Supercritical Fluid Applications in Novel Antimicrobial Materials

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The extraordinary properties of supercritical fluids such as high density, near-zero surface tension, and high diffusivities enable the uniqueness and numerous advantages of the materials obtained. The application of supercritical fluids is a powerful tool in the development of novel materials with antimicrobial activity desperately needed in the time of increasing bacterial resistance to antibiotics and the dramatic appearance and spread of not only multidrug-resistant (MDR) but also pandrug-resistant (PDR) bacterial strains. MDR is defined as the resistance to at least one antibiotic from at least three different categories, while PDR is defined as non-susceptibility to all drugs in all antimicrobial categories.

Keywords: supercritical fluid ; carbon dioxide ; antibacterial activity ; bacterial resistance ; multidrug resistance ; antibacterial materials

1. Introduction

The application of supercritical fluids is a powerful tool in the development of novel materials with antimicrobial activity desperately needed in the time of increasing bacterial resistance to antibiotics and the dramatic appearance and spread of not only multidrug-resistant (MDR) but also pandrug-resistant (PDR) bacterial strains. MDR is defined as the resistance to at least one antibiotic from at least three different categories, while PDR is defined as non-susceptibility to all drugs in all antimicrobial categories [1]. According to the World Health Organization (WHO), antibiotic resistance is one of the biggest threats to global health, food security, and development today and it can affect anyone, of any age, in any country [2]. As stated in the Centers for Disease Control and Prevention (CDC) Antimicrobial Resistance Threats Report for 2019 [3], more than 2.8 million antibiotic-resistant infections occur in the U.S. each year and more than 35,000 people die as a result. The report lists 18 antibiotic-resistant bacteria and fungi into three categories based on the level of concern to human health—urgent, serious, and concerning. Common to all urgent threats is that nearly all those infections happen in patients who recently received care in a healthcare facility, identifying hospitals as places where MDR strains occur and from which they spread to the community. According to the CDC, the main endangered categories are patients who have surgery (among them, 1.2 million women who had a caesarean section in 2017), chronic conditions (e.g., diabetes), organ transplant recipients, patients who receive dialysis treatment, and people receiving chemotherapy [4]. As reported by the WHO regional office in Europe, the health burden of infections caused by antimicrobial resistance in the European Union is similar to that of influenza, tuberculosis, and HIV/AIDS combined. In 2015, there were 670,000 antibiotic-resistant infections in the European Union, which resulted in 33,000 deaths [5]. The problem of bacterial resistance to antibiotics is not related to humans and hospitals only. It is also an urgent issue in veterinary hospitals and clinics and, among others, the question of how to treat companion animals is raised.

2. Supercritical Solvent Impregnation (SSI)

This technique provides broad possibilities for material design when active substances are soluble in $scCO_2$. In this process, the active substance is dissolved in $scCO_2$ and the supercritical solution is brought to contact with a solid phase to be impregnated. The process may be conducted in a batch or semi-continuous mode (**Figure 1**). The supercritical fluid easily penetrates the solid phase due to the absence of surface tension, carrying the active component into the matrix. If there is a possibility of hydrogen bonding between the active substance and the solid (e.g., polymer chains), high loadings of the active substance may be achieved [6]. At the same time, the solid phase can be impregnated through the whole volume, which is a significant advantage of SSI over the conventional impregnation techniques where surface tension prevents liquid penetration into the solid matrix. If there is no possibility of hydrogen bonding, the active substance can be deposited in the solid phase simply by the decompression. Decompression and CO_2 transfer from the supercritical into the gas phase lead to the decrease of the solubility of the active component in CO_2 and its precipitation in the solid phase. Besides, no liquid effluent is generated in this process and no drying step is needed, which makes SSI environmentally

friendly with considerably reduced energy requirements in comparison to the conventional impregnation processes. Thanks to these advantages, breakthroughs were made in the wood industry (Superwood, Hampen, Denmark) in spruce wood treatment as well as in the textile industry (DyeCoo, Weesp, Netherlands) in the dyeing of all kind of synthetic fabrics and yarn by applying this technology on the industrial scale.

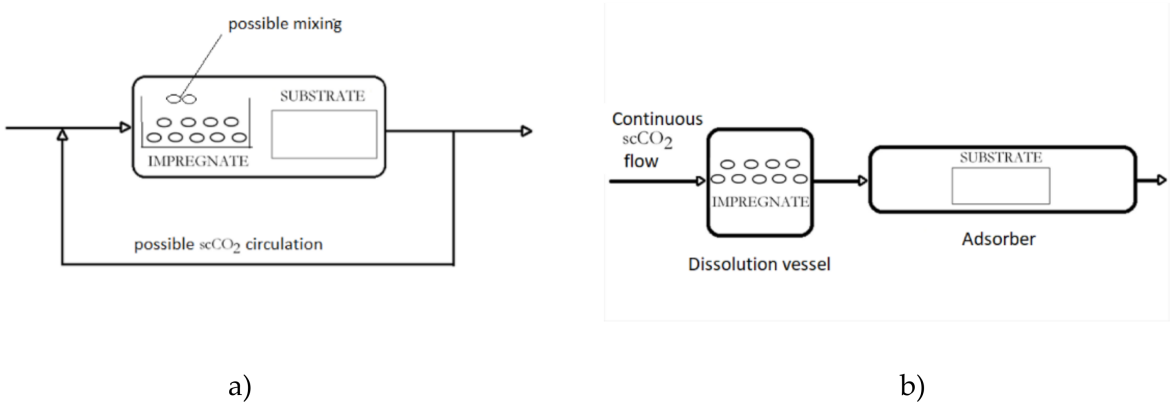

Figure 1. Simplified presentation of Supercritical Solvent Impregnation (SSI) modes: (**a**) batch; (**b**) semi-continuous.

