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1. PROJECT EXECUTION

1. Introduction

The main goal of SurfaceT project was the development of an innovative supercritical carbon dioxide ($SCCO_2$) surface technology, applicable to existing and new high performance functional products. SurfaceT project was covering the topics proposed by the European Commission in the Priority 3 "Nanotechnologies and nano-sciences, knowledge-based multifunctional materials and new production processes and devices" in the VI FP (2004), and, specifically the project was covering the topics of the call "Materials processing by radically innovative technologies". It was expected that by developing this technology, breakthroughs to come not only from the new method but also from the new obtained products, since surface control is very important for production of new high performance functional products. In this respect, the development of $SCCO_2$ technology has allowed to break some classical boundaries between types of materials related to processing methods, since a similar technology can be applied to both inorganic and organic matrixes or even to hybrid composites. This lead to the last very important point proposed in SurfaceT project: to show that $SCCO_2$ technology is a crosscutting technology serving multisectorial applications by developing general $SCCO_2$ procedures that enable the creation of complex surface structures and at the same time, enabling the production of unique product characteristics in relation to composition, purity, and effectiveness. The main goal of SurfaceT has been achieved using an end-product driven approach (retroprocessing) by developing generic $SCCO_2$ procedures integrated with advanced reaction engineering concepts, that enable the creation of complex-shaped structures with controlled size, morphology and surface or interface:

(i) Study case systems: A+A $\rightarrow$ A (or A') and A+B $\rightarrow$ C. Because of the additional degree of freedom on reaction control related to the density of $SCCO_2$, single step processes have been designed. Process and reactions have been carried out homogeneously in the solution bulk and heterogeneously on specific surfaces or confined into microreactors (microemulsions and zeolites).

(ii) $SCCO_2$ technology has been used for the production of existing and new high performance products with unique characteristics in regard to composition (purity, sterilization), size (micro or nanoscale) and architecture (fibers, foams). Materials with a large surface to volume ratio are generally extremely reactive since they have a high number of surface atoms with a low coordination number. It has been demonstrated that $SCCO_2$ is a non-aggressive solvent technology adequate to manipulate these kinds of materials without damage.

2. Achieved progress and innovation

The choice of solvent must be done in an early stage of chemical industrial development and is usually not questioned unless dramatic problems are encountered during up-scaling. Hence, environmentally friendly solvents must be considered in the used synthetic pathway already at a lab-scale. The organic solvents and additives presently used for the manufacturing of most products have well-known disadvantages like toxicity, flammability and environmental concerns. Furthermore, with increasing complexity and size

reduction to nanoscale in new materials, classical solvent approaches are destructive because the used liquids destroy the functional surfaces they are helping to create. This is due to viscosity and surface tensions. On top of this, obtaining end-products free of residual solvents becomes more and more important for several clinical (and non-clinical) applications. In general, there are two alternatives for the use of organic solvents. The first is to use no solvent at all. This involves physical techniques such as vapour-condensation, freeze drying and heat treatment, which are difficult to apply to the chemical synthesis of thermally labile organics. The second option is to replace the organic solvent by intrinsic benign solvents. Only water, carbon dioxide and nitrogen can be considered environmentally benign in all aspects. Technology based on $SCCO_2$ is an alternative to overcome the problems associated with the use of traditional organic solvents. $SCCO_2$ has a number of characteristics that allows processing a broad range of materials:

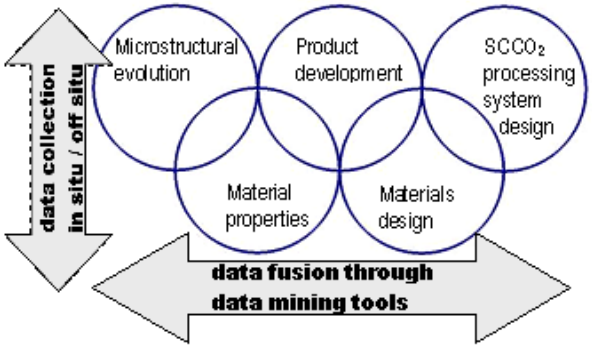
- Low critical temperature (31˚C) which allows the processing of thermally unstable compounds.
- Large quadruple moment which confers large solubility to organic reagents. High selectivity between reagents and products. Tunability of its solvent power through simple pressure changes.
- High diffusivity which enhances mass transfer and reaction kinetics.
- Low viscosity and surface tension. $SCCO_2$ is a non aggressive solvent adequate to manipulate complex functional materials and nanostructures.
- Low cost, no flammability and negligible toxicity. A reduction of the use of toxic products in the industry is foreseen, since the $SCCO_2$ based chemical technology avoids the production of organic waste with a net reduction of the released amount of CO_2 to the atmosphere.
- Gaseous at ambient conditions. Products are simply isolated by depressurization, resulting in dry compounds and reducing reaction/post-processing steps. This eliminates the cost of filtration (specially complicated for nanoparticulated matter) and the energy-intensive drying procedure.

SurfaceT project results are expected to accelerate transition towards a more efficient knowledge-based economy, since it addressed important environmental issues: towards a less intensive solvent-use industry. $SCCO_2$ used as a solvent for chemical processes or else as a reagent, may greatly expand in the short term to higher levels if several possible approaches to its use are implemented.

2.1. Processes

In SurfaceT, four main consecutive steps have been followed to advance in SCCO2 processes development.

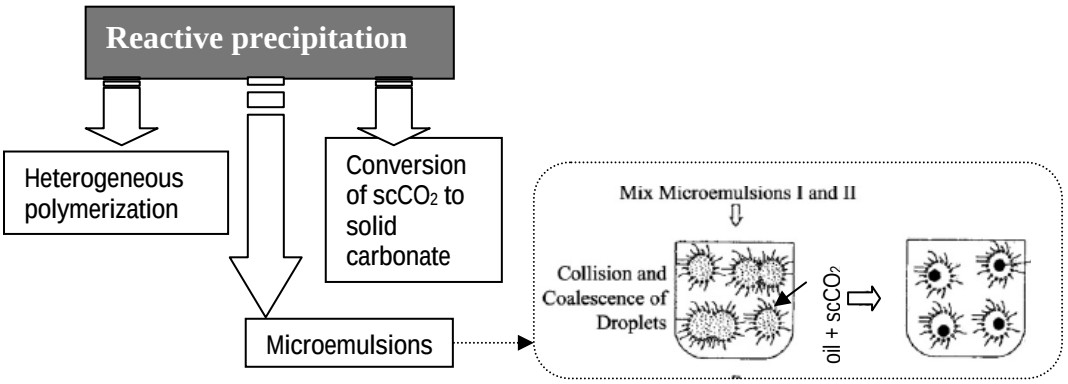
Step 1 Obtaining the fundamental (modeling) and practical (characterization) knowledge required for production of the desired products with the requirements for the specific applications. Developing validated predictive models aimed at reducing the efforts required for the production of new (nanostructured) product. Chemometric design of experiments.



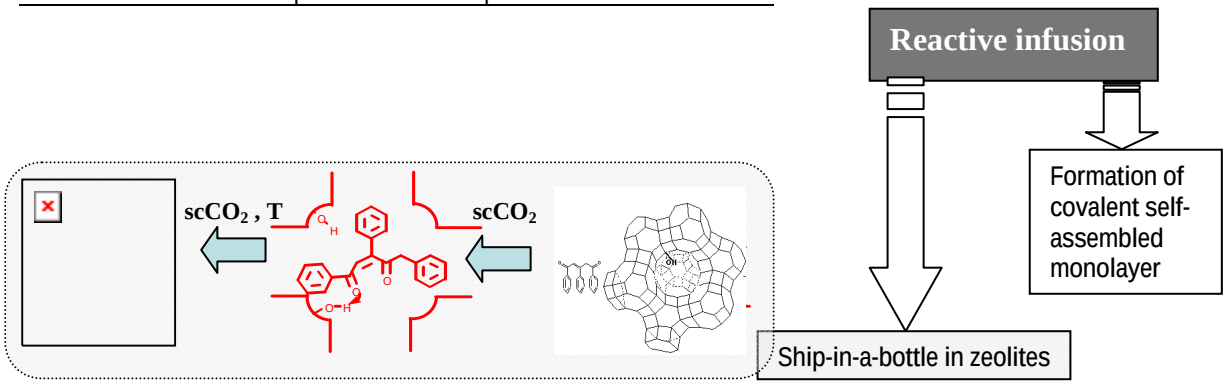
Role of modeling / characterization / chemometrics use, pervasive across all aspects of materials science engineering.

Step 2 Design and construction of batch and semicontinuous equipment.

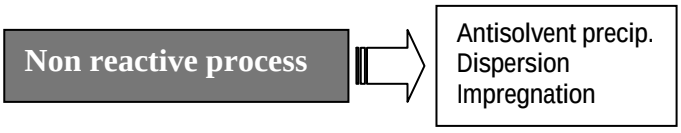
Step 3 Reactive and non-reactive processes design.



Process	Conventional	Supercritical
Carbonation	2 step	2 step. Better kinetics
Polymerization	2 step	1 step. Need new surfactants
Microemulsions	3 steps	2 steps. Better kinetics



Process	Conventional	Supercritical
Impregnation	3 steps	1 step. No solvent competition
Ship-in-a-bottle	3 steps	1 step. Better kinetics
Self assembled	2 step	1 step. Less side reactions

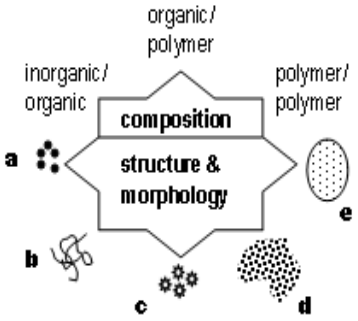


Process	Conventional	Supercritical
Antisolvent	2 steps	1 step
Dispersion	1 step	1 step. Better kinetics
Impregnation	2 steps	1 step

Step 4 Evaluation of obtained products, and demonstration of improved performance and cost (supercritical vs. conventional).

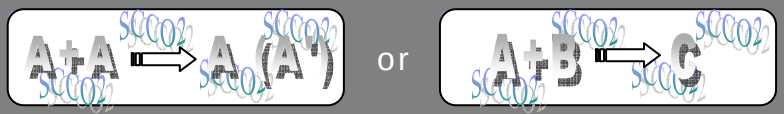
2.2. Products

The technology has been used for the processing of inorganic, organic and polymeric products. Prepared functional products were either monophasic (organic, polymeric or inorganic) or composite (organic/polymer, polymer/polymer or hybrid). Several fundamental structures have been produced: (a) nanoparticles, (b) fibers, (c) surface modified particles, (d) dispersed materials in a polymer matrix, and (e) internally impregnated porous systems.



This project was firstly centered in the production of complex functional composites for the biomedical sector, which are often synthesized by operating with toxic organic solvents and expensive additives. Technology based on $SCCO_2$ is an alternative to overcome those problems *[Toxic Solvents CD 97/C 76/01, 2006-ICH Guidelines for Good Clinical Practice]*. Moreover, technology transfer to other industries without the biomedical special requirements (fi, high-tech or commodity products manufacturers) has been attempted.

SurfaceT: integration of $SCCO_2$ technology



New shorter and more energy efficient processing. Less waste factor

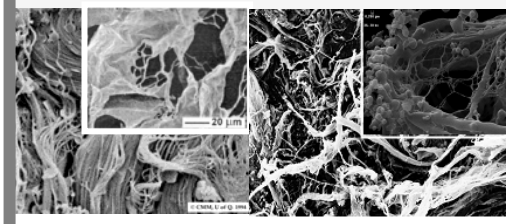
Composition control. High purity
Morphology control: size and shape

Higher Value Products

Biomaterials and drug delivery systems are complex composites composed of active ingredients (small active agents, enzymes, proteins, etc.) and a matrix. These composite materials often have internal structures in the nanometric scale and complex surfaces. The treatment or adjustment of these properties have been an essential step in the $scCO_2$ production routes in this project:

Porous (blend) scaffolds constituted by fibers of biopolymers, a Ca^{2+} source (HAP).

- Sponge scaffolds
- Drug delivery: nanoparticles, microspheres, impregnated biopolymers.
- Sterilized bonds and biopolymers
- Cosmetics: ointmers, UV-protectors.



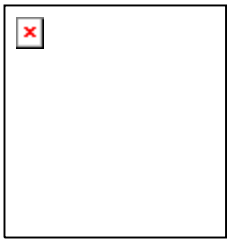
Human Collagen Type I

$scCO_2$ precipitated PLA (partner's laboratories)

High-Tech Products

The processing of biopolymer composites has similarities with the processing of many other applications, ranging from catalysts and sensors or even self-cleaning or antibacterian plastics, which have been also contemplated in this project:

- Functionalized micro/meso zeolites for applications in photocatalysis or biosensors.
- Impregnation of platics with antibacterians for food storage.

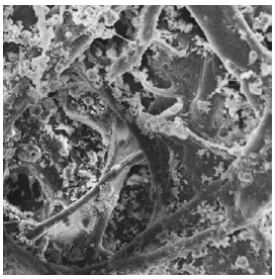


$scCO_2$ ship-in-a-bottle synthesis of zeolite@ photosensitizer (partner's laboratories)

Commodity Products

We have also focused on large-scale commodity products, since even small improvements would have a substantial impact on waste released to the environment. In today's global market, commodity products processing in Europe needs to be highly efficient, sustainable and flexible to keep up with competition from other parts of the world and to stand the pressures on supply of energy and raw material resources:

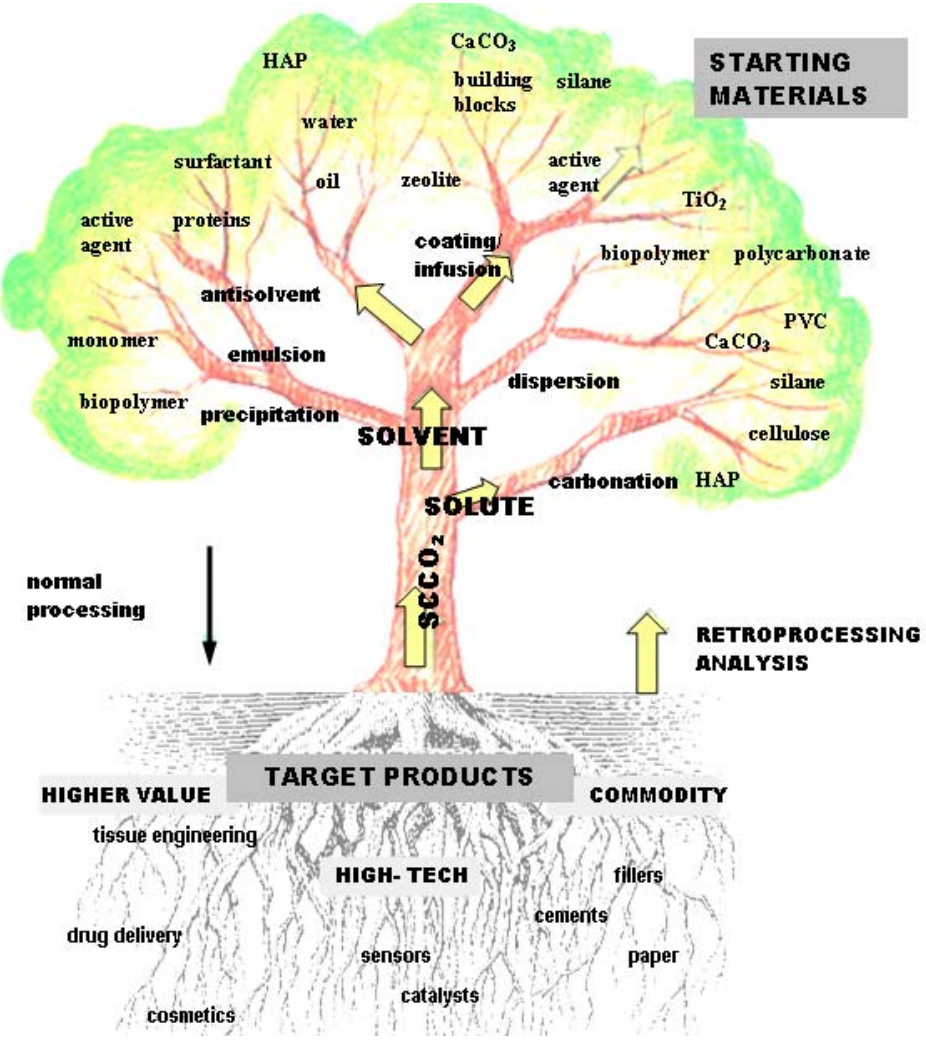
- Specialty papers: cellusose+$CaCO_3$
- Structure-Construction: carbonated cement



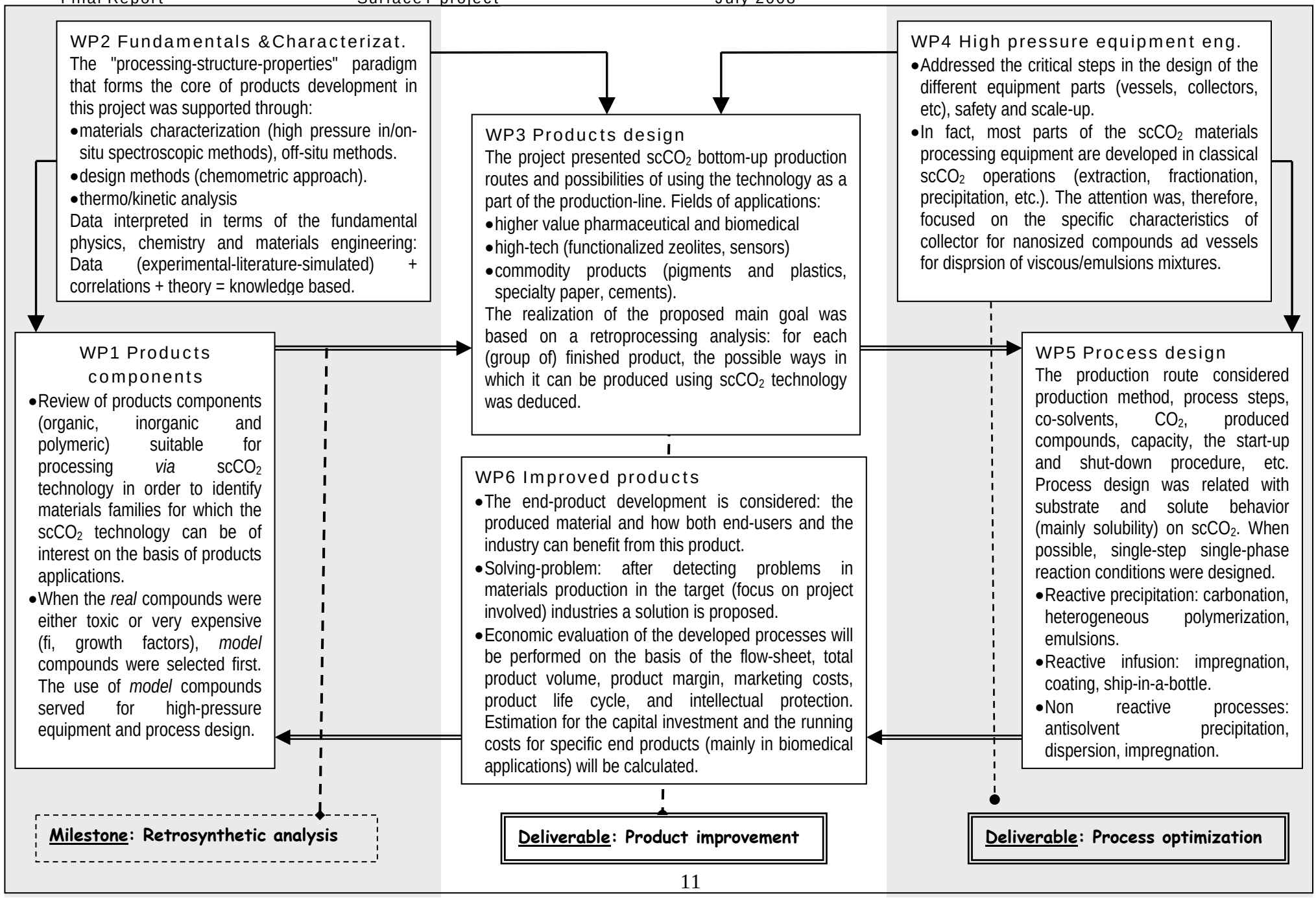
$scCO_2$ precipitation of nano CaCO into cellulose paper pores (partner's laboratories)

2.3. Methodology

SurfaceT was an ambitious project. It started with basic chemistry and went up to testing the efficiency of the products in terms of composition, size and morphology. Therefore, it has required fundamental and technological knowledge of several interrelated disciplines, covered by participants from complementary fields and industry. The project had considered the manufacturing process by applying bottom up nanotechnology approaches applied to $SCCO_2$ technology, in respect of molecular control of the surface and interface structures. A retrosynthetic/ retroprocessing analysis (going backwards from a target product to starting materials) have been applied, since the synthetic route using $SCCO_2$ technology to a target product have been developed in most of the studied processes.



