Fabrication, physico-chemical and pharmaceutical characterization of budesonide-loaded electrospun fibers for drug targeting to the colon

Giovanna Bruni[a]*, Lauretta Maggi[b], Loredana Tammaro[c#], Andrea Canobbio[b], Rosadele Di Lorenzo[a], Sharon D'Aniello[c], Chiara Domenighini[a], Vittorio Berbenni[a], Chiara Milanese[a], Amedeo Marini[a]

[a] C.S.G.I. - Department of Chemistry, Physical-Chemistry Section, University of Pavia, Viale Taramelli 16, 27100 Pavia, Italy

[b] Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

[c] Industrial Engineering Department, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Salerno, Italy

Present address: ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development), Brindisi Research Center, S.S. 7 Appia - km 706,00- 72100- BR- Italy

Abstract

The objective of this study was to fabricate and characterize electrospun fibers loaded with budesonide with the aim of controlling its release in the gastrointestinal tract. Budesonide is a non halogenated glucocorticosteroid drug, highly effective in the treatment of some inflammatory bowel diseases with local action throughout ileum and colon. At this aim, Eudragit® S 100, a polymer soluble at pH>7, commonly used for enteric release of drugs, has been successfully spun into ultrafine fibers loaded with Budesonide (B) at 9% and 20 % (w/w) using the electrospinning process. The physico-chemical characterization by scanning electron microscopy, X-ray diffraction, FT-IR spectroscopy and thermal analyses indicated the amorphous nature of budesonide in the electrospun systems. Dissolution rate measurements using a pH-change method showed negligible drug dissolved at pH 1.0 and sustained release at pH 7.2. Therefore, the pharmaceutical systems proposed, made of fibers, represent an effective method for drug targeting to terminal ileum and colon with the aim of improving the local efficacy of this drug.

Keywords

Colonic drug delivery, dissolution, FTIR, Calorimetry (DSC), Amorphous, Microscopy, Polymer drug carrier.

1. Introduction

Electrospinning is a straightforward, versatile and cost-effective technique for fabricating polymer nanofiber and microfibers with high surface area to volume ratio, high porosity and other outstanding properties making them excellent candidates for different applications, including filtration, catalysis, sensors and biomedical applications.[1-7] In recent years electrospinning has gained widespread interest as a potential polymer processing technique for application in drug delivery. [8-13] In this technique, a polymer solution or melt is ejected from a needle tip of a syringe toward a grounded collector plate by applying a high electric field. When electrostatic forces overcome the surface tension of the polymer solution, the electrified fluid forms a jet which extend along a straight line, stretches and, with the solvent evaporation, solidifies into nanofibers.[14-15] As a result, the film of nanofibers is formed on collector's surface. The properties of the fibers can be influenced by several processing parameters:[2] intensity of applied electrical potential, distance between two charged electrodes (needle and collector), flow rate of polymer solution and polymer characteristics (viscosity, conductivity, surface tension). In the pharmaceutical applications, the selection of the polymeric carrier for electrospinning is crucial since the polymer type and characteristics and the drug-polymer-solvent interactions influence the formation, morphology, mechanical properties of the fibers and the drug release in terms of rate and appropriate site of delivery in the body.

The aim of this work was to prepare and characterize, from a physico-chemical and pharmaceutical point of view, polymeric fibers for the controlled release of budesonide. Budesonide (scheme 1a), (a mixture of diastereoisomers 22R and 22S of 16α,17α-butylidenedioxy-llβ,21-dihydroxy-l,4-pregnadiene-3,20-dione), is a synthetic steroid of the glucocorticoid family, with potent anti-inflammatory action. It is mainly used by the inhalation route for the treatment of inflammation in the lungs and for the management of symptoms of allergic rhinitis and asthma. In addition, budesonide is used by the oral route to treat a recurrent inflammatory bowel disease, called Crohn's disease involving terminal ileum and proximal colon. Budesonide has high topical anti-inflammatory activity but low systemic activity because of extensive hepatic metabolism and has been demonstrated to be a safer alternative to conventional corticosteroids.[16-17]

The recommended daily dose is 9 mg orally for up 8 weeks.[18] The full effect is usually achieved within 2-4 weeks. For maintenance of clinical remission, the dosage of 6 mg daily is recommended.

