oil	PEG	-	200-400	40-50	191-373	[155]
PGSS	Limonene	Purity Gum Ultra®	-	120	60	5-9	[157]
PGSS	Salmon oil	PEG-6000	-	150-250	45-55	0.37-449	[158]
PGSS	n-3 PUFAs	Hi-Cap 100	-	100	110	10-100	[159]
PGSS	Rice bran oil	P.P.[f]/Maltodextrin	-	100	105	1.5-80	[160]
SAA	Curcumin	PVP	-	99, 1.5	80, 80	0.22-0.38	[138]
SAA	Indomethacin	CH	-	80, n.r.	80-95, n.r.	0.516-4.6	[184]
SAA	Olive pomace oil	Maltodextrin	-	n.r., 1.2	75, 75-95	0.603-0.858	[161]
SAA	Propolis	HPβCD	-	99, 1.5	80, 80	0.2-0.44	[162]
	Propolis	PVP	-	99, 1.5	80, 80	0.23-0.5	
SAA	Palmitoylethanolamide	PVP	-	80, n.r.	80, 65-70	0.4	[185]
SAA	β-Carotene	PVP	-	n.r., n.r.	n.r., n.r.	0.28-0.84	[163]
SAA-HCM	Insulin	TMC	-	95, n.r.	70, 90	0.2-3	[165]
SAA-HCM	DOX	Chitosan	-	80-120, n.r.	70, 90	0.12-0.25	[166]
SAILA	Diclofenac	PLGA	Acetone	80	60	2.96	[65]
	Piroxicam	PLGA	Acetone	85-100	60-80	0.28-2.50	
	Indomethacin	PLGA	Acetone	80	60	2.56	
SAILA	Luteolin	Zein	EtOH/Water	98-100	60	0.27-1.35	[172]
SAILA	α-Lipoic acid	PCL	Acetone	100	40-60	0.54-0.99	[173]
	Eugenol	PCL	Acetone	100	40-60	0.60-1.26	

[a] Disulfiram-Cu Complexes

[b] α-Tocopherol

[c] Passion fruit seed oil

[d] Curcuma mangga extract

[e] Laurel leaves

[f] Pea Protein

n.r.: not reported

* Effective hydrodynamic diameter

3.2 Aerogels (2016-2020)

As discussed in the second chapter, the drugs or bioactive compounds can be loaded in the solid structure before or after the formation of the aerogel. In this section, the discussion is focused only on those studies that performed the loading of the drugs/bioactive compounds mainly before the formation of the gel and the subsequent supercritical drying without using the supercritical solvent impregnation technique. The works where the compounds were loaded by means of the supercritical solvent impregnation technique are discussed in the paragraph 3.6.

The supercritical drying is a well-established practice for the last step of the formation of the aerogel, for the reasons already reported in the paragraph 2.2. However, only few studies focused on the loading of the drugs/bioactive compounds without the use of the supercritical solvent impregnation technique. They are discussed in the next paragraph and reported in table 3.3.

3.2.1 The loading of drugs/bioactive compounds before the formation of the aerogel

Tkalec et al. [71] investigated how the choice of the cross-linking ion in the formation of three different polysaccharide aerogels (pectin, alginate, and composite pectin/alginate aerogels) can affect the release of a model drug, the diclofenac sodium. The results showed that, if a prolonged release is desired, calcium ions are not the best solution, since the drug release in a PBS solution was completed in a hour. Conversely, by using the zinc ions, a more controlled release of the model drug was obtained. By comparing the results of all the combinations prepared, the pectin aerogels cross-linked with zinc ions showed the best features for a controlled release of the drug. However, when a rapid release was the target, the calcium ions offered the ideal solution.

Another nonsteroidal anti-inflammatory drug (NSAID) was loaded in other polysaccharide aerogels (barley and yeast β-glucans aerogels) [186]. So far, the β-glucans aerogels had rarely been studied for drug delivery applications, and the authors state that only another study had been published on the production of aerogels with barley β-glucans. The impregnation of the NSAID (the acetylsalicylic acid) [186] occurred at the same time of the supercritical drying step. By comparing the release behaviour of the barley- and yeast-derived aerogels prepared in a PBS solution, an initial lag time was found for the release of the drug from the barley β-glucan, which is interesting from an oral drug delivery point of view (when the drug release is desired after a certain administration time). The lag time was not observed in the case of the yeast β-glucan, but a more sustained release was obtained.

Horvat et al. [72] observed that the polysaccharides guar and xanthan are not ideal for a fast oral delivery of the nifedipine. In fact, they measured a prolonged release of the drug up to 14 days. Conversely, the nifedipine was totally released in the first five hours when it was loaded in pectin and alginate aerogels.

Iglesias et al. [187] investigated, for the first time, the use of chitosan aerogels to heal chronic wounds. They loaded the antibiotic vancomycin into the aerogel and measured a fast release of the drug from the solid matrix, which allows an efficient local therapeutic effect to occur. Moreover, the authors stated that, in the future, these types of systems could be applied to the wound in the form of a powder or could be incorporated into a multi-layered system and tested in an *in vivo* animal model of chronic wounds.

A novelty that has been proposed in the last five years is the preparation of aerogels microspheres by *thermal inkjet printing*, which is then followed by the supercritical drying [188]. As the authors report, the main advantage of this technique is the possibility to design automated deposition patterns of the compounds with a high spatial resolution, especially for the pulmonary administration. In fact, they loaded an alginate aerogel with a bronchodilator, the salbutamol sulphate, and obtained reproducible spherical particles of 23.8 μm with a narrow size distribution. Moreover, they stated that this unique technology can be extended to other pharmaceutical applications, where a high-resolution product design is required.

Finally, two groups of researchers [189,190] exploited the GAS mechanism to load the paracetamol into a silica aerogel [189] and a polysaccharide aerogel [190].

By analysing the articles that loaded drugs/bioactive compounds in aerogels before the supercritical drying, it emerged that there is not a well-established road to load the drug in the solid matrix. In fact, there is a large variety of different routes, which are summarized in table 3.2. More efforts should be focused on the comparison between this wide variety of routes used to load the active compound in the aerogel matrix, to understand which is the most promising alternative to the supercritical solvent impregnation. This could be of particular interest especially when the solubility of the active compound in SCF is low.

Tab. 3.2 The different routes used to load the active compound in the aerogel matrix.

Ref., authors, year	In what stage of the aerogel production the drug was loaded
[71], Tkalec et al., 2016	In the first part of the sol-gel process, when the "sol" is not already formed.
[186], Salgado et al., 2017	Simultaneously with the supercritical drying process.
[72], Horvat et al., 2018	Concurrently to the gelation step, after the formation of the "sol".
[187], Iglesias et al., 2019	Concurrently to the gelation step, after the formation of the "sol".
[73], Buratto et al., 2019	Direct wet impregnation in the first part of the sol-gel process, as [71]. Indirect wet impregnation after the aging process.
[191], Santos et al., 2020	Indirect wet impregnation after the aging process.
[189], Ulker et al, 2017	After the aging process, GAS-like method.
[190], Falahati et al., 2019	After the aging process, GAS-like method.

Summary of the conclusions

In this section, the main conclusions related to the active compounds-loaded aerogels, which have recently been obtained through the SCF technology, are listed:

1) a very large number of different methods were used to load the active compounds in the aerogel matrix. More efforts should be done to find the best alternative to the supercritical solvent impregnation, for those compounds that are not soluble or slightly in $scCO_2$.

2) The thermal inkjet printing is a promising technique for the pulmonary administration, but it can be extended to other pharmaceutical applications.

Tab. 3.3 List of the main properties and operative conditions of the preparation of aerogels in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the abbreviations.