The project was divided into six scientific workpackages (WP1-WP6). The project cycle started with specifying the requirements of the products and its components (WP1). To characterize the products performance, a firm knowledge of the raw materials and their mutual interaction was needed. This was achieved through WP2, where both theory (modeling) and characterization were addressed. With the knowledge of the raw materials and the design specifications, the product structure was designed (WP3). After that, the high pressure equipment needed for manufacturing the desired product was specified (WP4). In WP5, the products were made using $SCCO_2$ technology. Finally, improved product quality made by the developed $SCCO_2$ technology was demonstrated and a qualitative economic evaluation was made (WP6).


WP2 Fundamentals &Characterizat.
The "processing-structure-properties" paradigm that forms the core of products development in this project was supported through:
•materials characterization (high pressure in/on-situ spectroscopic methods), off-situ methods.
•design methods (chemometric approach).
•thermo/kinetic analysis
Data interpreted in terms of the fundamental physics, chemistry and materials engineering: Data (experimental-literature-simulated) + correlations + theory = knowledge based.
WP4 High pressure equipment eng.
•Addressed the critical steps in the design of the different equipment parts (vessels, collectors, etc), safety and scale-up.
•In fact, most parts of the scCO2 materials processing equipment are developed in classical scCO2 operations (extraction, fractionation, precipitation, etc.). The attention was, therefore, focused on the specific characteristics of collector for nanosized compounds ad vessels for disprsion of viscous/emulsions mixtures.
WP3 Products design
The project presented scCO2 bottom-up production routes and possibilities of using the technology as a part of the production-line. Fields of applications:
•higher value pharmaceutical and biomedical
•high-tech (functionalized zeolites, sensors)
•commodity products (pigments and plastics, specialty paper, cements).
The realization of the proposed main goal was based on a retroprocessing analysis: for each (group of) finished product, the possible ways in which it can be produced using scCO2 technology was deduced.
WP1 Products components
•Review of products components (organic, inorganic and polymeric) suitable for processing via scCO2 technology in order to identify materials families for which the scCO2 technology can be of interest on the basis of products applications.
•When the real compounds were either toxic or very expensive (fi, growth factors), model compounds were selected first. The use of model compounds served for high-pressure equipment and process design.
WP6 Improved products
•The end-product development is considered: the produced material and how both end-users and the industry can benefit from this product.
•Solving-problem: after detecting problems in materials production in the target (focus on project involved) industries a solution is proposed.
•Economic evaluation of the developed processes will be performed on the basis of the flow-sheet, total product volume, product margin, marketing costs, product life cycle, and intellectual protection. Estimation for the capital investment and the running costs for specific end products (mainly in biomedical applications) will be calculated.
WP5 Process design
The production route considered production method, process steps, co-solvents, CO2, produced compounds, capacity, the start-up and shut-down procedure, etc. Process design was related with substrate and solute behavior (mainly solubility) on scCO2. When possible, single-step single-phase reaction conditions were designed.
•Reactive precipitation: carbonation, heterogeneous polymerization, emulsions.
•Reactive infusion: impregnation, coating, ship-in-a-bottle.
•Non reactive processes: antisolvent precipitation, dispersion, impregnation.
Milestone: Retrosynthetic analysis
Deliverable: Product improvement
Deliverable: Process optimization

2.4. Project objectives and achievements

The main goal of the project was achieved through the development of the main project objective: Preparation of functional composites by using the clean, integrated $SCCO_2$ surface technology. To achieve this main objective we originally formulated the following specific objectives:

A. To develop a $SCCO_2$ surface technology that is able to produce complex structures and to improve product characteristics and effectiveness.

B. To engineer and scale-up the $SCCO_2$-processes for production.

C. To achieve a drastic reduction in waste generation.

D. To (re)design existing/new materials for applications in biotechnology and biomaterials with specific functionality and high performance.

E. To (re)design existing/new materials for applications in pigments and plastics related industries with specific functionality and high performance.

F. To convey the knowledge from researchers to manufacturing industries.

Based on this formulated objectives, SurfaceT main achievements are summarized as follow:

A. SurfaceT project has contributed To RESOLVE the reliability constrains of using $SCCO_2$ as a reaction and processing media in the production of higher value functional composites with improved effectiveness in the biomedical and cosmetic area. Objectives A and D.

B. SurfaceT project has contributed To CREATE basic information to overcome technical barriers between fundamental science and engineering scale-up of $SCCO_2$ processes, by validation and demonstration of the strategies and advantages of using $SCCO_2$ as a *i)* reaction solvent or dispersing media, *ii)* solute or reagent, and *iii)* antisolvent. Objective B.

C. SurfaceT project has contributed To EXTEND the state of knowledge in $SCCO_2$ technology and stimulate advanced research in new directions (different from biomedical) by exploring, developing and deploying efficient processes for the production of high-tech and commodity products: valuable carbon containing materials (carbonates), high-tech (photocatalysis) and commodity (reinforced plastics, concrete) products. Objectives A and E.

D. SurfaceT project has contributed To DEPLOYMENT of environmental technologies that drastically reduce waste generation by demonstration of a more efficient life cycle and lower waste factor for products obtained using $SCCO_2$ technology. Qualitative economic evaluation based on use of resources (raw materials), reaction steps and recycling costs. Objective C.

E. SurfaceT project has contributed To CONVEY the knowledge from researchers to manufacturing industries opening platforms to discuss the possible benefits for the sustainability of a process upon substitution of organic solvents by $SCCO_2$. Objective F.

Following, main SurfaceT achievements are described.

A. SurfaceT project has contributed To RESOLVE the reliability constrains of using $SCCO_2$ as a reaction and processing media in the production of higher value functional composites with improved effectiveness in the biomedical area.

1.1. Drug delivery systems

Improving the therapeutic properties of a drug is a key action for the pharmaceutical industry and a key benefit for the patient as well. The improvement come from a small size, a different crystal lattice, an increased purity of a drug or from its formulation with carriers that provide the desired delivery properties. As for improving the therapeutic action of drug through its formulation, two systems have received considerable attention in the last years: drug delivery products which aim at delivering the drug in a controlled slow way (avoiding the large fluctuations in drug concentration and reducing the need of several administrations) and/or in a given location in the body by the use of appropriated biocompatible or biodegradable polymers, and solid dispersions which aim at enhancing the bioavailability of poorly water-soluble drugs by the use of diverse types of carriers ranging from water-soluble to amphilic to lipid-soluble ones.

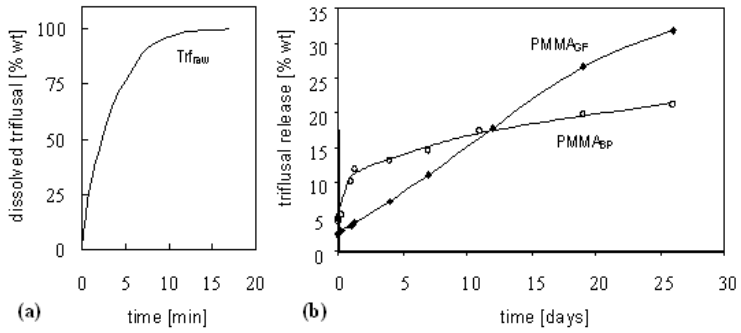
Controlled drug delivery systems comprise a drug dispersed in a polymer matrix and are designed either to release the drug in a predetermined manner or to target a specific site in the body. For most applications, in order to achieve an effective therapy, the drug should be dispersed in the matrix at a molecular level. Otherwise, crystallization of the drug in the polymer matrix could lead to a non-controlled impact on the dissolution and diffusion rate in the polymer. The preparation of drug release systems necessitates the use of a mobile phase to dissolve and carry the drug component into the polymer matrix, which also swells the polymer, facilitating the diffusion of the drug, and increasing the rate of impregnation. One of the main limitations that pharmaceutical industry encounters in the preparation of this kind of systems involves the use of organic solvents, since solvents residues get trapped in the polymer together with the drug and may be responsible of some toxic effects. Supercritical fluid technology using $SCCO_2$ as an alternative to organic solvents has already proved its applicability to the processing of polymers.

1.1.1. PMMA and Triflusal

Many polymers have a very limited solubility in $SCCO_2$, which results in an interesting feature for producing monolithic loaded microspheres, obtained by an impregnation process. Impregnation using $SCCO_2$ as a mobile phase has proven to be feasible when the pharmaceutical compound is soluble in $SCCO_2$ and the polymer can be swollen by $SCCO_2$. A high purity product, free of residual solvents, is thus obtained.

The model polymer used in SurfaceT as a matrix for impregnation was poly(methyl methacrylate) (PMMA). PMMA is an acrylic hydrophobic biostable polymer that is widely used in the biomedical field as implant carrier for sustained local delivery of anti-inflammatory or antibiotic drugs. The drug that has been used for impregnation was 2-acetyloxy-4-(trifluoromethyl) benzoic acid (triflusal). Triflusal is a drug patented by Uriach partner and is an irreversible inhibitor of the enzyme cyclo-oxygenase and is commonly used as platelet anti-aggregant preventers for the treatment of thrombosis diseases. An *in vitro* elution method coupled with high-performance liquid chromatography was used to evaluate the release behaviour from the prepared systems.

Homogeneously distributed loadings of *ca.* 20 wt% of active agent in PMMA were obtained. For impregnated systems, triflusal melting peak was not observed by DSC analysis, indicating that the triflusal did not crystallize inside the matrix. From a therapeutic point of view, it was concluded that impregnated samples had an excellent potential for the preparation of pharmaceutical formulations. Obtained delivery profiles were consistent with keeping stable levels of the dissolved drug over a long period of time, reducing the number of administrations and avoiding the initial sharp increase in drug concentration. Finally, the reported $t_{1/2}$ values were compatible with a sustained delivery for a period longer than 2 or 3 months.

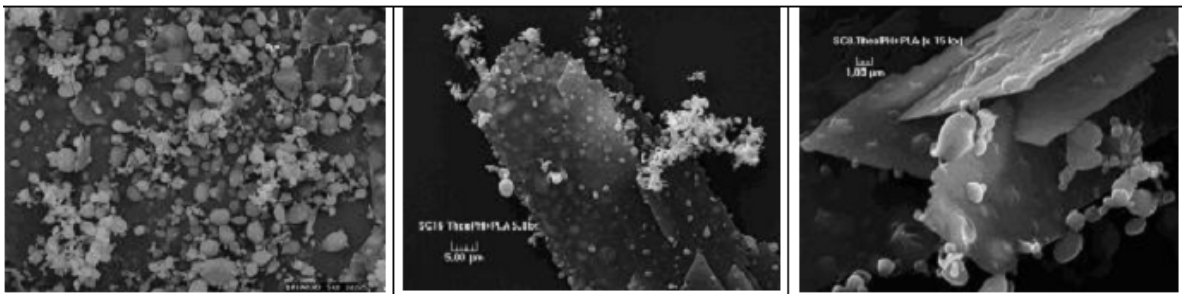


Profiles of triflusal release in acid medium: (a) raw triflusal (Trf) crystals from Uriach; and (b) Trf impregnated into PMMA using $SCCO_2$ technology.

1.1.2. Theophylline and L-PLA

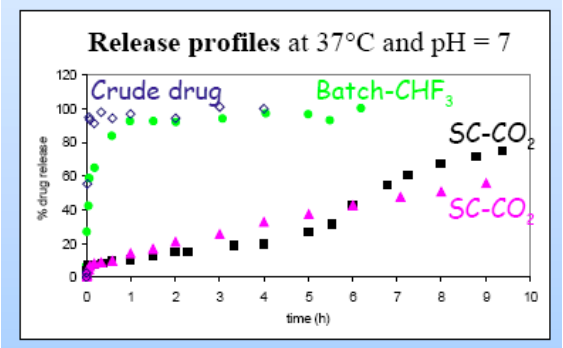
Theophylline (THEO) is a drug that has the disadvantage of a narrow therapeutic index (i.e. the therapeutic dosage and the toxicity level are much closed) and a poor patient compliance due to frequent administrations per day. THEO is a pharmaceutical compound used for its bronchodilator effect in the treatment of pulmonary diseases. To control THEO delivery in the body, and since it is quite soluble in water (below 0.9% at 23°C), it is necessary to use a hydrophobic polymer, like PLA, to sustain the release.

Theophylline formulations were produced by antisolvent supercritical techniques, using CO_2 as a precipitating agent when added to a liquid solution that contained both THEO and PLA species. Whatever the experimental conditions, powders were composed of two particle populations, with plate-like THEO coated or not, besides spherical particles of few microns in diameter. The presence of polymer slightly prevented the growth of the THEO particles, but was unable to reduce significantly their size down to limits adequate for pulmonary delivery, since smallest produced particles were in the range of 25µm. Regarding crystallinity, the "new" crystal lattice of THEO that was observed when single processed, was still obtained when PLA was co-precipitated.



SEM of various (THEO + PLA) coprecipitates

For this system the drug delivery is sustained



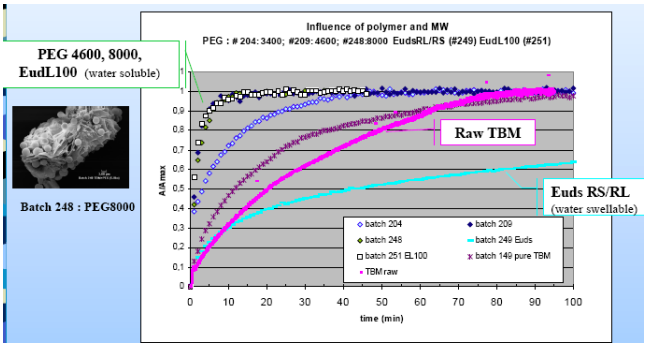
Release profiles of raw theophylline and teophylline+L-PLA prepared using $SCCO_2$ antisolvent

1.1.3. Mesalazine and Eudagrit

Mesalazine is and anti inflammatory drug which need specific delivery to the colon and, hence, drug delivery systems resistant to gastric juice. Encapsulation of mesalazine has been carried out using Eudagrit as a matrix and the $SCCO_2$ antisolvent process. Results lead to sustained release, similar to that obtained for theophylline. formulation of mesalalzine. Because of the target organs for specific delivery, formulations have to completely sustain the drug release in gastric fluids for 2 hours. The CO_2-produced formulations with eudragits did not completely sustain the release in acidic media.

1.1.4. Tolbutamide and PEG/Eudagrit

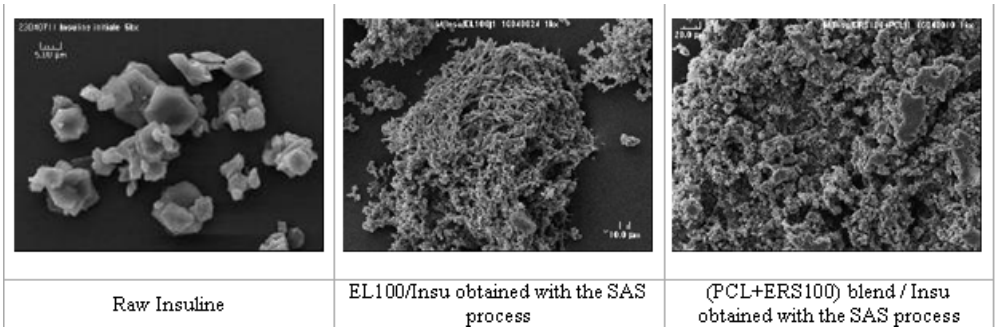
Tolbutamide is an antidiabetic with low water solubility, and, thus, low biodisponibility when administered. The interest in this case was to prepare a system that increase biodisponibility, ie., with a faster dissolution rate than the raw material. A water soluble or swellable excipients, such as PEG or Eudagrit, respectively, where chosen as a matrix. Encapsulated product was prepared using the antisolvent approach and dissolution rate was enhanced. Dissolution rate was enhanced when the drug was co-precipitated with hydrophilic excipients, or the release was sustained when the drug is coprecipitated with water-swellable polymers.



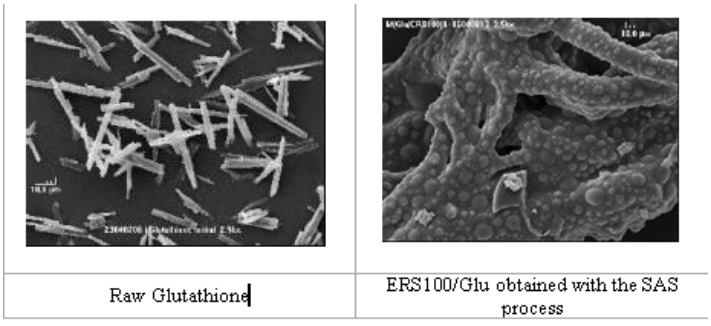
Release profiles of raw tolbutamide and tolbutamide+PEG or Eudagrit prepared using $SCCO_2$ antisolvent

1.1.5. Peptides and proteins

Encapsulation of peptides and proteins into polymeric particulate carriers provides several benefits over traditional formulations. Prior to release, the polymer protects the drug from degradation or premature metabolism. The choice of polymer matrix impacts several aspects of the carrier including the types of drug or protein that can be encapsulated, the mode of degradation and drug release, biocompatibility and physical properties. Polymeric film coatings are also frequently used to control drug release from solid pharmaceutical dosage forms. Several natural and synthetic macromolecules have proven to be suitable coating materials, providing different types of drug release behavior, e.g. zero order kinetics, pulsatile and sigmoidal patterns. For instance, too low and too high coating levels must be avoided to prevent accidental film rupturing (and subsequent dose dumping) and too long processing times. There is a large variety of polymers commercially available, among them copolymers of acrylic and methacrylic acid known as Eudragit. In all cases, carboxylic acid containing monomers inside those polymers trigger the polymer dissolution depending on the environmental pH. $SCCO_2$ technology was developed here for the production of microparticles aimed to be used as drug delivery systems. The technique used is the semicontinuous antisolvent process (SAS).
The first objective of the investigation was the establishment of proper solubility conditions to be used in the development of the drug delivery systems using the SAS strategy. The solubility of glutathione, insulin, hemoglobin and albumin were checked in solvents such as ethanol, dimethylsulfoxide, methylene chloride and so on. Conclusions indicated that small and medium peptides such as glutathione and insulin were reasonably soluble in organic solvents compatible with the SAS technique. Conversely, large proteins (hemoglobin and albumin) were soluble in water which, in principle, is not recommendable in the SAS techniques. Hence, glutathione and insulin were primarily selected for the preparation of prototypes of drug delivery systems. Several polymers such as polylactic acid (PLA), polycaprolactone (PCL), Eudragit RS100 and Eudragit L100 were utilized for the preparation of model drug delivery systems. Some of the obtained systems are shown following:



Raw Insuline | EL100/Insu obtained with the SAS process | (PCL+ERS100) blend / Insu obtained with the SAS process

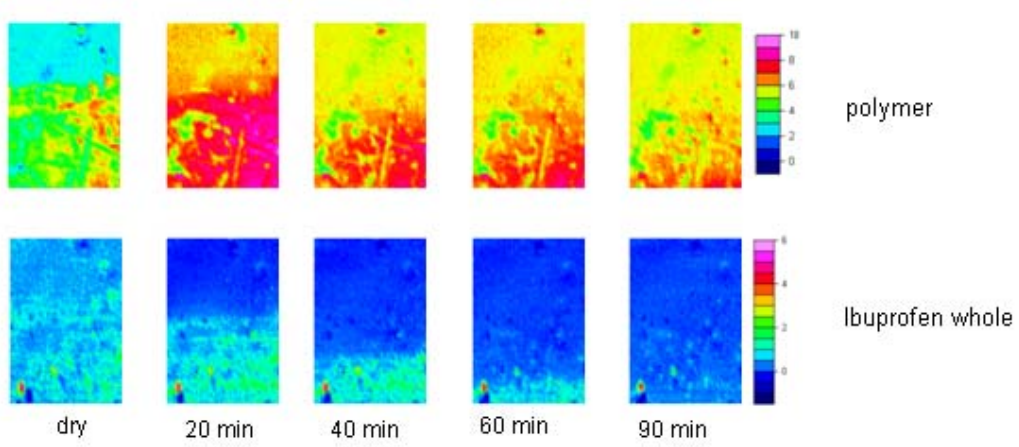


Raw Glutathione | ERS100/Glu obtained with the SAS process

1.1.6. New sensitive polymers

Conventional preparation of new acrylic stimuli sensitive (pH and temperature) polymers derived from morpholine and pyrrolidine using water as a solvent. Three new methacrylic monomers, *N*-ethyl morpholine metacrylate (EMM), *N*-ethyl morpholine methacrylamide (EMA) and *N*-ethyl pyrrolidine metacrylamide (EPA) have been synthesized by one step reaction. The corresponding monomers EMM, EMA and EPA were homopolymerized at 50º C in mixtures of water/isopropanol, characterized by spectroscopic techniques, evaluated their solubility and determined their thermal properties. The evaluation of the sensitivity to pH of the prepared polymers was performed by two different approaches: determining the pKa of the corresponding monomers and their respective linear homopolymers, and by swelling studies at different pH of the crosslinked polymers. pKb determination was carried out by acid-base titration in acidified aqueous NaCl solution titrated with NaOH. The presence of tertiary amine groups in the prepared systems confers the partial or total ionization of the polymer with pKb values that between 3.5 and 5.5. Similar polymers are now prepared using $SCCO_2$ fluid.

