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Chitosan microparticles for sustaining the topical delivery of minoxidil sulphate

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Abstract
Given the hypothesis that microparticles can penetrate the skin barrier along the transfollicular route, this work aimed to obtain and characterise chitosan microparticles loaded with minoxidil sulphate (MXS) and to study their ability to sustain the release of the drug, attempting a further application utilising them in a targeted delivery system for the topical treatment of alopecia. Chitosan microparticles, containing different proportions of MXS/polymer, were prepared by spray drying and were characterised by yield, encapsulation efficiency, size and morphology. Microparticles selected for further studies showed high encapsulation efficiency (~82%), a mean diameter of 3.0 μm and a spherical morphology without porosities. When suspended in an ethanol/water solution, chitosan microparticles underwent instantaneous swelling, increasing their mean diameter by 90%. Release studies revealed that the chitosan microparticles were able to sustain about three times the release rate of MXS. This feature, combined with suitable size, confers to these microparticles the potential to target and improve topical therapy of alopecia with minoxidil.

Keywords: microparticles, chitosan, minoxidil sulphate, follicular delivery, alopecia, topical delivery

Introduction

Polymeric nano- and microparticles have been widely studied as topical drug-delivery systems over the last few decades (De Jalon et al., 2001; Smijs and Bouwstra, 2010). Such particles are stable, are able to improve drug stability, are relatively simple to prepare and can sustain the delivery of drugs. In some cases, micro- and nanoparticles can also be used for targeting the delivery of some substances, such as DNA plasmids to the liver (Intra and Salem, 2010) and antibiotics to the intestinal epithelial cells (Gao et al., 2007). In the field of topical medicine, it has often been speculated that nano- and microparticles, depending on their sizes and surface properties, can target delivery to epidermal appendages, such as hair follicles and sweat ducts (Meidan et al., 2005; Smijs and Bouwstra, 2010; Prow et al., 2011). Although there are many studies that show follicular localisation of nano- and microparticulate systems in skin, the results are often contradictory (Smijs and Bouwstra, 2010; Wu et al., 2010; Lopez et al., 2011). Some authors, such as Toll et al. (2004), postulated that while microparticles larger than 10 μm in size cannot penetrate the skin and while smaller nanoparticles can preferentially cross the skin barrier through the epidermal route, following the intercellular route via the lipid envelope of the stratum corneum, microparticles between 1 μm and 5 μm in size can cross the skin through the follicular channels and then target the delivery of drugs to treat follicular disorders, such as acne and alopecia (Meidan et al., 2005).

Classical treatment against androgenic alopecia includes the topical application of ethanol/water solutions containing minoxidil (Bienová et al., 2005). Minoxidil acts directly and indirectly on the hair bulb, increasing local blood irrigation and stimulating the hair cells to spend more time in the anagen phase (growth period) of the hair growth cycle (Buhl et al., 1990; Messenger and Rundegren, 2004). Minoxidil is available today in the form of hydrogel or ethanol solutions for topical application (Rogaine®). Ethanol is used in both types of Rogaine®

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pharmaceutical formulations as a solubilising agent for minoxidil, and it might also present for permeation enhancement. These formulations, however, do not target minoxidil to the hair follicles and seem not to sustain drug release. The patient should thus apply the formulation at least twice a day to ensure the pharmacological effect. Microencapsulation, therefore, might be a good alternative for improving minoxidil therapy due to the potential of microparticles both for penetrating the skin via follicular appendages, thereby targeting drug delivery to the hair follicles, and for sustaining drug release, thereby reducing the number of applications of topical formulations throughout the day and thus increasing compliance of the patients to the therapy.

Polymeric nanoparticles of poly(-caprolactone)-block-poly(ethyleneglycol) containing minoxidil for topical delivery of the drug have already been described in the literature. The authors revealed that significant amounts of minoxidil from a formulation containing minoxidil loaded in 40 nm and 130 nm nanoparticles permeated the skin and reached the receptor solution of a Franz diffusion cell (Shim et al., 2004), which proved that these nanoparticles crossed the skin through follicular shunts. As minoxidil is a potent vasoconstrictor (Buhl et al., 1990; Messenger and Rundegren, 2004) and because its transdermal permeation could therefore bring about important side effects, we chose to study microparticles instead of nanoparticles to carry the drug. The microparticles might penetrate the skin because of their larger size but might also remain in superficial skin layers, thereby avoiding the systemic effects of the drug. Moreover, the microparticles should sustain minoxidil release over a longer time period than the smaller nanoparticles.

This work aimed to obtain and characterise chitosan microparticles loaded with minoxidil and to study their ability to sustain the release of the drug, attempting a further application utilising them in a targeted delivery system of minoxidil for the topical treatment of alopecia.