Active compound	Aerogel	Supercritical drying parameters			Drug loading, %	Ref.
		P, bar	T, °C	t, min		
Diclofenac Sodium	Pectin	100	40	n.r.	44.2-79	[71]
Diclofenac Sodium	Alginate	100	40	n.r.	40.5-79.6	
Diclofenac Sodium	Pectin/Alginate	100	40	n.r.	35.9-56.9	
Acetylsalicylic acid	Barley β-glucan	90-95	34	n.r.	8-15	[186]
Acetylsalicylic acid	Yeast β-glucan	90-95	34	n.r.	8-15	
Nifedipine	Xanthan	120	40	360	34.9	[72]
Nifedipine	Guar	120	40	360	25.7	
Nifedipine	Pectin	120	40	360	37.4	
Nifedipine	Alginate	120	40	360	21.7	
Vancomycin	Chitosan	120	40	210	8.5-12.9	[187]
Pulp oil	Silica	120	42	60 for 3 cycles	n.r.	[73]
Seeds oil	Silica	120	42	60 for 3 cycles	n.r.	
Slurry oil	Silica	120	42	60 for 3 cycles	n.r.	
P.P. extract of pulp[a]	Silica	120	42	60 for 3 cycles	n.r.	
P.P. extract of seeds[a]	Silica	120	42	60 for 3 cycles	n.r.	
P.P. extract of slurry[a]	Silica	120	42	60 for 3 cycles	n.r.	
Silymarin	GT-PVA	100	40	120	29.82-45.57	[192]
Resveratrol	Sodium Alginate	110	40	120	6.7-77.1	[191]
Salbutamol sulfate	Alginate	120	40	210	3	[188]
Paracetamol	Silica	90	40	30	17-41	[189]
Paracetamol	BSM	80-240	40-60	n.r.	11-26.5	[190]

[a] P.P.: polyphenols
n.r.: not reported

3.3 Microporous foams (2016-2020)

This paragraph discusses those recent works that have prepared drug- or bioactive-compound-loaded porous foams by using the SCF technology. As well as for the aerogels, the articles that have loaded the active compound by performing the supercritical solvent impregnation, after the foam formation, are discussed in the paragraph 3.6.

As it has already been reported in the second chapter, many efforts in this type of solid structures are focused in the field of the tissue engineering, i.e., in the production of supports for cell proliferation, eventually loaded with active compounds. However, the foams can also be used as drug delivery carriers, outside the field of tissue engineering [193,194,195].

3.3.1 Discussion

As far as the tissue engineering applications are concerned, Diaz-Gomez et al. [196] loaded the growth factors (GFs) obtained from platelet rich plasma, in hybrid PCL-starch scaffolds, to facilitate the bone tissue growth. The mild temperature conditions and the absence of an organic solvent in the supercritical CO_2 foaming process allowed the activity of the growth factors to be preserved. The starch was used as a GF control release component since it improves the homogeneity and hydrophilicity of the PCL-starch composites. Generally, the combination of polyesters (like the PCL) with other polymers (like the starch) allow tunable active compound-release profiles to be obtained. In fact, the hybrid scaffolds released the GFs three times slower than the GFs released from the PCL without starch. The authors stated the possibility to exploit these scaffolds to induce the bone tissue formation, with a higher efficiency with respect to the GF-containing devices that are currently used.

Salerno et al. [197] proposed a new scaffold preparation method by combining the freeze-drying and supercritical CO_2 foaming techniques, aiming at providing an alternative to the simple supercritical solvent impregnation method to load the active compounds in the scaffolds, since most of the employed active agents have low solubility in $scCO_2$. The process consisted in the preparation of PCL pellets impregnated with a solution of the drug in DMSO, followed by a freeze-drying process and a final supercritical foaming step. Salerno et al. [197] used this process to prepare PCL scaffolds loaded with 5-fluorouracil, an anticancer drug. Their work focused on finding the best operational conditions to maximize the performance of the anticancer release profile in a plasmatic medium. The authors demonstrated that stable 5-Fu/PCL scaffolds could be prepared by the proposed green technology, with a tunable long-lasting release profile, which is ideal for anticancer treatments.

Other scaffolds for tissue engineering applications were prepared by Asikainen et al. [198], who loaded bone-growth inducing active agents, the dexamethasone (DM) and 2-phospho-L-ascorbic acid trisodium salt (AS), in three copolymers of poly(ethylene glycol), ε-caprolactone, L- and D,L-lactide. According to the authors, there were not previous studies preparing PEG-P(CL-co-LA) scaffolds with bone-growth inducing agents. They found that the parameters that most affected the drug release profile in a Sörensen buffer solution were the polymer and drug compositions. Among the many combinations investigated, the most promising were P(CL30-LLA70) and PEG-P(CL30-LLA70) with DM as the model drug, since they allowed a controlled release up to 18-20 weeks to be reached.

Finally, scaffolds were prepared by Kravanja et al. [199], who prepared novel poly(ε-caprolactone)-based composites loaded with the enzyme transglutaminase (TGM). The novel composites consisted in PCL-based scaffolds containing (i)

chitosan (CS) to enhance the PCL's poor biological interactions and modulate the hydrophobicity of the PCL, and (ii) hydroxyapatite to improve the osteoconductivity of the product. Moreover, the results showed that, by adding glutaraldehyde to cross-link the CS groups with TGM, the TGM activity and release rate in a PBS solution could be altered, as the authors observed (a release of the TGM up to 30 days with a preserved activity was obtained).

In addition to the production of scaffolds for tissue engineering applications, there are also some authors who produced drug-loaded foams for other pharmaceutical applications [193,195,200,201]. For example, Campardelli et al. [195] prepared PCL patches impregnated with nimesulide, for a transdermal application. Due to the good solubility of the drug in $scCO_2$, a one-step loading of the drug was performed (i.e. the supercritical impregnation occurred simultaneously to the supercritical foaming). They obtained a successful nimesulide/PCL product, with a release rate in a PBS solution delayed up to 3.5 times when compared to that of the unprocessed drug, so stating its successful use for transdermal controlled release.

Ong et al. [193] demonstrated the possibility to obtain PLGA microporous foams loaded with either a hydrophobic (curcumin) or a hydrophilic compound (gentamicin). The results of their experiments showed that, by selecting different PLGA polymer blend and adding a biodegradable polymer (like the PEG) in the polymer matrix, the drug release profile in a PBS solution could be engineered.

Salerno et al. [194] developed a novel approach to prepare solid foams at saturation temperatures lower than the polymer melting point. In proposed process, a solvent was used to dissolve both the PCL and the drug (5-fluorouracil). Then, the solution was poured in a cylindrical mould, where the supercritical foaming was performed. The authors claimed that the addition of the organic solvent allowed the incorporation of additives to be optimized and the process to be more versatile and less time consuming, when compared to those that involve the melting of the polymer. Furthermore, the solvent residue was close to 1 % of the initial solvent weight.

As it can be observed from the tab. 3.4, the polycaprolactone (PCL) has been widely used. It is a biocompatible and biodegradable polyester that, in addition to its ability to form polymer blends, has a very slow degradation rate, which makes it ideal for long-term drug delivery applications. This is the main reason for which the PCL is mostly used for implants, like the scaffold for tissue engineering applications.

Summary of the conclusions

In this section, the main conclusions from the analysis of the most recent articles related to the incorporation of active compounds in microporous foams through the SCF technology are listed:

1) in the last five years, the extensive use of the microporous foams for tissue engineering applications has been confirmed.

2) Among the different researches, a new scaffold preparation technique has been proposed, which combines the freeze-drying and supercritical CO_2 foaming techniques. This technique is particularly promising especially when the solubility of the involved active compound in the SCF is too low.

3) A novel approach to prepare foams at temperatures lower than the melting point of the polymer has also been proposed. Although it introduces the use of a solvent, the experiments showed that the solvent residue in the final product was close to 1 % of the initial solvent weight. Furthermore, the authors claimed that the addition of the organic solvent allows the incorporation of additives to be optimized and the process to be more versatile and less time consuming with respect to the processes that involve the melted polymer.

4) The polycaprolactone is ideal for long-term drug delivery, due to its slow degradation rate, and, for this reason, it is widely used for implants in tissue engineering applications.

Tab. 3.4 List of the main properties and operative conditions of the preparation of microporous foams in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the abbreviations.