In this sense polymer matrices of poly-EMA were loaded with ibuprofen and analysed the release by ATR-IR spectroscopy. The most relevant results indicate that the poly-EPA polymer matrices avoid the ibuprofen crystallization at physiological and moderate acidic pH, an aspect that makes these polymer systems potential candidates as drug delivery carriers.

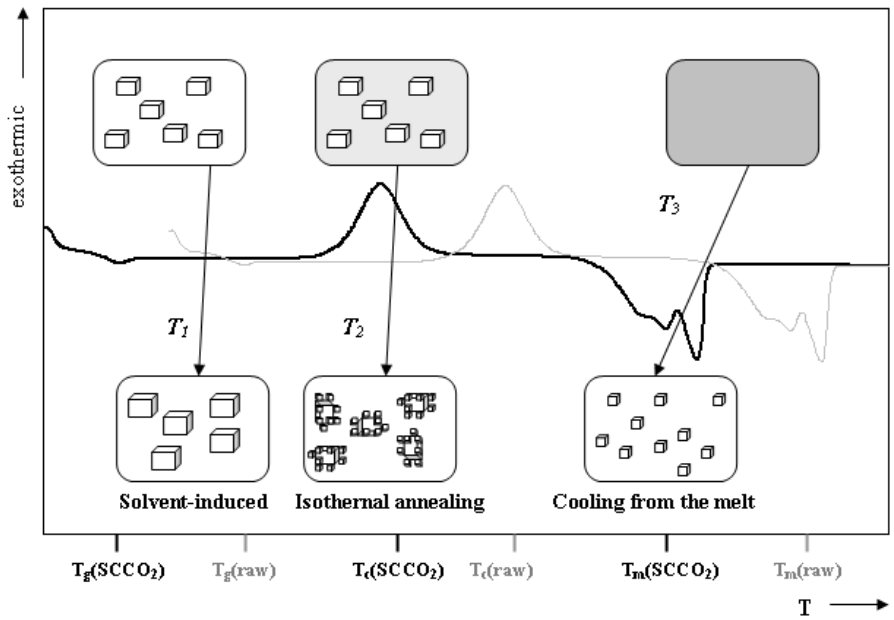


1.2. Modification of polymers/composites for hard tissue repair.

1.2.1. Biodegradable polymers (L-PLA)

PLA (polylactic acid) is an aliphatic polyester that has potential advantages for some specific uses, such as relative high mechanical strength, thermoplastic behavior, biodegradability and biocompatibility. Due to its high cost, PLA has been used mainly in the medical area and for specific purposes in the food industry to make plastic bags and bottles. The L-PLA isomer exists as a semicrystalline polymer, and its microstructural properties, mainly the molecular weight and degree of crystallinity, are of critical importance for its use in biomedical applications. The construction of loaded bearing materials, such as screws and plates or scaffolds for use as orthopedic implants, needs tough materials with high hardness and, therefore, L-PLA with high molecular weight in the range of 10^5-10^6 $gmol^{-1}$ and high crystallinity is desired. In conventional technology, various L-PLA microstructures and degrees of crystallinity have been described to be obtained by applying different processing conditions such as isothermal annealing, cooling from the melt or solvent-induced crystallization. Particularly, solvent-induced crystallization led to L-PLA materials with a high degree of crystallinity. However, the described process is performed using organic solvents, which are difficult to eliminate from the final product. Hence, the resulting product could not be appropriate for their use in medical applications. $SCCO_2$ induces depression in the glass transition (T_g), crystallization (T_c) and melting (T_m) temperatures of semicrystalline polymers, which has been reported to affect the crystallization kinetics. SurfaceT performed work was devoted to obtain general conclusions of the effect of L-PLA annealing under $SCCO_2$ and L-PLA crystallization behavior. The study was performed on various L-PLA polymers, ranging from low (2 $Kgmol^{-1}$) to high (350 $Kgmol^{-1}$) molecular weight processed at different crystallization conditions. We first analyzed the solid state processing at temperatures lower than the L-PLA melting point; and, second, we studied the effect of dissolving $SCCO_2$ in the molten polymer.

Treatment with $SCCO_2$ at different temperatures allowed obtaining L-PLA samples having different structures with respect to the crystallinity, crystallite size and crystal morphology. Three different behaviors were observed: (i) at temperatures near the glass transition temperature of the raw polymer a solvent-induced crystallization process was provoked by the presence of $SCCO_2$; (ii) at temperatures between the glass transition temperature and the cold crystallization temperature of the raw polymer, isothermal annealing leading to a seeded nucleation process of the polymer chains in the amorphous region was more likely to occur; and (iii) at temperatures between the cold crystallization and the melt of the raw polymer, nucleation and crystallization from the melt was provoked during simultaneous system depressurization and cooling. For the semicrystalline L-PLA, the induced nucleation and/or crystallization effect in the amorphous fraction prevented the molecular dispersion of organic compounds (such as triflusal) in the matrix.



Schematic description of the possible L-PLA annealing processes in the presence of $SCCO_2$ as a function of temperature.

1.3. Scaffolds for tissue engineering

Tissue engineering is evolving from the use of implants that repair or replace damaged parts, to the use of controlled three-dimensional scaffolds that induce the formation of new functional tissues either *in vitro or in vivo*. Many polymeric scaffolds are being evaluated for a possible role in tissue engineering. Biodegradable homopolymers and copolymers of lactic (L-PLA) and glycolic (PGA) acids are typically chosen for these applications. However, the degradation of these poly-α-hydroxyacids results in the generation of acidic species, which could cause local inflammation. Poly(ε-caprolactone) (PCL), had also attracted attention as a biopolymer due to the lack of toxicity and low cost, although it is a slow degradation polymer. Non-biodegradable materials such as polymethylmethacrylate (PMMA) are being also tested in tissue engineering to achieve long-term mechanical stability after implantation as well as high hydrophobicity that enhances cells adhesion. Along with the ongoing search for new materials, polymeric blends composed by a biostable polymer and a biodegradable one have recently gained significant attention in tissue engineering due to the possibility of provide them with specific advantages in morphological, degradation and mechanical properties. Not only the chemical composition, but also the architecture of a scaffold plays an important role in modulating tissue growth and response behavior of cultured cells. A correct architecture of a scaffold would be the one that mimics the natural extracellular matrix that surrounds cells in the body. Hence, required characteristics include high porosity and an interconnected 3D macroporous network necessary for cell proliferation, and high surface area to promote cell adhesion. Scaffolds can take forms ranging from complex monolithic 3D microcellular structures (macroporous sponges) to networks of fibers.

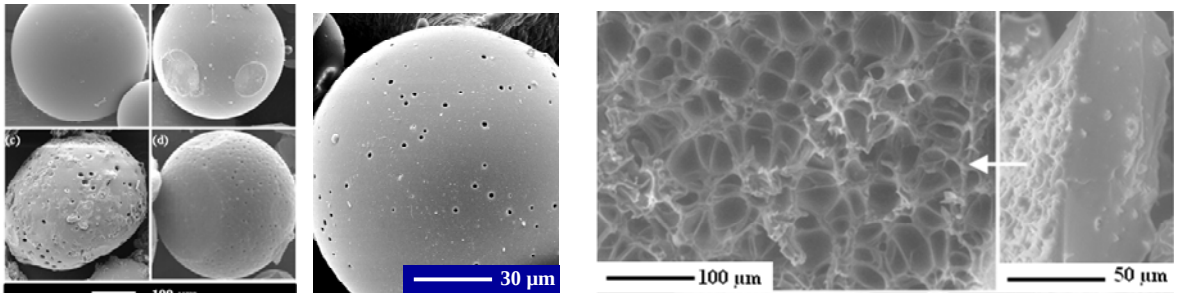
Scaffolds can be produced in a variety of ways, using either conventional techniques such as solvent casting, foaming, physical separation and freeze drying or advanced processing methods such as rapid prototyping technologies, where the scaffolds are built layer by layer from a computer-aided design model. The main drawbacks of conventional scaffolds production methods involve a reduced capability to control pore size and pore interconnectivity. Moreover, when using solvent approaches, the residual organic molecules left in the polymer after processing may be harmful to the transplanted cells and can inactivate biologically active growth factors. On the other hand, the preparation of blend systems presents a significant challenge using conventional solvent technology as the constituent polymers are highly immiscible. $SCCO_2$ technology has been already proposed for the production of a great range of biopolymers with excellent control on morphology, surface properties and purity. Many of these applications exploit the fact that $SCCO_2$ is an excellent non-solvating porogenic agent for amorphous polymers while being a poor solvent. It is used to produce polymer foams by pressure-induced phase separation. Furthermore, spray processes involving $SCCO_2$ have achieved considerable success in addressing polymer particles and fibers production. Antisolvent spray processes uses common organic solvents to dissolve the polymer and, thus, a large variety of these compounds can be processed. Finally, $SCCO_2$ acts as an interfacial tension and viscosity reducing agent, which facilitates the blending of immiscible polymers. $SCCO_2$ was used in SurfaceT for the preparation of foamed sponges and intermingled fibers of biopolymers. Solid monolithic porous scaffolds were prepared using $SCCO_2$ by simple swelling and foaming. Fibers were prepared using the semicontinuous antisolvent SAS technique. The development was focused on the processing of both biodegradable L-PLA and non-biodegradable PMMA homopolymers. Moreover, we established that the antisolvent supercritical technique can be used to prepare fibrous scaffolds of the blend PMMA/PCL.

1.3.1. Porous homopolymers (sponges)

Foaming

Research over the past years has demonstrated that simple swelling and foaming can be achieved using $SCCO_2$. The sorption of $SCCO_2$ in many polymers induces beneficial processing changes such as plasticization and swelling. It should be taken into account that using $SCCO_2$ for pore generation, porous foams can only be created in polymers with a relatively high amorphous fraction. By using $SCCO_2$ as a porogenic agent and a pressure quenching process, foams of the semicrystalline L-PLA and the amorphous PMMA have been prepared in SurfaceT. However, the obtained microcellular foams did not have an interconnected porous geometry. Most of the described $SCCO_2$ foaming processes have been only able to attain on average 10-30 % of interconnection between pores. Moreover, the small diameter of the pores often found using $SCCO_2$ foaming technology (from 0.1 μm to few μm) lead to pore occlusion by the living cells (with a few to tens μm in diameter). Finally, the porous structure of monolithic polymers obtained by pressure quenching make very difficult an effective seeding with cells due to their tortuous matrices. Similar problems are found using organic liquids as a porogen when preparing the scaffold by the more conventional temperature quenching process. Although obtained materials are not thoroughly adequate to distribute a high density of cells efficiently and uniformly throughout the scaffold volume, they have potential

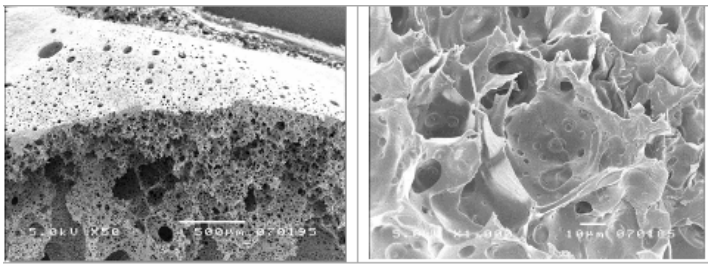
pharmaceutical applications as controlled drug delivery devices with drug relief characteristics depending on the pore morphology.



$SCCO_2$ foamed PMMA

Emulsion

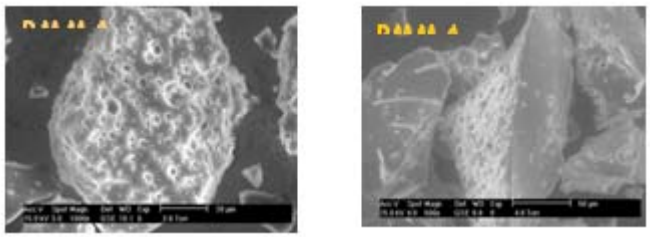
SurfaceT project have also made a process design for preparing polymer foams of low density via emulsification. Polyetherimide is a thermostable polymer with a high glass transition temperature (T_g). PEI, as it is also called, is composed of ftalic acid anhydride, DPP, and 1,3-aminobenzene. Foaming thermostable polymers to produce low density foams is difficult because of the high T_g (215-220 ºC). Blowing, as is used for example with polypropene, is not possible. As an alternative method, low density foam of PEI may be prepared with the help of micelles. In this project, micelles were first added to the system using the surfactant AOT and were drying wit supercritical CO_2. A very brittle material was obtained. In a second approach, surfactant Zonyl FSA (0,9 g), 8 ml PEI/$CHCl_3$ (15 %), 2 ml H_2O were mixed. In this case, sponges were obtained.



$SCCO_2$ emulsified PEI

Reactive precipitation of polymers

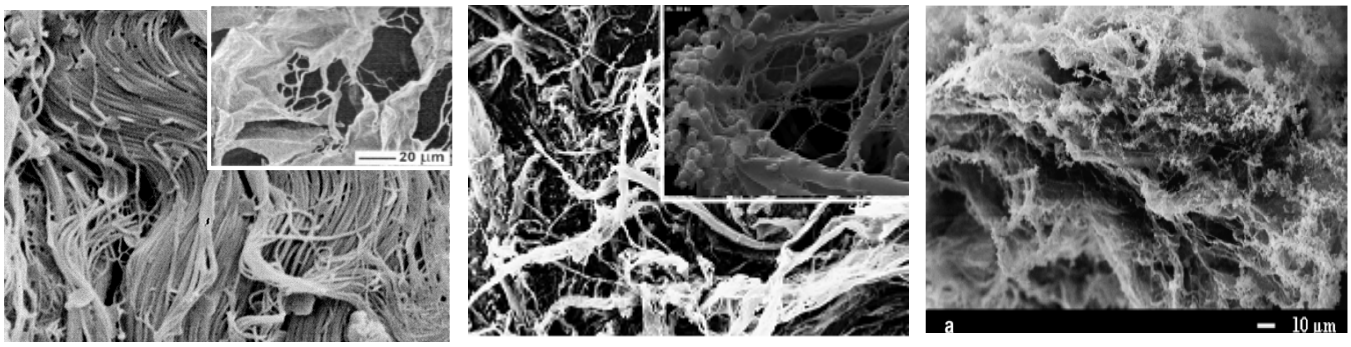
The aim of the polymerization in $SCCO_2$ was to obtain in one step reaction different polymer microparticles that will be also try to be loaded with bioactive molecules for their application as drug delivery systems. Polymerization reactions in $SCCO_2$ have been performed by using commercial monomers as MMA using the commercial surfactants Zonyl, PDMS with acrylic functionally and Krytox, incorporating in some cases small amounts of ibuprofen as a model drug. Similarly synthetic stimuli sensitive monomers such as *N*–ethyl pyrrolidine methacrylamide (EPA) has been copolymerized with a hydrophilic monomers such as *N,N*–dimethylacrylamide (DMA) in the presence of an amphiphilic synthetic surfactant, methacrylate derivative of Triton X-100 (MT). The polymerization reactions in $SCCO_2$ were all carried out at 250 bars and at 65º C for at least 20 h using AIBN as radical initiator, 2%-wt of the corresponding surfactant, followed by slow depressurization after desired time. ESEM micrographs showed compacted powder with some porosity of PMMA when PDMS was used as surfactant (see following Figure). When using Zonyl as surfactant similar morphology was obtained.



When incorporating the surfactant Krytox to the reaction microparticles of PMMA with homogeneous average diameter closed to 5 μm. The average molecular weight of the prepared systems resulted in polydispersity values lower than 2 in most of the cases, fact that could be attributed to the possible presence of chain transfer reactions in the polymerization process.

1.3.2. Porous homopolymers (fibers)

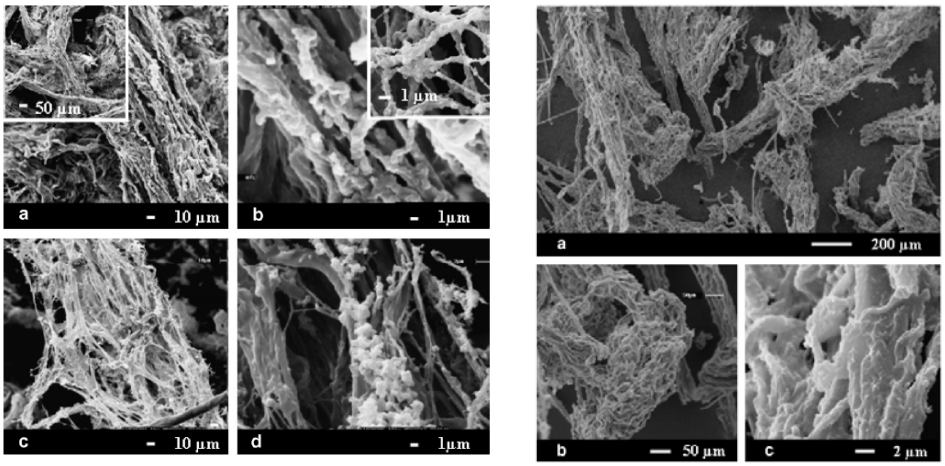
To overcome the difficulties derived from the use of monolithic foamed scaffolds, three dimensional meshes of polymer fibers are nowadays one of the preferred tissue scaffolds. The use of polymer fibers for biomedical and tissue engineering applications has some intrinsic advantages from a biomimetic approach, since a great variety of natural biomaterials are found with fibrous forms or structures (silk, keratin, collagen, cellulose, chitin, etc.). Therefore, polymer fibers can provide a proper route to emulate a biosystem such as the extracellular matrix. In the supercritical antisolvent precipitation (SAS) process, for systems involving high molecular weight macromolecules, viscous and inertial forces offer significant resistance to spray jet break-up. Polymer chain entanglements and viscosity build-up as liquid solvent leaved the polymer rich phase caused kinetic limitations for the formation of discrete polymer microspheres. The results presented in SurfaceT clearly suggested that the SAS technique can be used to process various biopolymers into fibers. The basic structure of these materials is made by small nanoparticles that either coalesced or fused. The morphological result is a rough textured surface with a high surface area to volume ratio. It should be taken into account that this is an essential requisite for effective cell seeding since it enhances cell adhesion onto the support. The obtained 3-D network structures showed two different types of internal porosity. First, there was a macroscopic (several microns in diameter) porosity formed by randomly oriented channels spread throughout the sample volume. Second, a continuous network structure of fused nanospheres composed the finest porosity of *ca.* 0.5-1 μm size. Therefore, SAS prepared fibrous scaffolds have a suitable macroporosity and interconnectivity to promote cell proliferation and to avoid pore occlusion by cell growth. Furthermore, the obtained scaffolds also exhibited adequate microporosity, necessary for capillary in-growth and neovascularization. Particles and fibrils of the amorphous PMMA polymer and the semicrystalline L-PLA were produced using the $SCCO_2$ antisolvent method.



