International Journal of Pharmaceutics 399 (2010) 71–79

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Poloxamer 407 microspheres for orotransmucosal drug delivery. Part I: Formulation, manufacturing and characterization

B. Albertini[a,*], N. Passerini[a], M. Di Sabatino[a], D. Monti[b], S. Burgalassi[b], P. Chetoni[b], L. Rodriguez[a]

[a] Department of Pharmaceutical Sciences, University of Bologna, Via S. Donato 19/2, 40127 Bologna, Italy
[b] Department of Pharmaceutical Sciences, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 10 May 2010
Received in revised form 26 July 2010
Accepted 2 August 2010
Available online 7 August 2010

Keywords:
Microparticles
Poloxamers 407
Formulation design
Release
Mucoadhesion
Solid state

ABSTRACT

The two-part article aimed to investigate poloxamer 407-based microspheres as a novel platform for enhancing and controlling the delivery of atenolol across the oromucosal tissue. In the Part I of the work, atenolol-loaded poloxamers 407 microparticles were prepared by the solvent free spray congealing technology. This approach was feasible upon the high viscosity of the systems allowing for high loaded (20% w/w) non-aggregated microspheres. Several formulations were studied and the results demonstrated that the drug release patterns, solubility data, mucoadhesion to buccal tissue and gelling properties in saliva could be modified by adding different amount of an amphiphilic polymer–lipid excipient (Gelucire® 50/13) to poloxamer 407. Particularly, microspheres based only on poloxamer 407 exhibited very high solubility, mucoadhesive strength and gelling behaviour. To assess their potential as matrix for buccal application, the gelling property and the drug release from tablets obtained from direct compression of the microparticles were further evaluated. The microspheres were then characterized by differential scanning calorimetry, X-ray powder diffraction and Fourier transform-infrared spectra analysis. No solid state modifications and chemical interactions were detectable in the microspheres after manufacturing and during storage, suggesting their stability and use as orotransmucosal delivery systems.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

In the last years, poloxamers, also known as Pluronics® or Lutrol®, a class of non-ionic surfactants with the triblock poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) [(EO)*x*(PO)*y*(EO)*x*] structure, have been object of increasing interest of technological and pharmaceutical researchers. Depending on the copolymer composition they may exhibit different functions: (i) high solubilizing capacity due to their ability to form self-assembling micelles (Oh et al., 2004), to decrease the drug crystallinity (Ahuja et al., 2007) and to form eutectic mixtures (Passerini et al., 2002a,b), (ii) thermogelling and thickening ability (Escobar-Chávez et al., 2006) and (iii) emulsification properties useful for the stabilization of nano/microemulsions (Alexandridis and Hatton, 1995). Recently, a great number of studies have shown that poloxamers L61, L62, L81, L92, F68, F127 and P104 (L, F and P indicate liquid, flakes and paste, respectively) may be advantageous for enhancing the drug adsorption through intestinal or nasal epithelial cells (Bromberg and Alakhov, 2003; Brüsewitz et al., 2007; Lin et al., 2007). This property is explained according to the permeation enhancement ability of surfactants by perturbing intercellular lipids and altering the tight junctions of those epithelia (via paracellular route) (Senel and Hincal, 2001; Bromberg and Alakhov, 2003; Lin et al., 2007).

Among poloxamers, poloxamer 407 (Lutrol® or Pluronic® F127) consisting of 70% w/w polyoxyethylene units, is a low toxicity excipient approved by FDA for different types of preparations as bioavailability enhancer (Dumortier et al., 2006). Lutrol® F127 has been shown to provoke neither skin irritation nor sensitivity, thus confirming its good tolerability and its safe application in topical, rectal and ocular formulations (Dumortier et al., 2006). Also evidence for the safety of poloxamer 407 for delivery to mice lungs was provided (Desigaux et al., 2005). Among buccal delivery systems, poloxamer 407 has been used in mucoadhesive gels (Shin and Kim, 2000; Morishita et al., 2001; Dhiman et al., 2008; Jones et al., 2009) and in mucoadhesive matrix tablets (Cafaggi et al., 2005). In these studies several strategies have been adopted to lengthen the residence time and to enhance the drug availability. The majority of the formulations have been developed by combining the poloxamer at a concentration ranging from 20 up to 50% w/w with other additives as chitosan or Carbopol® to obtain mucoadhesive systems (Dhiman et al., 2008; Jones et al., 2009; Cafaggi et al., 2005). Other strategies used the poloxamer together with some absorption enhancers like fatty acids as membrane permeability modifying agents (Morishita et al., 2001) and finally gels were developed using both carbopol 934 and poloxamer 407 with

* Corresponding author. Tel.: +39 0512095607; fax: +39 051245082.
E-mail address: beatrice.albertini@unibo.it (B. Albertini).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.08.004

Table 1
Composition, particle size distribution, viscosity data and drug content of microparticles.

Samples	Composition (w/w)			Particle size distribution, w/w (%)					Viscosity (mPa s)	Drug content (%, w/w) (100–750 μm
	AT	PF 127	Gelucire 50/13	<100	100 < x < 355	355 < x < 500	500 < x < 750	>750		
M1	–	100	–	0.16	47.31	22.40	18.46	8.08	5800	–
M2	10	90	–	0.75	26.95	19.2	33.09	20.04	9300	10.16 ± 1.07
M3	10	67.5	22.5	4.02	57.07	18.18	14.60	6.13	7500	10.65 ± 0.39
M4	10	45	45	8.41	65.05	13.27	9.20	4.07	6100	10.05 ± 0.54
M5	20	80	–	0.15	19.61	9.72	49.22	21.33	11,300	15.32 ± 0.68
M6	20	60	20	0.73	36.14	10.69	32.64	19.80	9800	16.87 ± 0.34

different enhancers, such as bile salts, glycols and non-ionic surfactants (Shin and Kim, 2000).

This study concerns on an original formulative and manufacturing approach for the preparation of a novel platform based on poloxamer 407 alone or in mixture with Gelucire® 50/13 (stearoyl polyoxylglycerides), for orotransmucosal drug delivery. In particular, the delivery system was designed to enhance the adhesion to the buccal tissue and to promote the permeation through the tissue of a therapeutic entity ensuring a sustained drug release. To this aim, atenolol, a cardio selective β-blocker with a spare systemic bioavailability, was selected due to its low permeability and high solubility (Class III drug according to the Biopharmaceutical Classification System) (Vogelpoel et al., 2004). Therefore the feasibility of poloxamer 407-based microparticles loaded with different amount of atenolol was first evaluated. As manufacturing method spray congealing was selected owing to its peculiarity of atomizing substances which melt between 40 and 90 °C; poloxamer 407, melting around 60 °C, exactly met this purpose. Secondly, the microparticles were compacted into tablets, which represent a simple dosage form for their buccal/sublingual administration. The in vitro release profiles of both free microparticles and tablets were performed. According to the displayed release behaviour, the microparticle formulation was then suitably modified to comply with the need of buccal administration. Finally, the microparticles were fully characterized for morphology, particle size, drug loading, solubility, bioadhesion to buccal tissue and physicochemical properties by means of FT-IR, DSC and X-RD.

