Macromolecular Nanotechnology

Adsorption and release of nicotine from imprinted particles synthesised by precipitation polymerisation: Optimising transdermal formulations

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ABSTRACT

Molecularly imprinted polymers (MIPs) are promising materials that can be used as advanced pharmaceutical excipients in the development of formulations for sustained release of drugs. In previous studies, we developed formulations for the transdermal administration of nicotine based on MIPs synthesised by bulk polymerisation that were able to control skin permeation rate of the drug for 24 h following a Higuchi kinetic. In this work, we optimise the synthesis protocol using the precipitation polymerisation technique. MIPs, including the hydrophilic monomer 2-hydroxyethyl methacrylate (HEMA), were selected for their improved adsorption capacity and selectivity in comparison with non-imprinted polymers (NIPs). Adsorption data were better fitted to Langmuir-Freundlich model, indicating heterogeneity of the binding sites. MIPs showed swelling 3–5 times larger than NIPs in aqueous media, while swelling studies in mineral oil (non-polar vehicle) did not indicate differences between these materials. Formulations prepared using mineral oil as vehicle indicated that MIP-based formulations showed a faster release than those based on NIPs, which was related to their increased swelling with the release medium. Skin permeation studies using ear porcine skin indicated that MIP-based formulations with high nicotine loading were able to control the skin permeation flux following zero order kinetic for prolonged times (48 h). The permeation rate from these MIP formulations was lower than from NIP formulations. Thus, these results were associated with limited swelling of MIPs during *in vitro* skin permeation assays, and the selective desorption of nicotine from MIP particles.

1. Introduction

Nicotine, (S)-3-(1-methyl-2-pyrrolidinyl) pyridine, is an alkaloid extracted from the leaves of *Nicotiana tabacum* L. The drug is a volatile base, liquid at room temperature, with pallid yellow colour. It is highly soluble in water, alcohols and oils, exhibiting a hydrophilic character (log_{Kow} = 1.17). Chemically, nicotine is a tertiary aromatic amine with two ionisable moieties: a pyridine (pKa_1 = 3.04) and a pyrrolidone (pKa_2 = 7.84). Due to its physicochemical characteristics, nicotine is considered a model drug for transdermal route. When this drug is directly administered on the skin, it exhibits high permeability and, according to the dose administered, it may achieve high plasmatic levels. Based on these properties, transdermal formulations should be able to control the drug release rate, regulating its permeation flux through the skin. The controlled release of nicotine is also limited due to several physical and chemical incompatibilities of the drug with many conventional polymeric materials used in developing this kind of formulation. Pressure-sensitive adhesives may lack their properties, and other polymers may be excessively loaded with nicotine, leading to a burst effect in the first step of the drug release. The volatility of nicotine is another factor that leads to loss of drug during storage [1–4]. Considering the difficulties in the development of these formulations, strategies must be investigated that hold the drug in the systems, avoid drug losses and assure controlled drug release and its compatibility with formulation components.

The design of pharmaceutical formulations that prolong release of nicotine may result in enhanced drug delivery systems, considering that the actual marketed transdermal devices regulate drug release up to 18–24 h. Novel polymers applied as drug delivery systems may offer an efficient strategy to achieve this aim. Molecularly imprinted polymers (MIPs) have been described as functional excipients for controlled release [5,6]. Nicotine has been studied as a template for MIPs synthesised by bulk polymerisation [7–10], precipitation polymerisation [11], polymerisation in the surface of TiO_2-modified electrodes [12] and

reversible addition fragmentation chain transfer polymerisation [13].

In a previous study [14], we synthesised bulk MIPs using nicotine as the template, and evaluated these materials as functional excipients for its transdermal release. Mineral oil was the most promising vehicle due to its hydrophobic characteristics, improving the molecular recognition of nicotine in MIP particles. MIP-based formulations using mineral oil were able to control its permeation flux up to 24 h following Higuchi kinetic. Now, we proposed to synthesise several MIPs using precipitation polymerisation technique, searching for optimised materials able to adsorb nicotine selectively and to sustain its skin permeation, resulting in functional excipients that prolong its transdermal absorption.

2. Material and methods

2.1. Materials

(-) Nicotine ($\geq$99%, liquid), methacrylic acid ($\geq$99%), glycidyl methacrylate ($\geq$97%), 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate ($\geq$98%) (EDMA) and 1,1′-azobis(cyclohexanecarbonitrile) (ABCN) were purchased from Sigma-Aldrich (USA). Acetonitrile, methanol, acetic acid, orto-phosphoric acid 85%, perchloric acid 70% and toluene were purchased from J.T. Baker (USA). Sodium hydroxide, hydrochloric acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, potassium chloride, phosphoric acid and triethylamine were purchased from Vetec Quimica Fina Ltda (Brazil). Mineral oil and propylene glycol were purchased from Mapric (Brazil). The glycerol monooleate (monoolein) of commercial grade was used (Myverol 18-99; Quest International, USA).

2.2. Methods

2.2.1. High performance liquid chromatography (HPLC) analysis

Samples were analysed using HPLC from Shimadzu (Kyoto, Japan). Analysis was carried out employing a C_{18} end-capping column 150.0 × 4.6 (i.d.) with 5 μm particle size (Phenomenex, USA). The mobile phase was a mixture of 20 mM sodium phosphate buffer with 0.2% of triethylamine (pH 6.9) and methanol (80:20, v/v). The flow rate of the mobile phase was 1.0 mL min^{-1}, injection volume was 50 μL and UV detection was carried out at 260 nm. The samples were filtered using a 0.45 μm syringe filter composed of hydrophilic polytetrafluoroethylene. The retention time of nicotine was approximately 4 min. The total run time was 12 min. Under these conditions, good linearity and reproducibility were shown over a range 0.5–300.0 μg mL^{-1} of nicotine. The analytical method was validated and sample dilutions were performed when necessary [15].