The oral formulations for the local treatment of such disease are coated with a pH-dependent polymeric film in order to prevent drug release in the stomach and the consequent first-pass metabolism while, on the contrary, the film should dissolve at pH values above 5.5, or even higher, to reach ileum and colon.[19] Thus, we selected Eudragit® S100, a copolymer based on methacrylic acids and methyl methacrylate (scheme 1b), soluble at pH>7, as polymeric carrier for the fabrication of electrospun fibers loaded with budesonide. After optimizing the experimental parameters of the electrospinning process, budesonide-loaded fibers with two different drug loads (9% and 20%, w/w) have been prepared and then characterized by means of scanning electron microscopy (SEM), X-ray powder diffraction (XRPD), FT-IR spectroscopy, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The in-vitro release profiles of the drug-loaded fibers were studied using a pH-change method: at pH 1.0 for two hours, to simulate the transit into the stomach, and then at pH 7.2, to simulate the intestinal environment.

2. Experimental procedures

2.1. Materials

Budesonide (*B*) was a kind gift of SICOR S.r.l., Rho, Italy and Eudragit® S100 (*EU*), methacrylic acid-methyl methacrylate copolymer (1:2), was obtained from Evonik, Essen, Germany. Their physical mixtures containing 9% and 20% (w:w) of drug (*B:EU9%pm* and *B:EU20%pm*, respectively) have been prepared by mixing the properly weighted amounts of the components by a Turbula (W.A. Bachofen AG, Basel, Switzerland) at 96 rpm for 15 minutes.

For the fabrication of electrospun fibers of pure Eudragit® S 100, named *EUel*, a 15% (w/w) polymeric solution has been prepared by dissolving the required amount of Eudragit® S 100 in hot acetone and stirring for about two hours. For drug-loaded fibers, coded as *B:EU9%el* and *B:EU20%el*, respectively, first the appropriate amount of budesonide has been dissolved in hot acetone, then the polymer has been added and the system stirred for about two hours.

The collected fibers have been placed in a vacuum oven overnight to fully eliminate any solvent residuals and moisture, then stored over silica gel in a dryer.

The fibers were analysed before and after manual milling of the fibrous fleeces in an agate mortar.

2.2. Instruments

2.2.1. Elettrospinning technique

The apparatus EC-CLI by IME Technologies (Spaarpot 147, 5667 KV, Geldrop, The Netherlands) has been used. The final experimental parameters were: applied high voltage 29 kV (charge applied to collector -4kV, charge applied to needle 25 kV), needle-collector distance 20 cm, needle tip diameter 0.8 mm, feeding rate 1.0 mL/h, temperature 24 °C, relative humidity (R.H.) 35%.

2.2.2. Microscopic analysis

SEM analysis was performed using a Zeiss EVO MA10 (Carl Zeiss, Oberkochen, Germany) on gold sputtered samples.

2.2.3. X-ray powder diffraction

XRPD measurements were performed using a D5005 Bruker diffractometer (Karlsruhe, Germany) (CuKα radiation, $\lambda(K\alpha_1)$ = 1.54056 Å; voltage of 40 kV and current of 40 mA) equipped with a θ-θ vertical goniometer, Ni filter, monochromator and scintillator counter. The patterns were recorded at room temperature in step scan mode (step size: 0.015°, counting time: 5 s per step) in the $3 < 2\theta° < 30$ angular range.

2.2.4. Spectroscopic techniques

FT-IR spectra were obtained using a Nicolet FT-IR iS10 Spectrometer (Nicolet, Madison, WI, USA) equipped with ATR (Attenuated Total Reflectance) sampling accessory (Smart iTR with ZnSe plate) by co-adding 256 scans in the 4000–650 cm^{-1} range at 4 cm^{-1} resolution.

2.2.5. Thermal analysis

Thermal characterization was carried out using a TGA Q2000 IR apparatus and a DSC Q2000 apparatus both interfaced with a TA 5000 data station (TA Instruments, NewCastle, DE, USA). The DSC instrument was calibrated using ultrapure (99.999%) indium (melting point = 156.6 °C; ΔH = 28.54 J g^{-1}) as standard. The calorimetric measurements were carried out in open standard aluminium pans under nitrogen flow (45 $mL \cdot min^{-1}$) at 10 $K \cdot min^{-1}$. All data from thermal measurements are the average of three or more experiments.