Active compound	Microporous foam	Supercritical foaming			Loading route	Ref.
		P, bar	T, °C	D.R., MPa/min[a]		
Growth factors[b]	PCL/Starch	100	37	1.5	two-step	[196]
5-Fluorouracil	PCL	200	45-50	1.8-120	two-step	[197]
Dexamethasone	Barley β-glucan	80-200	37	n.r.	two-step	[202]
Dexamethasone	Yeast β-glucan	80-200	37	n.r.	two-step	
Curcumin	PLGA	120	35	n.r.	two-step	[193]
Gentamicin	PLGA	120	35	n.r.	two-step	
5-Fluorouracil	PCL	200	50	1.2	two-step	[194]
Nimesulide	PCL	100-200	35-60	1	one-step	[195]
Thymol	PLA	75-150	25-50	30	one-step	[200]
Thymol	PLGA	75-150	25-50	30	one-step	
Transglutaminase	PCL	150	37	3	two-step	[199]
Transglutaminase	PCL/CS/HAp	150	37	3	two-step	
Gemcitabine	PLGA	120-200	25-40	n.r.	one-step	[201]
AS	PCL/PLLA	n.r.	90	n.r.	two-step	[198]
Dexamethasone	PCL/PLLA	n.r.	90	n.r.	two-step	
AS	PEG-PCL/PLLA	n.r.	90	n.r.	two-step	
Dexamethasone	PEG-PCL/PLLA	n.r.	90	n.r.	two-step	

[a] D.R.: depressurization rate

[b] Growth factors from platelet rich plasma

3.4 Liposomes (2016-2020)

The SuperLip technique was proposed in 2014 by Santo et al. [92], since the other SCF technologies had never achieved optimal results, especially for the low reproducibility and limited encapsulation efficiency of the drugs in the liposomes. Ever since it had been proposed, the SuperLip technique showed a great potential, since it successfully overcame the drawbacks of the other SCF technologies. For this reason, only the recent papers related to the SuperLip process are here discussed. The other SCF technologies employed to prepare drug-loaded liposomes are the RESS [203,204,205,206], SEDS [207], SAS [208], and scRPE [209].

3.4.1 Discussion

In the 2016-2020 period, the great potential of the SuperLip technique has widely been explored. Being it a very recent technique, each work has introduced a new active compound, which was encapsulated with the SuperLip process for the first time. For this reason, a chronological path is followed in this discussion, to document all the new steps that have contributed to make the SuperLip technique the most used to produce liposomes.

Campardelli et al. [210] explored the possibility to use the SuperLip process to produce liposomes with a different composition of the bilayer membrane, by employing a mixture of phosphatidylcholine (PC) and phosphatidylglycerol (PG), since until then, only PC-based liposomes had been produced with the SuperLip technique. To test the performances of the SuperLip process, the authors prepared the BSA-loaded liposomes with both the Bangham method and SuperLip. The results showed very high encapsulation efficiencies (between 92-98 %) obtained by using the SuperLip technique, and lower ones (between 2-57 %) obtained by the conventional Bangham method, so confirming the great potential of the SuperLip in terms of encapsulation efficiency. Furthermore, the functionality of the protein was preserved, due to the mild temperature conditions employed in the SuperLip process.

Trucillo et al. [211] exploited the SuperLip technique to optimize the lipid bilayer composition by adding natural lipids, such as cholesterol (Chol) and phosphatidylethanolamine (PE), in PC-based liposomes with entrapped theophylline. For the first time, the authors investigated the effect of PE and Chol on the mean diameter of liposomes, drug encapsulation efficiency and drug release. Moreover, they studied the effect of the water flow rate on the drug encapsulation efficiency. The results showed that the composition of the lipid layer did not affect the liposome size distribution and drug encapsulation efficiency, while, even at low concentrations, the Chol and PE, had relevant effect to prolong the drug release rate from the liposomes in water.

Until 2018, any lipophilic compound had never been entrapped in the liposomes by using the SuperLip process. The main reason why the encapsulation of lipophilic compounds had not been explored at the same extent as that of the hydrophilic ones is that the introduction of compounds in the lipidic layer (where the lipophilic compounds should be incorporated) can destabilize the liposomes integrity. Therefore, Trucillo et al. [212] used the SuperLip technique to encapsulate the eugenol (EUG) and α-lipoic acid (ALA), an amphiphilic and a lipophilic antioxidant, respectively. The high encapsulation efficiencies (EEs) obtained for both the EUG and ALA demonstrated the feasibility of the SuperLip in the encapsulation of lipophilic compounds in the lipidic layer. The same authors [213] exploited the mild conditions of the SuperLip technique to preserve the activity of other antioxidants. They successfully encapsulated aqueous phenolic compounds extracted from olive pomace into liposomes with mean particle size and EE of 265 nm and 58.1 %, respectively. The EE of 58.1 is very lower than the EE of the other compounds previously encapsulated by the SuperLip process (all of them had an EE higher than 90 %). However, the authors explained that the encapsulation of polyphenols contained in olive pomace extracts is a huge challenge, due to the high number of different compounds presents. In fact, they stated that their results are much better than those previously published in the literature.

The liposomes are considered the most valuable ophthalmic drug delivery systems [214]. However, the EEs of ophthalmic antibiotics in liposomes are generally low. Therefore, Campardelli et al. [215] encapsulated two ophthalmic antibiotics, the ampicillin and ofloxacin, into liposomes, by using SuperLip. The results showed encapsulation efficiencies up to 97 % and 99 % for ampicillin and ofloxacin, respectively. Moreover, the produced liposomes showed high stability since significant drug leakage was not observed for at least 3 months. The authors stated that the higher steric volume of these drug molecules with respect to others required different process parameter values, which would explain the unsuccessful encapsulations reported in previous literature works. In particular, the lipid-to-water ratio was a key parameter to improve the EE. The authors explained that, by increasing this ratio, the flying time of the water droplets inside the vessel was longer, and a better lipid coverage occurred. The versatility of the SuperLip technique was also tested by the simultaneous encapsulation of the two antibiotics. According to the results, the simultaneous encapsulation of both antibiotics did not affect the EE of each single compound, since the compounds remained confined in different water droplets, which behave like discrete volumes, without interfering with other water volumes. This was the first time that a simultaneous entrapment had been attempted with SuperLip.

In 2019 Trucillo et al. [216] performed an analysis of all the compounds previously incorporated into liposomes with the SuperLip technique and investigated the

incorporation of many other new ones. The authors highlighted the potential of the SuperLip process and its versatility for many different applications, since, counting both the already prepared composites and the ones produced for the first time in their work, the SuperLip had successfully been used for the encapsulation of dyes, proteins, essential oils, dietary supplements, antibiotics, antibodies and other lipids, as well as different molecules like polyinosinic-polycytidylic acid and hollow gold nanoparticles. Moreover, by choosing the amoxicillin as the model drug, the time needed to reach the steady state conditions of the process (i.e. the time required to reach a constant value of the liposomes diameter) was evaluated to be about 20 minutes from its start up, so proving that the SuperLip is scalable in terms of low costs of production, as well as for its continuous-flow configuration and good repeatability of the results.

The control of liposome diameter is a key aspect in their production. Some experiments [217] showed that the Gas to Liquid Ratio of the Expanded Liquid (GLR-EL, which is the ratio between the flow rates of the CO_2 and ethanol) is the key parameter to control the liposomes diameter, both in the micro- and nanometric scale, whose size depends on the desired application. Furthermore, the same parameter allows the amount of solvent residue to be modified.

Another step towards the scalability of the SuperLip process was proposed by Apicella et al. [218]. The process productivity was up to 1 g/h, which is a novelty if compared with the usual milligram scale productions. Moreover, they attempted to prepare an efficient triggered release from liposomes, which is a current challenge. The authors developed a continuous SCF-based process in which near-infrared (NIR) light sensitive liposomes loaded with Hollow Gold Nanospheres (HGNs) were prepared. In fact, according to the authors, to artificially trigger the drug release, the light activation is one of the most promising routes. Therefore, they prepared liposomes that contained both a triggering agent and two active agents (polyinosinicpolycytidylic acid and BSA). The results showed, in addition to the gram-scale production, high biocompatibility of the liposomes and high EE (> 95 % for each component), as well as good susceptibility of NIR-activated release.

Summary of the conclusions

In this section, the main conclusions from the analysis of the articles of the most recent active compounds-loaded liposomes obtained with the SuperLip process are listed:

1) among the SCF-based techniques developed to produce liposomes as drug delivery systems, the SuperLip, despite its being a novel technique (first proposed in 2014), has been the most used one in the last five years. This implies that the researchers believe in the great potential of the SuperLip process,

which explains the reason why they devoted so many efforts for its development and spread in many fields.