The in vitro delivery and ex vivo permeation experiments and in vivo absorption studies of the higher loaded–delivery systems were assessed in details in the Part II of the study (Monti et al., submitted for publication).

2. Materials and methods

2.1. Materials

Atenolol (AT) was purchased from Sigma–Aldrich (lot n. 075K1888) (Milan, Italy); Poloxamer 407 (PF127) was a gift of BASF (Germany) while Gelucire® 50/13 (stearoyl polyoxylglycerides) was kindly supplied by Gattefossè (France). Phosphate buffer saline (pH 6.8) was prepared as reported in Eur Phar; all components were from Sigma–Aldrich. Artificial saliva constituted of 0.5% w/v mucin (Kockisch et al., 2003) (from porcine stomach, Type III, purchased from Sigma–Aldrich) in pH 6.8 isotonic phosphate buffer was used in the mucoadhesion studies. Natural human saliva, obtained from a healthy donor using a procedure previously reported (Giannola et al., 2007), was used during the gelling studies. All other reagents were analytical grade and Milli-RX20 water (Millipore, Molsheim, France) was used throughout.

2.2. Preparation of the samples

Microparticles with a theoretical drug loading of 10% and 20% w/w were produced by the spray congealing process using the wide pneumatic nozzle (WPN), which has been fully described in recently published papers (Albertini et al., 2008, 2009; Passerini et al., 2010). Briefly, WPN is a two fluid atomizer equipped with a thermostated reservoir with a wide orifice opening. This nozzle configuration implies an external mixing of the fluid and of the gas (air) outside the nozzle orifice. As a consequence, atomization can be varied by changing the air pressure without affecting the liquid flow rate and high concentration or viscous products are better atomized. The microparticles solidify along the congealing chamber and are collected at the bottom. In particular the carrier was heated at a temperature at 70 °C, about 10 °C above its melting point. AT is added to the molten poloxamer alone or to the molten mixture of poloxamer and Gelucire® 50/13 and stirred to obtain a suspension, which was then loaded into the feeding chamber of the WPN, kept at 90 °C to avoid the solidification of the suspension in the nozzle orifice when the fluid encounter to the atomization air. The inlet air pressure was set at 3 bar. To facilitate the microparticles solidification the bottom of the collecting chamber was cooled using liquid nitrogen. Finally, the microparticles were collected and stored in polyethylene closed bottles at 25 ± 2 °C. The composition of the different formulations (M1–M6) is shown in Table 1. For comparison physical mixtures of the same composition of the microparticles were also prepared (PM-M5 and PM-M6).

The microparticles were then directly compacted using a single punch tabletting machine (Korsch type EKO, Berlin, Germany) to round flat tablets (11 mm in diameter) with different characteristics according to the formulation: TAB-M2 to M4 = around 500 mg and 4.80 mm in thickness; TAB-M5 and TAB-M6 = around 320 mg and 2.48 mm in thickness. All the tablets contained 50 mg of AT. The standard applied compression force was 16 kN.

2.3. Characterization of samples

2.3.1. Morphological analysis

The morphological characteristics of microparticles were observed by scanning electron microscopy. The samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards) and examined using a SEM (Philips XL-30) at 25 kV accelerating voltage.

The size distribution of microparticles was evaluated by sieve analysis, using a vibrating shaker (Octagon Digital, Endecotts, London, UK) and 4 standard sieves (Scientific Instruments s.r.l., Milano, Italy) of 100, 355, 500 and 750 μm.

Finally, to investigate the gelling properties of microparticles and tablets in saliva (3 ml) at 37 °C, an optical microscope (Nikon SNZ 2T) connected through a camera (Panasonic GP KR 222) to an image acquisition system (CV 9000, FKV s.r.l. BG, Italy) was used.

2.3.2. Viscosity measurements of the molten fluids

The viscosity determination was performed on the formulations mentioned in Table 1. The measurements were carried out on about 10 g of molten mass, prepared as described in the preparation of the microparticles and placed in the small sample adapter of the vis-

cosimeter (Visco Star-R, Fungilab S.A., Barcelona, Spain), which was previously heated to the temperature set for the spraying process (70 °C). After some preliminary tests, the measuring elements, spindle number TR11 and TR8 (only for Gelucire® 50/13), were selected and the spindle rotating speed was stated at 50 rpm; the results are expressed as mPa s.

2.3.3. Determination of the drug content

A variable quantity of microparticles (containing theoretically 10 mg of AT) was accurately weighed and then added to 100 ml of pH 6.8 phosphate buffer. The sample was heated to 60 °C to melt the carrier and then shaken 1 h. Finally, the solution was filtered and the drug content was assayed spectrophotometrically (UV-Vis spectrophotometer mod. UV2, Unicam) at 274 nm. Each formulation was analyzed in triplicate and the mean ± SD was reported.

2.3.4. In vitro dissolution studies

In vitro dissolution tests of pure drug and of microparticles were performed using a paddle apparatus (Erweka DT800, Germany) rotating at 50 rpm. As dissolution medium, 900 ml of pH 6.8 phosphate buffer were used at a temperature of 37 °C. Different amount of samples containing about 14 mg of AT to assure sink conditions (C < 0.2 Cs) were poured into the vessel. The aqueous solution was filtered and continuously pumped (12.5 ml/min) to a flow cell in a spectrophotometer (UV2 Spectrometer, Unicam, Cambridge, UK). At specific time intervals, the amount of drug dissolved was analyzed at 274 nm.

The dissolution tests of tablets were performed using the flow-through cell (USP apparatus 4), with a 22.6 mm internal diameter and a filter preventing undissolved solids from escaping. A single 5 mm glass bead was placed at the bottom opening of cell and 1 g of 1 mm glass beads were added on top of it. Tablets were fixed with a cyanoacrylate adhesive to a metallic disk placed at the bottom of the flow-through cell to have only one side exposed to dissolution media for drug release. The medium used for the test (pH 6.8 buffer) was placed in a constant temperature water bath, maintained at 37 ± 0.5 °C. The medium was pumped (12.5 ml/min) to a flow cell in a spectrophotometer (UV2 Spectrometer, Unicam, Cambridge, UK). At specific time intervals, the amount of drug dissolved was analyzed at 274 nm.

The dissolution tests were performed at least in triplicate and the mean ± SD was reported.

2.3.5. Solubility measurements

Solubility measurements of pure AT, M5 and M6 microparticles and of the corresponding physical mixtures were performed in pH 6.8 buffer. The samples were magnetically stirred at 37 °C for 72 h, then the suspensions were filtered several times through 0.20 μm membrane filters and the filtrates were analyzed spectrophotometrically at 274.0 nm. The measurements were performed in triplicate and the mean ± SD was reported.