3. Supercritical Assisted Impregnation (SAI) and High-Pressure Assisted Impregnation (HPAI)

These techniques may be applied to the impregnation of active substances, which are less soluble in $scCO_2$ or not soluble at all. In those processes, an active component is dissolved in an appropriate liquid solvent, and the liquid phase is brought to contact with a solid to be impregnated in the presence of supercritical or high-pressure carbon dioxide ($hpCO_2$—CO_2 under high pressure but not in the supercritical region). A simplified presentation of the process is presented in **Figure 2**. In this way, good transport properties of carbon dioxide in a liquid or supercritical state promote the contact between the liquid and solid. Quite often, swelling of the solid surface occurs, which also promotes the impregnation process. In the process, a considerably smaller quantity of the liquid phase to dissolve the active component is usually employed in comparison to the conventional impregnation from liquids. Also, a cosolvent may be added to $scCO_2$ to enhance the interaction between the active substance and the supercritical phase. The main difference between the SSI and SAI techniques is the following: in SAI, a contact between 3 phases exists (solid substrate, supercritical phase, and liquid phase) no matter what is the solubility of the active component in $scCO_2$. The active principle may be dispersible or soluble in $scCO_2$. Unlike, in the case of SSI, the substrate is in contact with the supercritical phase only.

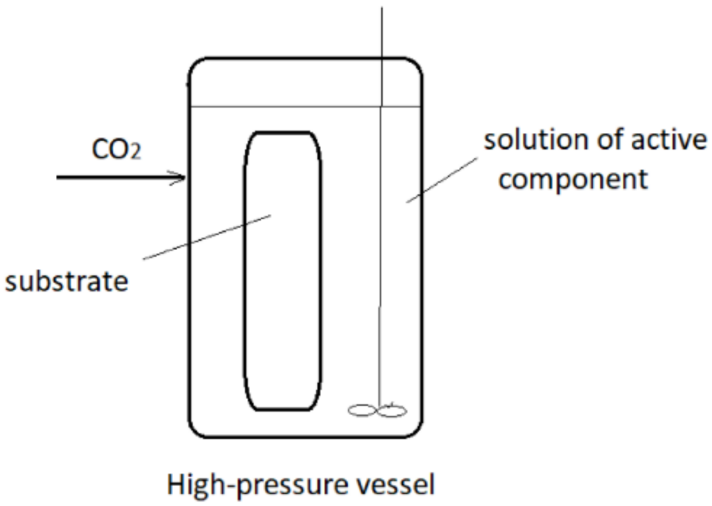

Figure 2. Simplified presentation of Supercritical Assisted Impregnation (SAI)/High-Pressure Assisted Impregnation (HPAI) process.

The first and essential application of HPAI was in leather tanning [7]. Leather is produced when an impregnate (tanning agent—usually chromium-III-salt) reacts chemically with collagens in pretreated animal hides in an aqueous solution. Leather manufacturing conventional process is exceptionally intensive concerning the consumption of resources, and an estimated overall amount of about 14 million m^3 of wastewater per year is generated worldwide [7]. In the new process, the skins are contacted with a tanning solution and subsequently contacted with $hpCO_2$ (>3 MPa) in rotating tanning drums. CO_2 is partly diffusing into the skin and in the tanning solution. The leather of high quality is obtained already after

2 h of contact with CO_2. There is no wastewater generation in the new process. In comparison to conventional method, consumption of the tanning agent is decreased for more than 50% and there is no need for the addition of sodium salt [7]. Further in text, results on the implementation of HPAI and SAI in the production of novel antibacterial materials will be presented.

Mölders et al. [8] applied carbon dioxide in a liquid (12 MPa, 20 °C) and supercritical state (12 MPa, 40 and 80 °C) to impregnate polycarbonate with silver nitrate as an antibacterial agent. The experiments were performed in a batch mode in a high-pressure view cell but also scaled up in a high-pressure vessel of 2 L. The samples were submerged in an ethanol solution of silver nitrate, heated, pressurized, and impregnated for 10 min. In parallel, submerging tests were performed under atmospheric pressure. Impregnation assisted by $scCO_2$ was superior in comparison to the impregnation in liquid CO_2 and far more superior than submerging at ambient pressure, providing silver content of around 23.4 mg/kg polymer. HPAI with liquid CO_2 provided silver content of 2.4 mg/kg polymer while submerging under atmospheric pressure and 80 °C resulted in a content of 0.2 mg/kg polymer. The samples impregnated by both supercritical and liquid carbon dioxide showed strong antimicrobial activity against *E. coli*. Abrasion as well as UV-radiation and led to a loss of antimicrobial activity of the samples impregnated at 20 °C. However, the samples impregnated at 80 °C resisted the tests. The leaching of the samples was analyzed to determine the toxicity on humans, and the toxicity could not be confirmed [8]. These excellent results opened a way towards production of antibacterial surfaces which could be applied to many elements in hospitals such as doorknobs, switches, handrails, buttons, surfaces for placement of medical devices, etc.

The subsequent studies deal with the development of a novel class of antibacterial mats based on carbon nanomaterials and silver nanoparticles (NPs) [9][10]. Carbon nanotubes and nanofibers wrapped by silver NPs were fabricated with the assistance of $scCO_2$ [9]. The SAI process was performed with the ethanol solution of the carbon materials, the silver precursor ($AgNO_3$), and glucose as a reducer at 12 MPa and 65 °C for 3 h. The TEM and SEM images revealed that carbon nanotubes/AgNPs hybrids possess a preferable assembled structure. Experimental results demonstrated considerable antibacterial activity of tested materials against *E. coli* [9]. In the following study, Haldorai et al. [10] reported results on graphene oxide treatment with silver NPs in the presence of $scCO_2$ to produce a material with photocatalytic and antibacterial activity. Graphene oxide was treated in an ethanol solution with $AgNO_3$ and glucose as a reducer at 12 MPa and 65 °C for 3 h. The graphene oxide modified with silver NPs displayed an excellent visible-light photocatalytic performance in degrading Rhodamine 123 dye and acetaldehyde as well as significant antibacterial activity against *E. coli*, *S. aureus*, and *Listonella anguillarum* [10].

