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Supercritical fluid polymerisation and impregnation of molecularly imprinted polymers for drug delivery

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Abstract

Herein the preparation of molecularly imprinted polymers (MIPs) using supercritical fluid technology is evaluated. Poly(diethylene glycol dimethacrylate), polyDEGDMA, was synthesised in supercritical carbon dioxide ($scCO_2$) using a carboxylic acid end-capped perfluoropolyether oil as stabiliser. Polymerisations were carried out in the presence of different concentrations of two different template drug molecules, salicylic acid and acetylsalicylic acid. Results suggest that molecular imprinted polymers were successfully prepared by supercritical polymerisation and then impregnated with the template in order to prepare controlled release systems.
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1. Introduction

Controlled drug delivery products, using biocompatible or biodegradable polymers, have received considerable attention in the last years. These substances provide in general a more controlled release rate and consequently assumption of the drug by the body improving its therapeutic action. In fact, there is a growing interest of the pharmaceutical industry in the development of these systems [1].

In many cases, conventional drug delivery products provide sharp increases in systemic drug concentration that can easily reach potentially toxic levels, followed by a relatively short period at the therapeutic level after which, drug concentration drops until new administration occurs [2]. Controlled drug delivery occurs when a polymer/drug system is designed to release the drug in a predetermined manner. One of the purposes of these controlled release systems is to achieve delivery profiles that yield the therapeutic systemic concentration of the drug over a longer period of time, avoiding the large fluctuations in drug concentration and reducing the need for frequent administrations [3].

Molecular imprinting is a new method to synthesise materials with sites of specific molecular arrangements that act as artificial receptors, on an otherwise uniform matrix [4]. The design of a precise macromolecular structure that is able to recognize specific molecules has indeed a large number of potential applications. There is a tremendous interest in analytical applications, such as biosensors, immunoassays, separation media and affinity supports among others [5]. Molecular imprinting has already given proofs of its enormous potential in the field of pharmaceutical applications [6]. In fact, in the last few years, the development of this technology has moved towards the preparation of new controlled release systems, where it may enhance the drug loading.

The generally accepted mechanism for the formation of the molecularly imprinted polymers (MIPs) is schematically presented in Fig. 1. One of the advantages of this technique is the ease with which highly specific, tailored polymer host can be prepared from simple building blocks [7]. According to this method a monomer is chosen to interact strongly with the template molecule. A network with specific conformational and structural sites is formed by polymerisation and cross-linking of the monomer around this complex [8,9]. Although the mechanism is relatively simple the optimisation of the process is more complicated due to the contribution of several variables involved in the process, such as the functional monomer(s), the type of cross-linker, the ration between monomer and
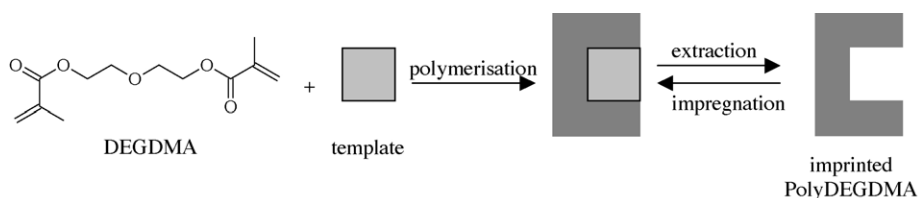
Fig. 1. Scheme of the imprinting process.

cross-linker and the ratio between monomer and template [10].

Different polymerisation processes are described in literature to prepare MIP. Bulk polymerisation was the first method employed to synthesise these polymers and it still is the most commonly used due to its simplicity. The polymerisation occurs in an organic solvent medium after basically, mixing all the components together. The polymeric matrix obtained has to be crushed and ground afterwards in order to obtain small particles. This method presents two major drawbacks, which are the large lost of product due to the grinding process and the heterogeneity of the matrix. In the case of suspension polymerisation a perfluorocarbon solvent is employed and the particles are synthesised with the aid of UV radiation. Another process is the two-step swelling polymerisation which requires several swelling steps of initial particles with the template molecule before polymerisation proceed. Emulsion core–shell polymerisation enables the production of particles with a specific property in the core without interfering with the imprinted shell. A different method is the so-called precipitation polymerisation which is based on the precipitation of the polymer chains as they grow more and more and become insoluble in the solvent medium [11]. More recently solid-state polymerisation has also been employed to prepare MIPs [12].

