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Research paper

Formulation and in vivo evaluation of chlorhexidine buccal tablets prepared using drug-loaded chitosan microspheres

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Abstract

This investigation deals with the development of buccal formulations (tablets) based on chitosan microspheres containing chlorhexidine diacetate. The microparticles were prepared by a spray-drying technique, their morphological characteristics were studied by scanning electron microscopy and the in vitro release behaviour was investigated in pH 7.0 USP buffer. Chlorhexidine in the chitosan microspheres dissolves more quickly in vitro than does chlorhexidine powder. The anti-microbial activity of the microparticles was investigated as minimum inhibitory concentration, minimum bacterial concentration and killing time. The loading of chlorhexidine into chitosan is able to maintain or improve the anti-microbial activity of the drug. The improvement is particularly high against *Candida albicans*. This is important for a formulation whose potential use is against buccal infections. Drug-empty microparticles have an anti-microbial activity due to the polymer itself. Buccal tablets were prepared by direct compression of the microparticles with mannitol alone or with sodium alginate. After their in vivo administration the determination of chlorhexidine in saliva showed the capacity of these formulations to give a prolonged release of the drug in the buccal cavity. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chitosan microspheres; Chlorhexidine; Spray-drying; Buccal tablets

1. Introduction

Topical delivery of anti-microbial agents to give prolonged active concentrations of drug in the oral cavity is an important goal in the field of controlled release formulations [1]. The control of *Candida albicans* is another area where drug delivery systems are being used to negate the need for repeated mouthwashes [2,3].

Chitosan is a hydrolyzed (deacetylated) derivative of chitin, a biopolymer widely distributed in nature and biologically safe [4,5]. The pharmaceutical application of chitosan microspheres as controlled drug delivery systems has attracted increasing attention in recent years [6]. This polymer exhibits several favourable properties such as biodegradability and biocompatibility [4]. It also has mucoadhesive properties due to its positive charges at neutral pH that enable an ionic interaction with the negative charges of sialic acid residues of the mucus [7,8]. On account of these characteristics chitosan has been used as an excipient in oral formulations (as a direct tabletting agent or as a component of buccal devices) [9,10]. Furthermore, chitosan inhibits the adhesion of *C. albicans* to human buccal cells and it can be used to prevent the development of mycosis [11].

In the present study, a drug delivery system has been developed, based on chitosan buccal tablets. Chlorhexidine diacetate was chosen as a model drug. This drug has a well known anti-plaque effect and chlorhexidine delivery systems have been proposed for the treatment of chronic *candidal* infections in immunosuppressed patients [1,10].

The preparation of the tablets was carried out by direct compression. To improve the flow properties of chitosan, drug-loaded chitosan microparticles were prepared before compression. Sodium alginate and/or mannitol were used as additional excipients.

A spray-drying method was used for the preparation of chitosan microspheres. In fact, in recent years, the spray-drying technique has been proposed, for the production of microparticles, as an alternative to emulsification methods [12–15]. In vitro characterization of microparticles was performed. Their anti-microbial activity was tested and compared to the activity of the drug alone and of drug-empty chitosan microparticles.

The in vivo behaviour of the tablets was tested after

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buccal administration and compared to a commercial mouthwash.

2. Materials and methods

2.1. Materials

The following materials were used: chitosan, deacetylation degree 75–85%, viscosity (Brookfield, 1% solution in acetic acid) 200–800 cps (Aldrich, Milwaukee, WI); chlorhexidine diacetate, $d_{vs} = 15.0$ μm (Coulter Counter model TA II, Coulter Electronics Ltd., Luton, UK) (Sigma, St. Louis, MO); sodium alginate, high viscosity, 2.0% w/v aqueous solution at 25 °C with viscosity of approximately 14,000 cps (manufacturer value) (Sigma, St. Louis, MO); mannitol (USP) (Sigma); and saccharine (FU) (Prodotti Crual, Italy). All solvents used were of analytical grade. The mother solution of chlorhexidine diacetate for microbiological assays was 10 mg ml^{-1} in water.