Based on the available literature survey, results on the SAI and HPAI applications are scarce but impressive. These techniques are a powerful tool yet to be applied to the design of novel materials. In the next section, the review will present combined processes of SAI/SSI and polymerization in $scCO_2$, which opened possibilities for the design of unique antibacterial mats.

4. Supercritical Solvent Impregnation or Supercritical Assisted Impregnation Coupled with Polymerization in $scCO_2$

The subsequent studies deal with the application of composite polymers known as interpenetrating polymer network (IPN) [11] in biomedical purposes. Solvent-free IPNs can be produced using $scCO_2$ [11]. In this process, one or more monomers are dissolved or dispersed in supercritical or near-critical carbon dioxide and brought to contact with a polymer to be impregnated (SSI or SAI). The polymerization and crosslinking of monomers can be performed by a radical starter that can be impregnated into the polymer matrix simultaneously with the monomer(s). The polymerization reaction may be triggered by the temperature increase in the supercritical conditions upon the impregnation, consequently leading to the formation of IPN. Because there is no chemical bonding between the polymer and the network (between two polymers), each material retains its individual properties in the blend. This allows for a variety of applications for the novel type of materials synthesized in an environmentally friendly way [11]. The different behavior of the polymers in IPN in combination with the solvent-free appearance of the final product makes these materials especially attractive for the design of medical devices.

Steffenson et al. [12][13] demonstrated that silicone elastomers used in catheter production could be modified to form an IPN material with a poly(2-hydroxyethyl methacrylate) (PHEMA)-based hydrogel. Extruded silicone [12] and poly(dimethylsiloxane) (PDMS) silicone elastomer [13] were impregnated with (2-hydroxyethyl) methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) in the presence of cosolvent(s) and a radical starter in $scCO_2$. The impregnation was performed at 40 °C and pressures 20–25 MPa for a time from 20 min to 16 h, depending on the contact between phases. The polymerization proceeded at 75 °C and 30–36 MPa for 3 h. Fabricated IPN materials retained mechanical properties similar to those of the original silicone elastomer while acquired the ability of the hydrogel to swell in aqueous

media. PHEMA content was in the range of 13–38% (*w*/*w*). It was shown that the hydrogel formed an interconnected hydrogel network in aqueous media when the content of PHEMA was at least 25%. The optimized IPN material was loaded with the antibiotic ciprofloxacin, and the resulting drug release inhibited bacterial growth of *S. aureus* when placed on agar [12]. In the further study [13], it was demonstrated that samples containing 25% (*w*/*w*) hydrogel loaded in a 5 mg/mL ciprofloxacin medium inhibited *S. aureus* growth upon incubation in broth with high efficacy for 29 days whereby no biofilm was observed on the material. These substantially significant results opened a possibility for the design of novel medical devices for long-term clinical use.

In the next study, Stenger et al. [14] produced IPN catheters by the polymerization and crosslinking of PHEMA in silicone elastomer in $scCO_2$ as previously described [12][13]. The system was loaded with dicloxacillin alone or in combination with thioridazine and tested against methicillin-sensitive *S. aureus* and MRSA. The drug-loaded IPN material was proven to be effective in in vitro experiments. Moreover, the IPN catheters were tested in a novel porcine model of central venous catheter-related infection, in which they were found to decrease the frequency of infection significantly [14].

The results presented on the preparation and application of IPN materials with controlled release of antibiotics are of the utmost importance bearing in mind that bacterial colonization with subsequent biofilm formation constitutes a severe and frequent problem associated with the use of many polymer materials commonly applied for medical devices [13]. Urinary tract infections are the most frequently occurring nosocomial infections [15]. During the long-term use of catheters, the risk of urinary tract infections increases rapidly over time and reaches 50% after 7–10 days [13][16]. To illustrate the significance, in the USA, approximately 250,000 of vascular catheter-related bloodstream infections occur annually associated with a mean hospital length stay of 22 days, increasing the hospital cost from US$ 3000 to 56,000 per patient, and with mortality rates of 12–25% for critically ill patients [13][17][18].

The use of carbon dioxide as a polymerization reaction medium has been investigated continuously since it is a green solvent with many advantages over conventional solvents [19][20]. Correia et al. [19] reported a method to obtain biocompatible 2-oxazoline-based oligomers quaternized with different amines using $scCO_2$ as a reaction medium. Oligo(2-methyl-2-oxazoline) and oligo(2-bisoxazoline) quaternized with N,N-dimethyldodecylamine were shown to be very efficient biocidal agents showing fast killing rates against *S. aureus* and *E. coli*. In a further study, Correia et al. [21] presented a novel approach to the design of antibacterial materials by combining plasma technology, SSI, and polymerization in $scCO_2$. In this study [21], oligo(2-methyl-2-oxazoline) quaternized with N,N-dimethyldodecylamine was grafted to a chitosan (CHT) scaffold. Chitosan scaffolds were prepared with the freeze-drying method, and subsequently, their surface was activated by argon plasma treatment. Upon the activation, the scaffolds were subjected to the SSI with the monomer 2-isopropenyl-2-oxazoline for 24 h at 18 MPa and 40 °C. After this grafting step, another monomer, together with an initiator, was introduced into the system, and the polymerization took place at 18 MPa and 65 °C for 20 h. In the final step, a tertiary amine was added to the reactor and the reaction was performed at 18 MPa and 40 °C for 20 h. The material obtained efficiently killed *S. aureus* and *E. coli* cells upon direct contact and prevented bacterial adhesion to the materials surface and biofilm formation. The material was shown to be suitable for water purification over ten cycles of reuse, efficient within minutes of contact and without leaching to the water [21].