Because of environmental regulations and the safety hazards associated with the use of organic solvents, the pharmaceutical industry has been moving away from the use of these solvents. Actual regulations force to remove all residual solvents to the extent possible in order to meet product specifications, good manufacturing practices, or other quality-based requirements [13].

Supercritical fluids, especially scCO$_2$, have been identified as prime candidates to develop alternative clean processes for the preparation of drug-loaded polymeric matrixes [14]. ScCO$_2$-based processes offer a lot of advantages over the traditional methods of preparing controlled release systems. CO$_2$ is readily available, environmentally acceptable and non-flammable; it has low critical constants ($T_c = 31\,°C$ and $p_c = 74$ bar) [15] and leaves no toxic residues.

Recently there has been great interest in processing polymers using supercritical carbon dioxide [16,17]. As a polymerisation medium, scCO$_2$ offers advantages over conventional solvents: the strong density dependence enables fine adjustments in polymerisation reactions, so that polymer chains will precipitate from solution after reaching a certain threshold molecular weight. Supercritical carbon dioxide can also be used to extract unreacted monomer, initiator, catalyst and some stabilizers from the polymer product, leading to highly pure materials; furthermore, as CO$_2$ is gaseous at ambient conditions the final product is a dry powder [18].

Polymeric microspheres of polyDEGDMA are already used to immobilize biomolecules such as antibodies, antigens, enzymes and hormones [19]. DEGDMA is a biocompatible cross-linking agent that belongs to the group of the most commonly used. It has also been proven that it provides stable networks in a wide range of pHs and temperatures under in vitro conditions which is fundamental for the preparation of molecularly imprinted polymers [6].

Salicylic acid and acetylsalicylic acid are commonly used as antipyretics. Salicylic acid is a drug with keratolytic properties being usually applied topically in concentrations between 2 and 20% (w/w) for the treatment of hyperkeratotic and scaling skin conditions such as dandruff and seborrhoeic dermatitis, psoriasis and acne. It is often found with pads as applicators. The solubility of salicylic acid in supercritical carbon dioxide was measured by Ke et al. [20]. Acetylsalicylic acid, aspirin, is a nonsteroidal anti-inflammatory drug (NSAID) effective in treating fever, pain, and inflammation in the body. Acetylsalicylic acid breaks down into salicylic acid 20 min after entering the bloodstream. It is the salicylic acid that is responsible for the beneficial effects of aspirin. Salicylic acid itself, it too caustic to be taken orally. The solubility of acetylsalicylic acid in supercritical carbon dioxide was measured by Huang et al. [21].

In this work, the preparation of molecularly imprinted polymers using supercritical fluid technology is described. MIPs were successfully prepared by supercritical polymerisation and then impregnated with the template (salicylic and acetylsalicylic acids) and the resulting material was evaluated as controlled release systems.

2. Experimental

2.1. Materials

Diethylene glycol dimethacrylate (DEGDMA) (99.96% purity, see Fig. 2) was purchased from Polysciences. Krytox 157 FSL was supplied by DuPont. AIBN (2,2′-azobis(isobutyronitrile), 98% purity), salicylic acid (99+% purity) and acetylsalicylic acid (99+% purity) were purchased from Aldrich. Ethanol (99.9% purity), was purchased from Panreac. Carbon dioxide (99.998% purity) was supplied by Air Liquid. All chemicals were used without further purification.

