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Spray-dried Amioca® starch/Carbopol® 974P mixtures as buccal bioadhesive carriers

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Abstract

In the present study, spray-dried Amioca® starch/Carbopol® 974P mixtures were evaluated as potential buccal bioadhesive tablets. Carbopol® (C 974P) concentrations from 5 to 75% were tested. All spray-dried mixtures showed a comparable or better bioadhesive capacity compared to a reference formulation (DDWM/C 974P 95/5). The bioadhesive capacities of Amioca®/Carbopol® 974P mixtures were improved by spray-drying. All spray-dried mixtures showed significantly higher work of adhesion values compared to their equivalent physical mixtures. The influence of Carbopol® concentration on the in vivo adhesion time of placebo tablets and in vitro miconazole nitrate release was tested. The ratio Amioca®/C 974P 70/30 showed the longest in vivo adhesion time (24.5 ± 8.5 h). Lower and higher C 974P concentrations had a shorter in vivo adhesion time. The mixtures containing between 15 and 30% C 974P could all sustain the in vitro miconazole nitrate release over 20h. Again, lower and higher C 974P concentrations showed a faster in vitro miconazole release. The drug loading capacity of a spray-dried mixture containing 20% C 974P was investigated in vivo in dogs using testosterone as model drug. The spray-dried mixture could be loaded with 60% drug without loosing its in vivo bioadhesive and pharmacokinetic properties.

Keywords: Spray-dried Amioca®/Carbopol® 974P mixtures; Buccal; Bioadhesion; In vitro/in vivo bioavailability

1. Introduction

In last years considerable attention has been focused on the development of novel bioadhesive polymers or platforms. Bioadhesion, in particular mucoadhesion, has been of interest for the development of controlled drug delivery systems to improve buccal, nasal, and oral administration of drugs. Typical polymers that have been used as mucoadhesive drug carriers are poly(acrylic acid) (PAA) (Thermes et al., 1992; Bouckaert and Remon, 1993; Voorspoels et al., 1996; Singla et al., 2000), poly(methacrylic acid) (Quintanar-Guerrero et al., 2001), cellulose derivatives (Suzuki and Makino, 1999), poly(ethylene oxide)

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(Tiwari et al., 1999; Di Colo et al., 2001), lectin (Lehr, 2000) and chitosan (Lehr et al., 1992). Of these, PAA and its crosslinked commercial forms, Carbopol® and Polycarbophil, exhibit strong mucoadhesive properties (Chun et al., 2002). However, their severe mucosal irritation potential limit their use as mucoadhesive drug carriers (Bottenberg et al., 1991; Adriaens et al., 2003). Many attempts have been undertaken to improve the mucoadhesive properties of polymers by preparing copolymers, polymer-conjugates or interpolymer complexes. Examples are starch-*g*-poly(acrylic acid) copolymers (Ameye et al., 2002), polycarbophilcysteine (Bernkop-Schnürch et al., 1999), chitosan-thioglycolic acid (Kast and Bernkop-Schnürch, 2001), carboxymethylcellulose-cysteamine and polycarbophil-cysteamine conjugates (Kast and Bernkop-Schnürch, 2002), PAA/chitosan polymer complexes (Ahn et al., 2002) and poly(vinyl pyrrolidone)/PAA interpolymer complexes (Chun et al., 2002). In the present study, a new bioadhesive platform was developed by spray-drying an aqueous mixture of Amioca® starch, an amylopectin corn starch, and Carbopol® 974P and was evaluated as a buccal bioadhesive tablet delivery system.

The buccal administration of drugs has gained interest as the oral cavity forms a convenient and easily accessible site for local and systemic drug delivery. Systemic drug administration via the buccal route has several advantages over peroral delivery: pre-systemic metabolism in the gastro-intestinal tract and hepatic first-pass elimination are avoided (Hoogstraate and Wertz, 1998). In a previous study, a non-irritating buccal bioadhesive drug carrier was developed containing a physical mixture of 5% crosslinked poly(acrylic acid), Carbopol® 974P, with a thermally modified starch (Bouckaert and Remon, 1993). This buccal drug carrier, used as reference formulation, has been shown to be effective for local (Bouckaert et al., 1993a) as well as for systemic drug delivery (Voorspoels et al., 1996). However, this carrier was limited in drug load (Voorspoels et al., 1996) and poly(acrylic acid) content (Bottenberg et al., 1991; Callens et al., 2001; Adriaens et al., 2003). Drug loads over 30% resulted in a significant decrease of bioadhesive capacities and a Carbopol® content above 10% induced severe mucosal irritation.