2.2.2. Extraction of nicotine from the vehicle (mineral oil) for HPLC analysis

Solutions of nicotine at concentrations of 100 μg mL^{-1} were prepared using mineral oil as diluent (n = 6). To extract the drug from the vehicle, 600 μL of the nicotine solution diluted in mineral oil was added to 600 μL of water, and extraction was performed using vortex agitation for 5 min. Next, the samples were centrifuged at 3400 rpm for 15 min, and 400 μL aqueous phase was withdrawn and analysed by HPLC (Section 2.2.1). The validation of the analytical procedure for nicotine extraction from the mineral oil was performed according to recommendations of the ICH Q2B guidance [16].

Selectivity was evaluated using extracted solutions from pure mineral oil (blanks). Linearity was evaluated to demonstrate the proportionality relationship between the concentration of the nicotine solutions and detector response (peak area). For this, a standard stock solution in mineral oil (1.0 mg mL^{-1}) was prepared and successive dilutions were performed to obtain standard solutions at concentrations of 25, 50, 100, 150 and 200 μg mL^{-1} (n = 3). These solutions were extracted with water according to the previous procedure described in this section. The correlation coefficient (r) and the calibration equation

($y = ax + b$) were calculated, where a corresponds to the slope and b is the linear coefficient (intercept). Precision (repeatability) was determined using nicotine solutions at concentration of 100 μg mL^{-1} (n = 6). Analyses were performed on two consecutive days. The intraday and interday precisions were established as the dispersion of the measurements around the average value, and expressed mathematically by the relative standard deviation (RSD), determined by Eq. (1):

$$RSD = \frac{Standard\ deviation}{Mean\ value} \times 100 \tag{1}$$

Accuracy was determined using standard solutions of nicotine at concentration of 100 μg mL^{-1} (n = 3). Analyses were performed on two consecutive days. The intraday and interday accuracy were presented as relative error (RE), according to Eq. (2):

$$RE = \frac{Experimental\ concentration - Theoretical\ concentration}{Theoretical\ concentration} \times 100 \tag{2}$$

To validate the procedure for dilution of concentrated nicotine samples (500 and 1000 μg mL^{-1}), the samples were diluted with the vehicle five to ten times before extraction with water, assuring that analysis is fitted into the linear range. Results were presented as RE.

2.2.3. Synthesis and preparation of nicotine-imprinted copolymers

Several imprinted copolymers were synthesised using the precipitation polymerisation technique (Table 1). The first step (prepolymerisation) of the synthesis of MIP-1, MIP-2, MIP-3, MIP-4, MIP-5 and MIP-6 was performed by adding MAA and nicotine in 150 mL of a mixture of toluene:acetonitrile (1:3, v/v), denominated as porogenic solvent. Other hydrophilic monomers (GMA and HEMA) were added in this step as shown in Table 1. After 30 min, EDMA (crosslinker) and 0.2 mmol of ABCN (radical initiator) were added to this solution. Next, the solution was purged thoroughly with nitrogen gas for 15 min, and the reaction flask was immediately sealed. Then, the mixture was continuously stirred, and polymerisation reaction was carried out overnight at 80 °C. The synthesis protocol was modified for MIP-7 and was performed in two steps. The first step was performed in porogenic solvent using 7.5 mmol of MAA, 4.0 mmol of HEMA, 4.0 mmol of EDMA, 1.875 mmol of nicotine and 0.2 mmol of ABCN. The solution was purged with nitrogen gas for 15 min, and stirred for 4 h at 80 °C. To this solution was added 26.0 mmol of EDMA and 26.0 mmol of HEMA. The mixture was continuously stirred, and polymerisation reaction was

Table 1
Synthesis of the copolymer particles.

Copolymer	MMA (mmol)	HEMA (mmol)	GMA (mmol)	EDMA (mmol)	Nicotine (mmol)
Poly-(EDMA)	–	–	–	30.0	–
MIP-1	7.5	–	–	30.0	1.875
MIP-2	7.5	7.5	–	30.0	1.875
MIP-3	7.5	–	7.5	30.0	1.875
MIP-4	7.5	7.5	7.5	30.0	1.875
MIP-5	7.5	–	–	30.0	0.625
MIP-6	7.5	–	–	7.5	0.625
*MIP-7**	7.5	30.0	–	30.0	1.875
*NIP-1**	7.5	–	–	30.0	–
NIP-2	7.5	7.5	–	30.0	–
NIP-3	7.5	–	7.5	30.0	–
NIP-4	7.5	7.5	7.5	30.0	–
*NIP-5**	7.5	–	–	30.0	–
NIP-6	7.5	–	–	7.5	–
*NIP-7***	7.5	30.0	–	30.0	–

MMA, methacylic acid; HEMA, 2-hydroxyethyl methacrylate; GMA, glycidyl methacrylate; EDMA, ethylene glycol dimethacrylate; MIP, molecularly imprinted polymer; NIP, non-imprinted polymer.

* NIP-1 and NIP-5 have the same composition.

** Synthesis was performed in two steps.