2.2.6 Solubility and Dissolution test

The drug solubility was measured at room temperature, in water, pouring an excess of *B* in volumetric flasks and left under magnetic stirring (200 rpm). After 2, 4, 24 and 30 hour, an aliquot of the supernatant was filtered (0.45 μm, Millipore), properly diluted, and the concentration was determined by UV detection (Lambda 25, Perkin Elmer, Monza, Italy).

The dissolution tests were performed in triplicate on the drug alone (*B*) 9 mg, used as reference, and on the proper amount of fibers corresponding to an active dose of 9 mg, using the USP 31 apparatus 2, paddle, (Erweka DT-D6, Dusseldorf, Germany), at 100 rpm, in 1000 ml of two different media: deionised water (pH=7.0) and phosphate buffer pH=7.2. Then, a pH-change method was applied: the samples were placed in 750 ml of 0.1N hydrochloric solution, pH=1, for the first two hours and then 250 ml of Na_3PO_4 0.2M were added to the medium to reach pH=7.2. The fluids were maintained at 37 °C. The drug concentrations were measured by the UV-Vis spectrophotometer, provided with automatic sampling and data analysis. The calibration curves of the drug absorbance were performed at 275 nm in the acidic environment, and at 247 nm in water and phosphate buffer pH=7.2.

3. Results and discussion

3.1. Fabrication of electrospun fibers

Several experiments have been performed with the polymer solution without drug in order to optimize the instrumental and ambient parameters of the electrospinning process. Trials have been made changing in sequence the applied high voltage (from 20 to 29 kV), the needle-collector distance (10, 20 and 30 cm), the feeding rate (1.0 and 2.0 mL/h) and the temperature (from 19 to 25 °C). Selected values, which guarantied the fabrication of fibers of good quality, are reported in paragraph 2.2.1..

3.2. Physico-chemical characterization of materials

3.2.1 Microscopic analysis

The SEM analysis reveals that the morphologies of *EUel*, *B:EU9%el* and *B:EU20%el* fibers are very similar (figure 1). The fibers of all these samples are randomly distributed, with no beads, and have smooth surface. No particles are visible indicating that in the loaded fibers the drug is dispersed homogeneously into the polymer and a solid dispersion of the two components has been created. Indeed, this behaviour is expected since the drug is completely dissolved in the solvent and, during the electrospinning process, the fast solvent evaporation does not allow its crystallization. The drug solidifies molecularly dispersed in the polymer without the possibility to create long-range order. This behaviour is confirmed by the experimental evidences described in the following paragraphs.

For each sample, 200-300 fibers were tested to determine the diameter distribution. The data have been fitted according to normal-log distribution.

$y = a \bullet \exp[-0.5 \bullet (\ln x / x_0)/b^2]$

where: x_0 is the mean value of diameter, *b* the standard deviation and *a* the regression coefficient.

In figure 2 the histograms of the particle size distribution and the curves of normal-log distribution are reported together with the mean value, the standard deviation and the regression coefficient. The parameters of the size distribution are reported in table 1. It is evident that the fibers mean size becomes bigger with increasing of the drug concentration.

3.2.2 X-ray powder diffraction

The X-ray powder diffraction pattern of budesonide (figure 3a) is typical of a crystalline compound with characteristic peaks appearing at angles of 2θ = 16.22°, 15.64°, 6.10°, 12.19° and 14.67°. Its main diffraction effects are still visible in the pattern of the physical mixtures *B:EU20%pm* and *B:EU9%pm* (figure 3b-c) although with low intensity in agreement with the system composition. On the contrary, the pattern of the fibers *B:EU20%el* and *B:Eu9%el* (figure 3d-e) show only the broad band characteristic of the amorphous nature of pure *EU* fibers (figure 3f). These experimental evidences support the idea that the drug is amorphously distributed in the fibers. The pattern does not change after milling the fibrous fleeces.

3.2.3 Spectroscopic analysis

The FT-IR spectra of pure budesonide, physical mixtures and electrospun fibers are drawn in figure 4. The spectrum of budesonide shows a lot of strong absorption peaks. The O-H stretching is observed at 3482 cm^{-1}, the C-H stretching at 2957, 2932 and 2872 cm^{-1}, the C=O stretching at 1722 cm^{-1}, the conjugated C=O stretching at 1665 cm^{-1} and the C=C stretching at 1625 cm^{-1}. The spectrum of *EUel* shows the wide adsorption band of O-H stretching, due to carboxylic acid group, superimposed by C-H vibrations at 3000-2900 cm^{-1}, the strong peak of C=O stretching esterified carboxyl group at 1724 cm^{-1} with the shoulder due to the acid C=O stretching, the CH_3 bending at 1436 cm^{-1} and 1388 cm^{-1}, the peak of the acid C-O stretching at 1246 cm^{-1} and the peaks of asymmetric and symmetric ester C-O-C stretching at 1191 cm^{-1} and 1149 cm^{-1}.