The spray-dried Amioca® starch/Carbopol® 974P mixtures of the present study were evaluated as potential buccal bioadhesive tablets. The objective was to investigate the influence of spray-drying compared to physical mixing on the bioadhesive capacities of the mixtures. Different ratios Amioca®/Carbopol® were tested on their ex vivo bioadhesive strength and compared with their equivalent physical mixtures. The influence of Carbopol® content on the in vitro drug release and in vivo adhesion time was compared. The drug loading capacity of a selected spray-dried mixture was investigated in vivo in dogs using testosterone as the model drug.

2. Materials and methods

2.1. Materials

Testosterone was purchased from Diosynth (Oss, The Netherlands). Miconazole nitrate was obtained from Janssen Pharmaceutica, Beerse, Belgium. Amioca® starch was from National Starch and Chemical Company, Bridgewater, New Jersey, USA. Carbopol® 974P (C 974P) was supplied by BF Goodrich (Cleveland, Ohio, USA). Drum Dried Waxy Maize (DDWM) was supplied by Cerestar (Vilvoorde, Belgium). Sodium stearyl fumarate (NaSF) was given by Edward Mendell Co. Inc. (New York, USA). Econazole was purchased from Sigma (Bornem, Belgium), methanol HPLC-S grade was purchased from Biosolve BV (Valkenswaard, The Netherlands) and tetrahydrofuran (THF) was purchased from BDH, Laboratory Supplies, Poole, UK. All other chemicals used were at least of analytical grade.

2.2. Preparation of the spray-dried Amioca®/Carbopol® 974P mixtures (SD)

The spray-dried Amioca® starch/Carbopol® 974P mixtures were prepared by National Starch and Chemical Company, Bridgewater, New Jersey, USA. First, Amioca® starch, an amylopectin corn starch, was pregelatinised by jet cooking in a custom made jet cooker. Temperature was set at 138 °C, at a pressure of 3.1–3.2 bar and a flow rate of 1.2–1.5 l/min. After jet cooking the obtained aqueous starch dispersion was mixed with an aqueous dispersion of Carbopol® 974P (C 974P), a highly cross-linked poly(acrylic acid). The aqueous Amioca® starch/C 974P mixture was

spray-dried using a Bowen spray-dryer model BE-1393 (Arnold Equipment Company, Cleveland, OH, USA) to obtain a powder. Different ratios were spray-dried ranging from 5 to 75% (w/w) Carbopol® 974P (Ameye et al., 2003).

2.3. Preparation of the Amioca®/Carbopol® 974P physical mixtures (PM)

The Amioca®/C 974P physical mixtures were prepared by blending granular Amioca® starch with C 974P in the ratios 25/75, 75/25, 90/10 and 95/5 (w/w).

2.4. Production of the tablets

Bioadhesion measurements were performed on 100 mg tablets. For the tablet production the powders were mixed with sodium stearyl fumarate (1%; w/w), as a lubricant and compressed on a Korsch compression machine (Type EKO, Berlin, Germany) equipped with 7 mm flat punches, at a pressure of 9.8 kN.

For the evaluation of the in vivo adhesion time placebo tablets (100 mg/7 mm) were used, prepared as mentioned above.

The tablets used in the in vitro dissolution study contained miconazole nitrate. For the tablet production the spray-dried powder was firstly mixed with miconazole nitrate (10 mg), next the lubricant (1% sodium stearyl fumarate) was added and mixed again. The tablets were compressed as described above with a tablet weight of 100 mg and diameter of 7 mm.

To produce the tablets for the in vivo drug loading study the powder was firstly mixed with micronised testosterone (60 mg), next the sodium stearyl fumarate (1%) was added and mixed again. The weight and the diameter of the tablets were 100 mg/7 mm (60% w/w drug concentration) and 200 mg/9 mm (30% w/w drug concentration) and the compression force was 9.8 and 14.7 kN, respectively.

2.5. Ex vivo determination of bioadhesion

The bioadhesion of the tablets was evaluated according to a previously described method (Bouckaert et al., 1993b). The adhesion force and the work of adhesion were determined as the height and the area under the curve of the force versus extension diagram. The apparatus consisted of a tensile testing machine (type L1000R, Lloyd Instruments, Segenworth, Fareham, UK), equipped with a 20 N load cell. Porcine gingiva was obtained at the slaughterhouse where they were excised directly after slaughtering. The mucosa were stored at −20 °C in isotonic buffered saline pH 7.4 (2.38 g $Na_2HPO_4 \cdot H_2O$, 0.19 g KH_2PO_4, 8.0 g NaCl made up to 1000 ml with demineralrzed water).