2.3.6. Evaluation of mucoadhesive strength

Mucoadhesive properties of the microparticles were evaluated using a procedure described in the literature to measure the bioadhesion ability of a single microsphere (Santos et al., 1999). In particular, a microtensiometer, usually applied to determine surface tension, was modified (Albertini et al., 2009) and the scheme is represented in Fig. 1. The substrate (porcine buccal mucosa), obtained from a local slaughter-house, was carefully separated from underlying tissue, washed with normal saline (NS) and cut into small pieces of adequate size. After rinsing, samples were frozen until required; immediately before the measurements, samples of mucosa were defrosted (bath of NS at 37 °C for 60 min), rinsed, stuck onto the bottom stage of the apparatus (thermostated at 37 °C) and hydrated with artificial saliva for 15 min. A single microsphere (>500 μm) is mounted on the top plate, fixed using cyanoacrylate glue using a needle to ensure the glue did not reach the upper side of the particle surface. The sample is then hydrated with artificial saliva for 2 min and the excess of fluid was carefully removed with absorbent paper; the bottom plate was then moved up until the minimum contact between the mucosa and the sample was established. After a 7-min rest, the top plate was moved up until the complete separation of the two surfaces. The force versus elongation was recorded (dyne/cm). This specific force (p) is then transformed into tensile force (T_F, dyne) using the following equation: $T_F = p \times 2\pi r$. The detachment force (D_F, dyne/cm^2) was then calculated assuming that the area of contact corresponds to the projected surface area of the sphere (πr^2), therefore $D_F = T_F/\pi r^2$. The results were then reported as mean values ± S.D (mN/cm^2) and at least 8 replicate measurements for each sample were performed.

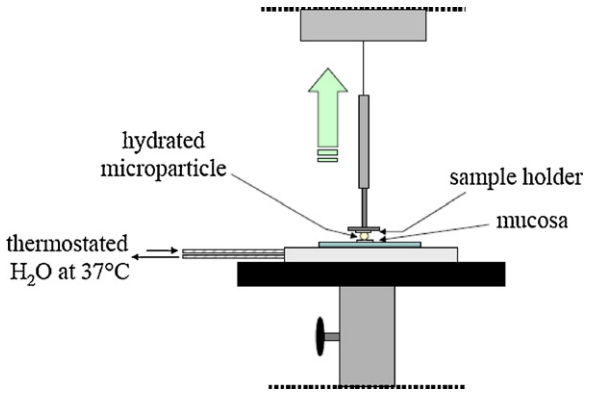

Fig. 1. Scheme of system used for the mucoadhesive tests (not in scale).

2.3.7. Differential scanning calorimetry (DSC) studies

DSC measurements were performed using a Perkin-Elmer DSC 6 (Perkin Elmer, Beaconsfield, UK). The calibration of the instrument was performed with indium and lead for the temperature, and with indium for the measurement of the enthalpy. The samples, weighting 6–10 mg, were placed into the DSC under a nitrogen flux (20 ml/min) and heated from 25 to 200 °C at a scanning rate of 10 °C/min. The microparticles (M5 and M6) were analyzed 48 h after the preparation. Each analysis was carried out in duplicated experiments. For comparison, the same procedure was followed for the raw materials and physical mixtures.

2.3.8. X-ray powder diffraction (XRD) analysis

Single components, physical mixtures (PM-M5 and PM-M6) and microspheres (M5 and M6) were studied by X-ray powder diffraction technique using a X'Pert PRO (PANanalytical, Almelo, NL) diffractometer with CuKα radiation (λ = 1.5418 Å) monochromatized by a secondary flat graphite crystal. The voltage was 40 kV and the current 40 mA. The scanning angle ranged from 5° to 45° of 2θ, steps were of 0.01° of 2θ and the counting time was of 2 s/step.

2.3.9. Fourier transform-infrared spectra (FT-IR) analysis

Studies of infrared spectra of raw materials, physical mixtures (PM-M5 and PM-M6) and microparticles (M5 and M6) were conducted with an IR spectrophotometer (Jasco FT-IR 200) using the KBr disc method. The samples were diluted with KBr and then compressed into a tablet, 10 mm in diameter and 2–3 mm in thickness, using a manual tablet presser (Perkin Elmer, Norwalk, USA) at 300 kg for 1 min.

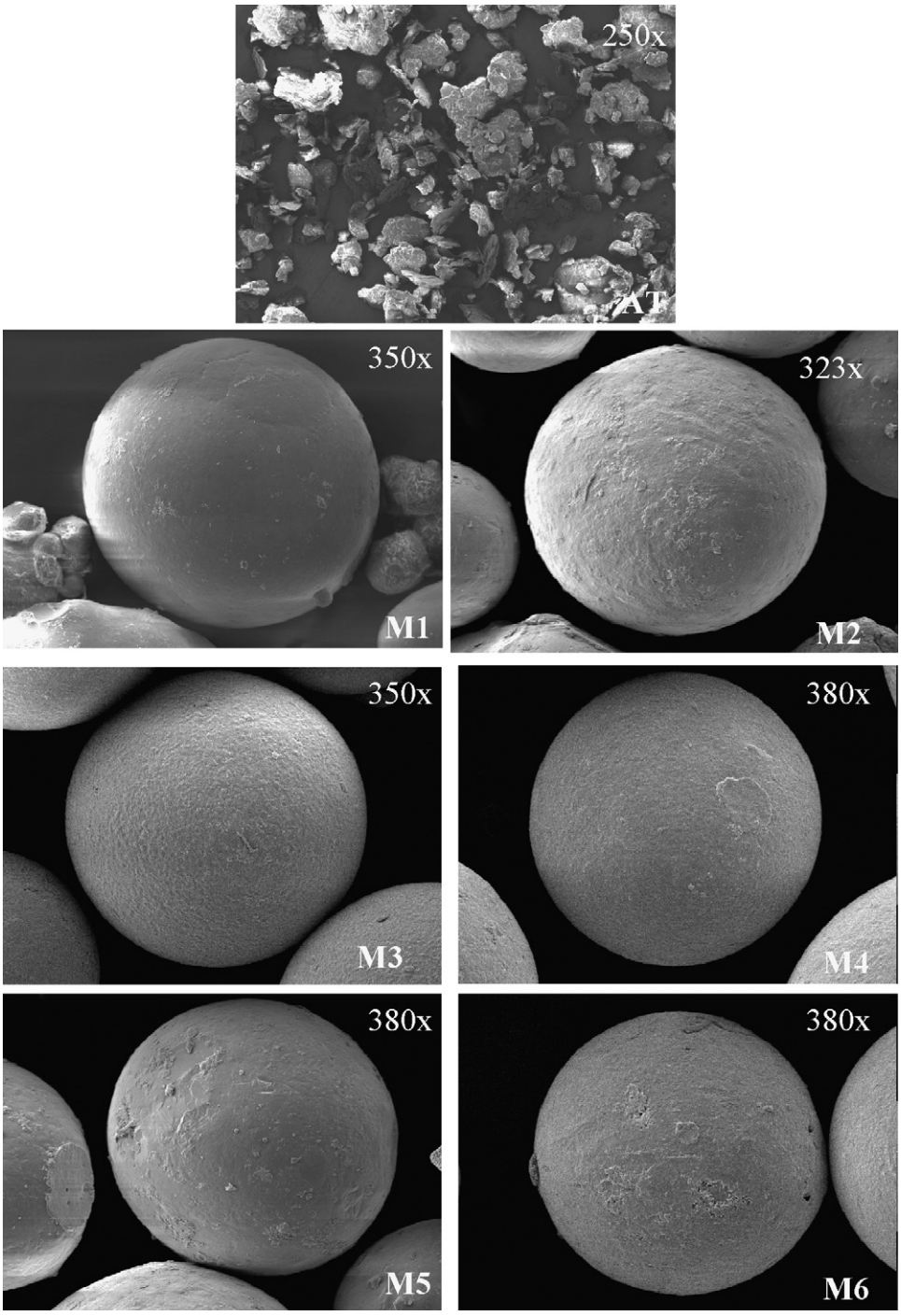

Fig. 2. SEM pictures of the pure AT and of microparticles (M1–M6) at different magnifications.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was selected to analyze the data of mucoadhesion and solubility; a post hoc comparison of the means of individual groups was performed by the Tukey–Kramer multiple-comparison test. A significance level of $P < 0.01$ denoted significance in all cases.