In this work, minoxidil sulphate (MXS, Figure 1a), an approved hydrophilic derivative of minoxidil, was selected for study because it has already been proved to be more active than the minoxidil base for topical purposes (Buhl et al., 1990). Moreover, its higher hydrophilicity compared to non-sulphated minoxidil might increase its loading efficiency in a hydrophilic polymer.

Chitosan (Figure 1b), a natural hydrophilic polymer obtained from chitin, was chosen to encapsulate MXS due to many advantages, such as low cost, biodegradability and nonimmunogenicity, and because chitosan has the ability to adhere to the mucous and the skin (Nafee et al., 2007; Shah et al., 2008), which could improve drug targeting.

Material and methods

Material

The MXS (99%) was kindly provided by Galena Química e Farmacêutica Ltda. (Campinas, Brazil). Chitosan (medium molecular weight, 190–310 kDa, 75–85% deacetylation) was purchased from Sigma-Aldrich (Steinheim, Germany) and cellulose acetate membranes were purchased from Fisher Scientific (Leicestershire, UK). HEPES and NaCl, used in buffer preparation, were obtained from Acros (New Jersey, USA). The solvents used were all of HPLC grade: acetonitrile and methanol were purchased from Fisher Scientific (Leicestershire, UK), ethanol from Synth (Diadema, Brazil) and acetic acid from Fluka (Steinheim, Germany). The water used in all preparations was of Milli Q grade (Millipore, France).

Preparation of microparticles

Microencapsulation of MXS using chitosan was performed by spray drying. Solutions containing certain amounts of chitosan and MXS (described in Table 1) were obtained by dissolving both components in an aqueous solution of 1% (w/w) acetic acid at a pH of 4.0. For each batch preparation, 200 mL of the solution was spray dried with a 1.0 mm pressurised atomiser at a feed rate of 6 mL/min in a Labmaq model MSD 0.5 spray dryer (Ribeirão Preto, Brazil). The atomising air flow rate was 6 m^3/min.

Table 1. Chitosan (Ch) and MXS content of solutions spray dried for the preparation of microparticles 01, 02, 03 and control.

MP	Ch:MXS proportion	Chitosan (g)	MXS (g)
01	1:1	3.0	3.0
02	2:1	3.0	1.5
03	3:1	3.0	1.0
Control	1:0	3.0	0.0

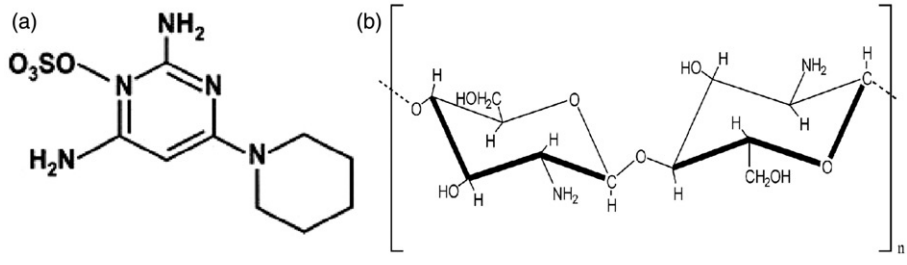

Figure 1. Chemical structures of (a) MXS and (b) chitosan.

The inlet temperature was maintained at 135°C, and the outlet temperature was 75–80°C. After preparation, the microparticles were kept at room temperature in a closed container until use.

Note that all the solutions described in this work were obtained by weighing all the components (water, ethanol and acetic acid) because of the differences in density among the solvents, in an attempt to facilitate the reproducibility of the experiments.

Characterisation

Yield

The microparticles obtained by spray drying were weighed, and the process yield was calculated as a percentage of the amount of solids added during the preparation process (Parikh et al., 2003), according to Equation (1):

$$R\% = \left(\frac{Q_f}{Q_i}\right) \times 100, \quad (1)$$

where $R\%$ is the yield of the process, Q_i is the amount of solids (chitosan plus MXS) initially added for the preparation of microparticles and Q_f is the quantity of microparticles obtained at the end of the process.

Efficiency of encapsulation

The encapsulation efficiency was determined by evaluating the amount of MXS entrapped in the chitosan microparticles. A 10 mg portion of each microparticle batch was dispersed in 10 mL of the aqueous solution of 1.0% (w/w) acetic acid under agitation at 500 rpm for 24 h. The dispersion was then left for 30 min in an ultrasonic bath and filtered afterwards, and the resulting solution was analysed for its MXS content, through which the amount of encapsulated MXS (Q_{obtained}) was recorded. The encapsulation efficiency was calculated from Equation (2):

$$EE\% = \left(\frac{Q_{\text{obtained}}}{Q_{\text{theorical}}}\right) \times 100, \quad (2)$$

where $EE\%$ is the encapsulation efficiency of the chitosan microparticles, Q_{obtained} is the amount of MXS extracted from the chitosan microparticles, and $Q_{\text{theorical}}$ is the amount of MXS initially added to prepare the chitosan microparticles.