Human collagen (left) and polymer fibers obtained using the SCCO2 SAS process of L-PLA (medium) and PMMA (right)

1.3.3. Porous blends (fibers)

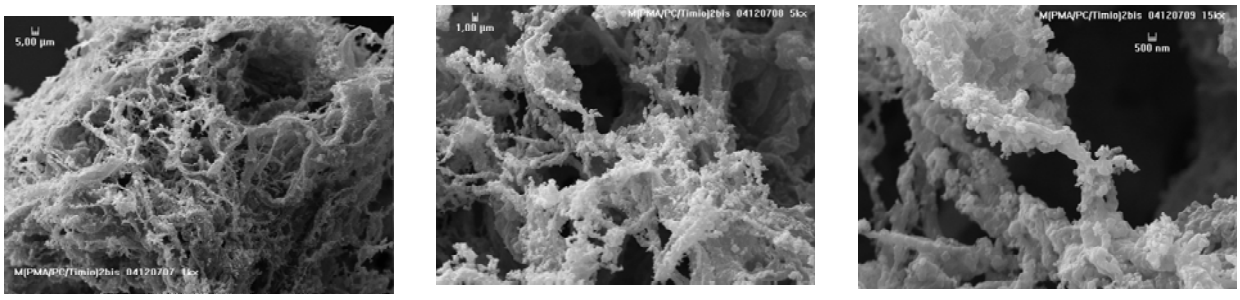
Polymer blends have been mainly prepared by batch mixing and extrusion with $SCCO_2$ as a plasticizing agent. The advantages of the SAS process described for SurfaceT project are based on the large diffusion coefficients of CO_2 into polymers and solvent into CO_2. These advantages were used in this work to prepare PMMA/PCL blends of several compositions: PMMA/15 wt% PCL and PMMA/30 wt% PCL. To trap the two components of a blend in a metastable state, without the need for a surfactant compatibilizer, they must be precipitated from solution rapidly. The time for polymer-solvent phase separation must be shorter than that for polymer-polymer phase separation. In this respect, compositional quenching by flash devolatilization of conventional liquids is used as a relatively new blending process after dissolving two incompatible polymers in a common solvent. In this process, the solvent is rapidly removed and the initial, single-phase mixture is plunged quickly into a multiphase region. In the SAS process, phase separation is accelerated because of the small length scale created by jet atomization and the rapid two-way mass transfer, leading to a fast solvent removal from the liquid polymer solution. $SCCO_2$ assisted viscosity reduction has been observed for PMMA associated with an increase in free volume of the polymer, which may allow more effective PCL polymer blending to occur. This phenomenon occurs via PMMA swelling resulting in a decrease in chain entanglement. A reduction in the interfacial tension caused by the presence of $SCCO_2$ at the interface may also contribute to facilitate polymer blending.



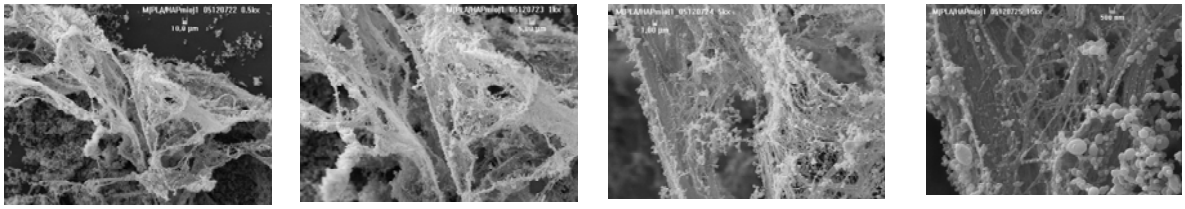
Fibers obtained using the $SCCO_2$ SAS process of PMMA/ 15 wt% PCL (left) and PMMA/ 30 wt% PCL (right)

1.3.4. Porous polymers (fibers) filled with surface treated nanoparticles

Owing to their extraordinary properties based on the combination of the easy-processing of polymers with the better mechanical properties of inorganic materials, inorganic/polymer composites, especially in the form of nanocomposites, are promising systems to substitute classical compounds for load bearing applications. Particularly nanohydroxyapatite (HAP) based composites, have gained much recognition as bone grafts due to their compositional and structural similarity with natural bone. First, our consortium carried out experiments based on the precipitation of fibrous matrices constituted by the biodegradable L-PLA or the blend PMMA/PCL by using the antisolvent $SCCO_2$ technique. However, prepared networks could be suited to the culture of only certain types of tissue, e.g. cartilage, but lack of the structural stability for many applications. Hence, the main aim of this work was to demonstrate that similar fibers composed of ultrafine mineral particles can also be prepared using the antisolvent $SCCO_2$ technique. In the developed process, the nonsoluble particulate filler was suspended in the polymer solution and both components were sprayed simultaneously into $SCCO_2$ Thus, a modification of the SAS technique was used to precipitate blends of PMMA/PCL loaded with TiO_2 powder and a composite of L-PLA and HAP nanoparticles. $SCCO_2$ technology was also used to silanize the surface of the nanometric powder previous to suspension in the polymer solution with the aim to facilitate the dispersion in the hydrophobic liquid medium and to further improve adhesion between the filler and the matrix during composite formation. Loadings of 20 wt% were achieved for the blend systems, and 15 wt% of HAP was incorporated to the L-PLA phase. Further loading of the composite systems with a model drug (ketoprofen) was not attained, due to the high solubility of the drug in the fluid phase.



PMMA/15%PCL & 20 wt% TiO2 nanometric



L-PLA & 15 wt% HAP nanometric

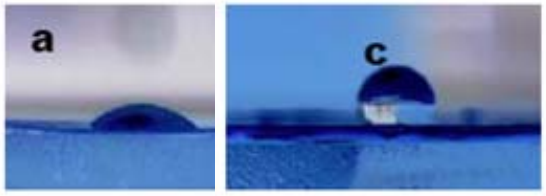
The study has been extended to other blends interesting as a biomaterials: PMMA/PEG, PMMA/PVP and PLA/PEG.

1.4. Cosmetics

1.4.1 Cosmetics for UV protection

Nowadays, there is a great demand of complex multiphase and multifunctional materials for smarter and smaller products. For most applications, hybrid composite materials are especially suitable, since the synergic combination of the characteristics of the dissimilar constituents provides a broad spectrum of properties, which can be engineered for each specific application. Composite systems, constituted by a nanometric inorganic phase homogeneously dispersed in a continuous organic phase, are becoming increasingly important for a multitude of applications, ranging from the development of lightweight structural materials to the advancement in medical devices. The degree of connectivity between the organic phase and the inorganic particles is of special relevance for determining the specific properties of the resulting composite. The interfacial interactions between both phases can be improved by means of the surface modification of the inorganic nanoparticles with bifunctional organic molecules acting as coupling agents. Among the different existing coupling agents, bifunctional silanes, a group of low toxicity and environmentally compliant chemicals, have been chosen in SurfaceT as the modifiers of the inorganic nanofillers.

The coating of the inorganic fillers is often carried out by applying organic solvents that are combined with surfactants and other additives. However, the coating of nanometric fillers cannot be easily performed by means of the classical liquid solvent route due to difficulties dealing with the viscosity and surface tension of the liquid solution. The use of $SCCO_2$, was envisaged in SurfaceT as an alternative for the surface functionalization of nanoparticles. Nanometric powder of titanium dioxide (TiO_2) was chosen as a model-real compound to be coated. TiO_2 is widely used in the cosmetics industry as an UV protector. The coating was performed with octyltriethoxysilane which confers a hydrophobic character to the TiO_2 surface. The silanization process in $SCCO_2$ media was optimized and characterized using a chemometric approach by means of a 2^3 factorial design. In contrast with conventional liquid solution-phase silanization, the TiO_2 surface-treated under $SCCO_2$ preserved the mesoporous character of the aggregates of TiO_2 particles. The supercritical silanization treatment increased the dispersibility of the silanized TiO_2 samples in oils, an, thus, in cosmetics bases (fi, oils, creams, etc).



Behaviour of a droplet of water on the surface of (a) non silanized TiO2 and (b) $SCCO_2$ silanized TiO_2.

1.4.2. Biopolymers for natural cures and cosmetics

SurfaceT has also worked on techniques for the production of new type of ointments that consist of nature high molecular combinations forming membrane and biologically active component. Medicamina products are multicomponent mixtures in form of liquids, ointments and creams which contain thickeners, preservatives (as ethanol), solvents (mainly water), bee-keeping products and biologically active components isolated from plant material. Among them, Hondra product has great importance for Medicamina since it takes around 20% of its turnover. Hondra is a medicament, used as a remedy in case of joint pain, blow pain, strains and pins and needles. Medicamina detected that this product shows oxidation and destructive processes during its isolation, and that the concentration of inactive compounds in its formulation is quite high. In order to remove these shortcomings, SurfaceT have worked with the objective of redesign Hondra product without changes in its actual compounds. The first objective of this work has been to increase the concentration of Hondra's active compound by improving the method of adding it to the base bend applying $SCCO_2$ impregnation or coating. Then, encapsulation techniques have also been tested, in order to avoid the degradation of the active compound during product manipulation and finished product preservation. HONDRA product contains carotene as an active ingredient in its formulation. Lycopene is known as one of the major carotenoids, and this is why it was selected as active compound of interest for the experimentation. However, lycopene is very sensible to oxygen, temperature and light, for that reason its stability is an important parameter to consider in formulations. Gelatine, pectin and cellulose were chosen as protected materials for being checked.

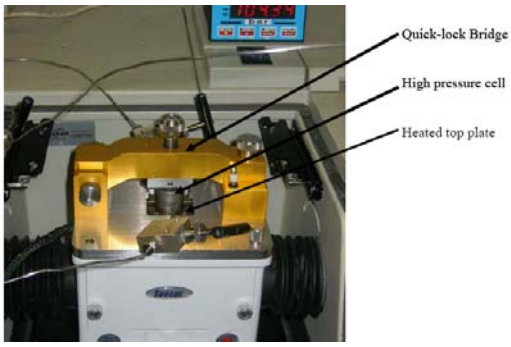
The impregnation of gelatine, pectin and cellulose with lycopene was studied under conventional conditions and using supercritical technology, with the aim of checking the advantages of the $SCCO_2$ compared to the conventional impregnation in regard to the concentration of lycopene in the final product. The average impregnation obtained using $SCCO_2$ was 1 wt%. The impregnation rate was not improved neither increasing the process duration nor increasing the system pressure. Therefore, in this case, the $SCCO_2$ technology could not be considered as a viable alternative to the conventional method.

With the aim of enhance lycopene stability and avoid its degradation, and according to the increasing interest in encapsulation of active compounds for the pharmaceutical industry, SurfaceT has studied the encapsulation of lycopene (core material) with gelatine (encapsulating agent). These compounds were selected in order to use some of the Hondra product components, and looking for an improvement of Hondra product formulation. The encapsulation set-up was not able to cat lycopene. Further work, will be performed using SAS $SCCO_2$ antisolvent approache.

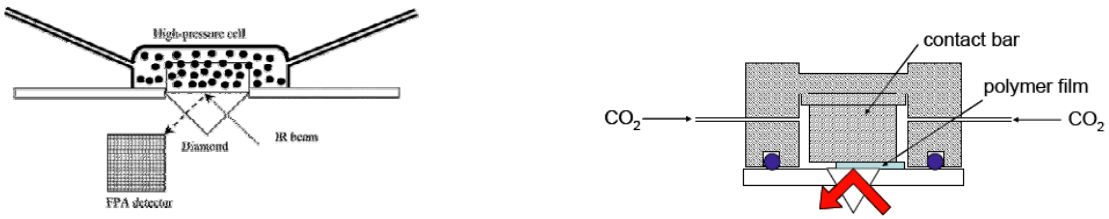
B. SurfaceT project has contributed To CREATE basic information to overcome technical barriers between fundamental science and engineering scale-up of $SCCO_2$ processes, by validation and demonstration of the strategies and advantages of using $scCO_2$ as a *i)* reaction solvent or dispersing media, *ii)* solute or reagent, and *iii)* antisolvent.

2.1 In situ spectroscopy

In situ spectroscopy is a powerful tool to probe fluids at a molecular level. This understanding helps engineers to utilize molecular level information for improving high-pressure processes or macroscopic properties of materials processed with supercritical fluids. Both Fourier transform infrared (FT-IR) spectroscopy and Raman spectroscopy have successfully been applied to elucidate the morphology and microstructure of polymers processed with supercritical CO_2. For infrared spectroscopy, a number of transmission optical cells have been designed for specific applications; nevertheless the challenges highlighted above often impose certain experimental limitations. Furthermore, it is very difficult to measure very short path lengths (less than 10 micrometers) using transmission cell. With a longer path length, polymers under supercritical CO_2 have been studied using overtones in near infrared region (smaller absorption) to measure spectra of polymers in-situ under supercritical CO_2. An alternative approach is to use ATR (attenuated total reflection)-IR spectroscopy for measuring the IR spectra of supercritical fluids and polymers. ATR-IR appears as a good alternative to circumvent the difficulties of transmission cells at elevated pressure. The evanescence wave in ATR-IR spectroscopy measures a very thin layer of the sample (from 0.1 μm to few micrometers) that is in contact with ATR crystal. Significant advantages of this approach are that there are no windows to seal, the chemical and physical properties of diamond are very favorable, and a small cell volume makes high-pressure experiments safe. The Supercritical Fluids Analyzer version of the Golden Gate™ Diamond ATR, was designed for operation at extreme temperatures and pressures. The high thermal conductivity of the diamond element is ideal for fast temperature equilibrium and accurate temperature measurement, reducing analysis time and increasing sample throughput.



The principle of this ATR imaging approach to study dynamic polymer systems in the presence of high-pressure and $SCCO_2$ is shown schematically in figure below.

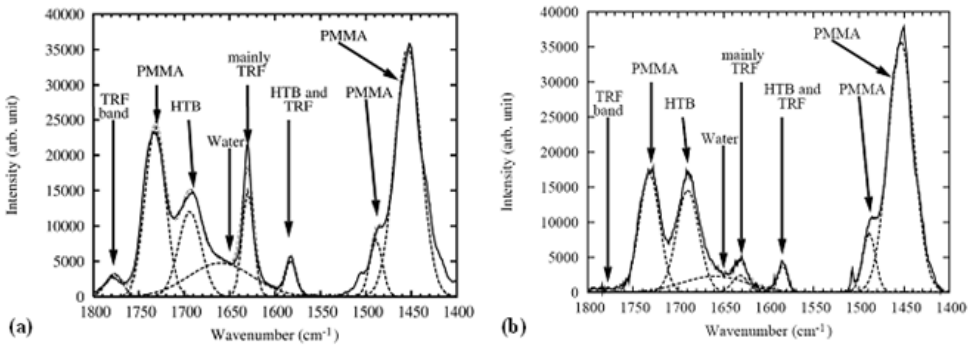


Schematic diagram of miniature high-pressure cell assembly. The polymer film, shown in blue, is sandwiched onto the diamond ATR crystal by the contact bar.

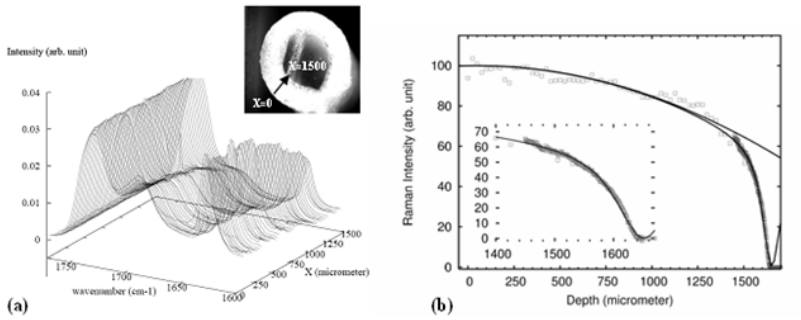
FT-IR imaging is a powerful new tool for characterizing the distribution of different chemicals in heterogeneous materials. The inherently rich information contained in the mid-infrared spectrum allows one to identify different components from their chemical structure. High spatial resolution is very important in revealing the true distribution of different components in heterogeneous materials. For samples with smaller domains of interest, other analytical methods are used, such as Raman microscopy, X-ray microscopy, or micro-thermal techniques.

Raman spectroscopy is viewed as a complementary technique to FT-IR since it can probe the IR-inactive vibrations of molecules. Raman spectroscopy also benefits from minimal sample preparation, and has proven to be very useful in characterization of supercritical fluids and polymers. Indeed, the analysis of vibrational bands of the fluid is very informative for the design and applications of high-pressure processes.

One example of application of these equipments in SurfaceT project is in the system PMMA/Triflusal. Drug concentration profiles and interactions between the polymer and the active agent molecules were analyzed using ATR Fourier transform infrared (ATR-FTIR) and Raman spectroscopy.

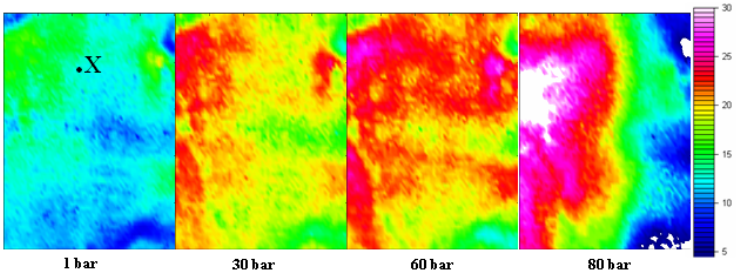


Decomposition by an 8 Gaussians functions of Raman spectra of PMMA impregnated with (a) triflusal TRF and (b) HTB metabolite of triflusal.



Evolution of the drug profile in a PMMA sample impregnated with triflusal as a function of the radius of the bar: (a) evolution of the Raman spectra in the range 1600-1750 cm^{-1}, (b) intensity of band at 1640 cm^{-1} related to impregnated drug (TRF/HTB) percentage.

A novel system to characterize coacervation using ATR-FTIR at atmospheric and high pressure CO_2 was constructed. FTIR images of elastin (20 mg/ml) under high pressure CO_2 were obtained and the distributions of amide II at different pressures could be detected and is shown in the following images. The images are of the same scale and the color denotes the absorbance of the assigned spectral peak which is proportional to the concentration of amide II. As the pressure increases, it can be shown that a higher concentration of Amide II was detected on the ATR diamond. ATR-FTIR spectroscopic analysis confirmed that CO_2 pressure did not change the secondary structure of α-elastin.



• In situ ATR-FT-IR single element detector can be used to obtain quantitative information about polymers, drugs, CO_2 with a time resolution and also monitor evolution of molecular interactions between them.
• In situ ATR-FT-IR spectroscopic imaging allows observing evolutions of concentrations (drugs, polymers, CO_2) with time and spatial resolution under supercritical conditions. In situ monitoring using FT-IR spectroscopic imaging helps to optimize high-pressure supercritical fluid processes.

• Confocal Raman microscopy is appropriate for studying molecular interactions and concentration. The time needed to realize a mapping (imaging) is usually too long for in-situ confocal Raman microscopy unlike ATR-FT-IR imaging. But confocal Raman microscopy allows one to measure depth profile of materials in non-destructive manner.
• In situ near-infrared (NIR) transmission spectroscopy can be complementary for ATR-FT-IR spectroscopy for studying liquid systems, especially when pressure dependence of the molar absorptivities of spectral bands in midinfrared is not negligible.

2.2. Scale up

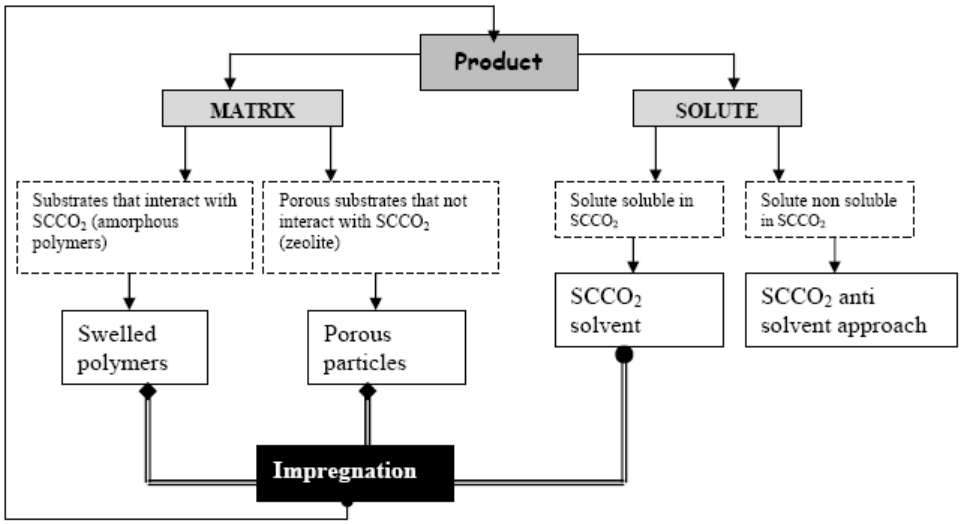
Partners Matgas and Feyecon have carried out the scale up of the equipments. The size of the up-scaled equipment at Matgas is characterized by the 16 liter pressure vessel in combination with a CO_2 feed pump with a capacity of 40 liter/hour liquid CO_2 (approx. 40 kg/hour CO_2) at 500 bars. Matgas has built the equipment multi-purpose (for impregnation, spraying, coating, etc.). FeyeCon has built an equipment with a pressure vessel of 10 liter, in combination with a feed pump with a maximal capacity of 60 kg/hour liquid CO_2 at 250 bar. The equipment has been designed to such extent that CO_2 can be recycled. CO_2 is circulated though the system using a high pressure circulation pump with a maximal capacity of 450 liter/hour, (at normal working conditions this is approx. 300 kg/hour CO_2). With this facility spraying can be performed with much higher CO_2 flow than with the single pass CO_2 system. The system was designed and built as single purpose, for particle formation by spraying. The production capacity of the system depends strongly on the specific product and process characteristics. For one specific product the system has a proven capacity of 350 gram powder per hour.