3. Results and discussion

3.1. Manufacturing and formulation's study

To investigate the potential of poloxamer 407-based systems for transbuccal drug delivery, the microparticles were prepared using the spray congealing technique. Spray congealing of a solution/suspension is a solvent free method of encapsulating actives into a solid solution/dispersion, which is then atomized into in a congealing chamber for the droplets solidification. The atomization of a molten poloxamer solution or suspension is not easy to achieve due to the high fluid viscosity which may affect the droplets formation; only one study is reported in literature concerning the preparation of poloxamer 188 microparticles by rotary spray congealing (Mackaplow et al., 2006). Poloxamer 407, due to its relatively low melting point (≅60 °C) appeared a suitable excipient for spray congealing process; nevertheless the high viscosity of its molten state could represent a limit in controlling the mean particle size. In this study the spray congealing system equipped with a very

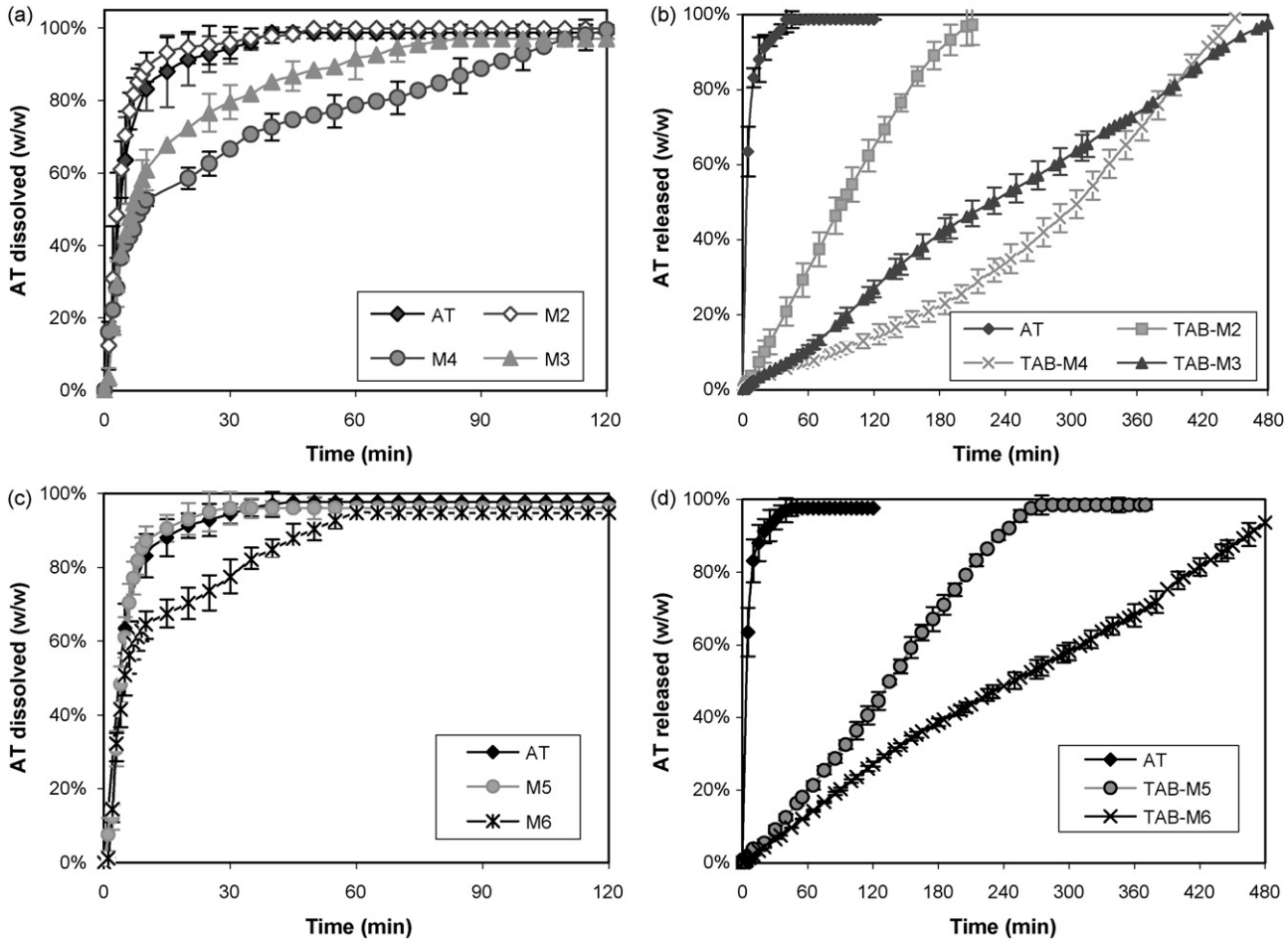

Fig. 3. In vitro dissolution profiles of (a) 10% w/w AT loaded-microparticles (M2–M4); (b) tablets made up of 10% w/w AT loaded-microparticles (TAB-M2 to M4); (c) 20% w/w AT loaded-microparticles (M5 and M6) and (d) tablets made up of 20% w/w AT loaded-microparticles (TAB-M5 and TAB-M6).

efficient nozzle (WPN), able to produce microparticles with high drug loading and good yields, upon the high viscosity of the melted mixture (Albertini et al., 2008), was employed. The composition of the obtained formulations (M1–M6) is summarized in Table 1.

AT is formed by irregular shape crystals (Fig. 2) with this particle size distribution: 6.5% w/w <50 μm; 72.7% w/w in the range 50–100 μm and 20.8% w/w in the range 100–355 μm. All the microparticles were non-aggregated, spherical and of smooth surface appearance, as shown in Fig. 2, even at higher AT amount (M5 and M6). The results showed that it was possible to nebulize the pure poloxamer solution (M1) using the described process parameters. The particle size analysis of M1 (Table 1) showed a unimodal size distribution with the prevalent particle size ranging from 100 to 355 μm. The introduction of 10% w/w of AT (M2) caused a bimodal size distribution, evidencing a great increase of the particle dimensions. This means that the suspension viscosity (Table 1) played an important role: the viscosity of M1 and M2 suspension was 5800 and 9300 mPa s, respectively. Therefore increasing the viscosity, the mean particle size increased as well. Furthermore, the drug loading of the fractions between 100 and 750 μm corresponded to the theoretical content. Fig. 3a displays the in vitro dissolution profiles of microspheres compared to the drug alone. The 90% (w/w) of AT dissolved in 30 min, indicating the high solubilization behaviour of both pure drug and M2.