Size distribution

The distribution of particle size was determined by laser diffraction in a Beckman Coulter LS 13 320 diffractometer. A 1 mg amount of microparticles was suspended in 2 mL of methanol and this dispersion was used for the measurement (Oliveira et al., 2006).

Morphology

The morphology of the obtained microparticles was verified by analysis in a scanning electron microscope (SEM). A sample of chitosan microparticles was coated with gold and analysed in a Phillips XL 30 FEG SEM at magnifications from 2000 to 20 000 times.

Characterisation of MXS-loaded microparticles in the vehicle (ethanol/water 60:40)

Zeta potential

For the zeta potential measurement, approximately 1 mg of microparticles was suspended in the vehicle in which the particles were to be topically administered, i.e. an ethanol/water 60:40 solution at a pH of 5.5 containing 10 mM NaCl. The zeta potential of the suspended microparticles was determined using a Malvern Zetasizer Nano ZS 4.0 instrument.

Swelling properties

The entry of water into chitosan microspheres containing MXS was determined by laser diffraction in a Beckman Coulter LS 13 320 diffractometer, according to the methodology proposed by Gavini et al. (2008). For this characterisation, 4 mg of chitosan microparticles was suspended in 8 mL of an ethanol/water 60 : 40 solution at a pH of 5.5 and was kept under agitation at 500 rpm. After 5, 30 and 60 min, samples were extracted from the suspension for size distribution analysis. The results were expressed as the swelling index (SI), calculated according to Equation (3):

$$SI\% = \left(\frac{D_2}{D_1}\right) \times 100, \quad (3)$$

where $SI\%$ is the swelling index of chitosan microparticles containing MXS, D_1 is the diameter of the microparticles before contact with the aqueous solution, and D_2 is the diameter of the microparticles after contact with the aqueous solution.

Release studies

The release rate of MXS from 300 μL of a dispersion containing 7.2% w/w of microparticles (equivalent to 2% MXS) in an ethanol/water 60:40 solution at a pH of 5.5 was determined *in vitro* by using a modified Franz-type diffusion cell (diffusion area = 1.85 cm^2) and a hydrophilic cellulose membrane (MW 12 000–14 000) over a period of 18 h. The amount of MXS that crossed the hydrophilic membrane from the microparticles was analysed by HPLC as described below, and the MXS release rate from this system was compared to MXS diffusion from an ethanol/water 60:40 solution at a pH of 5.5 containing 2% non-encapsulated MXS, used as a control. The diffusion coefficients (D) of MXS were calculated using the following equation (Higuchi, 1962; De Santana et al., 2010):

$$Q = 2C_o(Dt/\pi)^{1/2}, \quad (4)$$

where Q is the cumulative amount of drug released per unit area, C_o is the MXS concentration in the vehicle, D is the diffusion coefficient and t is time.

HPLC analysis

MXS concentrations were determined by a reversed-phase HPLC method as follows; 10 μL aliquots of the samples were injected into an LC (model LC-2010 A HT, Shimadzu, Kyoto, Japan) equipped with an automatic sample injector, and a reversed-phase C18 column (Dionex 4.0 × 125.0 mm, 5 μm). Elution was performed with a mobile phase consisting of a mixture of water/acetic acid/acetonitrile (70:0.1:30, v/v/v) at a pH of 3.5, and a flow rate of 1.0 mL/min. UV detection was performed at 285 nm. This method was validated in accordance to FDA guidelines (FDA, 2009), and was linear ($r = 0.999$) and highly selective for MXS over a concentration range from 0.05 to 1.00 μg/mL. The intra- and inter-day precision and accuracy of the method showed a coefficient of variation (% CV) and a relative error (% E) not greater than 1.8% and 2.4%, respectively.

Statistical studies

At least four replicates of each experiment were used. The results are presented in the text as means ± standard deviations. The data were analysed by ANOVA followed by a non-parametric Tukey's test. The statistical significance was fixed at $P < 0.05$.