The porcine gingival tissue was attached with cyanoacrylate glue (Bison International, The Netherlands) to a lower Teflon support, while the tablet was attached to an upper aluminium punch. After hydrating the mucosa with 15 μl of the isotonic phosphate buffered saline, the tablet was fixed on the mucosa applying a force of 0.5 N for 5 min. After the initial contact, the thermostatic beaker (37 °C) was filled with 125 ml isotonic buffered saline pH 7.4 at 37 °C. Next, the tablet and mucosa were pulled apart at a speed of 5 mm min^{-1} until a complete rupture of the tablet-mucosa bond was obtained. The bioadhesion results were compared to a reference formulation, a physical mixture of 5% C 974P, 94% DDWM and 1% NaSF (Voorspoels et al., 1996).

Statistical analysis was performed using a one-way analysis of variance. The data were transformed to their logarithm and were tested for normal distribution with a Kolmogorov–Smirnov test. The homogeneity of variances was tested with the Levene's test. To compare the different multifunctional polymers to a reference formulation, a Bonferroni test with $p < 0.05$ as significance level was used. To compare the different multifunctional polymers to each other, a multiple comparison was performed using a Scheffé test with $p < 0.05$ as significance level. The computer program SPSS version 10.0 was used for the statistical analyses.

2.6. In vivo adhesion time study

The in vivo adhesion time of each formulation was evaluated in seven castrated male dogs (weight 29.07 ± 3.25 kg). The dogs were conscious during the whole test period. One tablet was placed on the gingiva above the right upper canine (Bouckaert et al., 1993b). The in vivo adhesion time was followed visually. The in vivo adhesion time was defined as the time until loss or complete erosion of the bioadhesive tablet. The approval of The Ethics Committee was obtained.

2.7. In vitro drug release study (USP III)

To evaluate the in vitro drug release rate from the bioadhesive formulations based on the different spray-dried Amioca®/C 974P mixtures, miconazole nitrate was used as a model drug (Bouckaert and Remon, 1993; Bouckaert et al., 1993a). The dissolution tests were performed in an automatic reciprocating cylinder dissolution apparatus USP III (US Pharmacopeia XXIV, 2000) (VanKel BioDis III Release Rate tester, Cary, NC, USA). The dip speed was set at 21 dips/min and the temperature at 37 °C. The dissolution medium (250 ml) was a 0.1N HCl solution containing 0.5% hydroxypropyl-β-cyclodextrine (Janssen, Beerse, Belgium) in demineralised water (De Spiegeleer et al., 2001).

Quantitative analysis of miconazole nitrate in the dissolution samples was performed with a validated HPLC method with UV-detection using econazole as the internal standard (De Spiegeleer et al., 2001). Analysis was performed with a HPLC system consisting of a gradient HPLC pump (type L-7100, Merck-Hitachi, Darmstadt, Germany), a solvent degasser (type L-7612, Merck-Hitachi, Darmstadt, Germany), an autosampler (type L-7200, Merck-Hitachi, Darmstadt, Germany) equipped with a Rheodyne injector and an injection loop of 100 μl (Rheodyne, California, USA), a column oven (type L-7360, Merck-Hitachi, Darmstadt, Germany), a UV detector (type L-7400, Merck-Hitachi, Darmstadt, Germany) and a software interface (type D-7000, Merck-Hitachi, Darmstadt, Germany). Data were calculated with the software package 'HPLC System Manager' (Merck-Hitachi, Darmstadt, Germany). The column was a Lichrospher® 100 RP-18 column (125 mm × 4 mm) equipped with a Lichrospher® 100 RP-18 guard column (4 mm × 4 mm) (Merck, Darmstadt, Germany). The mobile phase, used as an isocratic eluent, consisted of 75% (v/v) methanol, 20% (v/v) sodium acetate buffer (2.5 mM, pH 5.0) containing 5 mM triethylamine and 5% (v/v) tetrahydrofuran. The eluate was monitored at 220 nm. The retention time of the econazole and miconazole nitrate peak was 4.5 and 7.0 min, respectively, at a flow rate of 1.0 ml/min. The analysis was performed at 25 °C.

Calibration samples were prepared in dissolution medium to obtain a standard curve ranging from 2.5 to 50.0 μg/ml. To 1.0 ml of dissolution sample, 1.0 ml of a methanolic solution of econazole (0.01 mg/ml) was added as internal standard, mixed and centrifuged at 2578 × *g* for 10 min (Tehtnica® Centric 322 A, Novolab, Belgium). Hundred microlitres of the supernatant was injected onto the HPLC column.

The exponential equation $M_t/M_\infty = kt^n$ which describes the Fickian and non-Fickian release behaviour of swellable systems that not swell more than 25% of its original volume, was used to evaluate the drug release mechanism (Ritger and Peppas, 1987). M_t is the amount released at time t, M_∞ is the overall released amount, k a release constant of the nth order. The exponent n gives information about the release mechanism, $n = 0.5$ indicates Fickian drug diffusion, while $n = 1.0$ for drug release controlled by polymer erosion.