As expected, considering the system composition, the spectra of *B:EU20%pm* and *B:EU9%pm* show domination of the polymer's bands. Only the strongest absorptions of the drug at 2932 cm^{-1} and at 1665 cm^{-1} are visible. In the spectra of *B:EU20%el* and *B:EU9%el* the peak at 2932 cm^{-1} disappears and the peak of the conjugated C=O stretching is shifted towards low frequency (these peaks are labelled by * in figure 4). These observations suggest that weak

interactions like hydrogen bonds between the drug and the polymer take place in the fibers indicating good compatibility between the components.

3.2.4 Thermal analysis

Budesonide begins to decompose slowly over 160 °C. *EUel* looses about 3% (by mass) of water before 85 °C, then decomposes at about 150 °C. *B:EU20%el* and *B:EU9%el* show a thermogravimetric behaviour similar to that of the unloaded fibers (figure 5).

Budesonide melts at T_{onset} 253.8 ±0.7°C (figure 6, curve a) with enthalpy change of 74.92 ± 2.92 $J \cdot g^{-1}$. *EUel* shows an initial endothermic shift caused by dehydration and two endothermic events partially overlapped between 160 °C and 250 °C due to decomposition (figure 6, curve f). The DSC curve of *B:EU9%pm* and *B:EU20%pm* are very similar to that of the pure polymer even if a new very low peak is present at about 240 °C (figure 6, curve b-c). This effect is likely due to melting of the drug anticipated for the presence of the polymer. The peak is absent in the DSC curve of *B:EU20%el* and *B:EU9%el* (figure 6, curve d-e) confirming that the drug in the fibers is in amorphous phase.

3.3. Solubility and dissolution rate

B solubility, measured after 2 hours of stirring, was 24.5 ± 0.4 mg/l, while the equilibrium solubility, after 32 hours, was 28.0 ± 1.0 mg/l. Due to this low water solubility also the dissolution rate of this drug is very slow in all the dissolution fluids tested (figure 7): less than 20% of the dose is dissolved in 1 hour. On the contrary the presence of the hydrophilic polymer and the amorphization of the drug in the electrospun fibers, strongly enhances the dissolution rate of the active, but only in the phosphate buffer at pH=7.2 where the carrier is soluble: about 90% of the drug content is dissolved in 5 minutes and the entire dose in less than 60 min, while a minimum quantity is detected in deionised water in the same time.

This enhancement is confirmed by the dissolution tests performed using the pH-change method (figure 8): the drug dissolution from the fibers is completely prevented at pH=1.0 where *B* alone can dissolve even if rather slowly (about 10% of the active content is detected after 2 hours at pH=1.0). Instead, the drug released from the two electrospun systems, *B:EU9%el* and *B:EU20%el*, is very fast and it is completed in few minutes after the pH change. Whereas, the dissolution rate of *B* alone is very slow at this pH: less than 40% of the drug content is dissolved in the first 5 minutes after the pH change and, in the following 3 hours, the dissolved active reached about 50% of the total sample content.

Conclusions

In this study, budesonide-loaded Eudragit® S100 fibers were successfully fabricated by electrospinning. The physico-chemical investigation indicates that the drug is incorporated in the polymeric fibers in an amorphous phase. In particular, the FT-IR measurements suggest that hydrogen bonds likely take place between budesonide and the polymer. The dissolution tests using a pH-change method put into evidence the potentiality of this technique to prevent budesonide dissolution in the acid environment, simulating the transit through the stomach, and to quick deliver the entire dose in the desired portion of the gastrointestinal tract. The suitable behaviour of budesonide at pH 7.2 is due to the presence of the hydrophilic polymer and to the amorphization of the drug in the fibers. Electrospinning proved to be a useful technique to obtain drug in the amorphous phase. This is an important result for low water-soluble drugs, like budesonide, that poorly transform into amorphous phase.