2.2. Preparation of microspheres by spray-drying

The microparticles were prepared with drug to polymer weight ratios of 1:2 and 1:4. Chitosan was dissolved in hydrochloric acid (0.1 M) that was then evaporated to dryness. The residual and the drug (2% total w/v concentration; chitosan concentration 1.33 and 1.66% in 1:2 and 1:4 preparations, respectively) were dissolved in distilled water, achieving a solution that was sprayed through the nozzle of a spray-dryer (model Mini Spray HO Pabisch, W.Pabisch S.p.A., Milan, Italy), co-current flow type, equipped with a standard 0.7 mm nozzle. The process conditions were: inlet air temperature 99–100 °C; outlet air temperature 74–75 °C; spray flow rate about 20 ml min^{-1} (approximate). Aqueous solutions of chitosan (2% w/v) were sprayed to obtain drug-empty microspheres. Each preparation was carried out in triplicate. The total amount of solid material used for the preparation of each batch was 15 g.

Table 1 reports the compositions of all types of microspheres prepared, and the corresponding production yields (shown as percent weight of microspheres obtained with respect to the initial amounts of drug and chitosan).

2.3. Scanning electron microscopy (SEM)

The shape and surface characteristics of spray-dried microspheres were studied by SEM (Zeiss DSM 962, Zeiss, Germany). Samples of microspheres were placed on a double-sided tape that had previously been secured on aluminium stubs and then analyzed at 20 kV acceleration voltage after gold sputtering under an argon atmosphere.

2.4. Particle size analysis

Particles were sized by laser diffractometry using a Coulter LS 100Q laser sizer (Beckman Coulter Particle Characterization, Miami, FL). Particle size analysis was carried out on blank and drug-loaded spray-dried microspheres suspended in soy oil and sonicated for 1 min. The average particle size was expressed as the volume-surface diameter (d_{vs}) [16].

2.5. Differential scanning calorimetry (DSC)

Temperature and enthalpy values were measured with a METTLER STAR^{e} system equipped with a DSC821^{e} Module on 3–5 mg (Mettler M3 Microbalance) samples in crimped sealed aluminium pans under a static air atmosphere. An empty pan was used as reference. The heating rate was 10 K min^{-1} over the 30–200 °C. Measurements were carried out in triplicate (relative standard deviation within approximately 5%).

2.6. Drug content determination

Drug-loaded microparticles were dissolved in ethanol 95%. The drug incorporated into the microparticles was determined spectrophotometrically at 262 nm (Hitachi, model U-2001 spectrophotometer; Hitachi Instruments Inc., Japan). Table 1 reports the actual drug contents of the different batches.

2.7. Microbiological assays (minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), killing time)

The anti-microbial activity of drug-loaded, drug-empty microparticles and chlorhexidine (as powder) was evaluated. Each experiment was carried out in triplicate. The anti-microbial activity has been evaluated as MIC (μg ml^{-1}) and MBC (μg ml^{-1}) by using a broth dilution technique [17] against selected strains of the following microorganisms: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *C. albicans* ATCC 10231. Briefly, twofold serial dilutions of mother solution of chlorhexidine diacetate or

Table 1
Compositions and production yields of the spray-dried chitosan microspheres ($n = 3$; SD within approximately 2%)

Formulation	Chlorhexidine-to-chitosan ratio	Theoretical drug content (% w/w)	Actual drug content (% w/w)	Encapsulation efficiency (%)	Production yield (%)
A	1:2	33.3	33.1	99.4	53.6
B	1:4	20.0	17.8	89.0	54.7
Blank microspheres	0:1	0	0	–	65.4

Table 2
MIC and MBC values expressed as μg ml^{-1} of chlorhexidine (powder) or of spray-dried microspheres (n =3; SD within approximately 3%)

Microbial strains	Chlorhexidine (powder)	Drug-empty microspheres (spray-dried chitosan)	Microspheres with a chlorhexidine-to-chitosan ratio of 1:2 (batch A)	Microspheres with a chlorhexidine-to-chitosan ratio of 1:4 (batch B)
Staphylococcus aureus	0.9 (3.9)	208 (1000)	2.4 (13.0)	3.9 (7.8)
Escherichia coli	1.9 (15.6)	1000 (>1000)	6.2 (11.7)	7.8 (31.2)
Pseudomonas aeruginosa	21.9 (125)	1000 (>1000)	62.5 (1000)	93.8 (>1000)
Candida albicans	7.8 (7.8)	1000 (1000)	15.6 (15.6)	31.3 (31.3)