2.2. Polymerisation in scCO$_2$

The polymerisation reactions were carried out as described previously [22], in a 33 mL stainless steel high-pressure cell equipped with two aligned sapphire windows. The cell is immersed in a thermostatted water bath with ±0.01 °C of stability. Temperature control was made with a RTD probe contacting the cell, connected to a *Hart Scientific* PID controller. The cell is equipped with a *Teflon* coated magnetic stir bar. An immersible stirrer magnet was placed under the cell. In a typical experiment the cell is loaded with the monomer (23% w/w of DEGDMA with respect to CO$_2$), stabiliser (10% w/w of Krytox with respect to monomer), initiator (2% w/w of AIBN with respect to monomer) and a certain amount of drug template (salicylic acid or acetylsalicylic acid). The cell is sealed and nitrogen is added to purge the cell and test leaks. The nitrogen is slowly released and liquid carbon dioxide is loaded into the cell using a high-pressure compressor (*NWA* GmbH). The cell is immersed in the water bath stabilised at 65 °C and temperature and pressure are allowed to rise to the required experimental conditions. Additional CO$_2$ may be added to reach the exact desired pressure (19 MPa). The reaction was allowed to proceed for 180 min under stirring. It could be seen through the sapphire windows that all polymerisations started as a completely homogeneous phase, with all reactants completely dissolved in scCO$_2$. At the end of the reaction, the resulting polymer was slowly washed with fresh high-pressure CO$_2$ in order to clean the remaining residues of stabiliser and unreacted monomer. Upon venting a fluffy, dry, white, free-flowing powder remained in the reaction vessel. SEM images (not shown) were obtained on a *Hitachi*, S-4100 instrument and particle diameters and their size distributions were determined in a dry powder dispersion system (*Aerosizer* LD API). Non-imprinted and imprinted polyDEGDMA are formed by disaggregated and discrete spherical polymer particles with 1.7 μm diameter (1.1 of particle size distribution). The presence of the drug templates does not affect the polymer morphology. From the SEM images obtained for the non-imprinted and the imprinted polymers it was not possible to see any differences in their morphology. The diameters of the particles and their size distributions were not affected by the presence of the small amounts of the template drugs (Fig. 2).
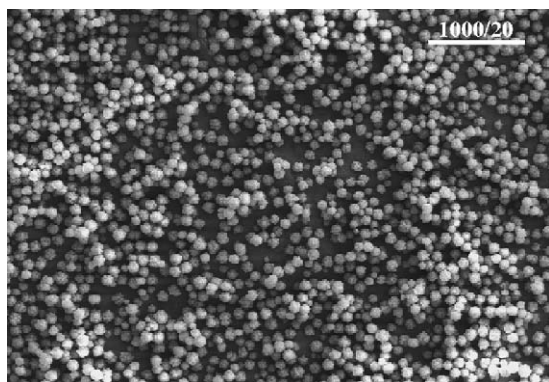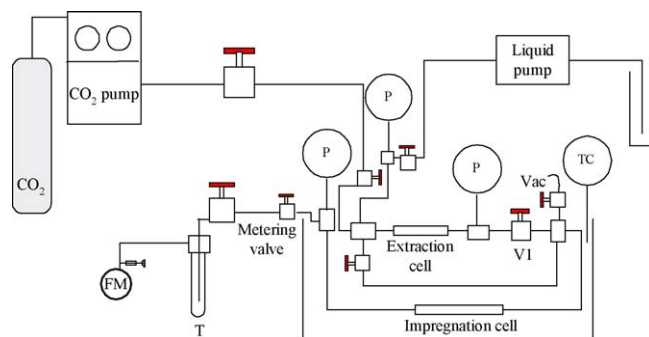

Fig. 2. SEM image of polyDEGDMA.

Fig. 3. Schematic representation of the impregnation apparatus.

2.3. Removal of the template

The template was removed from the imprinted polymers, before the impregnation process, to create the binding sites by repeated washes with ethanol and water.