In order to assess the potential of the microspheres to be used in matrix for buccal application, we evaluated the release of AT from tablets obtained from direct compression of the microspheres. The release profile of the tablets made up of M2 (TAB-M2) revealed that the drug release was characterized by a constant and slow release behaviour, being the 100% of AT released in 3 h, as shown in Fig. 3b. These results clearly indicated that the preparation of AT-loaded poloxamer mirospheres using spray congealing was promising, though it needed some improvements to decrease the width of the particle size distribution and to optimise the release behaviour. Moreover, Gelucire® 50/13 was added in the subsequent formulations as it might enhance the AT permeation across the buccal tissue. In particular, Gelucire® 50/13 was mixed with PF127 in two different ratios: 3:1 and 1:1 for M3 and M4, respectively. Actually the particle size distribution displayed more than 50% (w/w) of the microspheres in the range 100–355 μm with a great reduction of the particles in the higher fractions (Table 1). This behaviour was the consequence of the lower viscosity of M3 and M4 suspensions (Table 1), as pure Gelucire® 50/13 exhibited a viscosity of 50 mPa s. The drug loading resulted very close to the theoretical one. The in vitro dissolution data (Fig. 3a) demonstrated that the introduction of the co-carrier modified the dissolution behaviour of AT. The reason is due to lower hydrophilicity of Gelucire® 50/13 (HLB = 13) with respect to PF127 (HLB = 22) (Dumortier et al., 2006). The drug release from the tablets made up of M3 (TAB-M3) and M4 (TAB-M4) was strongly slower than that from TAB-M2, evidencing sustained release profiles. In particular, TAB-M3 exhibited the desired dissolution profile, since it released about the 100% of AT in 8 h with a dissolution performance suggesting a zero order release kinetic.

To improve the comfort of the tablets once applied to buccal mucosa, the following step consisted in the increase of the drug loading from 10 to 20% w/w keeping fixed the AT daily dosage

of 50 mg; consequently the tablet weight and thickness could decrease. Two formulations were processed: the first based only on PF127 (M5) and the second containing PF127 and Gelucire® 50/13 (M6) at the same weight ratio (3:1) of M3, as TAB-M3 showed the best in vitro release profile. However, as expected, the mean particle size distribution increased; in particular M5 showed a greater frequency in the fraction 500–750 μm with respect to M2, while M6 increased the amounts of particles in the higher fractions in comparison to M3 (Table 1). Anyway, despite the very high viscosity (M5 = 11,300 mPa s), the microparticles still had excellent spherical shape, while the drug loading was found lower than the theoretical (Table 1), probably due to the spare drug content uniformity between the fractions. M5 in vitro dissolution rate (Fig. 3c) remained similar to pure AT, while in M6 the presence of Gelucire® 50/13 slightly decreased the dissolution rate within 1 h. Then, both the tablets made up of M5 (TAB-M5) and of M6 (TAB-M6) evidenced a constant and controlled release with the 100% of AT released at 5 and 8 h, respectively (Fig. 3d).

As regard the solubility measurements, AT with a pK_a value of 9.6 (Vogelpoel et al., 2004) was expected to be sufficiently soluble in pH 6.8, although the high solubility of the copolymer could interfere and compete with AT solubilization. The results showed a significant difference between the solubility values of pure drug (AT = 2.32 ± 0.21 g/100 ml) and of microparticles (M5 = 2.47 ± 0.17 g/100 ml; M6 = 1.62 ± 0.09 mg/100 ml) compared to those of the corresponding physical mixtures (PM-M5 = 1.68 ± 0.11 g/100 ml; PM-M6 = 1.05 ± 0.23 g/100 ml). Therefore PF127 was able to promote the solubilization of AT dispersed within the microparticles, improving the wettability and micellization of AT. This behaviour is related to the better intimate contact between drug and excipient within the microparticles than the physical mixture.

3.2. Characterization of the microspheres

To fulfil the therapeutic requirements, beside sustained release profiles, buccal delivery systems should also present mucoadhesive characteristics. It is reported that bioadhesive force generally increases with the poloxamer gel strength, which depends on the temperature and polymer concentration (Dumortier et al., 2006).

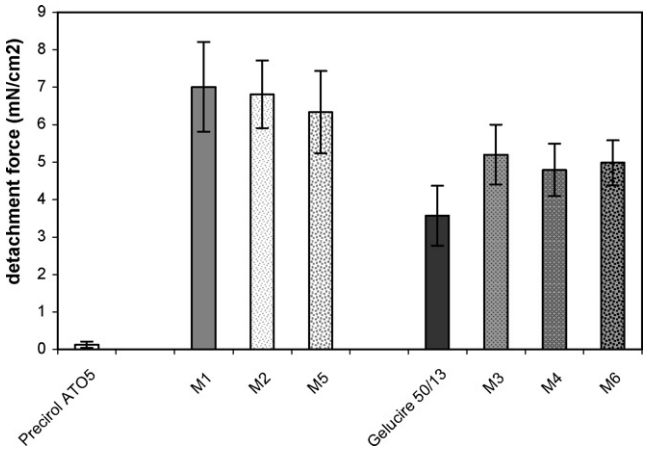

Fig. 4. Mucoadhesive strength of the microparticles.

Mucoadhesion in vitro experiments (Fig. 4) demonstrate that Precirol® ATO 5 (an atomized mixture of mono-, di- and triglycerides of stearic and palmitic acids, HLB = 2), the negative control, exhibited no affinity for the mucosal tissue. Relative to this negative control, the values of the detachment force were significantly greater for all microspheres. No significant differences were observed between M1, M2 and M5, suggesting that the amount of AT did not influence the detachment force. M3 formulation did not significantly differ from M1, M2 and M5, due to the low amount of Gelucire® 50/13, which shows a certain mucoadhesion ability. Conversely, the detachment force of M4 differed from the values of M1 and M2 and that of M6 was significantly different from the detachment force of M5. The results suggest that the gelling behaviour of the polxamer microparticles once hydrated at body temperature could ensure the adhesion of the samples to the tissue.

The major limit of the poloxamer 407 formulations (such as gels or in situ gelling solution) is generally due to the drastic in vivo dilution with the consequence loss of thermogelation and bioadhesion; whereas starting from a solid dosage form, this risk of being "washed away" due to the rapid dissolution is expected to be lower.