Cationic antimicrobial peptides are promising antibacterial agents [19] and, as presented, can be synthesized and grafted to solid carriers in $scCO_2$ [21]. Their mechanism of action is based on electrostatic forces and subsequent interaction between the cationic peptide and the anionic lipopolysaccharide in outer membrane of Gram-negative bacteria or the negatively charged teichoic acids attached to the thick layer of peptidoglycan present in the surface of Gram-positive bacteria [19][22]. It is believed that bacteria cannot develop resistance to these antibacterial polymers because the mechanism of action depends on the fundamental characteristics of the microbial cytoplasmic membrane. Therefore, the development of resistance would require bacteria to change their membrane structure completely [19][23].

5. Supercritical Foaming

Dissolution of $scCO_2$ in polymers may increase chain mobility and induce polymer swelling in amorphous and semi-crystalline polymers, at the same time decreasing their melting point under the supercritical conditions [24]. Optionally, a cellular structure of the polymer matrix (foam) can be formed by inducing phase separation with a pressure and/or temperature change. Supercritical foaming was previously mentioned in connection to SSI of PCL [25]. In this part, it will be commented more since it is not connected with the SSI technique only, as it will be seen from the next example.

García-González et al. [26] reported results on the preparation of PCL-chitosan scaffolds containing vancomycin as an antimicrobial agent by $scCO_2$ foaming, aimed for bone regeneration purposes. The foaming was performed from solid dispersions of PCL, chitosan, and the antibiotic. Powdered mixtures with different PCL, vancomycin, and chitosan

contents were introduced into cylindrical Teflon molds, compacted, and exposed to $scCO_2$ at 40 °C and 14 MPa for 1 h with subsequent decompression under the CO_2 flow rate of 1.8 g/min. The obtained scaffolds showed a suitable combination of morphological (porosity, pore size distribution, and interconnectivity), and vancomycin release behavior, as well as the biological properties (cell viability and proliferation, osteo differentiation, and tissue-scaffold integration). The scaffolds sustained vancomycin release in PBS for more than two weeks and showed considerable antibacterial activity against *S. aureus* and *E. coli* [26]. This study exemplifies a method for the incorporation of a substance poorly soluble in $scCO_2$ (vancomycin) into polymeric foams. Subsequent studies relate to the incorporation of a $scCO_2$ soluble substance into polymer matrix by foaming and SSI as a one-step process.

Ivanovic et al. [24] reported results on the impregnation and foaming of PCL and polycaprolactone-hydroxyapatite (PCL-HA) composites with thymol in $scCO_2$ for obtaining functional porous scaffolds. The effect of $scCO_2$ sorption kinetics on the swelling, foam morphology, and thermal behavior of the PCL and PCL-HA materials was studied, whereby sorption isotherms were determined using a magnetic suspension balance at 10–30 MPa and 35–40 °C and thermal properties using high-pressure differential calorimetry (HP-DSC) at pressures 4.6–17.0 MPa. In the next step, SSI of PCL and PCL-HA with thymol was performed simultaneously with the foaming to produce scaffolds with antimicrobial properties and controlled microstructure. The pressures in the range 13–17 MPa and 10% of HA were proven to be favorable for the creation of scaffolds with satisfying foam microstructure (mean pore size ~200–300 µm), filler distribution, and thymol loadings (12–18%) [24].

Milovanovic et al. [27] prepared foams loaded with thymol in a one-step SSI-foaming process from amorphous, medical grade poly(D,L-lactic acid) (PLA), and poly(D,L-lactic-co-glycolic acid) (PLGA). The impregnation performed with different CO_2 densities (273–815 kg/m3) and short processing times (2 and 4 h) enabled thymol loading of 0.92–6.62%. The process was optimized for each polymer to obtain stable microcellular foams upon the system decompression. The highest thymol loading (6.62%) was obtained for the copolymer PLGA, whereby the sample exhibited controlled thymol release within 72 h in media having pH values from 1.1 to 7.4 [27].

6. Supercritical Drying of Metal-Carrying Gels

Synthesis of metallic nanoparticles is of great importance for the application in catalysis, electronics, and optics and for the design of materials with antibacterial properties [28]. The preparation of metal colloids by the reduction is a simple reaction. Still, the control of particle size, shape, and dispersion stability requires careful control of the synthetic conditions because the process is sensitive to balances between nucleation and crystal growth [28][29]. One approach to facilitate both the synthesis control and immobilization is the use of porous materials as reaction medium, which might be a hydrogel [28]. The hydrogel can further be transformed into an alcogel by the solvent exchange and subsequently to an aerogel by supercritical drying resulting in a highly porous added value material for a wide range of applications [28][30][31]. Aerogels are characterized by the small bulk densities (0.017–0.021 g/cm^3), low thermal conductivities, big surface area (200–800 m^2/g), and proven capability for the controlled release of incorporated substances [30][32][33][34].

Cai et al. [28] synthesized silver, gold, and platinum nanoparticles in the cellulose hydrogel by hydrothermal reduction by the cellulose itself (for silver at 80 °C for 24 h) or by adding a reductant (for gold and platinum). To produce aerogels, the water of metal-cellulose hydrogels was exchanged to ethanol, and two-step batch drying in carbon dioxide was applied. First, the ethanol was replaced with liquid CO_2 at 5.3 MPa and 4 °C for 6 h and then supercritical drying took place at 10 MPa and 40 °C for 0.5 h, with subsequent slow decompression. The aerogels obtained were characterized by the high transmittance, porosity, and surface area as well as good mechanical strength [28].

Raman et al. [30] reported results on the synthesis of calcium-alginate aerogels augmented with zinc and silver for potential application in wound healing. By the combination of high-pressure gelation (room temperature, 50 MPa, for 24 h) and supercritical drying with a continuous flow of $scCO_2$ (at 50 °C and 12 MPa for 2 h and under 20 g/min CO_2 flowrate), hybrid Ca–Zn particles as well as hybrid Ca–Zn–Ag aerogel monoliths and particles were produced. The metal ions were released into supernatants upon the aerogels swelling in aqueous solutions in the amounts needed for a wound dressing [30].