2) Many advances have successfully been obtained in the SuperLip applications, like (i) the production of liposomes by employing a mixture of PC/PG, instead of only PC, (ii) the first encapsulation of a lipophilic compound into liposomes, (iii) the successful encapsulation in liposomes of two ophthalmic antibiotics for eye drug delivery system applications, (iv) the first simultaneous entrapment in liposomes of two different compounds, and (v) the gram-scale continuous production of liposomes loaded with Hollow Gold Nanospheres for a triggered release.

3) As it is shown in the tab. 3.5, many active compounds encapsulated in liposomes with the SuperLip process achieved EEs higher than 90 %.

4) The versatility of the SuperLip process was widely demonstrated, by the encapsulation of many different types of molecules, like dyes, proteins, essential oils, dietary supplements, antibiotics, antibodies and other lipids, as well as other molecules like polyinosinic-polycytidylic acid and hollow gold nanoparticles.

5) The SuperLip process was considered, ever since it had been proposed, a very promising scalable process. The main features of the SuperLip process are (i) its continuous configuration, (ii) the low time needed to reach the steady-state conditions, (iii) the repeatability of the results, and (iv) the successfully results obtained by Apicella et al. [218] for the gram-scale production of HGN-loaded liposomes.

Tab. 3.5 List of the main properties and operative conditions of the preparation of liposomes in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the abbreviations.

Active compound	Lipid	T, °C	P, bar	EE, %	D, nm	Ref.
BSA	PC	40	125-175	92-98	250-330	[210]
BSA	PC/PG	40-70	125-175	92-98	280-484	
Theophylline	PC+Chol+PE	40	100	up to 98	136.5-240.5	[211]
Eugenol	PC	35-40	100	80.4-94.2	139-260	[212]
α-Lipoic acid	PC	40	100	55.7-68.1	109-244	
Olive pomace extracts	PC	40	130-170	25-4-58.1	134-265	[213]
Ampicillin	PC	40	100	up to 99	281	[215]
Ofloxacin	PC	40	100	up to 97	202-1760	
Ampicillin+Ofloxacin	PC	40	100	86-99	957	

Olive pomace extracts	PC	40	100	58	264	[216]
Caffeine from SCG	PC	40	100	87	188	
A.M.[a] IgG Isotype Control	PC	40	100	93	142	
BSA	PC	40	100	93	245	
Albumin F.I.[b]	PC	40	100	99	379	
PIC	PC	40	100	95	1154	
Ofloxacin	PC	40	100	86	256	
Theophylline	PC	40	100	98	136	
Ampicillin	PC	40	100	99	313	
Amoxicillin	PC	40	100	79	198	
Vancomycin	PC	40	100	60	180	
HGN	PC	40	100	98	747	
Eugenol	PC	40	100	86-93	196-234	
Linalool	PC	40	100	55	128	
Lipoic acid	PC	40	100	63	109	
Limonene	PC	40	100	87	159	
Farnesol	PC	40	100	74	126	
Cholesterol	PC	40	100	96	140	
Vancomycin	PC	40	100	22.9-74	139-1729	[217]
Farnesol	PC	40	100	60-76.7	180-250	
HGN + PIC + BSA	PC	40	100	90-96	750-1760	[218]
FITC	PC	40	100	80	200	[219]
Farnesol	PC	40	100	22-74	126-146	[220]
Limonene	PC	40	100	35-87	116-197	
Linalool	PC	40	100	36-54	213-230	
Linalool+Farnesol	PC	40	100	61-70	181	
Limonene+Linalool	PC	40	100	61-90	121	
Amoxicillin	PC	40	100	2-84	183-246	[221]
Amoxicillin	PC+Chol	40	100	5-80	169-267	
Antioxidant from SCG	PC	40	100-200	26-100	168-191	[222]

[a] A.M.: antibody mouse

[b] F.I.: fluorescein isothiocyanate

3.5 Solid Lipid Nanoparticles (2016-2020)

The studies discussed in this paragraph are those that have prepared (in the last five years) solid lipid particles by using the SCF technology and have loaded a drug or bioactive compound in their structure.

3.5.1 Discussion

During the last five years, the most used SCF-based process for the production of drug-loaded solid lipid nanoparticles has been the PGSS process. Pedro et al. [223] used the PGSS technique to produce, for the first time, curcumin-loaded solid lipid particles. Although one of the main advantages of the PGSS technique is that it is solvent-free, the authors employed a solution of curcumin in DMSO (instead of pure curcumin) to increase the amount of the active compound in the solid lipid particles. This occurred because the solubility of the curcumin in the soy PC/tristearin mixture, which is used as the solid lipidic phase, is quite low. The experiments were performed both in the presence and absence of helium, which was used to create an inert environment to prevent the oxidation of the curcumin. The presence of helium was found to improve the drug loading and to have a key role in the control of the dimensional properties of the final product. Helium also helped preserve the integrity and stability of the curcumin, which is fundamental for the pharmaceutical applications of this active compound.

Ciftci et al. [224] prepared solid lipid particles from fully hydrogenated canola oil (FHCO), loaded with spearmint essential oil, by using the PGSS technique. The authors studied the effects of the process parameters on the quality of the final product. The results showed a production of spherical particles with a mean diameter of 1.27 μm with the smallest nozzle diameter (0.1 mm) and the lowest pressure (122 bar). Martín et al. [225], instead, loaded copper nanoparticles into glyceryl palmitostearate solid lipid particles by using the PGSS technology. This work is particularly interesting since the loading of metal nanoparticles in lipid particles for biomedical applications has rarely been studied. The authors claimed that their work was a preliminary study that proved that metal nanoparticles could be incorporated in solid lipid particles by using the PGSS process.

It must be pointed out that, as already discussed in the second chapter, the production of solid lipid particles with mean diameters lower than 1 μm is a challenge. In fact, the above-discussed works produced solid lipid particles with a mean diameter higher than 1 μm, as the table 3.6 shows. The GAMA process, which is a variation of the PGSS process (par. 2.5.1) was proposed to overcome this problem. However, to the best of my knowledge, no active compounds have been loaded in solid lipid particles by the GAMA technology in the last five years. However, Couto et al. [226] successfully prepared Vitamin B_2-loaded FHCO SLNs with a mean diameter lower than 1 μm, by employing a modified PGSS-process in which the decompression did not occur in air or in an inert gas but was performed in an aqueous solution of PEG. The production of smaller particles was guaranteed by (i) the increased shear rate generated by turbulence during the expansion step, (ii) the reduction of the growth

rate, due to the hydrophobic nature of the FHCO nanoparticles, and (iii) the stabilization of the nanoparticles in suspension, provided by the PEG. Due to the hydrophilic nature of the Vitamin B_2, its encapsulation is needed to achieve a sustained release in physiological fluids, so avoiding administering it every single day.

As the table 3.6 shows, the RESS and SAS processes have also been used to prepare drug-loaded SLNs. However, only one example of each technique can be found in the recent literature [227,228]. Ahmad et al. [227] encapsulated the trans-resveratrol (RVL) in PEGylated SLNs (Gelucire® 50/02 and Gelucire® 50/13), by using the RESS process. The purpose was to mitigate the radiation induced damages to human tissues during occupational radiology, radiotherapy, and diagnosis. Actually, even if this is not specified by the authors, their process should be classified as RESSAS (see section "RESS-derived processes"), since the expansion was performed into water. In fact, probably for the above-discussed reasons proposed by Couto et al. [226], the authors obtained SLNs with mean diameters lower than 1 μm. Saad et al. [228], instead, successfully prepared hesperidin-loaded SLNs by the SAS process. They optimized the process parameters to produce particles with a mean diameter of 175.3 nm with high EE (87.6 %), resulting in an aqueous solubility of the active compound improved up to 20 times, compared to the unprocessed drug.