Results and discussion

Polymeric nano- and microparticles have been widely studied as topical drug delivery systems due to their ability to protect drugs against degradation and thus stabilise formulations, to sustain drug release and to even restrict transdermal penetration (De Jalon et al., 2001; Cevher et al., 2006; Smijs and Bouwstra, 2010). For topical purposes, it is desirable that greater amounts of drugs are delivered to the skin and that a small amount or, if possible, no amount of drug diffuses through the skin and reaches the blood stream, thus avoiding systemic side effects. More specifically, for the treatment of follicular diseases, such as alopecia, it is very appropriate to target the delivery of minoxidil to the hair follicles. Because many works have suggested that the main skin penetration route for microparticles is transfollicular (Shim et al., 2004; Toll et al., 2004; Meidan et al., 2005; Münster et al., 2005; Pitaksuteepong et al., 2007), polymeric microparticles were obtained and characterised in this work, and their release rate was evaluated *in vitro* for further application in the treatment of androgenic alopecia.

Chitosan microparticles containing MXS were successfully prepared using a spray dryer. By drying small atomised drops of polymeric solutions containing the drug, solid microparticles were achieved in only one step. The three batches of microparticles obtained by changing the proportion of chitosan to MXS, according to Table 1, were first characterised with respect to yield, size, encapsulation efficiency and morphology. Based on these results, one microparticle formulation was chosen, and its zeta potential, swelling properties and *in vitro* release were evaluated within an ethanol/water 60 : 40 (w/w) vehicle.

Table 2. Characterization of chitosan (Ch) microparticles loading MXS: yield, encapsulation efficiency and mean diameter.

MP	Ch:MXS proportion	Yield (%)	Encapsulation Efficiency (%)	Mean diameter (μm)
01	1:1	34	81.8	4.2 (±1.7)
02	2:1	38	81.7	3.0 (±1.5)
03	3:1	45	44.7	2.9 (±1.5)
Control	1:0	46	–	4.1 (±2.1)

Characterisation

Yield

The yield of the MXS-chitosan microparticles varied between 34% and 45% (Table 2). This apparently low yield was obtained because chitosan is a polymer that forms films when dried under a smooth surface (Ji et al., 2009). Therefore, when the droplets of the polymer solution were sprayed and hit against the glass wall of the tub drying apparatus, the chitosan film formed and remained trapped in the equipment. Obviously, this loss of material is more significant when small quantities of the formulation are atomised (recall that only 200 mL of the formulation was dried per batch in this work). Note that this loss of material always occurs when particles of chitosan are obtained by the spray drying technique. Gavini et al. (2008) and Cevher et al. (2006) also obtained chitosan microparticles by spray drying and achieved yields of production between 30% to 50%. Despite the low yield, the spray drying method was chosen because it presents several advantages, such as simplicity of operation in that the microparticles are obtained already dried in one only step, good reproducibility from batch to batch unlike the majority of physicochemical methods used to prepare microparticles, ease of scale-up, and the low level of toxic residual organic solvent compared to other encapsulation methods (Bitz and Doelker, 1996; Cevher et al., 2006). Moreover, at larger scales of production, this relatively low yield could be avoided because this loss of material could be less significant than on a small scale. The material loss could also be decreased by using equipment coated with Teflon, for example.

Encapsulation efficiency

The encapsulation efficiency represents the percentage of the drug that can be encapsulated into the microparticles. In this work, this parameter was obtained from the ratio between the amount of MXS extracted from 10 mg of polymer microparticles and the amount of MXS initially added to the MXS/polymer suspension. Table 2 shows the MXS encapsulation efficiency for the three batches of microparticles obtained. These values ranged from approximately 45% to 82%. Larger proportions of MXS in relation to the polymer resulted in a higher percentage of drug

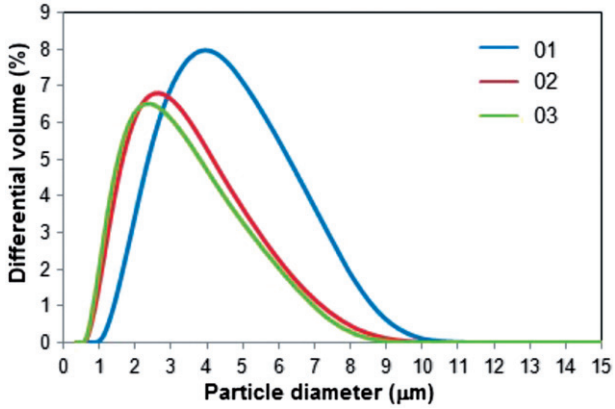

Figure 2. Distribution of the diameters of chitosan microparticles loaded with MXS.

encapsulation (see Table 2, microparticles 01 and 02). In spite of the fact that the medium size of the microparticles from the three different batches was not significantly different ($P > 0.05$), the polydispersity was not narrow (see discussion in the Section 'Particle size'), and the particles tended to decrease in size for higher polymer proportions (see Table 2). Therefore, higher proportions of the polymer tended to form smaller particles that were capable of encapsulating smaller proportions of MXS, resulting in a lower encapsulation efficiency (see Table 2, microparticle 03). The low encapsulation efficiency presented by the microparticles formed with larger chitosan amounts (microparticle 03) might also be related to the small amount of MXS added to this dispersion to be dried and to the method yield. More specifically, the adhesion of chitosan to the glass wall of the spray dryer during the atomisation process (see discussion in the Section 'Yield') might also drag MXS particles to the equipment wall. Because for microparticles 03 production, MXS was present in the dispersion in smaller concentrations, particles and the chitosan molecules that were possibly lost on the glass wall together meant that a lower number of particles were available for encapsulation, resulting in a low encapsulation efficiency.