The electrospun fibers could be simply filled into a capsule to protect the drug from degradation and to obtain a sustained and localized therapeutic effect.

Further studies are necessary to scale-up this promising technology from laboratory scale to the industrial production in order to obtain fibers with the desired and reproducible properties.

References

1) Huanga ZM, Zhangb YZ, Kotakic M, Ramakrishna S 2003. A review on polymer nanofibers by electrospinning and their applications in nanocomposites Compos. Sci. Technol. 63:2223–2253.

2) Sill TJ, von Recum HA 2008. Electrospinning: Applications in drug delivery and tissue engineering. Biomaterials 29:1989-2006.

3) Agarwal S., Wendorff JH, Greiner A 2008. Use of electrospinning technique for biomedical applications. Polymer 49:5603-5621.

4) Ma Z, Kotaki M, Inai R, Ramakrishna S 2005. Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue Eng 11:101–9.

5) Kumbar SG, James R, Nukavarapu SP, Laurencin CT 2008. Electrospun nanofiber scaffolds: engineering soft tissues. Biomed Mater 3:034002.

6) Szentivanyi A, Chakradeo T, Zernetsch H, Glasmacher B 2011. Electrospun cellular microenvironments: understanding controlled release and scaffold structure. Adv Drug Deliv Rev 63:209–20.

7) Meinel AJ, Germershaus O, Luhmann T, Merkle HP, Meinel L 2012. Electrospun matrices for localized drug delivery: current technologies and selected biomedical applications. Eur J Pharm Biopharm 81:1–13.

8) Tammaro L, Russo G, Vittoria V 2009. Encapsulation of Diclofenac Molecules into Poly(ε-Caprolactone) Electrospun Fibers for Delivery Protection. J Nanomater. DOI: 10.1155/2009/238206.

9) Paaver U, Tamm I, Laidmäe I, Lust A, Kirsimäe K, Veski P, Kogermann K, Heinämäki J 2014. Soluplus Graft Copolymer: Potential Novel Carrier Polymer in Electrospinning of Nanofibrous Drug Delivery Systems for Wound Therapy, BioMed Res Int, DOI: 10.1155/2014/789765.

10) Miao YE, Zhu H, Chen D, Wang R, Tjiu WW, Liu T 2012. Electrospun fibers of layered double hydroxide/biopolymer nanocomposites as effective drug delivery systems. Mater Chem Phy 134, 623-630.

11) Brettmann BK, Cheng K, Myerson AS, Bernhardt L 2013. Trout, Electrospun Formulations Containing Crystalline Active Pharmaceutical Ingredients. Pharm Res 30:238-246.

12) Sharma R, Singh H, Joshi M, Sharma A, Garg T, Goyal AK, Rath G 2014. Recent advances in polymeric electrospun nanofibers for drug delivery, Crit Rev Ther Drug 31:187–217.

13) Yu DG, Zhu LM, White K, Branford-White K 2009. Electrospun nanofiber-based drug delivery systems. Health 1:67-75.

14) Teo WE, Ramakrishna S 2006. A review on electrospinning design and nanofibre assemblies. Nanotechnology. 17, R89-R106.

15) Doshi J, Reneker DH 1995. Electrospinning process and applications of electrospun fibers. J Electrostat 35:151-160.

16) Seou CH, Benchimol EI, Steinhart AH, Griffiths AM, Otley AR, 2009. Budesonid for Crohn's disease. Expert Opin Drug Metab Toxicol 5:971-979.

17) Edsbäcker S, Andersson T 2004. Pharmacokinetics of budesonide (Entocort EC) capsules for Crohn's disease. Clin Pharmacokinet 43:803-21.

18) Dignass A, Stoynov S, Dorofeyev AE, Grigorieva GA, Tomsová E, Altorjay I, Tuculanu I, Bunganič I, Pokrotnieks J, Kupčinskas L, Dilger K, Greinwald R, Mueller R 2014. Once versus three times daily dosing of oral budesonide for active Crohn's disease: A double-blind, double-dummy, randomised trial. J Crohn's Colitis 8:970–980.

19) Pasupathi A, Palanisamy P, Jaykar B, Margret R, Chandira, Venkateswarlu BS 2013. Formulation and Evaluation of Gastro Resistant Extended Release Formulation for Colon Targeted Drug Delivery System. Int J Health Sci 1:20-35.