A novel SCF-based process was proposed by Yang et al. [229], which they defined as *atomization of the carbon dioxide-expanded lipid*, to produce hollow solid lipid particles. The production of hollow solid lipid nanoparticles allows the drawback of the low drug loading of the SLNs to be overcome. Until then, no hollow SLNs had been produced with the SCF technology. The proposed process exploits the decrease of the melting point of the lipids under pressurized CO_2. This allows a lower temperature to be used in the process, which is fundamental for minimizing the degradation of thermolabile active compounds. In particular, the melting point was diminished from 68.5 to 57 °C by increasing the pressure of CO_2 up to 120 bar. First, the lipid is warmed up by a heater, and enters a high-pressure expansion vessel. Then, the CO_2 is sent in the same stirred vessel to expand the liquid lipid. Finally, the CO_2-expanded liquid is atomized through a nozzle, and the formation of solid lipid particles occurs. The authors also proposed a mechanism to describe the formation of hollow SLNs. First, as a result of the atomization, droplets of lipid/CO_2 mixture are formed. The pressure drops that result from the expansion, then, allow the CO_2 to expand, so forming a spherical denser lipidic shell, which surrounds a core of CO_2. Finally, the cooling due to the Joule-Thomson effect allows the dense lipid shell to solidify, so forming the hollow SLNs. The same authors used the proposed SCF-based method to load, in the hollow SLNs, either the peppermint essential-oil [230] or the fish oil [231], and claimed that their technique allowed dry free-flowing products to be obtained, which makes their handling and storage more convenient.

Although the SLNs are very promising drug delivery systems, the use of the SCF technology has not been widely explored yet. Further studies need to be done to understand the SLNs potential for an industrial application.

Summary of the conclusions

In this section, the main conclusions from the analysis of the most recent articles related to active compounds-loaded SLNs by using the SCF-based processes are listed:

1) the PGSS has been the most used SCF-based process for the production of loaded solid lipid particles in the last five years. However, only a study reached the target of a mean diameter lower than 1 μm.

2) The GAMA process has never been used, in the last five years, to load active compounds in the solid lipid particles. Some attempts need to be done, since the GAMA process allows the issues of big mean particle size to be overcome.

3) In the PGSS process, by performing the decompression of the CO_2-saturated mixture into a water bath, instead of air, Couto et al. [226] prepared Vitamin B_2-loaded SLNs with a mean diameter lower than 1 μm. Many efforts should be done to try this approach with other formulations.

4) A novel SCF-based process was proposed by Yang et al. [229] to produce, for the first time, hollow solid lipid particles with the SCF technology. According to the authors, the hollow configuration of the SLNs should improve their loading capacity but no experimental evidence of this advantage has been provided yet.

Tab. 3.6 List of the main properties and operative conditions of the preparation of solid lipid particles in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the abbreviations.

Process	Active compound	Lipid	P, bar	T, °C	D, μm	Ref.
PGSS[a]	Curcumin	Tristearin/PC	150	55-75	0.4-2000	[223]
PGSS	Spearmint essential oil	FHCO	122-300	60	1.27-2.01	[224]
PGSS	Vitamin B_2	FHCO	100-250	65	0.061-0.772	[226]
PGSS	Copper	Precirol 5 ATO	100-150	60-80	32-74	[225]
RESSAS	Resveratrol	PEGylated SLNs	300-350	n.r.	0.2767	[227]
SAS[b]	Hesperidin	Stearic acid	80-200	35-45	0.153-0.385	[228]
Atom. of CO_2-exp. l.m.[c]	Pepperint essential oil	FHSO	120-300	57	2-40	[230]
Atom. of CO_2-exp. l.m.[c]	Fish oil	FHSO	200	57	5-18	[231]

[a, b] DMSO used as the solvent

[c] Atomization of CO_2-expanded lipid mixture

3.6 Supercritical Solvent Impregnation (2016-2020)

This paragraph discusses those recent works that employed the supercritical solvent impregnation technique to prepare drug- or bioactive-compound-loaded carriers. As it has already been reported in the second chapter, many types of matrices could be used as materials to be loaded. The following discussion is therefore divided in two different groups, porous and non-porous matrices, which are discussed in the paragraphs 3.6.1 and 3.6.2, respectively.

3.6.1 Preparation of active compound-loaded porous matrices by using the supercritical solvent impregnation technique

Among the wide variety of porous materials recently used as adsorbents to be impregnated with drugs or bioactive compounds, the *aerogels* have been the most employed, mostly due to their very high porosity and surface area. Mustapa et al. [108] compared two different impregnation methods: (i) the wet impregnation, during the aging process, by dissolving the active compound into the aging solution; (ii) the supercritical solvent impregnation, in a preformed aerogel. They used phytol and *Clinacanthus nutans* plant extracts (rich in phytols) as model active compounds, and alginate and silica aerogels as adsorbents. The supercritical solvent impregnation yielded higher loading content of the two active compounds with respect to wet impregnation, due to the swelling effect provided by the $scCO_2$. Moreover, the authors stated that the adsorbent surface area was the main factor influencing the drug loadings.

Vitamin D_3 is a very thermo-sensible active compound, therefore, its entrapment in a solid support is a huge challenge [232]. Pantić et al. [232] tested the stability of this compound by its impregnation in an alginate aerogel with both the sub-critical (with liquid CO_2) and supercritical impregnation techniques. They managed to pass from the first to the second technique by simply varying the temperature. According to the authors, the higher solubility of vitamin D_3 in $scCO_2$ provided a higher drug loading. However, as they expected, the subcritical impregnation allowed a more stable product to be obtained, due to the lower temperatures involved. In another work, the same authors [233] obtained an improvement of the solubility of vitamin D_3 in water, by its supercritical amorphization in aerogels. The solubility was enhanced up to 20 times with respect to that of the crystalline form of the same compound.

Other vitamins were impregnated in aerogels by means of the supercritical solvent impregnation technique [110,234]. De Marco et al. [110] loaded the vitamins E and K_3 in starch aerogels, so obtaining dissolution rates in a phosphate buffered saline (PBS) solution up to 3.5 and 16 times faster than the non-processed vitamins, respectively. Once demonstrated that the vitamin E could be successfully incorporated into

starch aerogels by using the supercritical solvent impregnation technique, the same authors [235] investigated the environmental impact of the entire process, from the corn cultivation up to the supercritical solvent impregnation, by performing a life cycle assessment (LCA) analysis. The results showed that, in most of the impact categories investigated (climate change, ozone depletion and many others), the supercritical drying process (used to transform the alcogel in aerogel) was the major contributor. Moreover, the supercritical impregnation had a contribution of at least 5 % in each impact category. According to the analysis, the high consumption of electrical energy related to the condensation and recycling of carbon dioxide is the reason that most affects the emissions of the two SCF-based processes. Another LCA analysis [236], performed by the same authors for the preparation of nimesulide-loaded starch aerogels, has further confirmed that the environmental impact of the two SCF-based processes can be ascribed to the above-cited electrical consumption.

Another type of porous adsorbents explored in recent years are the *mesoporous silica supports*. Bouledjouidja et al. [111] loaded fenofibrate in an ordered mesoporous silica (OMS-L-7) carrier by using both conventional and supercritical solvent impregnation. A higher drug loading resulted from the supercritical process. Moreover, supercritical impregnation allowed the degree of crystallinity of the drug to be lowered and the final product to be completely solvent-free, since the high solubility of the drug in $scCO_2$ did not require the use of a co-solvent. The low $scCO_2$-solubility of mangiferin, instead, led García-Casas et al. [237] to use an acetone/DMSO mixture as a co-solvent. The results showed a delayed release of the product in a simulated gastric fluid.

Finally, Banchero et al. [112] reported that their group is the only one who has used the zinc oxide (ZnO) as porous adsorbent to achieve drug amorphization by supercritical solvent impregnation. According to the authors, this should be due to the usual lack of mesoporosity of this material. They loaded ibuprofen (IBU), clotrimazole (CTZ) and hydrocortisone (HC), which can be considered a highly, a moderately, and a poorly CO_2-soluble drug, respectively, in a mesoporous nanostructured ZnO carrier. As far as the drug loadings are concerned, the highest one was observed for the ZnO-IBU system, with a drug loading that is double with respect to the ZnO-CTZ system. The drug loading of the HC, instead, showed a negligible value. It would be easy to conclude that the different drug loadings directly reflect the different CO_2-solubilities of the drugs. However, as recently stressed by Gurikov and Smirnova [107] the low solubility of drugs in $scCO_2$ not necessarily translates into low drug loadings in porous supports. Therefore, Banchero et al. [112], by investigating both the drug-drug and the drug-matrix interactions, proposed that the strong drug-drug interactions during the supercritical impregnation hindered the drug-matrix ones. In

fact, by performing the process in the presence of ethanol (in which HC is highly soluble) the amorphization of the drug did not occur.