Particle size

The mean diameter of microparticles dispersed in methanol (which is a solvent that inhibits chitosan microparticle aggregation and swelling) ranged from 2.9 to 4.2 μm (Table 2). Particles prepared with a ratio of MXS to chitosan of 1:1 were around 1 μm bigger than those prepared with lower drug proportions (1:2 and 1:3). These results are similar to those obtained by Cevher et al. (2006), who prepared chitosan microparticles containing vancomycin by spray drying. Like us, they observed a tendency of decreasing average particle size with increasing amount of polymer in the formulation, indicating that larger proportions of the polymer tended to form smaller particles with greater surface-to-volume ratio.

The size distributions of chitosan microparticles containing MXS (Figure 2) were monomodal, i.e. only one population of microparticles was formed (Gaumet et al., 2008). Although monomodal, the particles were not monodisperse because the size distribution was broad (Tran et al., 2011), varying by approximately ± 1.5 μm from the mean size of 3 μm; typically, 90% of the particles were smaller than 6 μm. These results were confirmed by the SEM analysis of the microparticles (see the next section). The influence of the vehicle, an ethanol/water 60:40 solution at a pH of 5.5, in which the microparticles would be administered, on the size distribution of microparticles will be discussed further in the Section 'Swelling properties'

Morphology

Scanning electron micrographs (SEM) were obtained to evaluate the microparticle morphology and to corroborate the laser diffraction results regarding the size distribution. Figure 3 shows that most of the chitosan microparticles had a diameter of approximately 3 μm, and the biggest microparticles had diameters of approximately 6 μm.

Regarding the microparticle morphology, all batches of drug-containing microparticles showed a fairly spherical, non-porous surface without grooves or fissures but with a scaly appearance. It is speculated that these scales are caused by deposition of the polymer on the particle surface, which might not interfere with their performance. These morphological characteristics were shown to be independent of the ratio of drug to polymer. Chitosan microparticles prepared by Cevher et al. (2006) by spray drying also presented spherical shapes, regardless of the proportion of drug to polymer. However, instead of superficial scales like those observed in our particles, Cevher et al. observed many pores in their microparticles. The differences in the chitosan microparticle morphology obtained using the same technique (spray drying) but encapsulating different drugs suggest an influence of the drug in the drying process. Therefore, depending on the interactions of the drugs with the polymer, i.e. chitosan, during the drying process, the morphology of the microparticles will be different. This influence can be proven by the differences presented by chitosan particles obtained in the presence and in the absence of MXS. In Figures 3g and 3h, it can be observed that most of the chitosan microparticles formed in the absence of MXS presented a non-spherical shape, with the appearance of ''deflated balls'', whereas in the presence of the drug, the morphology changed, as described above.

Despite the differences in morphology observed in the absence of MXS, when the drug was present, the microparticles were all very similar, independent of the ratio of drug to polymer. Therefore, the smallest particles with a high encapsulation efficiency, i.e. the microparticles obtained with a ratio of chitosan to MXS of 2:1, were selected for further studies for characterisation of the microparticles already dispersed in an appropriate vehicle for MXS topical administration, i.e. the ethanol/water solution.