The scale of production possible with the equipment available at this moment might be suitable for pharmaceutical products, where a total production is a relatively small quantity. For other product area's, like food materials, the quantities will be much larger. A further step in up-scaling will be necessary. An up-scaling by a factor 10, from 10 liter vessels to, at least, 100 liter vessels and related CO_2 capacity will be the next step.

2.3. Reaction solvent or dispersing media

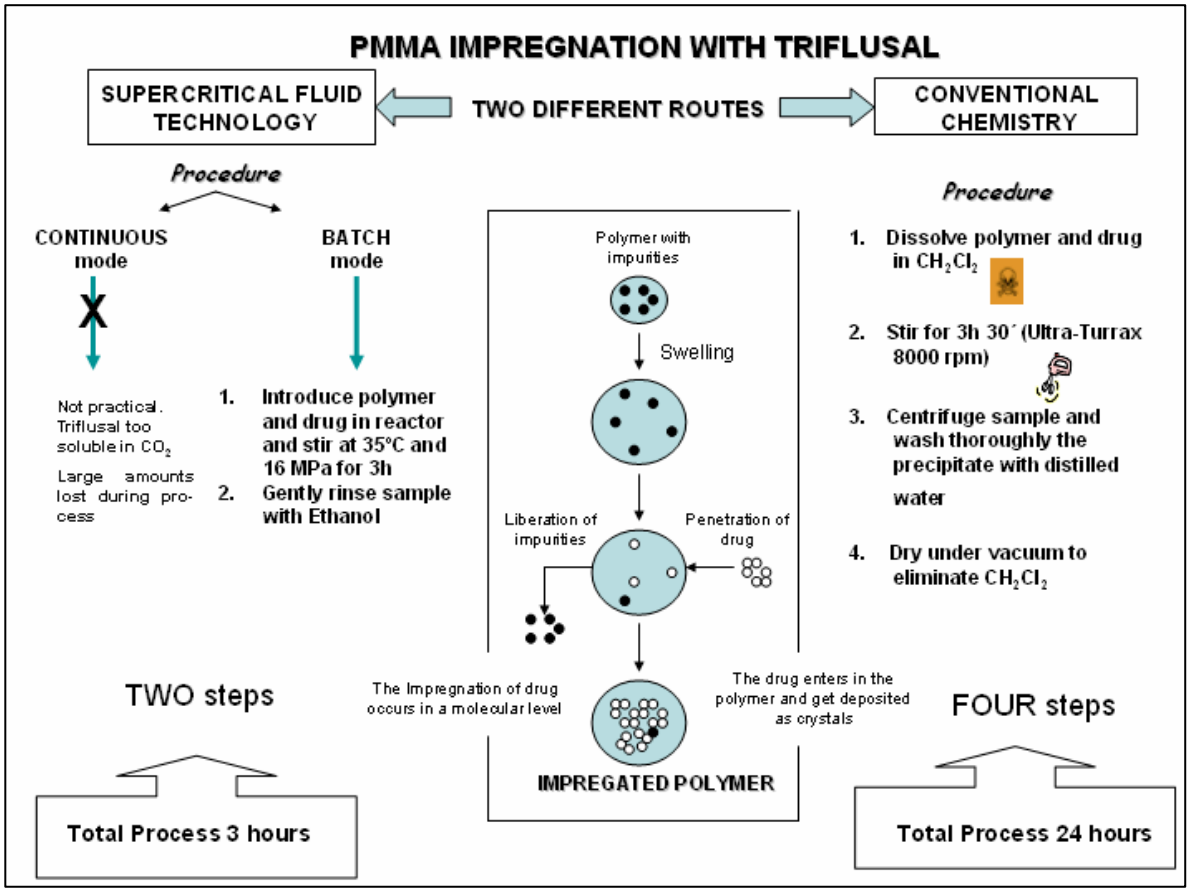
2.3.1. Impregnation processes

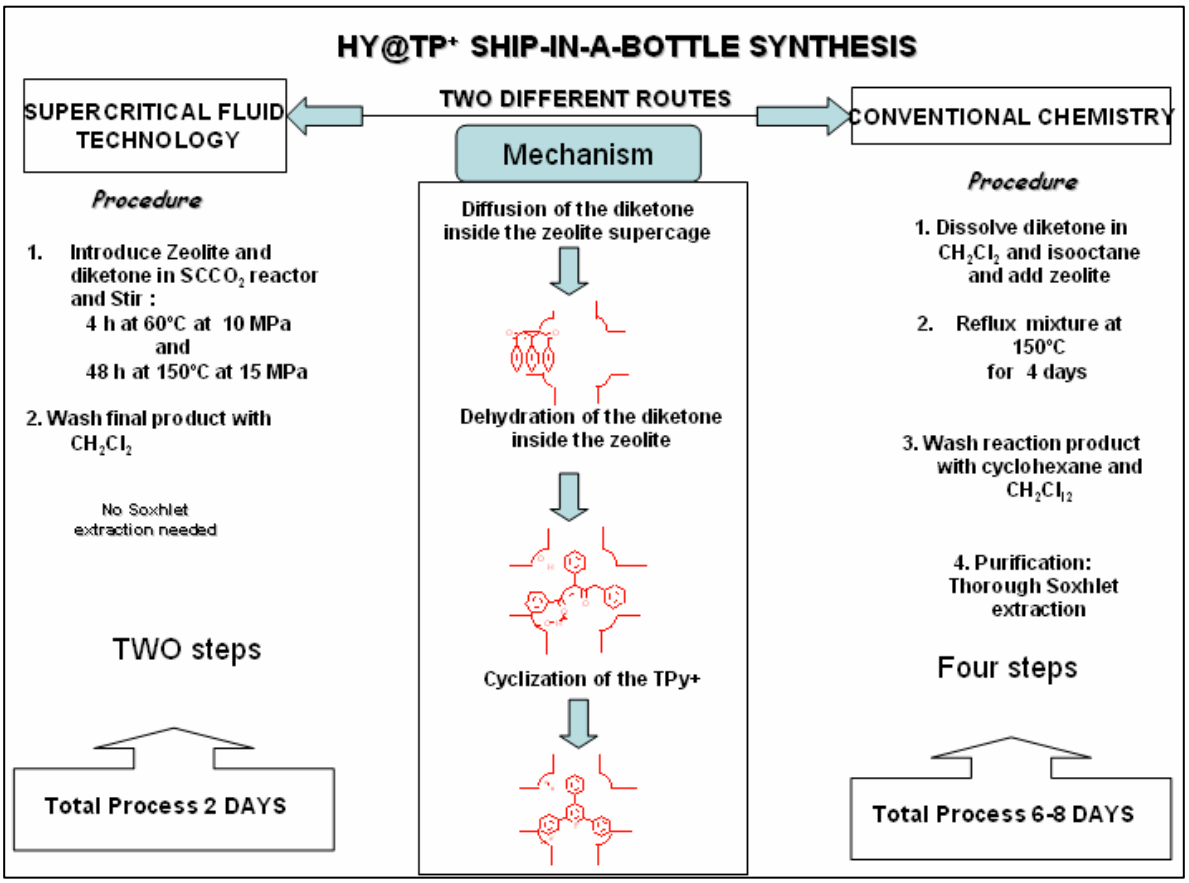
Supercritical impregnation of matrixes of organic (polymeric) and inorganic (zeolite) nature were developed in SurfaceT project using carbon dioxide as the solvent. These two different types of substrates were considered taking into account their interaction with $SCCO_2$: (i) substrates that interact with $SCCO_2$, where bulk adsorption of $SCCO_2$ into the polymeric matrixes leads to polymer swelling and increasing void volume; and (ii) porous substrates that do not interact with SCCO2, where the $SCCO_2$ is used as a solvent, facilitating the addition of organic molecules to internal surfaces since there is no competition for adsorption between the added reagents and solvent molecules.



Impregnation process design related with substrate and solute behaviour on $SCCO_2$

Below, it is shown two schemes comparing the polymer impregnation and the ship-in-a-bottle processes in both conventional and supercritical techniques. In both cases it can be seen that processing time significantly decreases, as well as the number of processing steps needed.

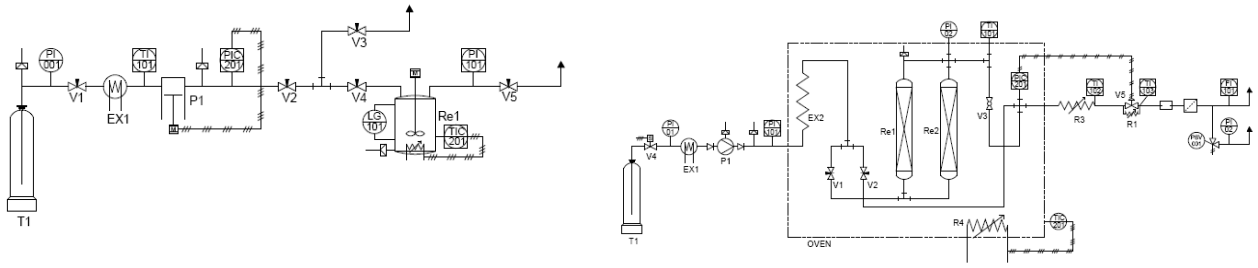

PMMA IMPREGNATION WITH TRIFLUSAL
SUPERCRITICAL FLUID TECHNOLOGY
TWO DIFFERENT ROUTES
CONVENTIONAL CHEMISTRY
Procedure
CONTINUOUS mode
BATCH mode
Not practical. Triflusal too soluble in CO_2
Large amounts lost during process
1. Introduce polymer and drug in reactor and stir at 35°C and 16 MPa for 3h
2. Gently rinse sample with Ethanol
Polymer with impurities
Swelling
Liberation of impurities
Penetration of drug
The Impregnation of drug occurs in a molecular level
The drug enters in the polymer and get deposited as crystals
IMPREGATED POLYMER
Procedure
1. Dissolve polymer and drug in CH_2Cl_2
2. Stir for 3h 30´ (Ultra-Turrax 8000 rpm)
3. Centrifuge sample and wash thoroughly the precipitate with distilled water
4. Dry under vacuum to eliminate CH_2Cl_2
TWO steps
Total Process 3 hours
FOUR steps
Total Process 24 hours


HY@TP+ SHIP-IN-A-BOTTLE SYNTHESIS
SUPERCRITICAL FLUID TECHNOLOGY
TWO DIFFERENT ROUTES
CONVENTIONAL CHEMISTRY
Mechanism
Procedure
1. Introduce Zeolite and diketone in $SCCO_2$ reactor and Stir :
4 h at 60°C at 10 MPa
and
48 h at 150°C at 15 MPa
2. Wash final product with CH_2Cl_2
No Soxhlet extraction needed
Diffusion of the diketone inside the zeolite supercage
Dehydration of the diketone inside the zeolite
Cyclization of the TPy+
Procedure
1. Dissolve diketone in CH_2Cl_2 and isooctane and add zeolite
2. Reflux mixture at 150°C for 4 days
3. Wash reaction product with cyclohexane and CH_2Cl_2
4. Purification: Thorough Soxhlet extraction
TWO steps
Total Process 2 DAYS
Four steps
Total Process 6-8 DAYS

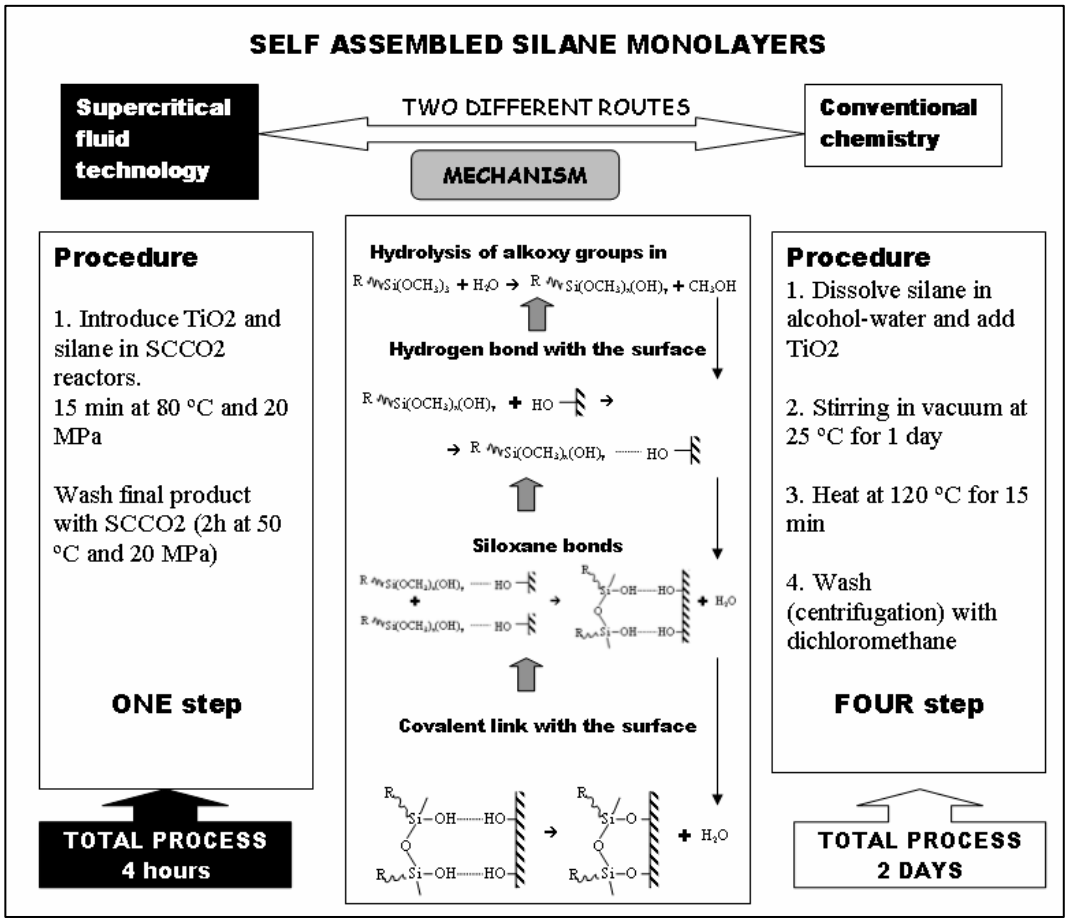
2.3.2. Coating processes

Silanization

One ob the important aims of surfaceT project has been to develop a generic method of silanization using $SCCO_2$, for the surface modification of inorganic nanoparticles with organic functional molecules, in order to obtain hybrid materials. The effective silanization process of TiO_2 nanoparticles in $SCCO_2$ has been presented. With respect to conventional methods it has several advantages. Supercritical fluid properties of gas-like diffusivity and liquid-like density combined with the tunability of these properties with pressure and temperature changes provide new solvent characteristics to these fluids. Moreover, the low viscosity and surface tension of $SCCO_2$ solutions allow the complete wetting of substrates with intricate geometries, including the internal surface of agglomerates of nanoparticles. Moreover $SCCO_2$ process is an anhydrous method, thus, avoiding multilayer formation; was carried out in a shorter period and at lower temperature than conventional method; and is more effective for the homogeneous coating of nanoparticles with mesoporous structure. According to experimental design results, operation variables of the supercritical silanization process play an important role in the properties of the silanized end material. Thereby, physicochemical and mechanical properties of the silanized TiO_2 material can be tuned by means of the proper selection of the operating conditions of the process. Depending of the necessity, two systems (batch and continuous) have been optimized.

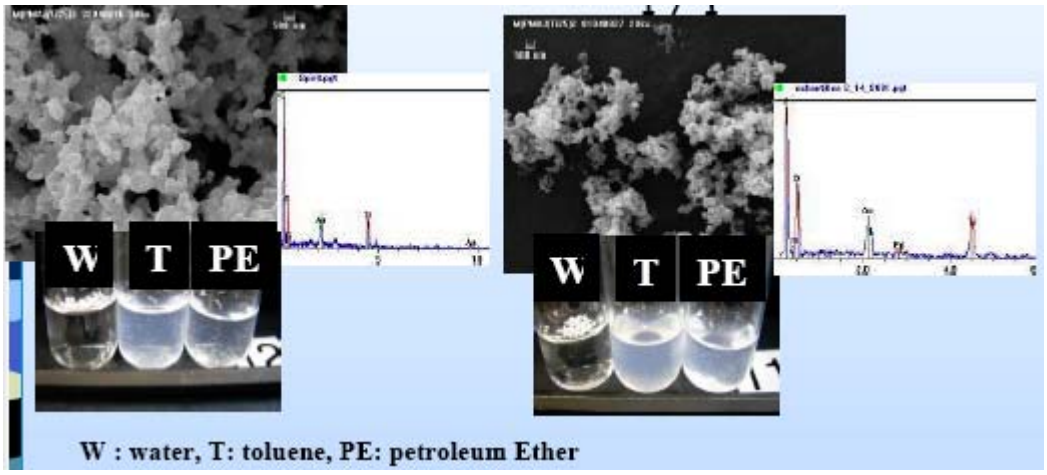


Equipment for batch (left) and continuous (right) supercritical processing



Polymer coating

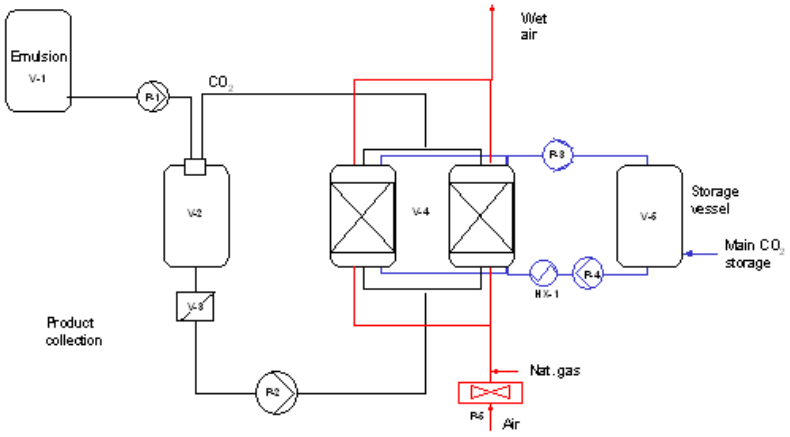
Nanometric particles (TiO_2) have been coated by coating by spraying the suspension in CO_2 antisolvent. PMMA polymer was used as the coating agent. After processing a hydrophobic coating was evidenced.



TiO2 nanoparticles coated with PMMA using $SCCO_2$ antisolvent approach, suspended in liquids with different polar behaviour

2.3.3. Emulsions

In the high-pressure equipment for the production of stable formulated drug particles, an emulsion containing the drug and excipients is dried under supercritical conditions. This process can be applied for drug formulation, encapsulation of actives and as an alternative to conventional drying methods for protein stabilization. In SurfaceT project, the process was also used to prepare polymer foams.