The ZnO was used in other two works [238,239] by the same group of researchers, to load clotrimazole [239] and ibuprofen [238]. Leone et al. [239] stated that their preparation route can be easily adapted to batch small-scale productions. Summarizing, the use of $scCO_2$ to load ZnO has recently been used, for the first time, to load ibuprofen, clotrimazole and hydrocortisone.

Tab. 3.7 List of the main properties and operative conditions of the preparation of compound-loaded porous matrices by using the supercritical solvent impregnation technique in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the abbreviations.

Active Compound	Support	Characteristics of the support			SSI parameters			Ref.
		S.a.[a], m^2/g	P.v.[b], cm^3/g	P.s.[c], nm	P, bar	T, °C	t, h	
Aerogels								
Fish oil	WPI	390-422	1.27-1.69	9.2-14	180	40	72	[258]
Fish oil	EWP	220-378	1.56-2.79	13.8-17.4	180	40	72	
Fish oil	NaCas	42	0.24	9.2	180	40	72	
Phytol	Cellulose	145-434	0.3-2.4	7.9-34	100	40	24	[259]
Ketoprofen	Silica	568.9-955-73	n.r.	9.53-24.44	120	40	72	[260]
GCO	Starch	30-185	0.04-0.85	n.r.	300	40	6-24	[261]
Phytol	Silica	881.5	2.9	12.9	200	40	24	[108]
C. n. plant extracts[d]	Silica	881.5	2.9	12.9	200	40	24	
Phytol	Alginate	125.9	0.8	25.5	200	40	24	
C. n. plant extracts[d]	Alginate	125.9	0.8	25.5	200	40	24	
Fish oil	WPI	154-354	0.33-1.55	7.7-14	100-180	35-60	72	[262]
Fish oil	EWP	232	2.28	41.7	100-180	35-60	72	
Fish oil	NaCas	48	0.28	13.4	100-180	35-60	72	
Vitamin E	Maize starch	90	n.r.	n.r.	150	40-60	24	[110]
Vitamin K_3	Maize starch	90	n.r.	n.r.	150	40-60	24	
Vitamin D_3	Alginate	418	0.87	11.4	80	35	n.r.	[232]
Vitamin K_3	Alginate	400-430	0.79-0.86	10.5-10.9	150-200	40	n.r.	[233]
Vitamin D_3	Alginate	400-430	0.79-0.86	10.5-10.9	150-200	40	n.r.	
Benzoic acid	Silica	580	2	15	200	55	5-10	[263]
Nimesulide	Maize starch	80	n.r.	n.r.	180	40-60	2-72	[264]
Ketoprofen	Maize starch	80	n.r.	n.r.	180	40-60	2-72	
Diclofenac sodium	Maize starch	80	n.r.	n.r.	180	40-60	2-72	
Nimesulide	Calcium alginate	277	n.r.	n.r.	180	40-60	2-72	
Ketoprofen	Calcium alginate	277	n.r.	n.r.	180	40-60	2-72	
Diclofenac sodium	Calcium alginate	277	n.r.	n.r.	180	40-60	2-72	
Mesoglycan	Calcium alginate	n.r.	n.r.	100	180	40-60	24	[265]
Ibuprofen	Alginate	316-420	1.2-1.8	30	200	45	6	[266]
Vitamin E	Silica	800	n.r.	n.r.	150	60	24	[234]
Vitamin E	Maize starch	90	n.r.	n.r.	150	60	24	
Ketoprofen	Alginate-based	330-548	1.7-5.9	n.r.	180	40	24	[267]
Quercetin	Alginate-based	330-548	1.7-5.9	n.r.	180	40	24	

Others								
Ibuprofen	ZnO	12-60	0.050-0.23	n.r.	100	35	12	[238]
Ibuprofen	ZnO	75	0.14	8	100	35	3-24	[112]
Clotrimazole	ZnO	75	0.14	8	250	100	12	
Hydrocortisone	ZnO	75	0.14	8	130	45	8	
Clotrimazole	ZnO	19-66	0.05-0.23	n.r.	250	100	12	[239]
Mangiferin	Silica	293-342	0.79-0.84	10.55-10.74	100-300	35-50	1.5-24	[237]
Quercetin	Silica	300	0.84	10.55	100-200	35-50	0.5-17	[119]
Carvedilol	Silica	281.6-345.9	0.298-0.377	3.35-3.83	241	70	24	[268]
Carvedilol	Silica MCM-41	1057	1.092	2.75	241	70	24	
Ibuprofen	Silica MCM-41	1030	1.09	3-6	150	40	72	[253]
Ibuprofen	Silica SBA-15	920	0.84	3.5-4.5	150	40	72	
Fenofibrate	Silica OMS-L-7	450-600	0.9-1.1	6-7.5	100-200	35		[111]
Ibuprofen	Kromasil-100	281	0.74	10	150	40	72	[253]
Ibuprofen	Kromail-300	63	0.72	30	150	40	72	
Copaiba oil	Spongostan®	29.8	13.6	18.3	132-317	45	n.r.	[252]
Copaiba oil	Promogran®	26.1	3.1	4.8	132-317	45	n.r.	
Rifampin	HMFN	48.5	0.27	22.12	100-200	30-50	2-4	[269]
Rifampin/Isoniazide	HMFN	48.5	0.27	22.12	100-200	30-50	2-4	
Thymol	PCL	n.r.	n.r.	n.r.	100-300	35-40	2	[256]
Thymol	PCL-HA	n.r.	n.r.	n.r.	170-300	35-40	2	
Thymol	PLA	n.r.	n.r.	n.r.	300	100-120	n.r.	[257]

[a] Surface area

[b] Pore volume

[c] Pore size

[d] Clinacanthus nutans

3.6.2 Preparation of active compound-loaded non-porous matrices by using the supercritical solvent impregnation technique

Marizza et al. [106] performed the supercritical impregnation method to load ketoprofen into microcontainers filled with the polymer PVP. The microcontainers consisted in cylinders with a cavity open on one side, which had a higher surface-to-volume ratio with respect to traditional tablets. The results showed that the drug loading could be controlled with a very high reproducibility, by varying temperature, pressure, contact time and drug concentration. Moreover, the solubility of the drug in CO_2 had not a key role on the drug loading, which was mostly affected by the drug-matrix interactions. Shi et al. [240], in fact, by modeling the drug loading of the ibuprofen/PMMA system showed that the relationship between the drug loading and the interaction parameters could be used to regulate the drug loading in the polymer. A further confirmation was provided by Coutinho et al. [109], that, for the first time, compared the impregnation of a compound and its co-impregnation with another compound (in particular, the three combinations obtainable from aspirin, ketoprofen and carvone) into PLLA and LLDPE. The authors claimed the following results: (i) the high solubility of compounds in $scCO_2$ hindered their impregnation in polymers during the partition between the two phases; (ii) a good polymer/drug affinity improved

the drug loadings in LLDPE as well as synergistic effect between different compounds (i.e. one solute helps the impregnation of others). Based on these results [106,109,240] the conclusion provided by the analysis of Gurikov and Smirnova [107], for whom the poor solubility of drugs in $scCO_2$ does not necessarily compromise their drug loadings in porous supports, could be extended to the polymers.

Many authors prepared polymer films impregnated with antibacterial compounds (mostly thymol). However, these works were mainly focused on the food packaging applications, and, for this reason, are not discussed in this thesis, even though some authors claimed some medical application of their products [241,242].

The supercritical solvent impregnation for antimicrobial applications has also been used for fibers such as sutures [243], dental floss [244,245] and fabric with medical properties [246,247], ophthalmic medical devices [124,248,249,250] wound dressing applications [251,252], as well as the preparation of drug-cyclodextrins inclusion complexes [113,253,254,255].