The time for 50% drug release, $t_{50\%}$, was calculated from the mean ($n = 6$) miconazole nitrate release profiles.

2.8. In vivo study

The bioavailability of formulations containing 30 and 60% testosterone was determined according to a previously described protocol (Voorspoels et al., 1996). The results were compared with two reference formulations (DDWM/C 974P; 95/5) containing the same amounts of testosterone [4]. 60 mg testosterone was incorporated in a 100 mg (60% drug concentration) or a 200 mg tablet (30% drug concentration). The formulations were tested in 6 castrated male dogs (weight 30.0 ± 2.5 kg). The dogs were conscious and fasted from 12 h before until the end of the experiment. Drinking water was available at libitum. One tablet was placed on the gingiva above the right upper canine and blood samples were collected before the administration and 0.5, 1, 2, 4, 8, 12, 16 and 24 h after the administration in heparinized tubes. The blood samples were centrifuged at 2000 × *g* and the plasma was kept at −20 °C until analysis. A time interval of at least 1 week was respected between each administration. To calculate the absolute bioavailability, 60 mg testosterone was administered intravenously to each dog. Blood samples were taken at 0, 2, 5, 10,20, 30 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 h after administration. The approval of The Ethics Committee was obtained.

The testosterone plasma concentrations were determined by chemiluminescent immunoassay (Immulite®

Total Testosterone, Diagnostic Products Corporation, Los Angeles, CA, USA). The analytical sensitivity was 10 ng/dl. The antibody was highly specific for testosterone, the cross reactivity was 0.79% for androstenedion and 9.1% for dihydrotestosterone. The interassay coefficient of variation varied between 6.5 and 16% depending on the concentration level.

The absolute bioavailability was calculated using the Kinbes® software (Proost and Meijer, 1992). The $T > 3$ ng/ml value (time during which the plasma testosterone concentration was above 3 ng/ml) was calculated from the individual graphs. The in vivo adhesion time of the bioadhesive tablet was determined visually. Adhesion was considered to be present until the complete erosion of the tablet. Statistical analysis was performed on the absolute bioavailability and $T > 3$ ng/ml values. As Pearson correlation coefficients showed that the data for absolute bioavailability and $T > 3$ ng/ml were independent ($p > 0.05$) of the subject (dog), statistically significant differences were determined using a one-way ANOVA post hoc Scheffé test with $p < 0.05$ as significance level. The data were tested for normal distribution with a Kolmogorov–Smirnov test. The homogeneity of variances was tested with the Levene's test. The computer program SPSS version 10.0 was used for the statistical analyses.

3. Results and discussion

3.1. Ex vivo bioadhesion measurements

The ex vivo bioadhesion values of the spray-dried Amioca®/C 974P mixtures and the reference formulation are shown in Fig. 1. All spray-dried mixtures showed a comparable or better bioadhesive capacity compared to the reference formulation. Increasing the C 974P concentration up to 15% (w/w) resulted in increasing bioadhesion values. The spray-dried Amioca®/C 974P mixtures containing 15% or more Carbopol® showed significantly higher work of adhesion values compared to the reference formulation (Bonferroni, $p < 0.05$). Increasing the C 974P concentration above 15 up to 75% did not result in significantly higher bioadhesion values (Scheffé, $p < 0.05$). This observation is in accordance with Bouckaert and Remon (1993) and Park and Munday (2002). The bioadhesive properties of the 95/5 and 90/10 mixtures were not significantly different compared to the reference tablet (Bonferroni, $p < 0.05$).

Mucoadhesion is, after the formation of an intimate contact between mucoadhesive and mucus, dependent on the hydration, swelling and interpenetration of the mucoadhesive polymers with the mucus macromolecules becoming so physically entangled. Secondly, polymer and mucus interact with each other via