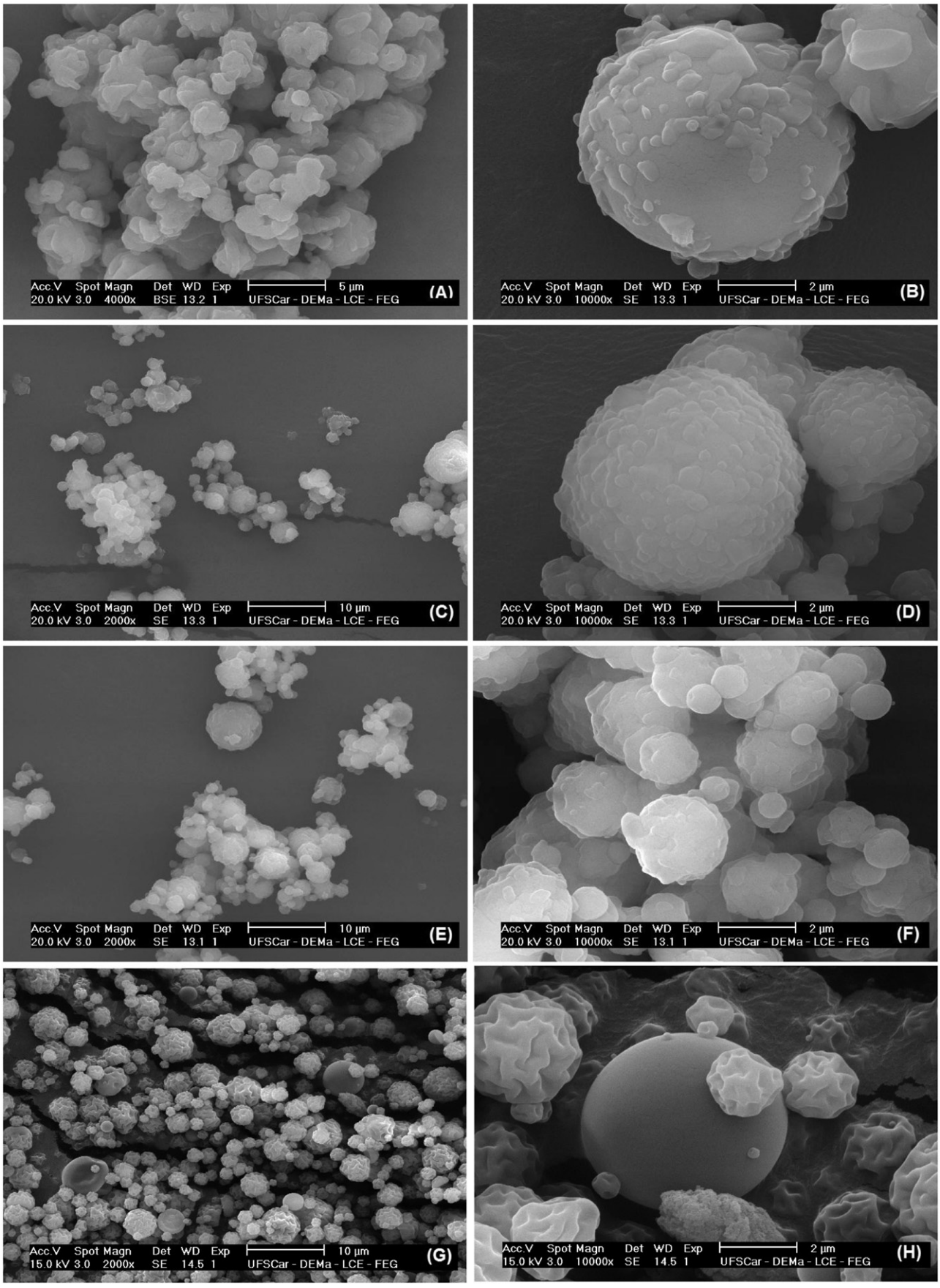

Figure 3. Electron micrographs obtained by SEM for the morphological analysis of microparticles 01 (A, B), 02 (C, D) and 03 (E, F) and the control microparticles (G, H).

Characterisation of MXS-loaded microparticles in the vehicle (ethanol/water 60:40)

Zeta potential

The value of the zeta potential obtained for the microparticle dispersion in an ethanol/water 60:40 solution at a pH of 5.5 was +5.9 (±5.2) mV. Considering that this value is near zero and also considering the standard deviation, the chitosan microparticles containing MXS can be considered neutral in charge when dispersed in the vehicle in which they will be topically administered (ethanol/water solution). Although it was expected that, due to the positive charge of chitosan in water (pKa ~ 6.5) (Gribanov and Sazanov, 2008), the zeta potential of the microparticles would be positive, it is possible that the decrease in the dielectric constant of the water caused by the presence of ethanol in the vehicle could change the chitosan pKa, decreasing chitosan chain ionisation and resulting in neutral microparticles.

Swelling properties

Chitosan is a hydrophilic polymer that presents amine groups in its chemical structure (Oliveira et al., 2006), which form hydrogen bonds with water. Therefore, in the presence of water, chitosan microparticles tend to undergo swelling, which is the formation of a gel layer around the particles due to chitosan hydration (Oliveira et al., 2006). The swelling obviously increases the diameter of these particles (Oliveira et al., 2006; Gavini et al., 2008). Together with the drug/polymer interaction, swelling might also influence the mechanism of drug release because to exit the particle, MXS is expected to solubilise in the water and diffuse through the swollen particle.