In the subsequent study [31], pectin-TiO_2 nanocomposite aerogels were prepared via the sol-gel process, consecutive solvent exchange step, and supercritical drying. The drying was performed at a temperature and pressure in ranges 50–60 °C and 11–13 MPa, respectively, for 5 h and with the $scCO_2$ flow rate of 0.2 kg/h. In the presence of TiO_2 nanoparticles, mechanical, thermal, and antimicrobial properties (against *E. coli*) of pectin-based aerogels were improved in comparison to the control ones. Thus, the aerogels may provide antibacterial protection and, to some extent, thermal protection due to the low thermal conductivity and may have a potential application in packaging for sensitive items [31].

7. Other Methodologies Applied to the Development of Antibacterial Materials

In this part, more ideas for the utilization of the extraordinary properties of supercritical fluids in the design of antibacterial materials will be presented. In the recent study, Li et al. [35] presented results on the synthesis of the hybrid Cu_2O/TiO_2 nanocomposites with the enhanced photocatalytic antibacterial activity against *Acinetobacter baumannii*. *A. baumannii* is Gram-negative bacteria, widespread, and multidrug resistant often found in intensive care unit, where it causes intra-hospital infections including sepsis, urinary tract infections, ventilator-acquired pneumonia, and wound infections [35][36]. In this study [35], a stable combined p-n Cu_2O/TiO_2 heterojunction was prepared by a supercritical solvothermal process in ethanol. The supercritical solvothermal process is regarded as a powerful tool for the synthesis of heterojunction materials with considerable advantages over conventional methods. Compared to the physical mixture, aqueous reduction, photochemical, and hydrothermal routes, all accompanied with weak combination, nonuniform size distribution, and easy aggregation, the application of supercritical fluids can provide a stable combination between Cu_2O and TiO_2 with the uniform dispersion and small crystal size resulting in the large special surface areas with a mesoporous structure and the expended visible-light absorption [35]. It is believed that the high rate of crystal nucleation without the easy crystal growth is the consequence of the high temperature and pressure applied in the supercritical state [35][37][38]. Cu_2O/TiO_2 composites were synthesized in supercritical ethanol at 243 °C and 6.4 MPa. In this process, $Cu(NO_3)_2{\cdot}5H_2O$ and *tetrabutyl* titanate were dissolved into absolute ethanol and kept at the operating conditions for 70 min to complete the synthesis. The bactericidal activity of the 5.0% Cu_2O/TiO_2 sample in the case of *A. baumannii* was 100% under the visible-light irradiation within 30 min. Moreover, the 5.0% Cu_2O/TiO_2 nanocomposite displayed the significant visible-light antibacterial activities (up to 100% mortality in 30 min) against other pathogenic bacteria including *P. aeruginosa*, *E. coli*, and *S. aureus*. Based on the experimental findings, it was presumed that the Cu_2O/TiO_2 composite first led to the leakage of K^+ ion with the disrupted permeability of the cell membrane and then induced the formation of inorganic compounds from the cell decomposition. The composites were shown to be durable due to the stable p-n Cu_2O/TiO_2 heterojunction obtained under the supercritical conditions. The durability and photocatalytic antibacterial activity of the composites present significant potential for the application in disinfection [35].

Bhartia et al. [39] used $scCO_2$ for grafting semiconductor surfaces with monolayers of alkylthiols. Hydrogen-terminated semiconductor surfaces were exposed to alkylthiols dissolved in $scCO_2$ at 100 °C and 10 MPa for the chemical reaction and establishment of the strong and nonpolar Si–S surface bond. The deposited monolayer on oxide-free silicon was stable, dense, and able to passivate the surface for more than 50 days (10 times than the conventional methods) without any oxide formation in the ambient atmosphere. The material resisted cell proliferation on the surface for more than 15 days and, besides the application in electronics, is envisaged for biomedical and antimicrobial applications. The inert nature of CO_2, as the ideal contamination-free isolated processing environment for grafting better-quality monolayers, allowed for the production of superhydrophobic and bio-resistant surfaces [39]. In this environmentally free process, drawbacks of conventional technologies are overcome and the product of better quality is obtained.

Katayama et al. [40] used $scCO_2$ to induce large pleat-like wrinkles on the surface of cotton fibers as support for nanoparticles. The cotton was immersed into the water first, and the treatment with $scCO_2$ followed. The process parameters were optimized to produce the appropriate wrinkles. The favorable conditions were found to be a temperature of 40 °C, the pressure of 20 MPa, the contact time of 60 min, and a fast decompression rate of 0.80 MPa/min^{-1}. It is assumed that the wrinkles occur due to the different degasification rates from the inner and surface parts of the fiber during the fast decompression. The material obtained was proven to be a suitable support for TiO_2 nanoparticles of average 35 nm in diameter without the presence of binders [40].

Cuadra et al. [41] used $scCO_2$ as an antisolvent to prepare a new adduct of isonicotinamide with copper(II) propanoate, a ligand complex with strong fungicidal properties. The precipitation was performed by introducing an ethanol solution of the components through a 100-µm nozzle into $scCO_2$ at 40 °C and 10 MPa at a flow rate of 1 mL/min. $ScCO_2$ dissolves in the ethanol, consequently decreasing the ligand complex solubility and leading to the precipitation. Applying the supercritical antisolvent (SAS) technique, crystals 100-fold smaller than those obtained by slow evaporation were produced, indicating a considerable bioavailability enhancement [41].

Imbuluzqueta et al. [42] employed liquid CO_2 (10 MPa, 25 °C) as an antisolvent to produce a novel bioactive hydrophobic gentamicin-filled carrier. In this process, gentamicin was ion-paired with the anionic surfactant Bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT) to obtain a hydrophobic complex (GEN–AOT). The solution of GEN–AOT in acetone was sprayed through a hollow cone nozzle into the CO_2, resulting in the precipitation due to the antisolvent effect and allowing for GEN-AOT micronization. In a further step, the encapsulation of the obtained complex in PLGA nanoparticles was performed by the emulsion solvent evaporation method. The procedure provided NPs with GEN–AOT encapsulation

efficiency of 100% and sustained release of the drug over 10 weeks. It was also shown that neither ion pairing, supercritical fluid processing, nor encapsulation in polymeric NPs affected the bactericidal activity of gentamicin against *E. coli* [42].