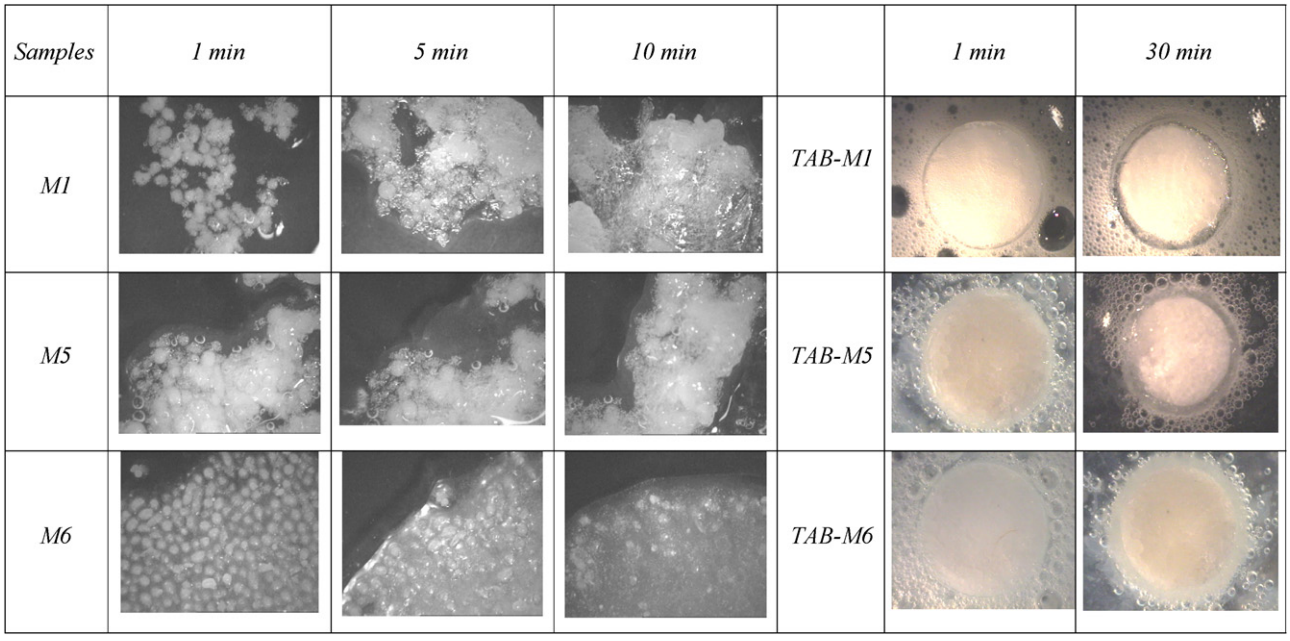

Fig. 5. Pictures of microspheres M1, M5 and M6 and of the corresponding tablets in saliva (T = 37 °C) at different times.

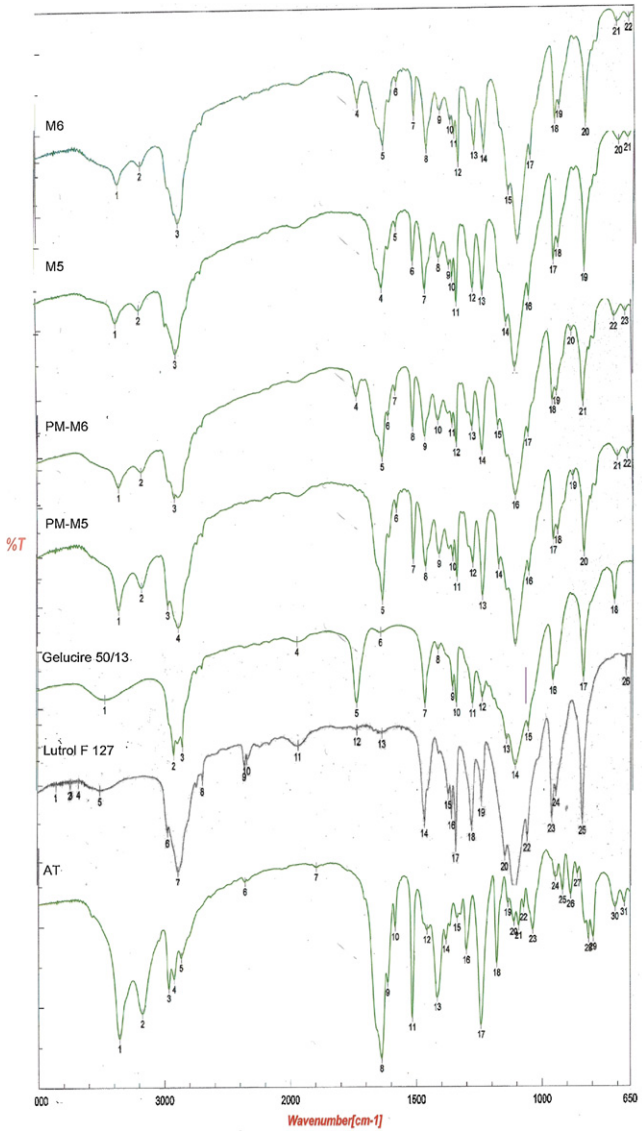

Fig. 6. FT-IR spectra of raw materials, physical mixtures and microparticles.

Fig. 5 shows the morphological changes occurring in the microspheres and tablets of formulation M1, M5 and M6 as a function of time after their addition in saliva at 37 °C. After few minutes both microparticles M1 and M5 underwent the gelling process and after 10 min a uniform gel with a firm texture was completely formed, whereas formulation M6 clearly evidenced a lower gel formation at the same time. As regards the tablets, their gelling ability confirmed the microparticle behaviour, even if a longer time is required for the gel layer formation, due to the lower surface area exposed to saliva. Therefore this qualitative evaluation suggested that the retention at the site of application of the microparticles (M5) and of the corresponding tablets (TAB-M5) and consequently their dissolution behaviour are closely related to the gelation properties of PF127 and probably these characteristics might enhance the extent of the drug permeation. However, it is important to underline that the times at which the pictures were taken do not correspond with the times of the dissolution process, due to the different hydrodynamic conditions.

In order to gather insight of possible interactions of AT with PF127 and Gelucire® 50/13 in the solid state during the manufacturing, physicochemical properties were then investigated. The infrared spectra of pure AT and of samples are reported in Fig. 6.

The IR spectrum of AT evidenced several characteristics vibrations: O–H/N–H stretching at 3356 cm^{-1}; aromatic C–H stretching at 3175 cm^{-1}; aliphatic C–H at 2964 and 2922 cm^{-1}; the primary amide bond at 1637 cm^{-1}; aromatic C=C stretching at 1613 and 1583 cm^{-1}; N–H bending at 1515 cm^{-1}; aromatic C–O stretching at 1242 cm^{-1}. The bands at 1180 and 1092 cm^{-1} were attributable to the C–O–C stretching vibration of the ether linkage, while the band 1036 cm^{-1} was caused by the C–N stretching vibration (Mundargi et al., 2007). Lutrol® F127 showed a large band between 3650 and 3300 cm^{-1} for free O–H stretching vibration; CH and CH_2 stretching vibrations between 2970 and 2885 cm^{-1}; and other bands at 1470 and 1120 cm^{-1} as singlets due to the vibration of the ether bond and at 963 cm^{-1} as doublet, also typical of PEGs. Gelucire® 50/13 showed the same peaks described for Lutrol® F127 and an additional band at 1737 cm^{-1} attributable to the C=O vibration. The spectra of the physical mixtures (PM-M5 and PM-M6) and of the microspheres (M5 and M6) appeared completely superimposable, showing the presence of all AT and excipients bands, thus suggesting the absence of interactions between AT and carriers in both systems. In fact, the possible interaction would occur between the amide hydrogen of AT and the OH of the polyglycol chain, inducing a shift of the N-H vibration with an extent that depended on the strength of the interaction. However, neither the simple mixing of drug and carrier, nor the incorporation of AT into microspheres modified the position of any of the above-mentioned peaks.