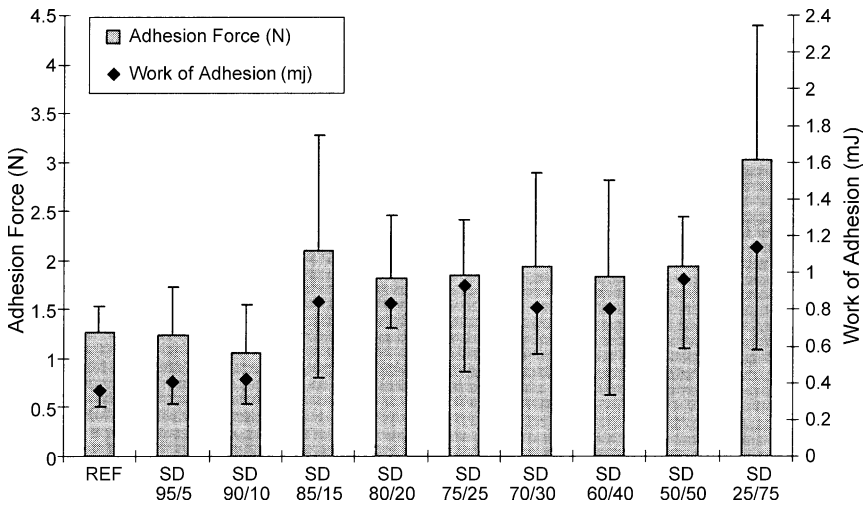

Fig. 1. Ex vivo bioadhesion results for the spray-dried Amioca®/Carbopol® 974P mixtures (SD) compared to the reference formulation (Ref) ($n = 10$, mean ± S.D.).

non-covalent bonds such as hydrogen bonds (Duchêne et al., 1988). Work of adhesion is suggested to be dependent on the interpenetration of the Carbopol® chains into the mucus, while the adhesion force is considered to be dependent on the formation of hydrogen bonds between the functional groups of the bioadhesive and the mucus (Park and Munday, 2002). Increasing the Carbopol® concentration and thus increasing the amount of functional groups resulted up to 15% C 974P in better bioadhesive properties, but over 15% more functional groups did not significantly increase bioadhesion.

Scanning electron microscopy and solid state NMR spectroscopy and relaxometry analysis showed that by spray-drying Amioca® starch/Carbopol® 974P mixtures, Carbopol® films are formed around the starch granules (Ameye et al., Polymer, submitted). From a molecular point of view, film formation can explain the performance of the different spray-dried Amioca®/Carbopol® 974P mixtures in the ex vivo bioadhesion test. The significantly increased adhesion properties, starting from a Carbopol® concentration of 15% (SD 85/15), can probably be explained by an optimal balance between Carbopol® coated and non-coated surface areas on the Amioca® granules. Amioca® granules are thought to be completely surrounded by Carbopol® in the spray-dried mixtures containing 25% or more C 974P. Increasing the C 974P concentration above 15% and thus complete C 974P coating of the Amioca® granules did not significantly increase the bioadhesive properties of the spray-dried mixtures. The slightly, but not significantly, increased bioadhesion values for the SD 25/75 mixture can be explained by individual Carbopol® 974P nanoparticles in addition to an increased film thickness. Film thickness is probably also increased with increasing C 974P contents in the mixtures SD 85/15–SD 50/50, but a complete coating or an increased film thickness had no additional positive effect on bioadhesion.

Fig. 2 shows the bioadhesive properties of the spray-dried mixtures compared to their equivalent physical mixtures. From the graph it is clear that the bioadhesive capacities of Amioca®/Carbopol® 974P mixtures were improved by spray-drying. All spray-dried mixtures showed significantly higher work of adhesion values compared to their equivalent physical mixtures (Scheffé, $p < 0.05$). The 5% C 974P physical mixture (PM 95/5) showed significantly lower adhesion values than the reference, also containing 5% Carbopol® (Bonferroni, $p < 0.05$). This can be explained by the difference in used starch. DDWM, used in the reference formulation, and Amioca®, used in the physical mixtures, are both waxy corn starches, but DDWM is a pregelatinised starch, while the Amioca® was a granular starch. In water, pregelatinised starches will hydrate and swell faster than granular starches. As hydration and swelling of the polymer is an important step in the formation of bioadhesive bonds between

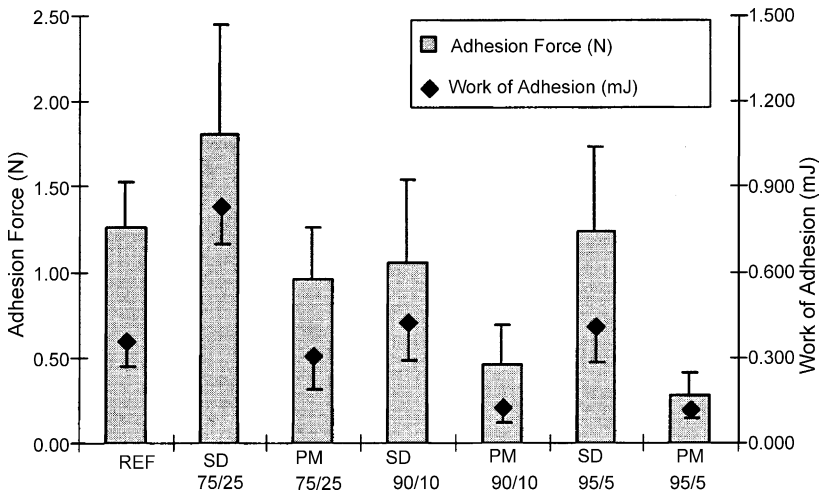

Fig. 2. Ex vivo bioadhesion results for the spray-dried Amioca®/Carbopol® 974P mixtures (SD) compared to their equivalent physical mixtures (PM) and the reference formulation ($n = 10$, mean ± S.D.).

mucus/mucosa and polymer, it could be expected that DDWM showed better bioadhesive properties than the granular Amioca® (Bouckaert, 1994). It should be noticed that the Amioca® in the spray-dried mixtures was pregelatinised by jet cooking. After pregelatinisation not only the bioadhesive properties are increased, but the starch can also be easier dispersed in water, which was required before spray-drying.