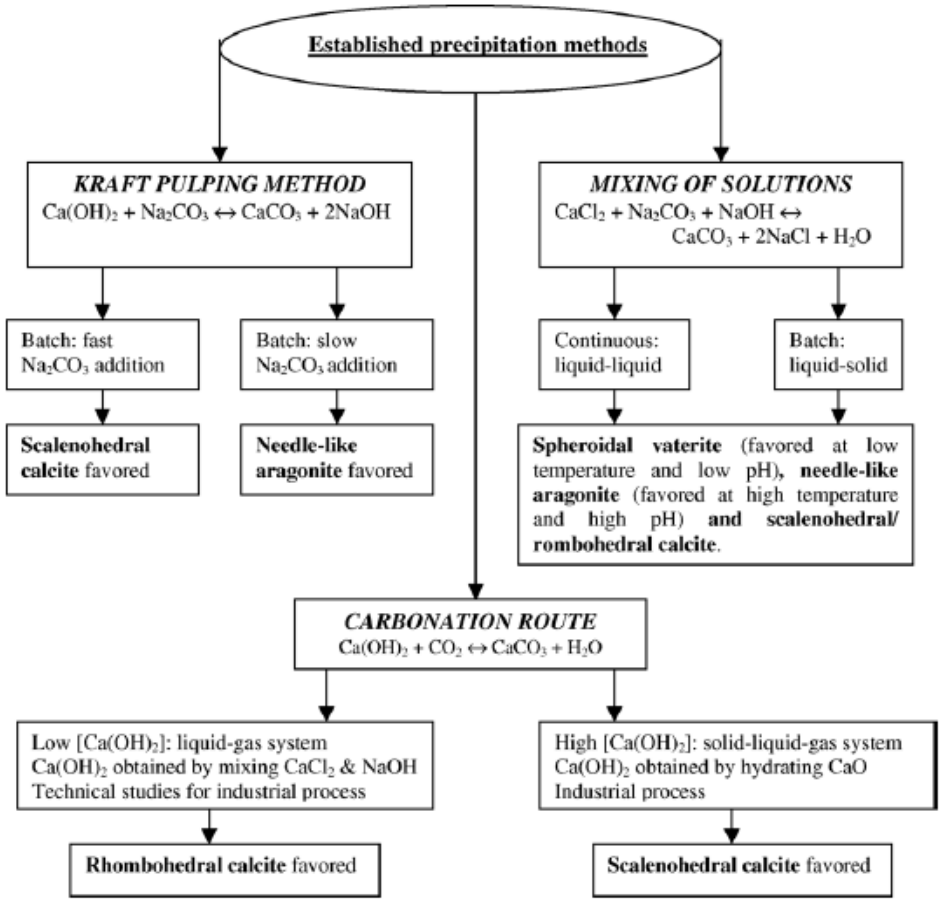


Flow Scheme of the process of drying emulsions with $scCO_2$

2.4. Solute or reagent

2.4.1. Carbonation

The formation of solid calcium carbonate (CaCO3) from aqueous solutions or slurries containing calcium and carbon dioxide is a complex process of considerable importance in the ecological, geochemical and biological areas. In the figure, the most common established procedures for CaCO3 precipitation are schematically depicted.



Most common established procedures for the controlled precipitation of CaCO3 powders

In the conventional methods, the morphologic control is most of the times provided by adding certain expensive additives. The development of new methods for the production of calcite by carbonation of slaked lime with different morphologies, avoiding agglomeration, is actually of great interest. The low viscosity and high diffusivity occurring in near-critical or $SCCO_2$ result in enhanced reaction rates for process carried out involving these media. Therefore, compressed CO_2 has many properties which make it an attractive solvent for chemical processing and/or reactions on an industrial scale. The conditions and mechanism for producing calcium carbonate particles with various shapes and sizes was first investigated in a reaction between $Ca(OH)_2$ and compressed CO_2 (carbonation route). The developed process is efficient in regard of running time, energy, and morphological control of the obtained calcite. Specific process applications in the papermaking industry and in the concrete field were also envisaged and tested.

2.4.2. Sterilization of biomaterials

The most common methods of bacterial inactivation (sterilization) consist of superheated steam, ethylene oxide treatment, UV-irradiation, or gamma-irradiation. None of these methods is well-suited to the sterilization of synthetic, polymer-based materials, which are increasingly being used in modern medicine, especially for controlled-release drug delivery, tissue implants, and orthopedic devices. For example, the high temperatures required for autoclaving can decompose encapsulated drugs or other sensitive biologics. Similar losses in activity result from UV-irradiation. Sterilization with gaseous ethylene oxide may leave residual material in the polymer, which can cause hemolysis or other toxic reactions. Ethylene oxide can also react with proteins and nucleic acids, which are increasingly being developed as drugs. Gamma radiation may alter the elasticity or tensile strength of polymers, and damage other labile biological materials Gentle alternatives to existing sterilization methods are called for by rapid advances in biomedical technologies. Supercritical fluid technologies have found applications in a wide range of areas and have been explored for use in the inactivation of medical contaminants. In particular, supercritical CO_2 is appealing for sterilization due to the ease at which the supercritical state is attained, the non-reactive nature, and the ability to readily penetrate substrates. However, rapid inactivation of bacterial endospores has proven a barrier to the use of this technology for effective terminal sterilization.

Cleaning human bone

SurfaceT objective in this area was to develop a process for cleaning human hard tissue for implantation purposes with superior sterilization and antiviral potential in addition to extraction of fat using $SCCO_2$ technology. Currently $SCCO_2$ is used to extract fat from human bones (mainly femoral heads). The advantage over chemical extraction is the lack of chemical waste products compared to conventional extraction of fat from femoral heads. As $SCCO_2$ is a promising technique to sterilize products that are sensitive to radiation or heat, of which bone is one. For reconstructive surgery the use of autologous (the patient is receiving bone from a different location from its own body) human bone is a gold standard up to now. In addition allogenic bone (patient receives bone from a donor) is frequently being used as autogenic bone is not always sufficiently available and requires an additional operation procedure with all its associated risks (blood loss, infection, additional care etc). The main problems encountered with the use of allogenic material are the transmission of bacteria and viruses and immunogenic responses to the donor bone. SurfaceT is therefore developing a process using $SCCO_2$ to inactivate bacteria and viruses. As outlined before $SCCO_2$ is currently used to extract fat from human femoral heads. The advantage of using $SCCO_2$ above other processes is its use at low temperature (up to 40^{o}C) and the absence of chemical waste generation. Other processes use chemical treatment and high temperature regimes or gamma irradiation to eradicate bacteria and viruses. These processes have been shown to negatively affect mechanical properties of the bone. In addition to bacteria, $SCCO_2$ has the potential to inactivate viruses. In the framework of SurfaceT project possible additives that can increase antibacterial and antiviral properties of $SCCO_2$ have been investigated. These additives will also be tested on its antibacterial and antiviral properties and mechanical properties of human bone. The additives we have determined are EtOH, H_2O_2 and O_3. The used equipment for experiments is an EMCM plant where the $SCCO_2$ equipment is integrated in the clean room facility. Therefore, bacteria and viruses cannot be introduced into the production plant.

***1. Bacteria inactivation*:** not achieved to EU requirements (on going work). Based on these results it is concluded that $SCCO_2$ either by itself or with additives tested cannot achieve the level of bacterial and endotoxin removal as required for medical implants. Based on these results additional action must be performed to prevent presence of bacteria and endotoxins in human bone based products. Gram negative bacteria (containing endotoxins) must be excluded as an implant. Therefore incoming inspection must include microbiological testing. $SCCO_2$ cannot be used as a final sterilization method as a microbiological reduction could not be shown. Therefore a final sterilization with g-radiation must be performed to guarantee a sterile end product even though the mechanical products are negatively affected.

Process	Endotoxin level (EU/device)	Endotoxin Specification (EU/device)	Sterility achieved	Species identified
$SCCO_2$ 250 Bar; 40 ºC; 30 kg CO_2 /h	17,4	≤ 1	No	Bacillus spp
$SCCO_2$ 250 Bar; 40 ºC; 30 kg CO_2 /h + Ethanol-*R* 1.3%	2,5	≤ 1	No	Bacillus spp
$SCCO_2$ 250 Bar; 40 ºC; 30 kg CO_2 /h + Ethanol-*R* 2.0%	12,8	≤ 1	No	Bacillus spp
$SCCO_2$ 250 Bar; 40 ºC; 30 kg CO_2 /h + Ethanol-*R* 2.6%	43,8	≤ 1	No	Bacillus spp
Pressure drop $SCCO_2$ (75-150-300 Bar, 2½ x)	15,0	≤ 1	No	Bacillus spp
$SCCO_2$ 250 Bar; 40 ºC; 30 kg CO_2 /h + O_3 (1 puls = 100 mg)	24,0	≤ 1	No	Bacillus spp
$SCCO_2$ 250 bar; 40 ºC; 30 kg CO_2 /h + O_3 (2 pulses = 200 mg)	24,1	≤ 1	No	Bacillus spp
$SCCO_2$ 250 Bar; 40 ºC; 30 kg CO_2 /h + O_3 (3 pulses = 300 mg)	1,9	≤ 1	No	Bacillus spp

***2. Virus inactivation*:** partially (on going work). The overall conclusion of this study is that the supercritical CO_2 fluid extraction results in considerable clearance for the lipid-enveloped viruses (BVDV, HIV and PSR) tested. In case of the non-lipid-enveloped viruses (CPV and HAV), the clearance is limited. In the bench controls of the cortical bones also limited clearance was observed indicating that clearance is caused by inactivation rather than removal of virus. In the spike controls of the cancellous bones viral reduction was observed, but the mechanism of this unexpected reduction remains unclear.

Virus	Clearance factor (log)
Cortical bone	
BVDV	3.8 – 6.3
CPV	1.2
HAV	0.9
HIV	>6.4
PSR	>6.9

Cancellous bone	
BVDV	Ongoing
CPV	0.5
HAV	2.6 – 4.6
HIV	Ongoing
PSR	5.7 – 6.5

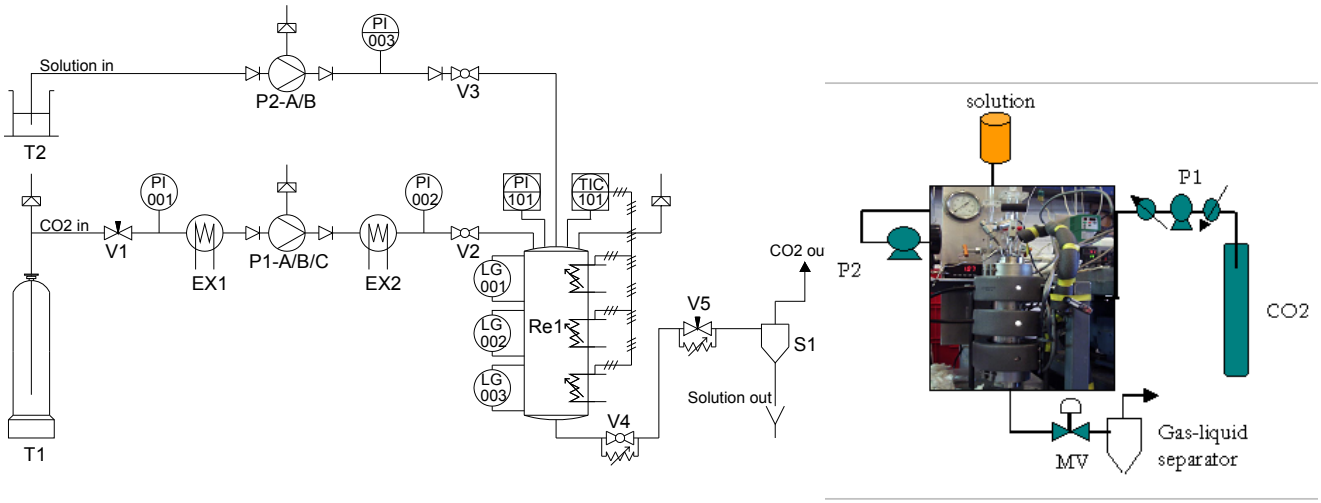
Alginates (seewead)

Alginates are derived from brown seaweed, a source of alginic acid which is treated with appropriate basic or acidic compounds to produce the potassium, sodium, ammonium or calcium salts, and propylene glycol and glyceryl alginates. Alginates (seewead) are used as a raw material for pharmaceuticals and for medical devices. Alginates have contamination with endotoxins (mainly Lipopolysaccharides (LPS) with a Lipid A that is the "active" pyrogen) from Gram negative bacteria and with polyphenols. Endotoxins cause fever, septic shock or even death. Medical Devices must have less than 20 Endotoxin Units (EU) per device, while pharmaceuticals must have less than 0,25 EU/mL (in parental solutions). Hence, endotoxin removal is essential. The current methods for endotoxin removal are (i) liquid – liquid extraction (Class II organic solvents – hexane); (ii) hydrolysis (loss of strength – decrease chain length), (iii) filtration (debris on filter – pressure difference – membrane damage). In general, current methods have the drawbacks of a need of production throughput of several days, high bioburden, and necessity of preservatives (e.g. antibiotics).
In the framework of SurfaceT project, a method for the inexpensive production of low endotoxin biopolymers has been developed and is being optimized. The method is a fully non-degradative method, utilizing exclusively relatively harmless class III organic solvents (ethanol) and volatile (compressed carbon dioxide) solvents. Laboratory scale batch can easily be processed within one day which reduces overall costs and makes the addition of antibiotics superfluous. The laboratory scale investigation showed that the method can easily compete with established methods. However, the process has only been tested on a laboratory scale and is not ready for commercialization.

Detoxified alginate can be used for: (i) antiadhesiveness gel, slow release fleece (e.g. gentamycin), aneurysm therapy and resorbable invasive device for bone therapy. A prospect of market value indicated that (a) worldwide, annual current market is of 30 million € for GMP alginate, (b) it exits an increasing demand of approximately 6% per year, and (c) $SCCO_2$ product can be produced with a compatible price with respect to conventional-competitor price.

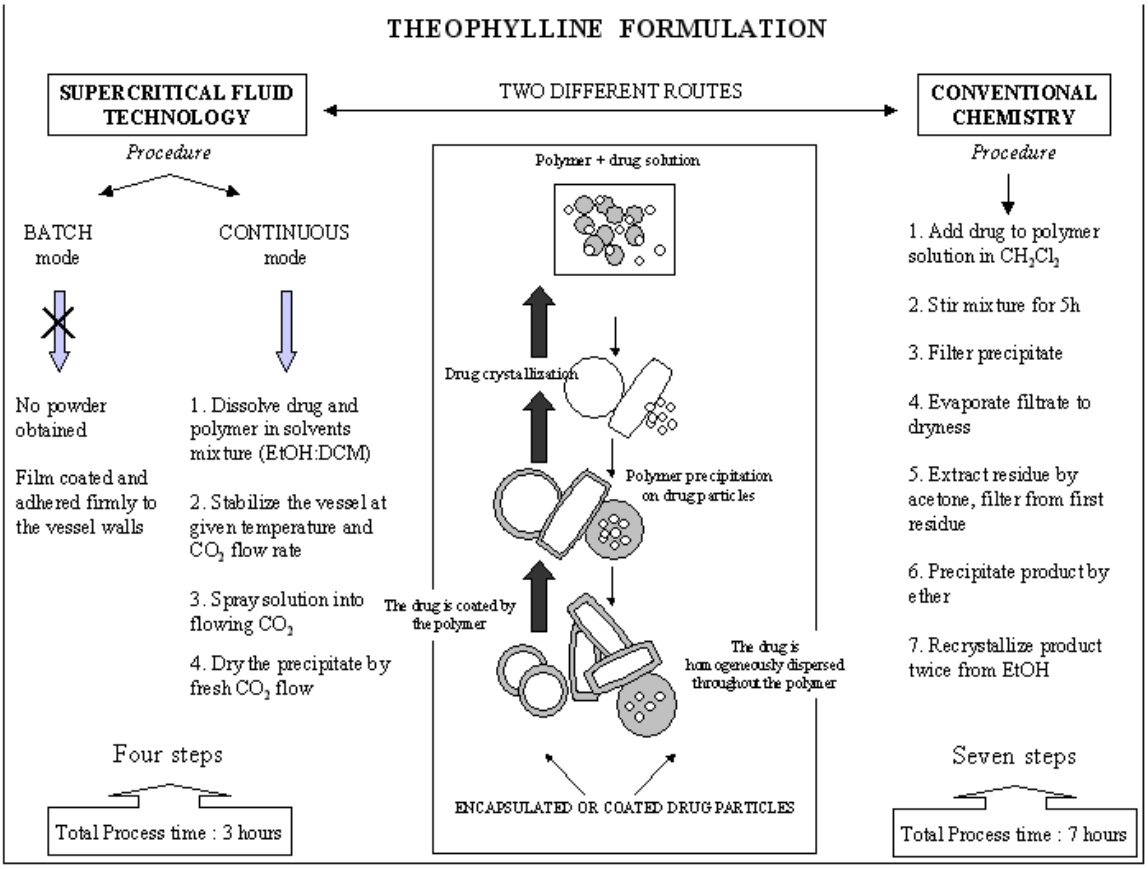
2.5. Antisolvent

Antisolvent equipment has been optimized for both the production of particles (microspheres) and fibers (composites). The used equipment is schematized following:



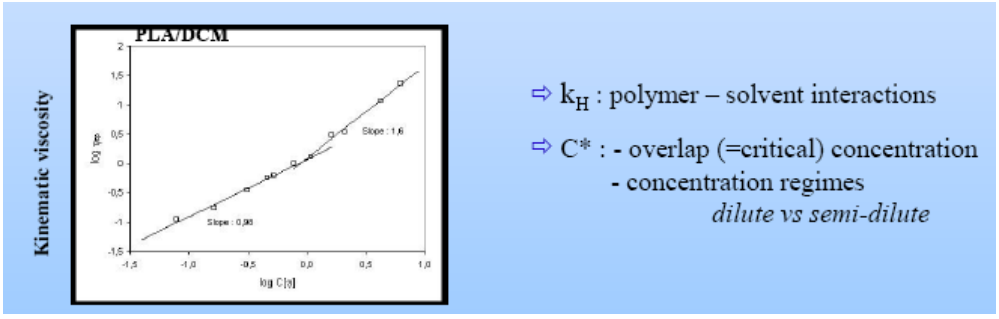
$SCCO_2$ SAS equipment to work either in batch or in continuous.

Crystallization or formulation of drugs with carriers aim at producing a drug with specific characteristics, either for therapeutic benefit (bioavailability, controlled release…), or for engineering benefit (flowability, anti-aggregation, handling…). The definition of the specificities of the final material pinpointed the targets, but producing it by as cleanest route as possible defines the challenge. The retroprocessing approach was initiated at the early stage of the antisolvent process design, with the therapeutic problem that drove the selection of the different carriers and/or crystal characteristics, and the selection of the most appropriate solvent for developing new and clean routes for formulation. The next figure compares the formulation by both conventional and supercritical routes for THEO. The supercritical route provides a significant reduction in the number of steps, specially of filtration, sieving, washing and drying, that are known to generate a large volume of liquid wastes (to be further recycled or safely destroyed), of solid dusts and emission of vapors in atmosphere.



The semi-continuous antisolvent process has been deeply analyzed in regard of:
- Spray processes: viscosity is a highly significant parameter
- Polymer solutions: strong dependence of viscosity on its concentration

In order to carry out and evaluation of rheological parameters and in order to assess a possible correlation between the polymer solution properties and the different morphologies obtained by SAS a viscosimetry study of solutions of L-PLA and PLGA in various solvents was performed.

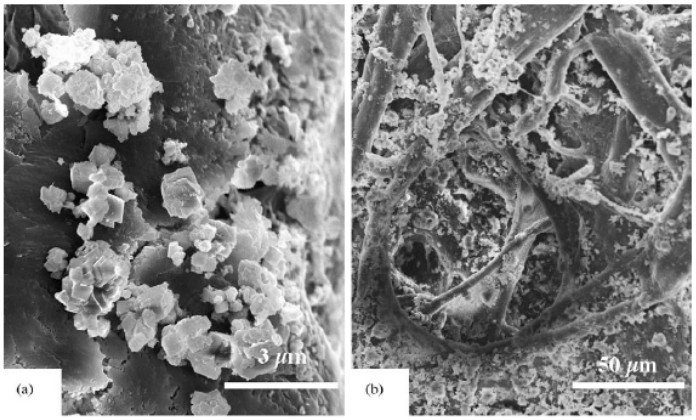


C. **SurfaceT project has contributed** To EXTEND **the state of knowledge in $SCCO_2$ technology and stimulate advanced research in new directions (different from biomedical) by exploring, developing and deploying efficient processes for the production of high-tech and commodity products: valuable carbon containing materials (carbonates), high-tech (photocatalysis) and commodity (modified plastics, concrete) products.**

3.1. Carbonates

3.1.1 Paper industry

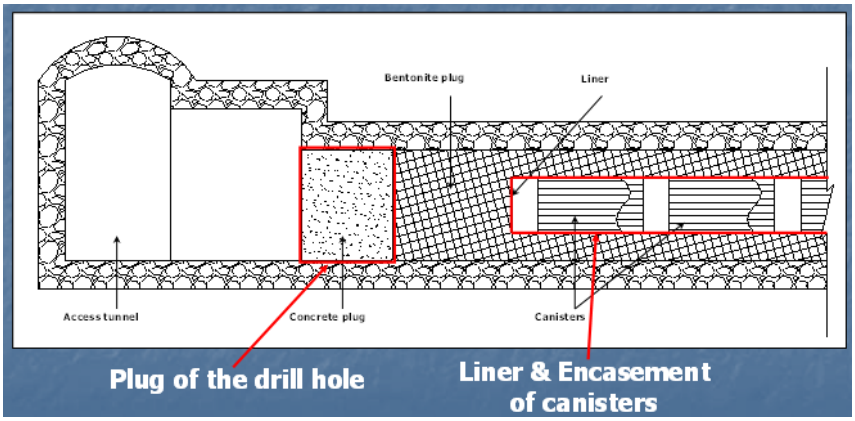
In general, the currently utilized manufacturing processes of precipitated calcium carbonate are slow, with low carbonation efficiencies. Scalenohedral forms are usually obtained and agglomeration cannot be avoided without the use of additives. First results of SurfaceT showed the possibility of precipitate rhombohedral calcite with a low degree of agglomeration by using $SCCO_2$ as a reagent. The use of high pressure CO_2 is also desiderable for increasing reaction rate and carbonation efficiency. Thus, manufacturing plants require smaller equipment, resulting in low capital costs per unit of calcium carbonate production, which can compensate the large initial investment inherent to high pressure equipment. Preliminary results showed also the possibility of rhombohedral calcite precipitation within the pores of a fibrous material, allowing the increase of the total loading without unacceptable deterioration on paper properties. This process can be more efficient than the conventional papermaking method due to its high consistency *on line* reactor operation, which could eliminate the need for a satellite plant to manufacture powdered calcium carbonate slurry. Applications can range from those related to the preparation of high added value papers (fi, light weight high-opacity bible paper) to others related to conservation of cultural paper heritage. Process is now extended for the precipitation of $CaCO_3$ into the biopolymer fibers to prepare biomaterials for tissue engineering.