The DSC scans of pure AT, raw materials, physical mixtures (PM-M5 and PM-M6) and of microspheres (M5 and M6) are represented in Fig. 7a. The DSC curve of commercial AT showed only a single sharp endothermic peak at 156.27 ± 0.21 °C. PF127 exhibited the endothermic peak at 61.34 ± 0.39 °C. The DSC trace of pure Gelucire® 50/13 indicated the presence of the stable form I, exhibiting a very small pre-transition (around 32 °C) and a main transition at 48.84 ± 0.33 °C (Passerini et al., 2002a,b). The thermogram of the physical mixtures showed the endothermic peak of PF127 at 61.67 ± 0.59 °C (PM-M5) and of PF127 and Gelucire® 50/13 (PM-M6) at 61.18 ± 0.41 °C and 48.51 ± 0.48 °C, respectively, while the endotherm of AT was difficult to detect in both mixtures. It can be observed only a very small peak around 136 °C, probably corresponding to the melting of the drug. As regards microspheres, DSC curve of M5 was similar to that of PM-M5, even if the melting endotherm of PF127 shifted to 59.51 ± 0.28 °C and the small AT peak moved to 134 °C. The DSC curve of M6 exhibited the small pre-transition at 32.24 ± 0.30 °C (corresponding to the shoulder in the DSC curve of raw Gelucire® 50/13) and the main transition at 43.67 ± 0.51 °C (Passerini et al., 2002a,b). These results suggested that during the congealing phase, a certain amount of the Gelucire crystallized in the lowest melting point modification. The absence of AT peak in DSC curves of microparticles and the appearance of a very small peak at 20 °C lower then the AT melting point might suggested the partial transformation of crystalline AT into an amorphous form. However, the similarity of physical mixture and microspheres DSC behaviour also suggested the hypothesis of the dissolution of the drug into the molten carrier during DSC scan. To clarify this point, XRD analysis were performed on the samples.

The X-ray diffraction patterns of the samples are presented in Fig. 5b. Diffractogram of AT shows many characteristics sharp peaks; the main crystalline AT peaks occurred at 2θ of 6.07°, 9.30°, 17.45°, 18.00°, 18.97°, 20.32°, 22.01°, 23.55° and 24.23° (Caplar et al., 1984). Both Lutrol® F127 and Gelucire® 50/13 displayed two peaks at 2θ of 19.08° and 23.18°. All these peaks were detectable in both physical mixtures and microparticles, indicating the crystalline state of AT within the samples.

Therefore the results of the solid state characterization of microparticles indicated that the encapsulation of AT within poloxamer 407 by means of spray congealing did not induce chemical or physical modifications of the components, hence providing the sta-

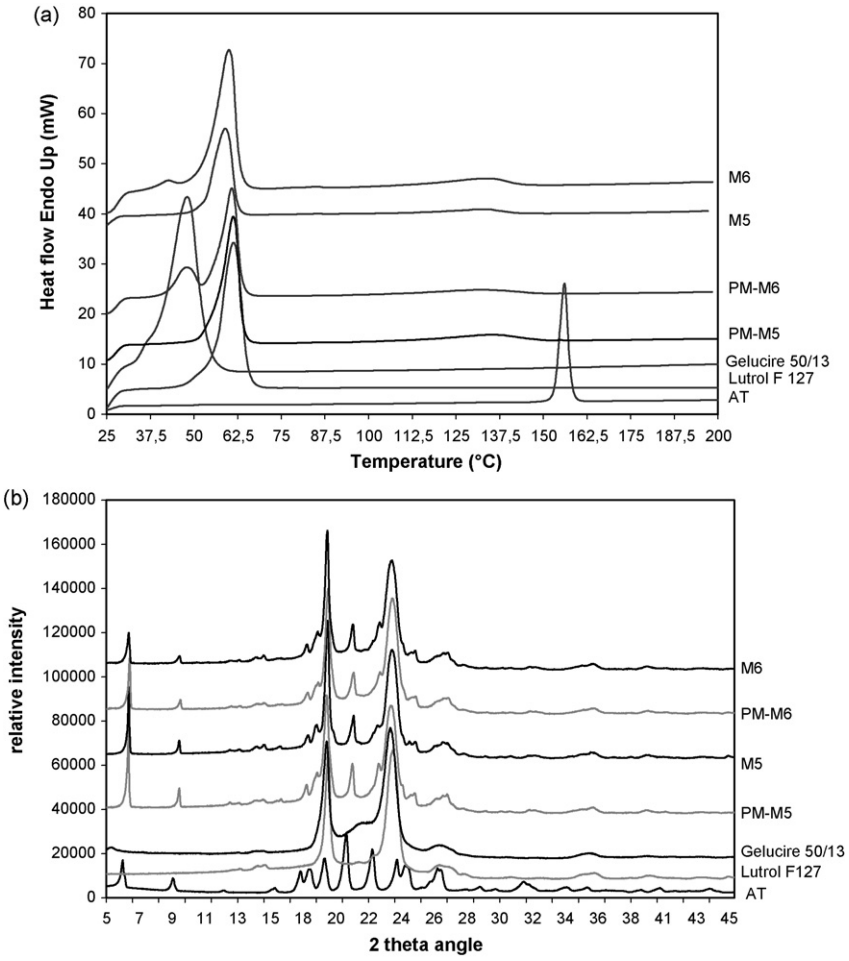

Fig. 7. Solid state analysis of raw materials and samples by means of DSC (a) and X-RD (b).

bility of the designed delivery systems. Actually, the FT-IR and XRD analysis performed after 6 months on M5 and M6 confirmed the previous results.

4. Conclusions

In light of the results, the spray congealing technology allowed the production of poloxamers 407-based microspheres loaded with 20% w/w of atenolol, used as model drug because of its poorly permeable characteristic through the buccal mucosa. This strategy gave way for developing a novel platform having mucoadhesive ability, high gelling behaviour and in vitro sustained release characteristics. Moreover, as the manufacturing technology is prevalently based on heating process, which can cause chemical or physical changes of the drug and/or of the excipients, considerable attention was given to the characterization of such systems to assess their stability over time. The results evidenced the absence of both interactions between components and confirm that the carriers and the drug existed as their original solid state in the microparticles.

To verify the potential usefulness of both microspheres and tablets in orotransmucosal delivery, the ex vivo and in vivo behaviour, until now rather unexploited, were assessed in the subsequent part of the study.

References

Ahuja, N., Katare, O.P., Singh, B., 2007. Studies on dissolution enhancement and mathematical modeling of drug release of a poorly water-soluble drug using water-soluble carriers. Eur. J. Pharm. Biopharm. 65, 26–38.