Saelo et al. [43] applied Rapid Expansion of a Supercritical Solution into a Liquid Solvent (RESOLV) process to obtain caffeic acid phenethyl ester (CAPE) nanoparticles. The mixture of CAPE, ethanol, and $scCO_2$ at 17.3 MPa and 50 °C was expanded through a nozzle at 80 °C into distilled water. The obtained CAPE NPs were incorporated into methylcellulose films in the process of film preparation by the solvent casting method. Films containing 0.5% of CAPE NPs exhibited antimicrobial properties against *P. aeruginosa*, *C. albicans*, and *Listeria monocytogenes* [43].

Varona et al. [44][45] applied high-pressure techniques PGSS (Particles from Gas Saturated Solutions) and PGSS-drying to encapsulate lavandin (*Lavandula hybrida*) essential oil known for its antibacterial and antiviral properties. Carrier materials investigated were soybean lecithin, n-octenyl succinic anhydride (OSA) modified starch, PCL, and polyethylene glycol (PEG). PGSS was applied to the oil encapsulation into PCL [44] and PEG [45]. In this process, the lavandin oil and polymer were filled together in a high-pressure cell and intensively mixed in the presence of $scCO_2$ for 2 h (the polymer was in a molten state) to reach phase equilibrium. Then, the mixture was depressurized, and due to the rapid expansion through a nozzle to ambient pressure, small particles were formed. The driving force for particle formation is the strong cooling as a consequence of the Joule Thomson effect produced during the expansion. It results in the polymer solidification and a covering layer formation around the essential oil droplets. PGSS-drying was applied to the oil encapsulation into OSA modified starch and soybean lecithin [44][45]. In this process, an oil-in-water emulsion was prepared in which the essential oil constitutes the dispersed phase and OSA-starch/soybean lecithin acts as a surfactant. The emulsion saturated with CO_2 was contacted with the $scCO_2$ in a static mixer and subsequently expanded through a nozzle. The expansion facilitated the formation of extremely fine droplets which dried very fast, while the polymer solidified encapsulating the essential oil. The results showed an enhancement of the antibacterial activity of lavandin oil against *E.coli*, *S. aureus*, and *Bacillus cereus* by the encapsulation due to the protection and control release provided by the carrier [44]. PGSS processes may provide polymer particles of micron size, filled with an antimicrobial agent, and suitable for spraying onto different surfaces.

Recent studies [46][47][48][49] demonstrated the feasibility of $scCO_2$ application in liposome production. Conventional methods of liposome production suffer from drawbacks, such as the difficulty of controlling particle size distribution, micrometric dimensions, low stability, and high solvent residue [48]. To overcome these deficiencies, Santo et al. [46] and Trucillo et al. [47] developed a continuous supercritical assisted process called SuperLip (Supercritical assisted Liposome formation), characterized with good control of particle size distribution, possibility to produce liposomes on a nanometric or micrometric level, liposome stability of over one year, and solvent residue in liposomes lower than FDA limits [47]. The SuperLip process was successfully applied to the production of liposomes with antimicrobial activity loaded with vancomycin [47], amoxicillin [48], ampicillin, and ofloxacin [49]. In this process, an ethanol solution of phospholipids is brought to contact with $scCO_2$ at 10 MPa and 40 °C in a saturator vessel first. The expanded liquid ethanol-$scCO_2$ mixture is subsequently introduced into a high-pressure formation vessel operating at the same temperature and pressure conditions as the saturator. An aqueous solution with an active substance is injected through a nozzle into the formation vessel as well. The atomized droplets of water solution are quickly captured by the phospholipids contained in the fluid phase, creating lamellae around the inner core containing the drug. This is the key step of the $scCO_2$-assisted process and an inversion of the traditional liposome production [48]. These inverted micelles, falling in a water bulk formed at the bottom of the vessel, are covered by a second lipids layer, completing the double-layer structure. The results showed that it was possible to control particle size distribution at the nanometric level, with an encapsulation efficiency of the drug up to 84% [48].

In the following study, Trucillo et al. [50] applied two scCO2-assisted techniques to load alginate aerogels with liposomes containing amoxicillin. The SuperLip process was used first to obtain amoxicillin loaded liposomes. In the next step, liposomes were entrapped in alginate hydrogels. After the water replacement with ethanol, obtained alcogels were subjected to supercritical drying to obtain aerogels. The results demonstrated that ampicillin release time from these meta-carriers was about four days or twice its release time from liposomes alone [50].

References

1. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria:

An international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 2012, 18, 268–281.