2.4. Supercritical impregnation process

The impregnation is performed in a semi-continuous apparatus described schematically in Fig. 3. A tubular batch extractor is initially loaded with the pharmaceutical compound and packed between a sequence of a filter paper, cotton and net metallic discs that work as filters of small particles. The polymer is loaded, in the same manner, in the impregnation cell. The cells are then immersed in a thermostatted water bath heated by means of a controller that maintains temperature within ±0.1 °C (TC) (Ero Electronic LMS-491-13). Carbon dioxide is pumped into the cells using a high-pressure piston pump (Haskel model MCPV-71), until the operational pressure is attained. In the intervening time the cells are not connected to each other, however they are at the exact same pressure. The pressure inside the cells is measured with a pressure transducer (P) (LEO2 0.300 bar). The solubilization of the drug in the supercritical fluid and the swelling of the polymer due to the presence of carbon dioxide take place during 1 h, a typical equilibrium time. Preliminary experiments with other contact times were performed and, similarly to other reticulated polymers and common model drugs, one hour was found to be the adequate contact time for both the swelling of the polymeric matrix and solubilisation of the drug in scCO2. Opening valve V1 the cells are placed in contact and the continuous process of impregnation starts. A saturated stream of the pharmaceutical compound in carbon dioxide passes through the polymeric matrix, for a predetermined period of time and at a very slow rate so that the impregnation can occur. The impregnation took place within 2 h:30 min. The outflow is regulated by a micrometric valve (Hoke 1315G4Y), in order to maintain a constant pressure in the system and a slow carbon dioxide flow, which is measured with a flowmeter (FM) (Alexander Wright DM3C). The solid that might be solubilized in the gas is collected in a small glass trap (T). At the end of this period the system is quickly depressurized. All experiments were performed at 20.0 MPa and 40 °C.

2.5. In Vitro drug release studies

Drug impregnated microspheres (20 mg) were suspended in 40 ml of phosphate buffer solution (pH = 7.4) and stirred at 150 rpm at 37 °C. A 0.5 ml aliquots were withdrawn at time intervals and the same volume of fresh medium was added to the suspension. The samples were filtered (0.45 μm, milipore filters) and analysed by HPLC (ThermoFinnigan Surveyor consisting of a quaternary pump, autosampler and diode array detector). A C_{18} column (Merck Lichrospher 250-4, 5 μm) kept at 30 °C, was used in isocratic conditions with a mobile phase consisting of water:acetonitrile:acetic acid (500:490:10). The flow rate was 1.2 ml/min and the volume of injection was 20 μl. Quantification was performed at 280 nm by external standard calibration.

The total mass of released drug in each moment of the experiment was calculated taking into account the aliquots taken and the dilution produced by addition of fresh buffer.

3. Results and discussion

In the work reported two pharmaceutical compounds were used to test this new imprinting technique using supercritical technology. Salicylic acid and acetylsalicylic acid are soluble in supercritical carbon dioxide, which is fundamental for the impregnation process. PolyDEGDMA was synthesised in $scCO_2$ in the presence of different percentages of drug (template). The amount of drug present as the polymerisation takes place is relatively low to make sure that the polymer is not impregnated during this step. Table 1 presents the initial percentage of drug template with respect to the monomer in polymerisation reaction, and the relative quantity of drug in an impregnated sample, expressed in w/w percentage.

The release profile of the imprinted polymers prior to the impregnation process was evaluated in order to determine if some of the drug was still impregnated after the template removal step. The concentration of the templates in the polymers was less than 1×10^{-4} mg/ml, in all cases.

Non-imprinted polyDEGDMA was successfully impregnated with salicylic acid, however, when the same polymer is polymerised in the presence of a residual amount of drug the concentration of impregnated drug is higher (Fig. 4). These experiments were repeated to validate the results. In Fig. 5, the
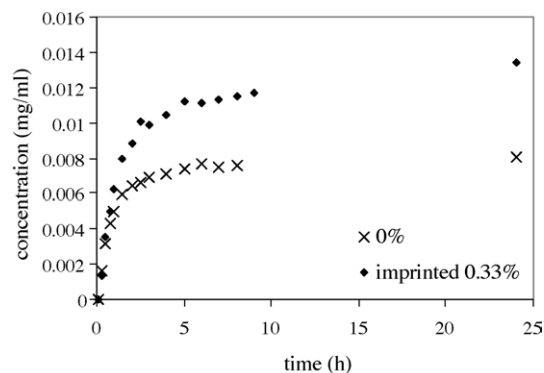

Fig. 4. Release profiles of the systems polyDEGDMA + salicylic acid, pH = 7.4.

kinetics of the release is not influenced when the template was present during the polymerisation. The trends of the curves are similar and the initial slopes are the same.

MIPs of the same polymer were prepared with another template, acetylsalicylic acid, to further test this technology. Different percentages of the template were used in the polymerisation process in order to check the dependence of the degree of impregnation with the amount of drug present in the polymerisation. Acetylsalicylic acid was successfully impregnated in all cases, even when non-imprinted polymer was used. In fact, there is a correlation between the quantity of template present in the preparation of the MIPs and the percentage of impregnation. A higher concentration of the drug during the polymerisation step leads to higher percentages of impregnation (Fig. 6). Acetylsalicylic acid-loaded polyDEGDMA miscrospheres were able to sustain drug release for several hours maintaining the drug concentration within 8 h.