microparticles suspensions (1 mg ml^{-1} to 0.0039 mg ml^{-1} in Mueller Hinton broth, Oxoid) were prepared in triplicate in the same medium and inoculated with 5×10^5–1×10^6 organisms ml^{-1}. After 24 h of incubation at 35 °C, test tubes were checked for bacterial growth. MICs were defined as the lowest concentration that completely inhibited bacterial growth. Suspensions from tubes not showing bacterial proliferation were subcultured onto Mueller Hinton Agar (Oxoid) plates and incubated at 35 °C. MBCs were recorded, after overnight incubation, as the lowest concentration at which no growth was detectable. MIC and MBC values are reported in Table 2.

Killing time was determined as the exposure time required to kill a standardized microbial inoculum. *Escherichia coli* in the logarithmic phase of growth were suspended at a density of 5×10^5–1×10^6 cfu ml^{-1} in 10 ml of phosphate buffer (pH 7.3) containing appropriate concentrations of chlorhexidine (as a solution) or microparticles (as a suspension). The concentration of drug used was 15.6 μg ml^{-1}, which corresponds to its MBC value against *E. coli*. A quantity of microspheres corresponding to the same drug concentration was used. A control tube (bacteria suspended in phosphate buffer at the same density) was included in each experiment. At regular intervals, 0.5 ml of suspension was removed, subjected to serial tenfold dilutions in buffer and seeded on Tryptone Soya Agar (Oxoid) plates. The number of viable bacteria at each time was evaluated counting colonies after incubation for 24 h at 35 °C.

2.8. Preparation of the tablets

Drug-loaded chitosan microparticles and excipients were mixed in a Turbula apparatus (W.A.Bachofen, Basel, Switzerland) at 90 rev./min for 10 min for the preparation of two different formulations: TAB1 and TAB2 (compositions in Table 3). The tablets were prepared by direct compression (1000 kg compression force, hardness 6–8 kP) using a hydraulic press (Perkin-Elmer, Bucks, UK) equipped with 13 mm flat punches.

Table 3
Composition (mg) of tablets prepared from drug-loaded microspheres (batch A): weight of the tablets 100 mg corresponding to 13 mg of drug

Tablet components	TAB1	TAB2
Microspheres (batch A)	40	40
Mannitol	58	38
Sodium alginate	–	20
Saccharine	2	2

2.9. In vitro drug release tests

In vitro tests were carried out to characterize the drug release behaviour from the chitosan microspheres, in comparison with the dissolution behaviour of chlorhexidine (as powder). Microspheres containing 2 mg of drug or 2 mg of chlorhexidine as free powder were suspended in 200 ml of USP 24 phosphate buffer (pH 7.0) at 37 °C. The tests were carried out using the USP apparatus n.2 (100 rev./min). The drug was analyzed spectrophotometrically at 262 nm. Each experiment was performed in six replicates.

The in vitro release tests of the tablets prepared from the drug-loaded microparticles (corresponding to 13 mg of drug) were carried out using the USP apparatus n.1 (50 rev./min), using 300 ml of USP phosphate buffer (pH 7.0). Each experiment was performed in six replicates.

2.10. In vivo evaluation of buccal tablets

In vivo experiments were performed after approval of the protocol by the scientific-ethics committee of the University of Sassari. The tablets were placed on the buccal mucosa (cheek) of one volunteer. For each formulation the experiments were carried out in triplicate. At each time point samples of saliva were collected and the chlorhexidine concentration was determined by HPLC assay. The following instruments were used: Hewlett-Packard 1050 Series quaternary pump and variable-wavelength detector operating at 257 nm (Hewlett-Packard, Waldbronn, Germany). The standards and samples were injected through a 20 μl autosampler injection. The peak areas determined with a 3390 integrator (Hewlett-Packard, Avondale, PA) were used for quantitation. Reverse-phase HPLC was performed at room temperature. The column used was a Nucleosil RP-18 (150 × 4.6 mm I.D.) from Alltech with a 5 mm average particle diameter. The mobile phase was acetonitrile-buffer (0.1 M disodium hydrogen phosphate, 0.005 M 1-heptanesulfonic acid and 0.05 M triethyleneamine) 35:65 v/v. The pH of the buffer was adjusted to 2.5 with phosphoric acid. An extraction procedure of chlorhexidine from saliva was carried out as follows: a 200 μl sample of clear saliva was introduced into a test tube and 400 μl of 4.5 M sodium