3.2. *In vivo adhesion time and in vitro dissolution tests*

Fig. 3 gives an overview of the in vivo adhesion time in dogs of placebo buccal bioadhesive tablets based on the different spray-dried mixtures. Up to 30% C 974P the in vivo adhesion time increased with increasing C 974P amounts in the spray-dried mixtures. Over 30% C 974P content the in vivo adhesion time decreased with increasing C 974P concentrations in the spray-dried mixtures. The ratio Amioca®/C 974P 70/30 showed the longest in vivo adhesion time (24.5 ± 8.5 h) and is apparently the optimal ratio of Amioca® starch and Carbopol® 974P in terms of adhesion time. Although the SD 25/75 mixtures showed the highest ex vivo bioadhesive properties, it did not show the longest in vivo adhesion time. The ex vivo bioadhesion test can be used to evaluate the intrinsic bioadhesive properties and allows to compare different bioadhesive polymers or formulations, but the test seemed unable to predict the in vivo adhesion time probably because the tablet is not subjected to frictional forces and erosion. The SD 25/75 formulation has high intrinsic bioadhesive capacities, but the polymer matrix eroded relatively fastly in vivo. This is in accordance with a previous study showing that a higher Carbopol® concentration did not result in a longer in vivo adhesion time (Bouckaert, 1994).

The in vitro drug release rate was evaluated by incorporating 10 mg miconazole nitrate as a marker. Miconazole nitrate is a poorly water soluble antifungal drug. It is frequently used in local oral drug delivery systems such as oral gels or bioadhesive buccal tablets (Bouckaert and Remon, 1993; Bouckaert et al., 1993a,b; De Spiegeleer et al., 2001; Nafee et al., 2003). Testosterone, a lipophilic molecule which is known to be systemically absorbed over the buccal mucosa, was used to investigate the in vivo bioavailability and drug loading capacity of the spray-dried mixtures (Voorspoels et al., 1996).

The in vitro dissolution profiles are shown in Fig. 4. The spray-dried mixtures containing between 15 and 30% C 974P could all sustain the drug release over 20 h ($t_{50\%} = 8$ h). As well lower as higher C 974P

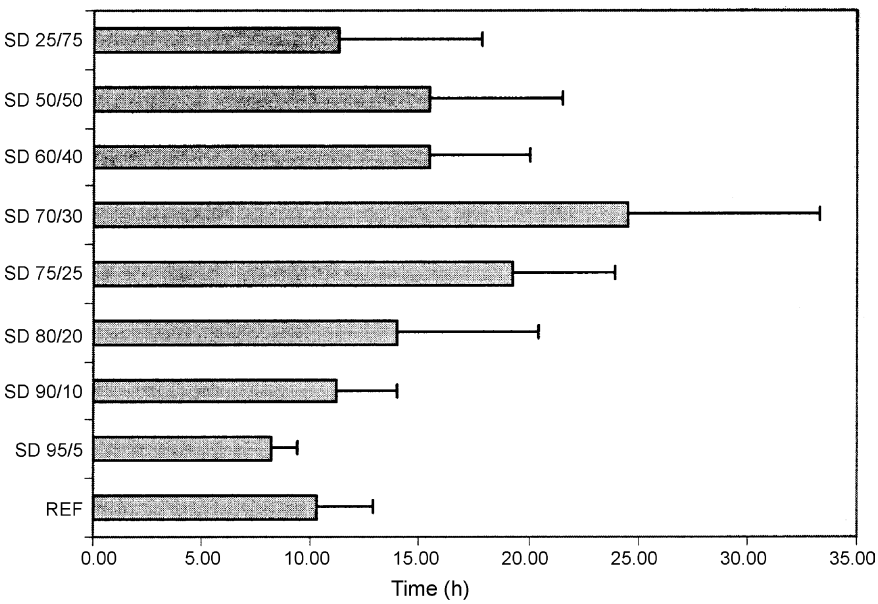

Fig. 3. In vivo adhesion time for the spray-dried Amioca®/Carbopol® 974P mixtures (SD) and the reference formulation ($n = 7$, mean ± S.D.).