Albertini, B., Passerini, N., Pattarino, F., Rodriguez, L., 2008. New spray congealing atomizer for the microencapsulation of highly concentrated solid and liquid substances. Eur. J. Pharm. Biopharm. 69, 348–357.

Albertini, B., Passerini, N., Di Sabatino, M., Vitali, B., Brigidi, P., Rodriguez, L., 2009. Polymer–lipid based mucoadhesive microspheres prepared by spray congealing for the vaginal delivery of Econazole nitrate. Eur. J. Pharm. Sci. 36, 591–601.

Alexandridis, P., Hatton, T.A., 1995. Poly(ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. Colloids Surf. A: Physicochem. Eng. Aspects 96, 1–46.

Bromberg, L., Alakhov, V., 2003. Effects of polyether-modified poly(acrylic acid) microgels on doxorubicin transport in human intestinal epithelial Caco-2 cell layers. J. Control. Release 88, 11–22.

Brüsewitz, C., Schendler, A., Funke, A., Wagner, T., Lipp, R., 2007. Novel poloxamer-based nanoemulsions to enhance the intestinal absorption of active compounds. Int. J. Pharm. 329, 173–181.

Cafaggi, S., Leardi, R., Parodi, B., Caviglioli, G., Russo, E., Bignardi, G., 2005. Preparation and evaluation of a chitosan salt-poloxamer 407 based matrix for buccal drug delivery. J. Control. Release 102, 159–169.

Caplar, V., Mikotic-Mihun, Z., Hofman, H., Kuftinec, J., Kajfez, F., 1984. Atenolol. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 13. Academic Press, New York, pp. 1–25.

Desigaux, L., Gourden, C., Bello-Roufai, M., Richard, P., Oudrhiri, N., Lehen, P., Escande, D., Polard, H., Pitard, B., 2005. Nonionic amphiphilic block copolymers promote gene tranfer to the lung. Hum. Gen. Ther. 16, 821–829.

Dhiman, M., Yedurkar, P., Sawant, K.K., 2008. Formulation, characterization, and in vitro evaluation of bioadhesive gels containing 5-fluorouracil. Pharm. Dev. Technol. 13, 15–25.

Dumortier, G., Grossiord, J.L., Agnely, F., Chaumeil, J.C., 2006. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm. Res. 2, 2709–2728.

Escobar-Chávez, J.J., López-Cervantes, M., Naïk, A., Kalia, Y.N., Quintanar-Guerrero, D., Ganem-Quintanar, A., 2006. Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations. J. Pharm. Pharm. Sci. 9, 339–358.

Giannola, L.I., De Caro, V., Giandalia, G., Siragusa, M.G., Tripodo, C., Florena, A.M., Campisi, G., 2007. Release of naltrexone on buccal mucosa: permeation stud-

ies, histological aspects and matrix system design. Eur. J. Pharm. Biopharm. 67, 425–433.
Jones, D.S., Bruschi, M.L., de Freitas, O., Gremiao, M.P.D., Guimaraes Lara, E.H., Andrews, G.P., 2009. Rheological, mechanical and mucoadhesive properties of thermoresponsive, bioadhesive binary mixtures composed of poloxamers 407 and carbopol 974P designed as platforms for implantable drug delivery systems for use in the oral cavity. Int. J. Pharm. 372, 49–58.
Kockisch, S., Rees, G.D., Young, S.A., Tsibouklis, J., Smart, J.D., 2003. Polymeric microspheres for drug delivery to the oral cavity: an in vitro evaluation of mucoadhesive potential. J. Pharm. Sci. 92, 1614–1623.
Lin, H., Gebhardt, M., Bian, S., Kwon, K.A., Shim, C.K., Chung, S.J., Kim, D.D., 2007. Enhancing effect of surfactants on fexofenadine HCl transport across the human nasal epithelial cell monolayer. Int. J. Pharm. 330, 23–31.
Mackaplow, M.B., Zarraga, I.E., Morris, J.F., 2006. Rotary spray congealing of asuspenction: Effect of disk speed and dispersed particle properties. J. Microencapsul. 23, 793–809.
Monti, D., Burgalassi, S., Rossato, M.S., Albertini, B., Passerini, N., Rodriguez, L., Chetoni, P., Poloxamer 407 microspheres for orotransmucosal drug delivery. Part II: in vitro and in vivo evaluation, Int. J. Pharm., submitted for publication.
Morishita, M., Barichello, J.M., Takayama, K., Chiba, Y., Tokiwa, S., Nagai, T., 2001. Pluronic F-127 gels incorporating highly purified unsaturated fatty acids for buccal delivery of insulin. Int. J. Pharm. 212, 289–293.
Mundargi, R.C., Patil, S.A., Agnihotri, S.A., Aminabhavi, T.M., 2007. Evaluation and controlled release characteristics of modified xanthan film for transdermal delivery of atenolol. Drug Dev. Ind. Pharm. 33, 79–90.
Oh, K.T., Bronich, T.K., Kabanov, A.V., 2004. Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic Pluronic block copolymers. J. Control. Release 94, 411–422.
Passerini, N., Albertini, B., Gonzalez-Rodriguez, M.L., Cavallari, C., Rodriguez, L., 2002a. Preparation and characterization of ibuprofen-polaxamer 188 granules obtained by melt granulation. Eur. J. Pharm. Sci. 15, 71–78.
Passerini, N., Perissutti, B., Moneghini, M., Voinovich, D., Albertini, B., Cavallari, C., Rodriguez, L., 2002b. Characterization of carbamazepine-gelucire 50/13 microparticles prepared by a spray-congealing process using ultrasound. J. Pharm. Sci. 91, 699–707.
Passerini, N., Qi, S., Albertini, B., Grassi, M., Rodriguez, L., Craig, D.Q.M., 2010. Solid lipid microparticles produced by spray congealing: influence of the atomizer on microparticle characteristics and mathematical modeling of the drug release. J. Pharm. Sci. 99, 916–931.
Santos, C.A., Jacob, J.S., Hertzog, B.A., Freedman, B.D., Press, D.L., Harnpicharnchai, P., Mathiowitz, E., 1999. Correlation of two bioadhesion assays: the everted sac technique and the CAHN microbalance. J. Control. Release 61, 113–122.
Senel, S., Hincal, A.A., 2001. Drug permeation enhancement via buccal route: possibilities and limitations. J. Control. Release 72, 133–144.
Shin, S.C., Kim, J.Y., 2000. Enhanced permeation of triamcinolone acetonide through the buccal mucosa. Eur. J. Pharm. Biopharm. 50, 217–220.
Vogelpoel, H., Welink, J., Amidon, G.L., Junginger, H.E., Midha, K.K., Möller, H., Olling, M., Shah, V.P., Barends, D.M., 2004. Biowaiver monographs for immediate release solid oral dosage forms based on biopharmaceutics classification system (BCS) literature data: verapamil hydrochloride, propranolol hydrochloride, and atenolol. J. Pharm. Sci. 93, 1945–5196.