In both cases, the quantity of drug impregnated is higher when the template molecule was present in the polymerisation step, this result is more relevant in the case of acetylsalicylic (Table 1).

The template molecule to be used in the imprinting polymerisation and afterwards in the impregnation procedure should be the same or as similar as possible, namely, in terms of molecular weight, molecular structure and geometry. The polymerisation of molecularly imprinted systems involves the formation of a pre-polymerisation complex in which the template and the functional monomer establish interactions either cova-

Table 1
Initial concentration of the template drugs in the polymerisation reactions

Template	Percentage of template[a]	Percentage of impregnation[b]
Salicylic acid	0	1.7
	0.33	2.7
Acetilsalicylic acid	0	1.2
	0.05	0.8
	0.2	2.2
	0.5	3.8

[a] Initial w/w percentage of the template drug with respect to the monomer in the polymerisation reaction.

[b] Relative quantity of drug in an impregnated sample, expressed in w/w percentage.
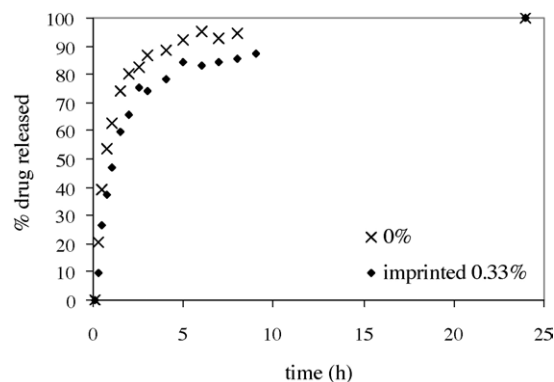

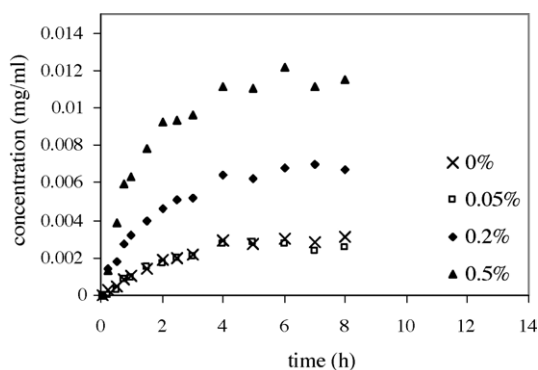Fig. 5. Release profiles of the systems polyDEGDMA + salicylic acid in terms of % drug released, pH = 7.4.

Fig. 6. Release profiles of the systems polyDEGDMA + acetylsalicylic acid, pH = 7.4.

lent or non-covalent followed by the polymerisation reaction itself. Once the template is removed the polymeric matrix is a macroporous structure with specific recognition sites for the template molecule [6,9]. These sites are directly proportional to the amount of template initially present in the polymerisation reaction as it is confirmed by the drug release profiles obtained in this work.

PolyDEGDMA, being highly cross-linked, prevents the polymer network to change the conformation of the imprinted cavities, due to the rigidity of the polymeric chains. This can create some difficulties in the re-uptake of the template in the cavity. Nevertheless, the results obtained from the release profiles of the impregnated samples prove the success of this technique.

4. Conclusions

Very interesting results can be taken from the work developed. A new clean and environmentally friendly technique is proposed to prepare molecularly imprinted polymers for controlled drug delivery. In this first attempt to prepare MIPs using supercritical fluid technology a highly cross-linked polymer was prepared and two different templates were tested with successful results. The release profiles of the systems studied show that there is a correlation between the amount of template present during the polymerisation step and the percentage of impregnation.

Further work on this subject requires the preparation of less cross-linked polymers in order to study this effect on the release profiles of the impregnated systems. A compromise between the cross-linking agent and the functional monomers has to be established so that the structure of the imprinted cavities is stable enough to maintain the conformation in the absence of template, but flexible into some extend to ease the impregnation and release of the drug.

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