2. World Health Organization. Antibiotic Resistance. Available online: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (accessed on 4 March 2020).
3. Centers for Disease Control and Prevention. Antibiotic/Antimicrobial Resistance (AR/AMR). Available online: https://www.cdc.gov/drugresistance/biggest-threats.html (accessed on 4 March 2020).
4. Antibiotic-Resistant Infections Threaten Modern Medicine. Available online: https://www.cdc.gov/drugresistance/pdf/threats-report/Threat-Modern-Medicine-508.pdf (accessed on 4 March 2020).
5. World Health Organization Regional Office for Europe. Moving Towards a Multisectorial Approach to Tackling Antimicrobial Resistance. Available online: http://www.euro.who.int/en/countries/france/news/news/2020/3/moving-towards-a-multisectoral-approach-to-tackling-antimicrobial-resistance (accessed on 20 March 2020).
6. Milovanovic, S.; Stamenic, M.; Markovic, D.; Ivanovic, J.; Zizovic, I. Supercritical impregnation of cellulose acetate with thymol. J. Supercrit. Fluid. 2015, 97, 107–115.
7. Renner, M.; Weidner, E.; Brandin, G. High-pressure carbon dioxide tanning. Chem. Eng. Res. Des. 2009, 87, 987–996.
8. Mölders, N.; Renner, M.; Errenst, C.; Weidner, E. Incorporation of antibacterial active additives inside polycarbonatesurfaces by using compressed carbon dioxide as transport aid. J. Supercrit. Fluid. 2018, 132, 83–90.
9. Niu, A.; Han, Y.; Wu, J.; Yu, N.; Xu, Q. Synthesis of One-Dimensional Carbon Nanomaterials Wrapped by Silver Nanoparticles and Their Antibacterial Behavior. J. Phys. Chem. C 2010, 114, 12728–12735.
10. Haldorai, Y.; Kim, B.K.; Jo, Y.L.; Shim, J.J. oxide nanocomposite as an efficient visible-light plasmonic photocatalyst for the degradation of organic pollutants: A facile green synthetic approach. Mater. Chem. Phys. 2014, 143, 1452–1461.
11. Karthäuser, J. Method of Producing an Interpenetrating Polymer Network (IPN), the IPN and Use Thereof. U.S. Patent No. US7687585 B2, 30 March 2010.
12. Steffensen, S.L.; Vestergaard, M.H.; Groenning, M.; Alm, M.; Franzyk, H.; Nielsen, H.M. Sustained prevention of biofilm formation on a novel silicone matrix suitable for medical devices. Eur. J. Pharm. Biopharm. 2015, 94, 305–311.
13. Steffensen, S.L.; Vestergaard, M.H.; Moller, E.H.; Groenning, M.; Alm, M.; Franzyk, H.; Nielsen, H.M. Soft hydrogels interpenetrating silicone-a polymer network for drug-releasing medical devices. J. Biomed. Mater. Res. B Appl. Biomater. 2015, 104, 402–410.
14. Stenger, M.; Klein, K.; Grønnemose, R.B.; Klitgaard, J.K.; Kolmos, H.J.; Lindholt, J.S.; Alm, M.; Thomsen, P.; Andersen, T.E. Co-release of dicloxacillin and thioridazine from catheter material containing an interpenetrating polymer network for inhibiting device-associated Staphylococcus aureus infection. J. Control. Release 2016, 241, 125–134.
15. Maki, D.G.; Tambyah, P.A. Engineering out the risk for infection with urinary catheters. Emerg. Infect. Dis. 2001, 7, 342–347.
16. Schumm, K.; Lam, T.B.L. Types of urethral catheters for management of shortterm voiding problems in hospitalized adults: A short version cochrane review. Neurourol. Urodyn. 2008, 27, 110–121.
17. Raad, I.; Hanna, H.; Maki, D. Intravascular catheter-related infections: Advances in diagnosis, prevention, and management. Lancet Infect. Dis. 2007, 7, 645–657.
18. Dimick, J.B.; Pelz, R.K.; Consunji, R.; Swoboda, S.M.; Hendrix, C.W.; Lipsett, P.A. Increased resource use associated with catheter-related bloodstream infection in the surgical intensive care unit. Arch. Surg. 2001, 136, 229–234.
19. Correia, V.G.; Bonifacio, V.D.B.; Raje, V.P.; Casimiro, T.; Moutinho, G.; da Silva, C.L.; Pinho, M.G.; Aguiar-Ricardo, A. Oxazoline-Based Antimicrobial Oligomers: Synthesis by CROP Using Supercritical CO2. Macromol. Biosci. 2011, 11, 1128–1137.
20. Sheldon, R.A. Green solvents for sustainable organic synthesis: State of the art. Green Chem. 2005, 7, 267–278.
21. Correia, V.G.; Ferraria, A.M.; Pinho, M.G.; Aguiar-Ricardo, A. Antimicrobial Contact-Active Oligo(2-oxazoline)s-Grafted Surfaces for Fast Water Disinfection at the Point-of-Use. Biomacromolecules 2015, 16, 3904–3915.
22. Brogden, K.A. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. 2005, 3, 238–250.
23. Zasloff, M. Antimicrobial Peptides, Innate Immunity, and the Normally Sterile Urinary Tract. J. Am. Soc. Nephrol. 2007, 18, 2810–2816.
24. Ivanovic, J.; Knauer, S.; Fanovich, A.; Milovanovic, S.; Stamenic, M.; Jaeger, P.; Zizovic, I.; Eggers, R. Supercritical CO2 sorption kinetics and thymol impregnation of PCL and PCL-HA. J. Supercrit. Fluid. 2016, 107, 486–498.

25. Fanovich, M.A.; Ivanovic, J.; Misic, D.; Alvarez, M.V.; Jaeger, P.; Zizovic, I.; Eggers, R. Development of polycaprolactone scaffold with antibacterial activity by the integrated supercritical extraction and impregnation process. J. Supercrit. Fluid. 2013, 78, 42–53.

26. García-González, C.A.; Barros, J.; Rey-Rico, A.; Redondo, P.; Gómez-Amoza, J.L.; Concheiro, A.; Alvarez-Lorenzo, C.; Monteiro, F.J. Antimicrobial Properties and Osteogenicity of Vancomycin-Loaded Synthetic Scaffolds Obtained by Supercritical Foaming. ACS Appl. Mater. Interfaces 2018, 10, 3349–3360.

27. Milovanovic, S.; Markovic, D.; Mrakovic, A.; Kuska, R.; Zizovic, I.; Frerich, S.; Ivanovica, J. Supercritical CO2-assisted production of PLA and PLGA foams for controlled thymol release. Mater. Sci. Eng. C 2019, 99, 394–404.

28. Cai, J.; Kimura, S.; Wada, M.; Kuga, S. Nanoporous Cellulose as Metal Nanoparticles Support. Biomacromolecules 2009, 10, 87–94.

29. Rao, C.N.R.; Muller, A.; Cheetham, A.K. The Chemistry of Nanomaterials: Synthesis, Properties and Applications; Wiley-VCH Verlag Gmbh & Co. KGaA: Weinheim, Germany, 2004.