Because chitosan is a pH-sensitive polymer, the pH and the nature of the dispersive medium can affect the swelling properties of chitosan microparticles (Gaumet et al., 2008). Therefore, for these experiments, the microparticles were dispersed in an ethanol/water solution, and the pH of the dispersion (pH 5.5) was monitored over time. This ethanol/water medium was chosen to be the microparticle vehicle instead of pure water because chitosan microparticles tend to aggregate in water. The ethanol/water solution prevents this aggregation. Moreover, a similar ethanol/water solution has been used as the vehicle for the minoxidil base in commercial topical formulations for alopecia.

MXS-loaded microparticle swelling was assessed by dispersing microparticles in the ethanol/water solution at a pH of 5.5 and keeping them in this medium for different periods of time (5, 30 and 60 min). After each period of time, a sample of the dispersion was withdrawn, and its size distribution was rapidly determined by laser diffraction. The pH of the dispersion was also measured and was found to be constant over time.

The microparticle size distribution as a function of the time in the ethanol/water solution is shown in Figure 4. After 5 min, the swelling index (SI) was calculated to be 90.5%, i.e. the microparticle mean size increased by almost twice after immersion in the ethanol/water solution (3.0 μm to 5.7 μm). After 30 and 60 min, the mean size of the particles remained almost the same, i.e. SI was practically constant after at least 5 min in the ethanol/water solution, indicating that hydration of the MXS-loaded chitosan microparticles is almost immediate.

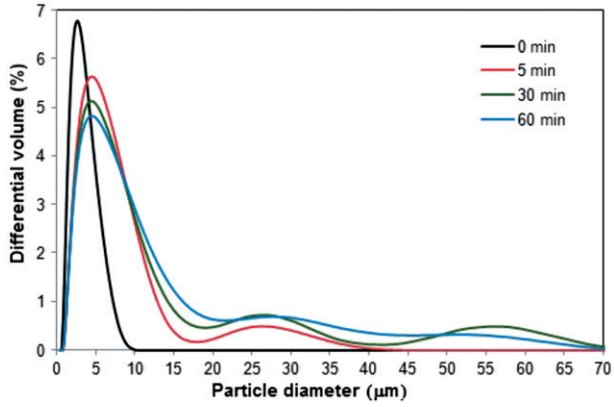

Figure 4. Distribution of diameters of the MXS-loaded chitosan microparticles as a function of time in an aqueous solution.

In summary, after 5 min in an ethanol/water solution, chitosan microparticles swelled, and their diameters increased from 3.0 μm to 5.7 μm. In addition, the emergence of a second small peak around 25 μm was observed (Figure 4), which probably represents agglomeration of these particles. After 30 min, the number of clusters of microparticles start increasing until 60 min. These results are in agreement with what has been described in the literature for other chitosan microparticles. Gavini et al. (2008) obtained an SI equal to 91% after 5 min in water for metoclopramide-loaded chitosan microparticles, which is very similar to our results.

It is important to take into consideration this increase in size when proposing MXS-loading chitosan microparticles as a drug delivery system to target the topical delivery of MXS to hair follicles; however, it appears that even with this increase, microparticles approximately 6 μm in size still might be able to cross the skin through hair appendages (Toll et al., 2004). Even if these particles were not to enter into the follicles, we believe that the chitosan microparticles described herein might accumulate around the hair follicles, targeting drug release to this area. This hypothesis is based on nanoparticle skin penetration experiments that have shown that hair follicles might be one of the main penetration routes of particles (Wu et al., 2010; Lopez et al., 2011). Therefore, microparticles, which are larger than nanoparticles, might not enter into the follicles as easily as the nanoparticles but are expected to accumulate around this follicular area. As MXS release from the microparticles was sustained and reproducible (see the Section 'MXS *in vitro* release studies'), a targeted, sustained release of MXS is predicted from these microparticles to the hair follicles.

MXS in vitro *release studies*

In vitro release of MXS from chitosan microparticles was performed using ''Franz'' diffusion cells and a cellulose

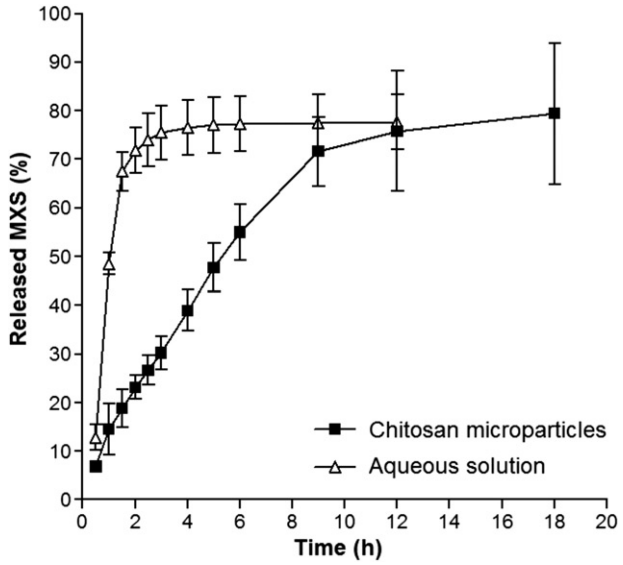

Figure 5. Release profile of MXS from the chitosan microparticle formulation (2% w/w of MXS) in comparison to the diffusion rate of MXS through a cellulose membrane from an aqueous solution of MXS at 2% w/w. Values are presented as mean ± S.D. of 4 determinations.