Tab. 3.8 List of the main properties and operative conditions of the preparation of compound-loaded non-porous matrices by using the supercritical solvent impregnation technique in the (2016-2020) period. Please see the list of symbols and abbreviations for the meaning of the

Active compound	Support	SSI parameters			Ref.
		P, bar	T, °C	t, h	
Polymers					
Lansoprazole	PVP	150-250	35-55	1-3	[121]
Lansoprazole	HPMC	150-250	35-55	1-3	
Aspirin+Ketoprofen	PLLA	90-300	60-80	3	[109]
Aspirin+Carvone	PLLA	90-300	60-80	3	
Carvone+Ketoprofen	PLLA	90-300	60-80	3	
Aspirin+Ketoprofen	LLDPE	90-300	60-80	3	
Aspirin+Carvone	LLDPE	90-300	60-80	3	
Carvone+Ketoprofen	LLDPE	90-300	60-80	3	
Ketoprofen	PVP	100-200	40-60	1-4	[106]
Flurbiprofen	PMMA/β-TCP	85-115	40-60	24	[270]
Flurbiprofen	PMMA	115-148	40-60	24	[271]
Biopolymers					
Lycopene	Hydrolysed collagen	150-250	50-60	0.75	[272]
Chia Oil	Soy protein	100-160	40-60	4	[273]
CoQ_{10}	β-glucan	300	40	0.25-1.5	[114]
CoQ_{10}	Gum arabic	300	40	0.75	[274]

Cyclodextrins					
Ibuprofen	β-Cyclodextrin	150	40	72	[253]
Ibuprofen	PA β-CD	250	35	24	[254]
Linalool	β-Cyclodextrin	100	40	3	[255]
Carvacrol	β-Cyclodextrin	100	40	3	
Daidzein	HPβCD	200	200	8	[275]
(+)Catechin	β-Cyclodextrin	90	40	1	[276]
Flurbiprofen	Methyl-β-cyclodextrin	100-200	35-45	n.r.	[277]
Baicalin	HPβCD	100-300	45-65	3-14	[113]
Polymer Fibers					
Eugenol	Polyamide	80-120	60	2	[244]
Eugenol	Polyamide	100-120	40-60	0.5-4	[245]
Ketoprofen	PLLA	300	75	3	[243]
Mango leaf extract	Polyester	400	35-55	22	[246]
Mango polyphenols	Cotton fabric	100-300	35-55	3	[247]
Intraocular Lenses					
Gatifloxacin	Hydrophobic acrylic[a]	80-250	35-55	0.5-4	[248]
DXP	PMMA	80-200	35-60	2	[124]
Ciprofloxacin	PMMA	80-200	35-60	2	
Methotrexate	Acrylate-methacrylate cop.[b]	80-250	35	0.5-4	[250]
DXP	P-HEMA	80-200	35	0.5-4	[249]
Ciprofloxacin	P-HEMA	80-200	35	0.5-4	
Wound Dressing					
Borage oil	Polyurethane	90-300	35-55	0.5-16	[251]

[a] Benzyl methacrylate/methyl methacrylate copolymer

[b] Copolymer

3.6.3 The role of the supercritical solvent impregnation in the formation of drug-loaded aerogels and microporous foams

The supercritical solvent impregnation technique was used as a downstream treatment of the supercritical foaming process only two times [256,257]. Moreover, as shown in table 3.4 (see paragraph 3.3) only few times the one-step loading route (which is the simultaneous supercritical foaming and impregnation) has been used. This could be a consequence of the limited understanding of the role of the solubility of the active compounds in $scCO_2$ in the drug loading process. Therefore, more attempts should be done to load microporous foams by means of $scCO_2$.

As far as the aerogels are concerned, instead, the 67 % of the works published in the last five years loaded the active compound, by using supercritical solvent impregnation, in a pre-formed aerogel. Moreover, as highlighted in the paragraph 3.2.1, the remaining 33 % used a wide variety of loading routes.

Summary of the conclusions

In this section, the main conclusions from the analysis of the articles that loaded active compounds in carriers by using the supercritical impregnation method are listed:

1) the aerogels have been the most used supports loaded by the supercritical impregnation technique, probably due to their very high porosity and surface area. The 67 % of the works published in the last five years loaded the active compound in a pre-formed aerogel. The wide variety of loading routes employed by the remaining 33 % of works (discussed in the par. 3.2.1) further confirms the importance of the supercritical solvent impregnation technique in the preparation of drug-loaded aerogels.

2) The two LCA analyses performed by De Marco et al. [235,236] showed that the supercritical drying and supercritical solvent impregnation processes are the ones that most affects the environmental impact of the preparation of the aerogels. According to the authors, this is mostly due to the high consumption of electrical energy related to the condensation and recycling of carbon dioxide.

3) The zinc oxide (ZnO) has been, for the first time, used to achieve drug amorphization by means of the $scCO_2$. Ibuprofen and clotrimazole have been successfully loaded into ZnO mesoporous carriers; however, many different types of active compounds should be employed in the future to test the potentiality and expand the applicability of this adsorbent material.

4) In 2018, Gurikov and Smirnova [107] highlighted the fact that the drug loading in porous supports is not necessarily limited by the poor $scCO_2$-solubility of the drugs. Many results [106,109,240] allowed me to state that this could be extended to the polymers.

5) The supercritical impregnation technique has been compared with the wet impregnation [108], subcritical impregnation [232], and conventional solvent impregnation [111] ones. In each case, the $scCO_2$ impregnation method provided the highest drug loadings.

6) As far as the microporous foams are concerned, the use of the supercritical impregnation method to load drugs in their carrier is still limited. This could be a consequence of the limited understanding on the role of the drug solubility in $scCO_2$ on the economy of the process. Therefore, more attempts should be done to load microporous foams by means of $scCO_2$.

CONCLUSIONS

The potential of the supercritical fluid technology in the production of drug delivery systems has been widely explored over the years. The analysis of the studies published in the 2016-2020 period has showed that many efforts have been devoted to this purpose, especially for the production of polymeric micro- and nanoparticles, aerogels, microporous foams, liposomes, solid lipid nanoparticles, as well as for using the supercritical impregnation to load already-formed carriers with drugs.

The large variety of the characteristics of the drug delivery systems that could be produced with the SCF technology does not allow a generalized conclusion to be drawn as far as the "which is the best system" is concerned. In fact, each system displays different advantages and disadvantages. Therefore, the choice of the most suitable carrier, or the most advantageous process to achieve its drug loading, should be done case-by-case. However, the analysis of the most recent advances of the above-mentioned drug delivery systems provides a general overview that may help the researchers decide on which technologies they should invest their efforts. My personal opinion is below reported, in order to propose some guidelines in this choice.

Among the many possible parameters that could be used to discriminate one process from another, two of the most important are (i) the solvent-free nature of the process and (ii) its scalability, which is favoured by a continuous configuration and/or its simplicity (including the number of steps required).

The two best candidates to produce polymeric micro- and nanoparticles are the SAS and PGSS processes. Based on the two above-cited parameters, the SAS process, which has been the most used technique in the last years, has a continuous configuration. However, it is not solvent-free. The PGSS process, instead, has a batch configuration, but is completely solvent-free. It must be pointed out that the solubility of the involved drug plays a key role in the choice between the two processes. In fact, the SAS process is limited to solutes that are both not soluble in $scCO_2$ and hydrophobics, so in the other cases the choice would fall on the PGSS technique. Given the successfully results of many pharmaceutical formulations, more efforts should be devoted to the possible scalability of these two processes (especially in the case of the PGSS since in the pharmaceutical industry the batch small-scale productions are often employed).

As far as the aerogels are concerned, the SCF technology is involved both in the supercritical drying and supercritical impregnation processes. Both processes are fundamental to produce aerogel drug delivery systems. The supercritical drying, in fact, is the only technique that preserves the mechanical stability of the dried alcogel. Moreover, the supercritical impregnation is the only consolidated route to load solutes into

an aerogel. On the other hand, the LCA analyses performed by De Marco et al. [235,236] have highlighted the huge environmental impact that the SCF-based processes may have on the entire aerogel production process, which complicates its launch into the marketplace. It must be pointed out, however, that the aerogels are the most used carriers to load a drug with the supercritical impregnation technique, probably due to their high porosity and surface area. Therefore, it is important to try to improve its layout in order to reduce its environmental impact.