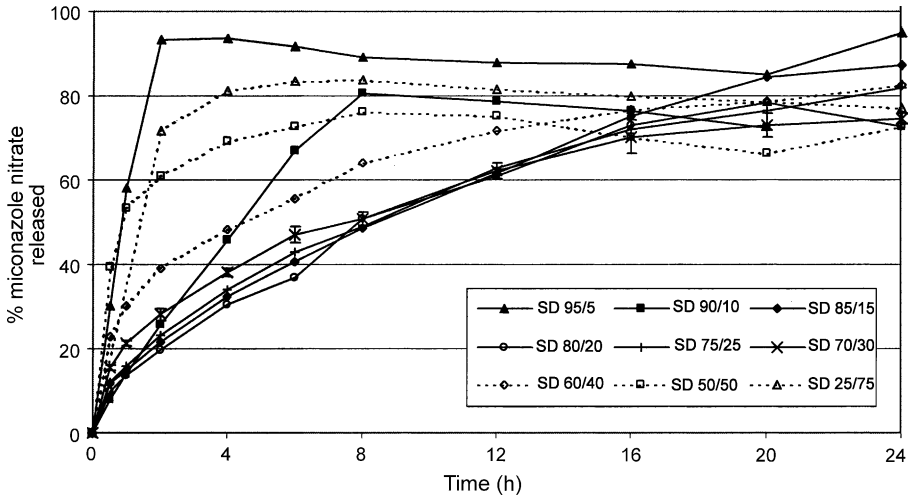

Fig. 4. In vitro dissolution profiles for the spray-dried Amioca®/Carbopol® 974P mixtures (SD) ($n = 6$, mean).

concentrations in the spray-dried mixtures showed a faster in vitro miconazole release. The $t_{50\%}$ was for SD 95/5 and SD 90/10 45 min and 4.5 h, respectively. SD 60/40, 50/50 and 25/75 had a $t_{50\%}$ of 4.5 h, 45 min and 60 min, respectively. The drug release from the spray-dried mixtures with the lowest C 974P content, SD 95/5 and 90/10, is controlled by polymer erosion, as the diffusional exponents, n, were 1.01 ± 0.02 and 1.04 ± 0.05, respectively. The drug release was almost constant in relation to time. The release mechanisms from the spray-dried matrices containing 15% C 974P or more could not be described by the exponential equation $M_t/M_\infty = kt^n$ as the higher C 974P concentrations resulted in matrices which swelled more than 25% of its original volume. The miconazole release from these matrices was mainly controlled by diffusion as at the end of the dissolution an almost intact swollen translucent tablet gel matrix was found. The drug diffusion from the matrices with the highest C 974P concentrations (SD 60/40, SD 50/50 and SD 25/75) was faster than from the matrices containing between 15 and 30% C 974P, which sustained the drug release over the longest period. Drug diffusion through the swollen gel layer took the longest time for the polymer matrices of spray-dried combinations of 15 to 30% C 974P with Amioca® starch, resulting in the longest sustained release profiles. By increasing the C 974P concentration to 40% or more the diffusion rate of the drug through the polymer matrix was increased. It is well known that polymer matrices with high contents of Carbopol® exhibit short dissolution times (Khan and Zhu, 1999).

These results were in good correlation with the in vivo adhesion times of placebo tablets. The in vitro USP III dissolution test can be used to predict the in vivo adhesion time of buccal bioadhesive tablet formulations based on spray-dried Amioca®/C 974P mixtures.

3.3. In vivo drug loading—bioavailability study

From the above mentioned results it is clear that by spray-drying different ratios of Amioca® starch/Carbopol® 974P a whole range of potential bioadhesive carriers can be prepared with improved bioadhesive properties. By modifying the C 974P concentration the in vivo adhesion time of the bioadhesive formulations can be influenced. As bioadhesive powder formulations are intended to stick to mucous membranes it is important to evaluate their mucosal irritation potency. In a previously reported study the biocompatibility of the different spray-dried Amioca®/C 974P mixtures was evaluated using an alternative mucosal irritation test using slugs (Adriaens et al., 2003). Spray-dried mixtures containing up to 20% C 974P induced no irritation of the mucosal tissue of the slugs and can be considered as safe bioadhesive carriers. On the other hand, mixtures containing higher amounts of C 974P induced mucosal irritation and membrane dam-