acetate hydrophilic membrane with controlled pore size (approximately 4 nm). As the main purpose of this work was to develop microparticles to topically deliver MXS, the release rate was determined using vertical diffusion cells, very commonly used *in vitro* to evaluate the permeation profile of formulations to be applied to the skin (Gelfuso et al., 2008, 2011). The release rate of MXS from the microparticles was compared to the diffusion rate of MXS in the ethanol/water solution (MXS at a 2% concentration dissolved in an ethanol/water 60:40 solution) through the cellulose membrane, used as a control (Figure 5).

Figure 5 shows that the chitosan microparticles were able to sustain the release of MXS for at least 9 h, compared to MXS in solution. While only 2 h were required for 68% of the MXS to be released from the simple solution, almost 12 h were necessary for the same percentage of encapsulated MXS to be found in the receptor solution of the diffusion cell.

It is interesting to note in Figure 5 that the total amount of MXS that crossed the membrane and reached the receptor solution was about 80%. There might be some interaction between the drug and the membrane used to separate the donor and the receptor compartments of the Franz diffusion cells, which prevented the diffusion of 100% of the MXS placed in the donor compartment during the 18 h of the experiment. This hypothesis can be supported by the fact that when non-encapsulated MXS in solution was placed under the same conditions as the encapsulated drug, only 80% of the drug diffused through the membrane and reached the receptor solution.

The diffusion coefficient (D) of MXS from both formulations was calculated from Equation IV, considering the first 9 h of the experiment for the microparticles [when the MXS release profile was linear ($r > 0.98$)] and only the first two hours for the drug in aqueous solution (when practically all the drug content had already diffused through the cellulose membrane). These data are presented in Table 3.

Table 3. Diffusion coefficient calculates for MXS from the formulation containing chitosan microparticles (2% w/w MXS) and from the aqueous solution of MXS 2% w/w.

Formulation containing MXS	Diffusion coefficient (cm^2/s)	Lienar correlation coefficient (r)
Aqueous solution	1.0×10^{-6}	0.98
Chitosan microparticles	0.3×10^{-6}	0.99

From the analysis of the data (Table 3), it is evident that the microparticles dispersed in the ethanol/water solution decreased the diffusion rate of MXS through the membrane by approximately three times compared to the non-encapsulated drug. The sustained MXS release from the chitosan microparticles can be explained by the swelling and slow MXS diffusion through the gelled particles. More specifically, due to the small size of the microparticles and the physicochemical properties of chitosan at a pH of 5.5 (the chitosan chains were elongated due to the acidic pH) (Gaumet et al., 2008), the chitosan/MXS microparticles almost completely gelled after 5 min of contact with the ethanol/water solution (see Figure 3) and remained in this condition during the 18 h of the experiment (most of chitosan microparticles from the donor compartment remained with mean diameter smaller than 10 μm). The loaded MXS diffused slowly through these swollen particles, leading to a constant drug release as a function of the square root of time.

These results show that chitosan microparticles have the potential to sustain the release of MXS after the application on the skin. The dosage of topical solutions containing minoxidil currently on the market requires topical application at least twice a day, which causes some discomfort to the patient, compromising the success of the therapy. The chitosan microparticles obtained in this work, by sustaining the release rate of MXS, might maintain therapeutic drug concentrations in the hair follicles for longer periods and, therefore, have the potential to decrease the number of applications of the formulation throughout the day, thereby increasing patient compliance to alopecia therapy.

Conclusion

Chitosan microparticles loaded with MXS were prepared by spray drying and presented a mean diameter of 3.0 μm. The particles were morphologically spherical, regardless of the proportion of MXS in relation to chitosan, although small proportions of MXS to chitosan decreased the encapsulation efficiency. These microparticles were shown to sustain a 3-fold release of the drug; even after undergoing swelling, they presented diameters adequate for targeting MXS delivery to the hair follicles. Further studies evaluating the follicular bioavailability of MXS from this formulation are being conducted in our laboratory in an attempt to prove the potential use of such microparticles in

targetting drug delivery for the topical treatment of alopecia.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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