30. Raman, S.P.; Keil, C.; Dieringer, P.; Hübner, C.; Bueno, A.; Gurikov, P.; Nissen, J.; Holtkamp, M.; Karst, U.; Haase, H.; et al. Alginate aerogels carrying calcium, zinc and silver cations for woundcare: Fabrication and metal detection. J. Supercrit. Fluid. 2019, 153, 104545.

31. Nešić, A.; Gordić, M.; Davidović, S.; Radovanović, Ž.; Nedeljković, J.; Smirnova, I.; Gurikov, P. Pectin-based nanocomposite aerogels for potential insulated food packaging application. Carbohydr. Polym. 2018, 195, 128–135.

32. Tewari, P.H.; Hunt, A.J.; Lofftus, K.D. Ambient-temperature supercritical drying of transparent silica aerogels. Mater. Lett. 1985, 3, 363–367.

33. García-González, C.A.; Alnaief, M.; Smirnova, I. Polysaccharide-based aerogels—Promising biodegradable carriers for drug delivery systems. Carbohydr. Polym. 2011, 86, 1425–1438.

34. Subrahmanyam, R.; Gurikov, P.; Meissner, I.; Smirnova, I. Preparation of biopolymer aerogels using green solvents. J. Vis. Exp. 2016, 113, e54116.

35. Li, H.; Zhong, J.; Zhu, H.; Yang, Y.; Ding, M.; Luo, L.; Huo, Y.; Li, H. Hybrid Cu2O/TiO2 Nanocomposites with Enhanced Photocatalytic Antibacterial Activity toward Acinetobacter baumannii. ACS Appl. Bio Mater. 2019, 2, 4892–4903.

36. McConnell, M.J.; Actis, L.; Pachon, J. Acinetobacter baumannii: Human infections, factors contributing to pathogenesis and animal models. Fem. Microbiol. Rev. 2013, 37, 130–155.

37. Sui, R.H.; Charpentier, P. Synthesis of metal oxide nanostructures by direct sol-gel chemistry in supercritical fluids. Chem. Rev. 2012, 112, 3057–3082.

38. Sahraneshin, A.; Takami, S.; Hojo, D.; Minami, K.; Arita, T.; Adschiri, T. Synthesis of shape-controlled and organic-hybridized hafnium oxide nanoparticles under sub- and supercritical hydrothermal conditions. J. Supercrit. Fluid. 2012, 62, 190–196.

39. Bhartia, B.; Puniredd, S.R.; Jayaraman, S.; Gandhimathi, C.; Sharma, M.; Kuo, Y.C.; Chen, C.H.; Reddy, V.J.; Troadec, C.; Srinivasan, M.P. Highly Stable Bonding of Thiol Monolayers to Hydrogen-Terminated Si via Supercritical Carbon Dioxide: Toward a Super Hydrophobic and Bioresistant Surface. ACS Appl. Mater. Interfaces 2016, 8, 24933–24945.

40. Katayama, S.; Zhao, L.; Yonezawa, S.; Iwai, Y. Modification of the surface of cotton with supercritical carbon dioxide and water to support nanoparticles. J. Supercrit. Fluid. 2012, 61, 199–205.

41. Cuadra, I.A.; Martínez-Casado, F.J.; Cheda, J.A.R.; Redondo, M.I.; Pando, C.; Cabañas, A. Production and Characterization of a New Copper(II) Propanoate-Isonicotinamide Adduct Obtained via Slow Evaporation and using Supercritical CO2 as an Antisolvent. Cryst. Growth Des. 2019, 19, 620–629.

42. Imbuluzqueta, E.; Elizondo, E.; Gamazo, C.; Moreno-Calvo, E.; Veciana, J.; Ventosa, N.; Blanco-Prieto, M.J. Novel bioactive hydrophobic gentamicin carriers for the treatment of intracellular bacterial infections. Acta Biomater. 2011, 7, 1599–1608.

43. Saelo, S.; Assatarakul, K.; Sane, A.; Suppakul, P. Fabrication of Novel Bioactive Cellulose-Based Films Derived from Caffeic Acid Phenethyl Ester-Loaded Nanoparticles via a Rapid Expansion Process: RESOLV. J. Agric. Food Chem. 2016, 64, 6694–6707.

44. Varona, S.; Rodríguez Rojo, S.; Martín, Á.; Cocero, M.J.; Serra, A.T.; Crespo, T.; Duarte, C.M.M. Antimicrobial activity of lavandin essential oil formulations against three pathogenic food-borne bacteria. Ind. Crop. Prod. 2013, 42, 243–250.

45. Varona, S.; Kareth, S.; Martín, Á.; Cocero, M.J. Formulation of lavandin essential oil with biopolymers by PGSS for application as biocide in ecological agriculture. J. Supercrit. Fluid. 2010, 54, 369–377.

46. Santo, I.E.; Campardelli, R.; Albuquerque, E.C.; de Melo, S.V.; Della Porta, G.; Reverchon, E. Liposomes preparation using a supercritical fluid assisted continuous process. Chem. Eng. J. 2014, 249, 153–159.
47. Trucillo, P.; Campardelli, R.; Scognamiglio, M.; Reverchon, E. Control of liposomes diameter at micrometric and nanometric level using a supercritical assisted technique. J. CO2 Util. 2019, 31, 119–127.
48. Trucillo, P.; Ferrari, P.F.; Campardelli, R.; Reverchon, E.; Perego, P. A Supercritical Assisted Process for the Production of Amoxicillin Loaded Liposomes for Anti-microbial Applications. J. Supercrit. Fluid. 2020.
49. Campardelli, R.; Trucillo, P.; Reverchon, E. Supercritical assisted process for the efficient production of liposomes containing antibiotics for ocular delivery. J. CO2 Util. 2018, 25, 235–241.
50. Trucillo, P.; Cardea, S.; Baldino, L.; Reverchon, E. Production of liposomes loaded alginate aerogels using two supercritical CO2 assisted techniques. J. CO2 Util. 2020, 39, 101161.