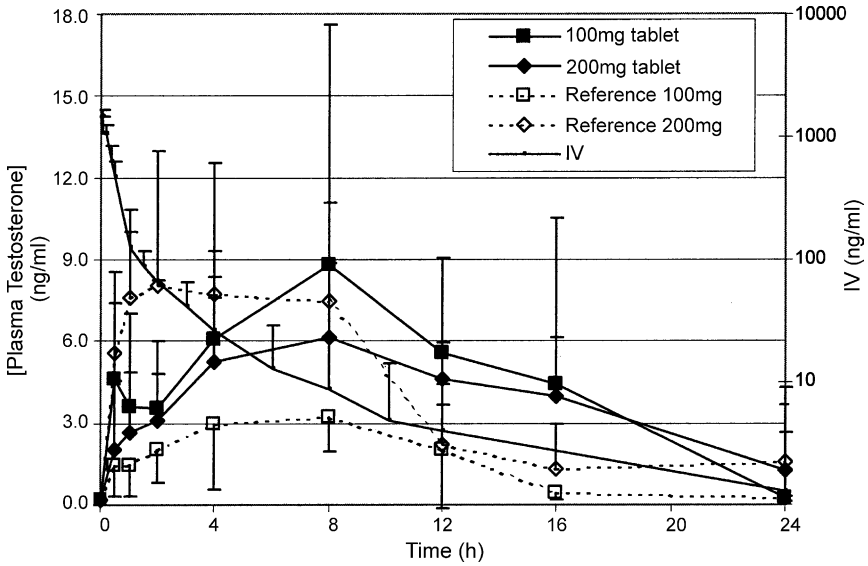

Fig. 5. Plasma concentration time profiles for the 30 and 60% loaded spray-dried Amioca®/Carbopol® 974P 80/20 mixture compared to the reference formulation ($n = 6$, mean ± S.D.).

age. This makes only the mixtures containing up to 20% C 974P useful as bioadhesive carriers. Nevertheless, by changing the C 974P content between 5 and 20% the in vivo adhesion time of 100 mg placebo tablets can be varied between 8 and 17 h (Fig. 3) and the in vitro miconazole release between 2 and 20 h (Fig. 4).

The drug loading capacity of the non-irritating spray-dried mixture SD 80/20 was investigated in vivo in dogs. Fig. 5 shows the mean testosterone plasma concentration time profiles for the 30 and 60% loaded SD 80/20 and the reference formulation (DDWM/C 974P; 95/5). The absolute bioavailability (F_{abs}), the T >3 ng/ml and the in vivo adhesion time are shown in Table 1. For both formulations the in vivo adhesion time decreased with a higher drug load, but the SD 80/20 formulation adhered for both drug concentrations longer compared to the reference formulation and resulted in a higher absolute bioavailability for the SD formulations. The F_{abs} was for the 60% loaded SD 80/20 formulation significantly higher than for the similar loaded reference formulation. Also the T >3 ng/ml value, which gives a therapeutic indication (Mazer et al., 1992), was for the spray-dried 60% loaded formulation significantly higher than for the 60% loaded reference formulation. From these results it is clear that using SD 80/20 as bioadhesive platform the buccal testosterone delivery was improved compared to a DDWM/C 974P 95/5 formulation (Voorspoels et al.,

Table 1
Absolute bioavailability (F_{abs}), time during which the plasma testosterone concentration was above 3 ng/ml ($T > 3$ ng ml^{-1}) and the in vivo adhesion time for 30 and 60% loaded spray-dried Amioca®/Carbopol® 974P 80/20 mixture compared to a reference formulation ($n = 6$, mean ± S.D.)

	SD 80/20		Ref	
	100 mg[a] 60%[b]	200 mg[a] 30%[b]	100 mg[a] 60%[b]	200 mg[a] 30%[b]
F_{abs} (%)	14.28 ± 5.12[c]	11.31 ± 2.77	4.59 ± 2.16	6.97 ± 3.33
T >3 ng ml^{-1} (h)	14.00 ± 1.67[c]	15.83 ± 5.53	5.00 ± 5.06	11.25 ± 3.86
Adhesion time (h)	15.25 ± 2.56	24.80 ± 5.31	13.00 ± 3.29	15.67 ± 18.62

[a] Total weight.
[b] Drug load.
[c] Significantly higher compared to the similar loaded reference formulation.

1996). Moreover the spray-dried formulation could be loaded with 60% drug without loss of its bioadhesive capacities and without major changes in plasma concentration time profiles (F_{abs} and T >3 ng/ml).

4. Conclusion

By spray-drying Amioca®/Carbopol® 974P mixtures a range of potential bioadhesive carriers was obtained with excellent bioadhesive properties. Up to 20% C 974P could be incorporated without any risk of mucosal irritation. By ranging the C 974P concentration between 5 and 20%, the in vivo adhesion time of placebo tablets could be varied between 8 and 17 h. The data from the in vivo adhesion time study correlated well with the in vitro miconazole release profiles (USP III dissolution). A spray-dried Amioca®/C 974P 80/20 mixture could be loaded with 60% drug without loosing its in vivo bioadhesive and pharmacokinetic properties.

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