Precipitated CaCO3 particles using $SCCO_2$: (a) rhombohedral calcite on the surface of cellulose paper, and (b) hollow cellulose fibers impregnated with calcite.

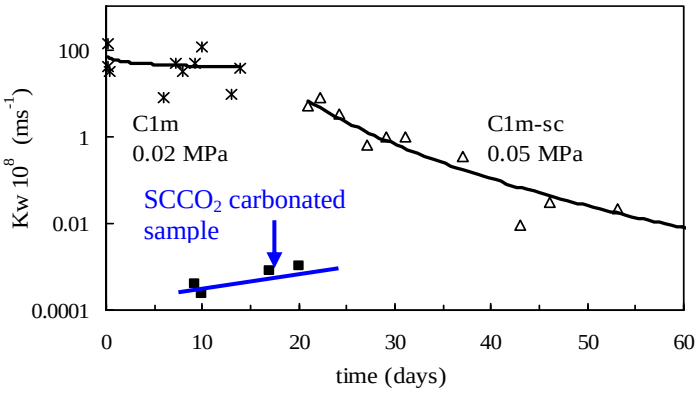
3.1.2. Cement/Concrete industries

Concrete has been proposed in Europe for its use as a barrier in long-term deep geological repositories of nuclear wastes. Nevertheless, leaching processes take place when concrete is in contact with groundwater, leading to some chemical and physical transformations on the barriers of the repository with a subsequent decrease in its confinement capacity of radionuclide. Improvements on concrete properties for this specific application must be performed.



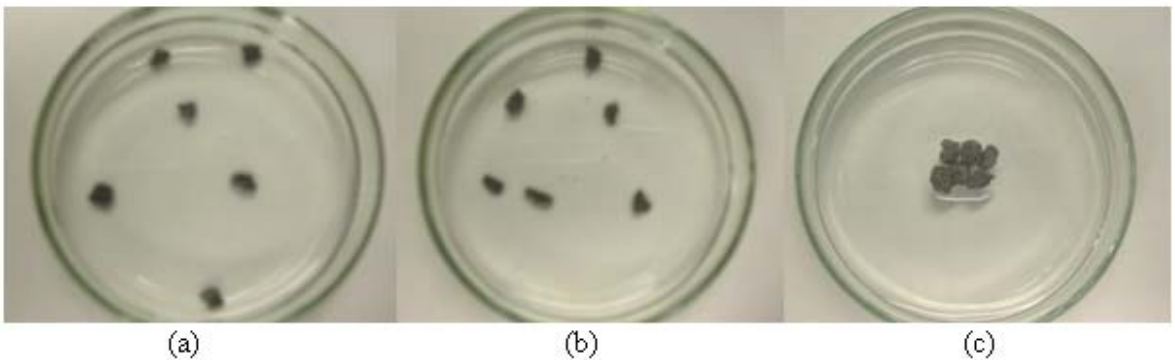
Geological repository

In SurfaceT, concrete carbonation processes were assessed in order to increase the initial cement paste density, to reduce the pH of the cement pores and, thereby, to decrease permeability and total porosity in cementitious materials. Carbonation reaction was strongly speeded up by using $SCCO_2$ as the carbonating atmosphere, rather than using natural carbonation. The first and most important effect of $SCCO_2$ treatment was a significant reduction of the cement permeability to water.



Water permeability analysis for 2 h $SCCO_2$ samples and 2 months naturally carbonated samples

Enhancement in durability of cement-based materials was also performed by means of a hydrophobic treatment with silane using $scCO_2$ as solvent. Finally, optimization and scale-up of the process were studied.

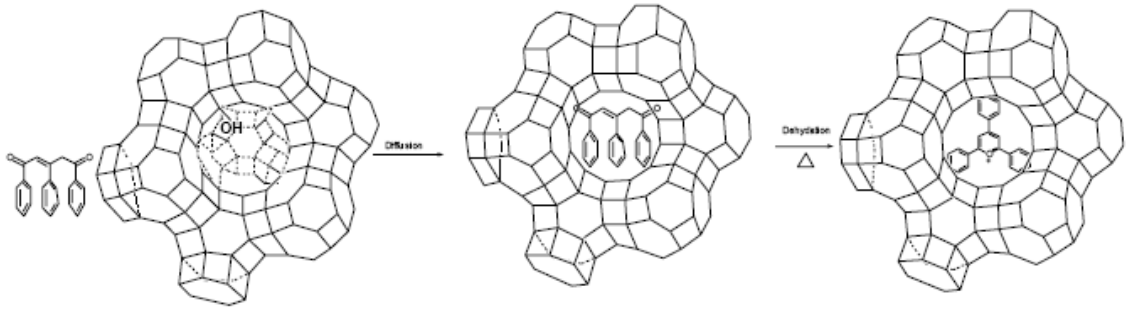


Photographs of monolithic mortar pieces added to a layer of water: (a) raw mortar, (b) supercritically carbonated mortar, and (c) supercritically silanized mortar samples (hydrophobic).

3.2. Photocatalists

SurfaceT aims at developing a generic impregnation supercritical method, extended to microporous (zeolite) substrates that do not interact with $SCCO_2$: in this case the $SCCO_2$, used as a solvent, facilitates addition of organic molecules to internal surfaces because there is no competition for adsorption between the added reagents and solvent molecules. The work aims at establishing a new methodology using $SCCO_2$ as solvent for the encapsulation of large cations inside zeolite cavities using a ship-in-a-bottle approach.

Zeolites are porous nanostructured solids that can incorporate guest molecules in their open framework without a need of being swelled. The resulting composite materials possess a wide range of prospective applications, mainly in the areas of sensor devices, catalyst design, or optical data storage. The rigid porous of zeolites also serve as excellent matrices for stabilizing otherwise reactive or unstable cations. Our interest regarding the stabilization on cations inside zeolites lies behind the importance of triarylpyrilium cations, which are well-known electron-transfer photosensitizes. In particular, we focus our attention on 2,4,6-triarylpyrilium cations (TP+). The introduction of these cations inside the zeolite voids appears to be the solution for increasing the life time of these cations. Previous studies have shown that (TP+) cation fits inside the zeolite cage; however its size is larger than the channel where it has to enter. For this reason, the synthesis needed to be carried out introducing by diffusion the necessary building blocks (in our case, the diketone) through the narrow channels of the zeolite. The synthesis of the entrapped cations proceeds by a two step sequence: (i) diffusion of the organic starting material inside the zeolite and (ii) thermal dehydration of the diketone inside the zeolite, leading to the cyclisation of the compound.



The experiment of encapsulation of TP+ cations in zeolite cages was extensively studied using conventional chemistry techniques. The use of $SCCO_2$ as a processing solvent, besides improving the diffusivity of the organic materials has the advantage, in comparison to conventional organic solvents, of not presenting competition with the starting material. In this way, all of the zeolite cavities are going to be available for the organic material to enter, whereas when using conventional solvents, these could fill in the zeolites pores and remain inside as guests impeding the entrance of the target molecule. By using the advantages of $SCCO_2$ properties the synthetic method can be improved by modulating the amount of cation charge and decreasing the reaction times. Although more research needs to be done, this methodology appears to be promising for the synthesis of this type of complexes.

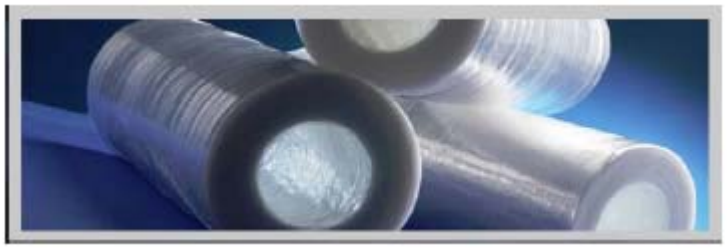
3.3. Modified plastics

Packages are essential for maintaining food in suitable preservation conditions. In packaging sector, the possible contamination of food with compounds which are present in packages has always been considered, and this contamination could be physical, chemical or biological. On the SurfaceT objectives has bee develop an advanced modified polymer for food applications. Smart packaging is one of the future trends in the food market, as a way to add value improving the conservation of products (meat, vegetables, precooked meats…). They are produced introducing an active compound like antibacterial, antioxidants, thermal indicators, oxygen content sensor, etc by $SCCO_2$ impregnation. To achieve this objective, it was defined an active compound of interest for food industry (triclosan) and several polymers of current use for packing (PE, PP, PET, PA, ABS and PS). Technical viability of the impregnation using $SCCO_2$ and optimal working conditions were studied at lab scale and then scaled up in order to produce at least 1.5 Kg of sample. Packing film was produced from developed polymers and check for antibacterial properties at laboratory scale. Applying the $SCCO_2$ impregnation process to the system low density polyethylene (LDPE)-triclosan, the load depended on working conditions, and, thus, on solubility of triclosan into CO_2. In conclusion, higher is the triclosan content in the CO_2 higher is the impregnation achieved. Samples of LDPE were also impregnated with triclosan by using the conventional extrusion process. It can be concluded that impregnation rates are similar for LDPE pellets impregnation process with triclosan by extrusion or under supercritical conditions, $SCCO_2$. A light major loss of active compound is observed in the impregnation of LDPE by extrusion.

The antimicrobial activity of the films obtained from LDPE impregnated pellets was tested, in order to check if these films could be suitable due to their antimicrobial activity for packaging sector. The analysis method employed was "*Antimicrobial products-Test for antimicrobial activity and efficacy*" based on the standard JIS-Z-2801:2000(dynamic method); and two model microorganisms, Escherichia coli CECT 516 and Staphylococcus aureus CECT 239, were selected for the tests. Films were cut in square samples (5 x 5 cm) for analysis.

(left) Films samples for the antimicrobial activity tests; (medium) Triclosan at 0,1% (w/w) in H2O. Antimicrobial activity test against Escherichia coli; (right) Triclosan at 0,1% (w/w) in H2O.

The results obtained for the microorganisms tested show that the antibacterial activity is the same for films obtained from LDPE pellets impregnated by extrusion or under supercritical conditions, $SCCO_2$.



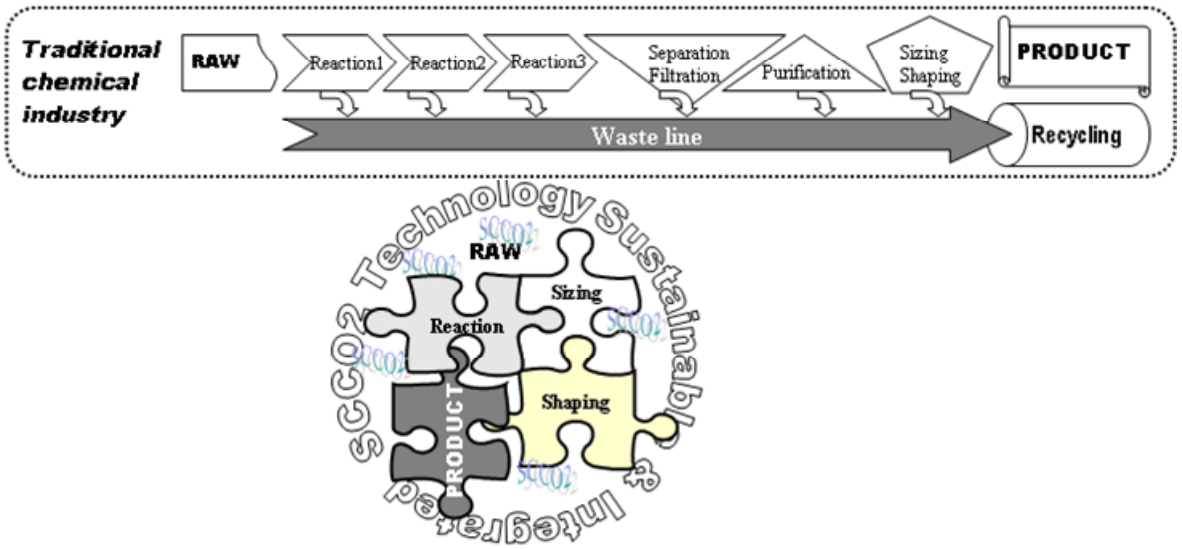
Food film obtained after pellets SCCO2 impregnation with Triclosan

D. SurfaceT project has contributed To DEPLOYMENT of environmental technologies that drastically reduce waste generation by demonstration of a more efficient life cycle and lower waste factor for products obtained using $SCCO_2$ technology. Qualitative economic evaluation based on use of resources (raw materials), reaction steps and recycling costs.

Commercialization of $SCCO_2$ technology has been limited to food and textiles and a few pharmaceuticals. The potentials of $SCCO_2$ technology (clean, fast, flexible, compact, integrated and sustainable) remain largely unrealized, because of various obstacles that hinder its implementation and market penetration:

- Industry lacks awareness of the potentials of $SCCO_2$ technology and has insufficient expertise in this area. Fostering scale-intensive research in $scCO_2$ technology, directed to specialized suppliers and potential user industries, will promote the adoption and integration of this clean technology.
- Risk aversion for replacement of current "matured technology" by insecure technology. Development of sustainable $SCCO_2$ technology will contribute to transforming traditional industry and must increase competitiveness and productivity through simultaneous innovation in ecoefficient processes and high-added value products
- Insufficient established evidences that $SCCO_2$ processing lead to products with added value obtained with shorter and more benign pathways. $SCCO_2$ is expected to play a key role in establishing a high-value European science-based chemical industry by demonstrating that breakthroughs will come not only from the processing method but also from the new products with improved performance.
- Cost of pressure equipment. The advantages of this benign medium need to be balanced against the installation and use of pressure equipment, which is generally perceived as costly and safety intensive. Cost reduction is achieved by using more direct synthetic pathways with respect to existing methodologies and by avoiding the generation of side-products and waste.
- Scarce research in the possibilities of $SCCO_2$ technology as a bottom up approach for nanosized products and composites preparation. It should be taken into account that the development of nanostructured matter can not be effective, when no large scale production technology is available. Moreover, simultaneous control of composition and morphology is not easily achieved using conventional solvent technologies, but is viable using $SCCO_2$ technology.

SurfaceT has aimed at the development and validation of $SCCO_2$ technology that can be integrated into the design operation of manufacturing processes for an ecoefficient and sustainable new industrial model. Project realization is expected to lead to the development of a $SCCO_2$ cross-cutting technology serving multisectorial (traditional and new) applications, thus, facilitating continuous innovation.



Traditional discontinuous model *vs.* sustainable and integrated SCCO2 processes.

SurfaceT demonstrated that $SCCO_2$ is an environmentally friendly solvent that leads to innovative and more benign pathways in synthesis of chemicals and materials processing. The project has examined several processes and gives special attention to technologies that might be replaced, including qualitative cost estimation and EU regulations. Life cycle assessments have indicated that the waste factor increases with the number of reaction steps necessary for the synthesis and processing of a determinate product. Moreover, the waste factor increases by a factor of 10 for pharmaceutical and biomedical products. In this project, advanced reaction engineering concepts based on retrosynthetic/retroprocessing approaches have been applied. The development has focused not only on the reduction of the number of steps, achieved through a smart design of the processing pathways, but also in the reduction of the waste factor by an optimal use of reagents and solvent. $SCCO_2$ technology has facilitated the easy recovery of the remaining high value raw materials. No final washing and drying steps are required in most of the developed processed. The development and implementation of $SCCO_2$ surface technology is expected to move production activities from incremental to breakthrough strategies and, thus, from short to long term innovation.

The Environmental and Climate Program asks for technological solutions in the chemical industry that incorporate both prevention and minimization of pollution. Hitherto, manufacturing industry efforts to meet environmental requirements have focused on implementing (expensive) end-of-pipe solutions such as waste treatments. SurfaceT project has had as a priority objective to promote the shift from old-polluting-technologies to new-clean-technologies, incorporating environmental considerations into products design from the beginning, minimizing the use of persistent organic solvents and preventing pollution from arising. On the longer run, an intrinsic clean process will eliminate those that have damaging effects on environment and health. The CO_2 used is a wasted-product of other industries. Therefore, the application of $SCCO_2$ as a solvent does not increase the anthropogenic greenhouse gas emission, as it does not generate any additional CO_2. Moreover, a net reduction of CO_2 emissions to the atmosphere is expected by using CO_2 in chemical processes that produce valuable carbon containing products. In general, the use of recycled CO_2 in industries will mitigate the CO_2 detrimental effect on climate change.

The development of the $SCCO_2$ clean technology will bring significant benefits to the quality of life and well-being of citizens per se. Specifically, the project had intended to lead to a new generation of safety products and services across a range of applications, while minimizing any potential adverse environmental and health impacts. Public health can be improved due to the fact that the materials produced using $SCCO_2$ technology contains less or no toxic solvents. Health is improved by increasing efficiency and safety in medical therapies using the solvent-free produced drug delivery systems, biomaterials and scaffolds. $SCCO_2$ is a friendly solvent, improving working conditions when replacing toxic solvents. Even in large-scale use, no human/environmental risk would arise in case of accidental contamination with the nonexplosive, nonflammable and nontoxic $SCCO_2$ solvent. On the other hand, higher operation pressure leads to additional risks. These risks have to be limited by implementing intrinsic safe equipment.

The (near)zero waste $SCCO_2$ technology will not only reduce pollution by organic solvents substantially, but also economize on the consumption of raw materials. Both raw materials and products can be recovered easily, while none organic solvents (or only small amounts) are used. The energy usage is decreased due to fewer reaction steps and minimization of waste disposal. Post-processing treatments such as filtration, washing and drying are eliminated. $SCCO_2$ technology produces high-quality products free of residual solvents, which also reduce the rate of degradation of systems when stored (especially important for the pharmaceutical industry). Strengthening the EU position in world markets for environmental technologies will contribute to sustainable consumption, new business opportunities and improved competitiveness.

A common paradigm is that new applications will be initially more costly than existing technologies. On the other hand, new and innovative environmental technologies would add economic growth, provided they reduce the costs of environmental protection and avoids end-of-pipe solutions. $SCCO_2$ processes are an economical alternative, since it uses more direct synthetic pathways, decreases organic waste disposal, and improves the conservation period of products. These advantages should offset investment costs for high pressure technology. Next to this, there is a growing tendency, by both consumers and investors, to award products or companies that apply clean technology. $SCCO_2$ technology goods will probably be introduced earlier in those markets where performance characteristics are especially important and price is a secondary consideration, traditional examples are pharmaceutical and medical applications. When more research in the area of $SCCO_2$ is done, a disruption in scientific assumptions will lead to new discoveries, and the technology will be applied to new economic sectors. When no action is taken the productivity remains stable. In the long run, the aim of SurfaceT is to come to the point where $SCCO_2$ technology will be considered as Best Available Technique in some applications, by being identified as a win-win technology. The development of knowledge-based $SCCO_2$ technology will accelerate the creation of new markets, products, services and jobs. Finally such transformation from a resource-intensive-solvent industry to a highly competitive and sustainable knowledge-based industry will reduce the risk of chemical industry delocalization to BRIC countries.

The scale-up of some of the proposed materials have been performed at least up to the first stages.