When the desired pharmaceutical application falls in the field of tissue engineering, the PCL-based microporous foams are the ideal carriers and supercritical foaming is a good candidate to overcome the drawbacks of the conventional techniques. From an industrial point of view, the extrusion foaming offers the best opportunities, due to its continuous configuration and high productivity.

The lipid-based carriers, i.e. liposomes and solid lipid nanoparticles, are innovative and emerging drug carriers. The use of physiological lipids makes them much more attractive when compared to the polymers of the inorganic supports employed in the production of polymer micro- and nanoparticles, aerogels, microporous foams, which are biocompatible but not biodegradable. If the attention is focused on the carrier itself, the solid lipid nanoparticles seem to overcome some drawbacks of the liposomes, such as the drug leakage and instability during storage. However, only few studies have been published. It is expected that, in the next years, the SLNs will be more and more investigated. If the attention is, instead, focused on the industrial applicability, the SuperLip technique to produce liposomes, despite its young age, seems to have a great potential in the next future.

Although the supercritical impregnation is a well-established method to load active compounds in a wide variety of carriers, it is important to consider that it requires an already-produced carrier. Therefore, it should not be necessarily considered as an alternative to the other discussed carriers, but a possible integration. From a process point of view, the supercritical impregnation could be integrated (as already attempted with the aerogels) to the other SCF processes, so including an optimized recirculation of the $scCO_2$.

List of symbols and abbreviations

μ	Dynamic viscosity, μPa · s
5-Fu	5-Fluorouracil
ACN	Acetonitrile
ALA	α-lipoic acid
AMOXI	Amoxicillin
AMPI	Ampicillin
API	Active Pharmaceutical Ingredient
AS	2-phospho-L-ascorbic trisodium salt
ASES	Aerosol Solvent Extraction System
BCS	Biopharmaceutical Classification System
BSA	Bovine serum albumin
BSM	Balangu seed mucilage
CAN-BD	Carbon Dioxide Assisted Nebulization with a Bubble Dryer
CAP	Cellulose acetate phthalate
C_b	Bulk concentration of a drug in an aqueous solution, kg · m^{-3}
CH	Chitosan hydrochloride
Chol	Cholesterol
CO_2	Carbon dioxide
CoQ_{10}	Coenzyme Q_{10}
CS	Chitosan
C_s	Solubility of a drug in an aqueous solution, kg · m^{-3}

CTZ	Clotrimazole
d	Diameter of a particle, μm or nm
D	Diffusion coefficient, $m^2 \cdot s^{-1}$
DCM	Dichloromethane
DELOS	Depressurization of an Expanded-Liquid Organic Solution
DESAM	Depressurization of an Expanded Solution into Aqueous Media
DM	Dexamethasone
DMF	N-dimethylformamide
DMSO	Dimethylsulfoxide
DOX	Doxorubicin hydrochloride
DXP	Dexamethasone 21- phosphate disodium salt
EA	Ethyl acetate
EC	Ethyl cellulose
EE	Encapsulation efficiency
EUG	Eugenol
EWP	Egg white protein
F	Absolute bioavailability of a drug
FHCO	Fully hydrogenated canola oil
FHSO	Fully hydrogenated soybean oil
FITC	Fluorescein Iso-Thiocyanate
FO	Fucoxanthin-rich oil
F_{rel}	Relative bioavailability of a drug

GAMA	Gas-Assisted Melting Atomization
GAS	Gas Antisolvent Crystallization
GCO	Green coffee oil
GFs	Growth factors
GLR-EL	Gas to Liquid Ratio of the Expanded Liquid
GT-PVA	Gum tragacanth-polyvinyl alcohol
H	Hydrogen
h	Thickness of the diffusion layer of a bio-membrane, m
HC	Hydrocortisone
HCPT	10-Hydroxycamptothecin
HGNs	Hollow Gold Nanospheres
HMFNs	Hollow mesoporous ferrite nanoparticles
HPMC	Hydroxypropyl methylcellulose
HPβCD	Hydroxypropyl-β-cyclodextrin
IBU	Ibuprofen
IUPAC	International Union of Pure and Applied Chemistry
J	Diffusive flux, $kg \cdot m^2 \cdot s^{-1}$
K	Mass transfer coefficient
LA	Lauric acid
LCA	Life cycle assessment
LLDPE	Linear low-density polyethylene
LUVs	Large unilamellar vesicles

M	Mass of a drug, kg
MCMF	Mixture critical mole fraction
MCP	Mixture critical point
M_d	Mass of a drug in a drug/polymer system, g or kg
MEK	Methyl ethyl ketone
MLVs	Multilamellar vesicles
M_T	Total mass of a drug/polymer system, g or kg
N_A	Avogadro constant, 6.02214076×10^{23}
NaCas	Sodium Caseinate
NIR	Near-infrared
NLCs	Nanostructured lipid carriers
NSAID	Nonsteroidal anti-inflammatory drug
OMS	Ordered mesoporous silica
p	Permeability coefficient, $m \cdot s^{-1}$
P	Pressure, bar or MPa
PA-β-CD	Peracetylated β-cyclodextrin
PBS	Phosphate buffered saline
P_c	Critical Pressure, bar
PC	Phosphatidylcholine
PCL	Polycaprolactone
PE	Phosphatidylethanolamine
PEG	Polyethylene glycol

PG	Phosphatidylglycerol
PGSS	Particle from Gas-Saturated Solutions
PHBV	Poly(hydroxybutyrate-co-hydroxyvalerate)
P-HEMA	Poly-2-hydroxyethyl methacrylate
PIC	Polyinosinic-polycytidylic acid
PLA	Polylactic acid
PLGA	Poly-lactic-co-glycolic acid
PLLA	Poly(L-lactic) acid
PMMA	Poly(methyl methacrylate)
PSD	Particle size distribution
PUFAs	Polyunsaturated fatty acids
PVP	Polyvinylpyrrolidone
r	Radius of a particle, μm or nm
R	Universal gas constant, $J \cdot mol^{-1} \cdot K^{-1}$
RESOLV	Rapid Expansion of Supercritical Solutions into a Liquid Solvent
RESS	Rapid Expansion of Supercritical Solution
RESSAS	Rapid Expansion of Supercritical Solutions into an aqueous solution
RESS-N	Rapid Expansion of Supercritical Solution with a Non-Solvent
RESS-SC	Rapid Expansion of Supercritical Solutions with a Solid Co-solvent
RSM	Response Surface Methodology
RVL	Trans-resveratrol
S	Surface area, $m^2 \cdot g$

SAA	Supercritical-Assisted Atomization
SAA-HCM	Supercritical-Assisted Atomization with a Hydrodynamic Cavitation Mixer (SAA-HCM)
SAILA	Supercritical Assisted in a Liquid Antisolvent
SAS	Supercritical Antisolvent
SAS-DEM	Supercritical Antisolvent with Drug-Excipient Mixing
SAS-EM	Supercritical Antisolvent with Enhanced Mass Transfer
$scCO_2$	Supercritical carbon dioxide
SCF	Supercritical Fluid
SCG	Spent coffee ground
scRPE	Supercritical Reverse Phase Evaporation
SEA	Supercritical-Enhanced Atomization
SEDS	Solution Enhanced Dispersion by Supercritical Fluids
SFEE	Supercritical Fluid Extraction of Emulsions
SLN	Solid Lipid Nanoparticle
SSI	Supercritical Solvent Impregnation
SuperLip	Supercritical Assisted Liposome Formation
SUVs	Small unilamellar vesicles
T	Temperature, °C or K
t	time, s or h or min
T_c	Critical Temperature, °C or K
T_g	Glass transition temperature o a polymer, °C or K
TGM	Transglutaminase

t_{JB}	Time of jet break-up, s
T_m	Melting point of a polymer, °C or K
TMC	N-trimethyl chitosan
t_{STV}	Time of surface tension vanishing, s
UVs	Unilamellar vesicles
VLE	Vapor-liquid equilibria
WPI	Whey protein isolate
ZnO	Zinc oxide
β-TCP	β-tricalcium phosphate
γ	Surface tension, $kg \cdot s^{-2}$
ΔG_{homo}	Energy barrier to achieve bubble nucleation, J
ΔP	Difference between the pressure in the nucleus of a bubble of a gas and the pressure in a polymer/gas solution, Pa
π	Mathematical constant, 3.141592653589793

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