4.1 PMMA/TRF scale-up

The impregnation of PMMA with triflusal was first performed in SurfaceT, using 1 g of polymer and 0.5 g of drug in a 100 ml vessel, using as sample holding a 5 ml glass beaker where drug and polymer were kept separated by mesh or glass wool. Between all experimental conditions tested, it was defined as best conditions to scale up the process to work at 180 bars, 40 ºC and 3 hours of contact time. Batches of 100 g of PMMA were successfully produced and reproducing the similar triflusal load as 1 and 10 g. After the cleaning stage, the mean loads were kept constant between 4-5 % of triflusal into PMMA. This load is smaller that the maximum load obtained on lab scale, but optimizes the ratio between the impregnated and non-solved triflusal and minimize the cleaning stage. In all cases, it was observed that all PMMA trended to agglomerate and making lumps, these lumps can be easily disaggregated and recover the original particles. A batch of 500 g of polymer was carried out but first results were disappointing. The agglomeration of PMMA, observed in previous experiments as lumps, finished forming a cylinder of several centimeters of diameter, easily to disaggregate but with non-homogenous triflusal load. More research is needed to understand the cause of this aggregation and how to prevent it

4.2 LDPE/Triclosan scale-up

For pilot scale, the 16 liters vessel mounted during the project was used. Scaling of the process was done using LDPE at best results: 45 & 90 ºC and 250 bars. Ratio of triclosan:polymer in the feedstock blend was defined by estimation of the amount of triclosan needed to keep saturated both CO2 and polymer plus 20%. Triclosan is highly soluble in CO_2 at supercritical conditions and could be easily impregnated on the target polymers achieving good loads. The process has been easily scaled and more than 5 Kg of impregnated polymer has been produced to test its antibacterial properties.

E. SurfaceT project has contributed To CONVEY the knowledge from researchers to manufacturing industries opening platforms to discuss the possible benefits for the sustainability of a process upon substitution of organic solvents by $SCCO_2$.

SurfaceT project has the intention of transferring new methods, techniques and practices to important European industries involved in the project as end users, enabling them to foster competitiveness and to create added value processes and products, thus, leading to economic growth. SurfaceT has tried to increase European leadership in key (but nowadays scattered) sectors of $SCCO_2$ technology application through trans-national cooperation of industries and research institutions. The knowledge-based $SCCO_2$ technology has been applied to create complex chemicals that are traditionally produced with organic solvents. Moreover, the technology holds the exceptional promise of creating new markets for the emerging chemical manufacturing industries of nanoparticles, fibers and polymers. Hence, innovation has been a combination of existing and new knowledge. Analyzing the profile of the market in terms of key industries, a similar development in $SCCO_2$-technology is needed to produce different products. Hence, when this technology is implemented in advanced chemistry, it is expected to provide new possibilities in diverse industrial sectors, such as those manufacturing higher value, high-tech and commodity producers.

5.1. Process and products development

Process and products development in Surfacet have been driven by the interest of participant end-users companies. Some examples are as follow:

5.1.1 EMCM / FeyeCon

The SurfaceT project allowed EMCM to develop a bone cleaning process that was expected to have strong microbiological and viral inactivation properties. This is considered advantageous as is in the future bone used for implantation must require to strict regulatory requirements in order to achieve higher quality and safety standards for products of human origin. During the project the lab scale $SCCO_2$ setup was successfully developed and used. A realistic downscale process was used for several studies. First the penetration of CO_2 into bone was measured. With these experiments it was shown that the dye dissolved in CO_2 was able to penetrate into the dense structures of cancellous bone. The dye partially penetrated cortical bone. In addition the equipment was used to measure microbiological inactivation. Unfortunately $SCCO_2$ and some additives could not achieve the level of inactivation to use $SCCO_2$ as a final sterilization process at low temperature. Therefore, final sterilization with existing sterilization methods is still required. Also endotoxin inactivation could not be demonstrated with the lab scale $SCCO_2$ process developed. The presence of gram negative bacteria on procured human bone must be prevented. Finally, the viral inactivating properties of $SCCO_2$ were investigated. It was shown that lipid enveloped viruses could be efficiently inactivated in cortical bone. For cancellous bone addition treatments are preferred in order to achieve maximum safety of allogenic bone implantation. It is therefore concluded that $SCCO_2$ was able to increase safety of human bone. In the near future in vivo animal experiments will be performed to investigate the effectively of $SCCO_2$ treated human bone in bone augmentation.

5.1.2. BACTIMM / FeyeCon

Inexpensive production of low endotoxin biopolymers to develop essential medical aids. We recently investigated a low-cost method to remove endotoxins from alginate. Our method is a fully non-degradative method, utilizing exclusively relatively harmless class III organic (ethanol) or volatile (compressed carbon dioxide) solvents. One laboratory scale batch can easily be processed within one day which reduces overall costs and makes the addition of antibiotics superfluous. The laboratory scale investigation showed that the method can easily compete with established methods and a patent is pending. However, the process has only been tested on a laboratory scale and is not ready for commercialization. Our activities will not be limited to solely alginate. We will adapt the successful alginate depyrogenisation method to make it applicable for other biopolymers, such as chitosan, Carrageenan or Hyaluronan, or recombinant biopolymers like collagen. In the following stage of the project, the purified biopolymers will be formulated into new medical products. An end product has been developed and currently a production plant is being built. The quality of the end product will be verified using validated procedures to guarantee low endotoxin levels, low polyphenopl levels, and a good overall quality alginate (as per European Pharmacopoeia monograph). In conclusion the objective of this project is to develop a low-cost full scale process for the production of low

endotoxin and ultrapure biopolymers within a pharmaceutical grade setting. The produced biopolymers will be used to develop new medical products.

5.1.3. Uriach / CSIC

The therapeutic value of a drug delivery system is largely dependent on the homogeneity of the dispersion of the drug in the matrix as well as on the physical state of the dispersed drug. In general, crystallization of drugs embedded in a polymer matrix should be avoided due to erratic dissolution rates. For systems formulated with molecularly dispersed drugs, nonporous swellable polymers are mainly used as matrixes. In this work, the molecular impregnation of PMMA matrixes with Triflusal (a drug patented by Uriach) was studied using a $SCCO_2$ process. Homogeneously distributed loadings of ca. 20 wt% of active agent in PMMA were obtained. For impregnated systems, triflusal melting peak was not observed by DSC analysis, indicating that the triflusal did not crystallize inside of the matrix. Raman spectroscopy indicated hydrogen bonding interaction only between the hydrolyzed metabolite of triflusal and the PMMA, but not between the triflusal and the polymer. For triflusal drug, the formation of dimmers was inferred. Due to the low affinity between the Trf and the PMMA, a $SCCO_2$ continuous flowing method could not be used for impregnation. From a therapeutic point of view, it was concluded that impregnated samples had an excellent potential for the preparation of pharmaceutical formulations. Obtained delivery profiles were consistent with keeping stable levels of the drug over a long period of time, reducing the number of administrations and avoiding the initial sharp increase in drug concentration. Finally, the reported t1/2 values were compatible with a sustained delivery for a period longer than 2 or 3 months. Results for triflusal or other drug of interest can be transfer to Uriach in a near future.

5.1.4. Gaiker / MATGAS

On the SurfaceT objectives has bee develop an advanced modified polymer for food applications. Technical viability of the impregnation using $SCCO_2$ and optimal working conditions were studied at lab scale and then scaled up in order to produce at least 1.5 Kg of sample. Packing film was produced from developed polymers and check for antibacterial properties at laboratory scale. Applying the $SCCO_2$ impregnation process to the system low density polyethilene (LDPE)-triclosan, the load depended on working conditions, and, thus, on solubility of triclosan into CO_2. In conclusion, higher is the triclosan content in the CO_2 higher is the impregnation achieved. Samples of LDPE were also impregnated with triclosan by using the conventional extrusion process. It can be concluded that impregnation rates are similar for LDPE pellets impregnation process with triclosan by extrusion or under supercritical conditions, $SCCO_2$. A light major loss of active compound is observed in the impregnation of LDPE by extrusion. Possible transfer to plastic industry is under scrutiny.

5.1.5. FeyeCon /Bactimm

Together with participant 5-Feyecon, Bactimm has developed the THC preparation. The active ingredient is the cannabinoid dronabinol or tetrahydrocabinol (THC). THC introduces a new approach to relieving pain and possibly brain diseases. The product possesses unique characteristics and its production is also much simpler and less expensive using a $SCCO_2$ approach (extraction + PGS -particles form a gas saturated solution). Improved product characteristics consist of stability, solubility and bioavailibity. Further technology transfer to Echo Pharmaceuticals B. V. is under scrutiny.

2. DISSEMINATION AND USE

(More information can be found in the Final plan for using and dissemination the knowledge)

5.2 Exploitation and dissemination of knowledge

5.2.1. Patents

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- H.P. Pellikaan, P.S. Vermeulen, J.C.M. Bender, M.V. Fernandez Cid; Granulate containing a pharmaceutically active substance and method for its manufacture; WO2008033023 (Echo Pharmaceuticals B.V./FeyeCon B.V.) **(2008)**

5.2.2. Articles

- C.A. García-González, A. Hidalgo, C.Andrade, M.C. Alonso, J. Fraile, A.M. López-Periago, C. Domingo; Modification in composition and microstructure of Portland cement pastes as a result of natural and supercritical carbonation procedures; Ind. Eng. Chem. Res., 45(14) **(2006)** 4985-4992.
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- I. Pasquali, J.-M. Andanson, S.G. Kazarian, and R. Bettini, **"**Measurement of CO2 sorption and PEG 1500 swelling by ATR-IR spectroscopy**",** Journal of Supercritical Fluids, 45(3) **(2008)** 384-390
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- C. Roy, A. Vega-González, P. Subra-Paternault, Theophylline formulation by supercritical antisolvents, Int. J. Pharmaceutics **(2008)** Accepted.
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- J.-M. Andanson, A. López-Periago, C. A. García-González, C. Domingo, S.G. Kazarian, Spectroscopic analysis of triflusal impregnated into PMMA from supercritical CO_2 solution, Vibrational Spectroscopy **(2008)** On-line.
- A. López-Periago, A. Argemí, J.-M. Andanson, V. Fernández, C.A. García-González, S.G. Kazarian, J. Saurina, C. Domingo, Impregnation of a biocompatible polymer aided by supercritical CO2: evaluation of drug stability and drug-matrix interactions, J. Supercrit. Fluids **(2008)** Accepted.
- A. Vega-Gonzalez, P. Subra-Paternault, Viscometric study of solutions of l-PLA and PLGA in different solvents, J. Supercrit. Fluids **(2008)** accepted.
- C.A. García-González, J.M. Andanson, S.G. Kazarian, C. Domingo, J. Saurina, Application of principal component analysis to the thermal characterization of silanized nanoparticles obtained at supercritical carbon dioxide conditions, Anal. Chim. Acta **(2008)** submitted.

5.2.3. Books chapters

- C. Domingo, E. Loste, C. García, A.M. López Periago; Self-Assembled silane monolayers on the surface of nanometric particles obtained using supercritical treatment; In: 10th European Meeting on SCFs: Reactions, Materials and Naturla Products Processing. ISBN: 2-905-267-47-X, Mn1, **(2005)**.
- C. García, J. Fraile, C. Domingo, A. Hidalgo, C. Andrade, C. Alonso; Low pH concretes processed using SCCO2: applications in nuclear waste storage devices; In: 10th European Meeting on SCFs: Reactions, Materials and Natural Products Processing. ISBN: 2-905-267-47-X, Pr4, **(2005)**.
- C. Roy, A. Vega-Gonzalez, P. Subra-Paternault, Batch et semi-continuous techniques for the precipitation of theophylline, In: 10th European Meeting on SCFs: Reactions, Materials and Natural Products Processing. ISBN: 2-905-267-47-X, Mo19, **(2005)**.
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- C. Garcia, C. Domingo, J. Saurina; Self-assembled silane monolayers obtained using a supercritical treatment: Exploratory analysis and characterization of silanized materials by using chemometrics; in 8th Conference on Supercritical Fluids and Their Applications. ISBN: 88-7897-010-7, (2006).
- *O.S. Fleming and S.G. Kazarian*. "Supercritical Carbon Dioxide: in Polymer Reaction Engineering", Chap. 10 "Polymer Processing with Supercritical Fluids", 205-238. IBSN 3527310924 **(2006)**
- A. Hidalgo, C. Domingo, C. García, S. Petit, C. Andrade, C. Alonso, J.L. García-Calvo; Microstructure of cement-based materials processed with supercritical CO2. Comparison with natural carbonation processes; Sixth International Conference on the Environmental and Technical Implication of Construction with Alternative Materials. Science and Engineering of Recycling for Environmental Protection; ISBN 86-9008815-0-6, pag. 689-700 (**2006)**.
- A. Hidalgo, C. Andrade, C. Alonso, C. Domingo, C. García; Microstructural characterization of Portland cement based materials processed using SCCO2; In: Accelerated Carbonation for Environmental and Materials Engineering. ISBN-13 978-0-9553296-0-9, ISBN-10 0-9553296-0-4 **(2006)**
- C. A. García-González, A. M. López-Periago, J. Saurina, C. Domingo; Surface modification of inorganic nanoparticles using supercritical silanization process. ISBN 2-905267-58-5 **(2008).**
- A.M. López-Periago, X. Saurina, C. García, C. Domingo; Supercritical CO_2 impregnation of zeolites with organic building blocks – The "ship-in-a-bottle" technique. ISBN 2-905267-58-5 **(2008)**.
- P. Subra-Paternault, A. Vega-Gonzalez, Ch Roy, Encapsulation assistée par fluides supercritiques, Chapter of a book (in french) " Microencapsulation: Des sciences aux technologies', Coordinateurs: Vandamme, Poncelet, Subra, Edt. Lavoisier, ISBN 978 – 2 – 7430 – 0976 – 2 (France) **(2008)**

5.2.4 Congresses

The 10th European Meeting on SCFs, December **2005**, France.
"Batch and semi-continuous techniques for the precipitation of theophylline" by CNRS
"Viscosity measurements of various polymer/solvent solutions" by CNRS
"Self-assembled silane monolayer on nanometric particles using supercritical treatment" by CSIC

The 8th Conference on SCFs & their Applications, May **2006**, Italy.
"Supercritical antisolvent process technique for the production of biopolymer scaffolds" by CNRS&CSIC
"Co-precipitation of theophylline and polymer using $SCCO_2$" by CNRS
"Impregnation of porous polymeric polyesters using SCFs" by CSIC
"Self-assembled silane monolayer obtained using SCFs: chemometric analysis" by CSIC&UB
"FTIR imaging of polymer processes under $SCCO_2$" by Imperial College

9th National Congress on Materials, June **2006**, Vigo (Spain)
"Carbonatación y silanización de cementos utilizando CO2 supercrítico. aplicaciones en el almacenamiento de residuos" by CSIC
"Polimetilmetacrilato Impregnado Con Triflusal Utilizando Fluidos Supercríticos. by CSIC

EMLG / JMLG (European/Japanese Molecular Liquid Group), September **2006**, Spain.
"Supercritical enhanced processing of polymeric material" by Imperial College
"In situ ATR-IR spectroscopy of polymers subjected to high-pressure gases and fluids" by Imperial College

The 8th International Symposium on Supercritical Fluids 5-8 November **2006** , Kyoto (Japan)
Aminosalicylate formulation using compressed CO2 (CNRS)

Xarxa de Quimiometria, December **2006**, Tarragona (Spain)
"Characterization of unstable drugs by means of continuous flow spectrophotometric titrations and further chemometric data analysis" by UB
"Determination of antiretroviral drug mixtures by pH-gradient flow injection analysis and chemometric data treatment " by UB
"Characterization of wines through the contents of biogenic amines using PCA and PLS" by UB
"Application of MCR-ALS to the quantification of reverse transcriptase inibitors in sample mixtures of interest in pharmaceutical and clinical fields" by UB

I Iberoamerican Conference on Supercritical Fluids, April **2007**, Brazil
"Supercritical processing of polymers for the production of scaffolds and impregnated drug delivery systems" by CSIC & CNRS

1st International Congress on Green Process Engineering, 24-26 April **2007** Toulouse
Crystallization of drugs using supercritical CO_2 as antisolvent : from phase equilibria to products (CNRS)

The 5th International Symposium in Chemical Engineering and High Pressure Processes. June **2007**, Segovia.
"SCCO2-Based Process for Encapsulation of Target Compounds (I)" by Gaiker.
"Synthesis of Nanostructured Multiphase Materials Using Supercritical Fluid Technology" by CSIC&UB
"Cement Processing for Nuclear Waste Storage Using scCO2 Technology" by CSIC
"Impregnation Aided by $SCCO_2$: from Drug Delivery to Ship-in-a-Bottle" by CSIC

Doctoral Master: "Química Avanzada. Preparación y Caracterización de Materiales". June **2007.** Malaga University.
"Technological applications of supercritical fluids" by Gaiker.

8th ULLA Summer School, July **2007**, Netherland
"STUDY OF THE TERNARY SYSTEM PEG 1500/SUPERCRITICAL CO2/DIAZEPAM" by Imperial College

Eurofillers 2007: Functional Fillers for Advanced Applications, August **2007**, Zalakaros (Hungary)
"Preparation of Nanostructured Organic-Inorganic Hybrid Materials Using Supercritical Fluid Technology" by CSIC&IC&UB

ESOPS 17 (European Symposium on Polymer Spectroscopy), September **2007**, Austria
"In situ ATR-IR spectroscopy of polymers subjected to high-pressure gases and fluids" by Imperial College
"Applications of FTIR imaging to polymers" by Imperial College

Euroanalysis XIV, September **2007**, Antwerp (Belgium)
"Liquid chromatographic characterization of delivery profiles of triflusal impregnated on polymeric matrices" by CSIC and UB
"Liquid chromatographic determination of AIDS drugs in clinical samples" by UB

12th Scientific Meeting on Inorganic Chemistry / 6th Scientific Meeting on Solid State Chemistry, September **2007**, Barcelona
"Utilización de la tecnología supercrítica para la modificación de materiales inorgánicos", by CSIC
21st European Conference on Biomaterials. ESB 2007. Brighton, UK . 9 - 13 September **2007**.
"Novel stimuli-responsive polymers derived from morpholine and pyrrolidone" by CSIC

XV international workshop on Bioencapsulation, 6-8 september **2007** , Viena (Austria)
Supercritical CO2 to produce drug and polymer formulations (CNRS)

1st Conference on Inoovation in Drug Delivery from Biomaterial to devices, October **2007**, Italy
Diazepam/PEG1500 phase behaviour under supercritical CO2" by Imperial College

AAPS Annual Meeting, November **2007**, U.S.A.
PEG 1500/diazepam phase behaviour under supercritical CO2 by ATR-IR spectroscopy"
by Imperial College

HTC-10, International Symposium on hyphenated techniques, February **2008**, Bruges (Belgium)
"Optimization scheme for the development of analytical methods based on hyphenated systems" by UB
"Determination of biogenic amines in wines by pre-column derivatization and high-performance liquid chromatography coupled to mass spectrometry" by UB
"Characterization of wines through the biogenic amine contents and chromatographic profiles using chemometric techniques for data analysis" by UB

8th World Biomaterials Congress Amsterdam (Holland), May **2008**.
"Preparation in supercritical CO2 of polymer porous scaffolds incorporating bioactive molecules" by CSIC.

The 11th European Meeting on Supercritical Fluids. May **2008**. Barcelona
"SCCO2-Based Process for Encapsulation of Target Compounds" by Gaiker.

10th National Congress on Materials, June **2008,** San Sebastián (Spain)
"Preparación de materiales compuestos tipo polímero – nanopartículas inorgánicas utilizando tecnología supercrítica" by CSIC&CNRS
"Preparación de zeolitas funcionalizadas utilizando técnicas de impregnación supercrítica" by CSIC&UB

ICORS (Internaltional conference on Raman Spectroscopy), August **2008**, London (U.K.)
"Trifusal Diffusion in PMMA under Supercritical CO2 using Confocal Raman Microscopy"
by Imperial College
"Confocal Raman microscopy applied to diffusion of solutes in polymeric materials" by Imperial College

11th European Meeting on Supercritical Fluids **2008**, Barcelona
"Surface modification of inorganic nanoparticles using supercritical silanization process", by CSIC&UB.
"Supercritical CO2 impregnation of zeolites with organic building blocks – The "ship-in-a-bottle" technique" by CSIC&UB
"Method Comparison for the Investigation of Phase Behaviour of Systems C02 + Lipid Carrier Behaviour of Systems CO2+ Lipid Carrier" by Imperial College