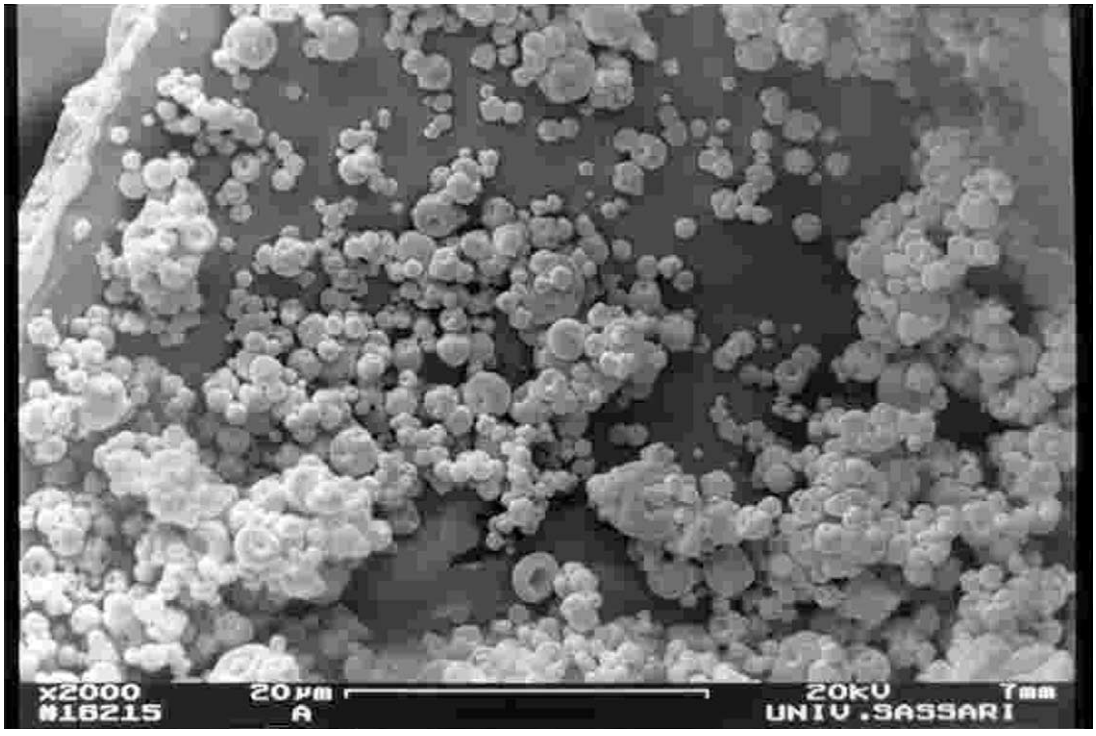

Fig. 1. SEM micrograph of microspheres with a chlorhexidine-to-chitosan ratio of 1:2.

hydroxide and 500 μl of acetonitrile were added. The tube was vortex-mixed for 1 min and centrifuged for 1 min at 14,000 rev./min. A 20 μl aliquot was injected onto the HPLC system.

3. Results and discussion

Spray-drying is a good technique for the preparation of chitosan microparticles. Other techniques such as emulsification/solvent evaporation involve different steps and the use of surfactants for the stabilization of the emulsion. Spray-drying is easy and rapid as it is a one-step process that involves only the preparation of a solution containing drug and polymer. As shown in Table 1, the yield of spray-dried microspheres is relatively low, independent of their composition: about 55% for drug-loaded and 65% for drug-empty microparticles. These low values are quite frequent when the spray-drying method is used for the production of microparticles, as pointed out previously [18,19]. This can be attributed both to the small amount of materials processed in each batch (15 g), and to the loss of the smallest and lightest particles through the exhaust of the spray-dryer apparatus if it is not equipped with a trap to recover the lighter and smaller particles. The actual drug content is approximately 33% for the microspheres with the chlorhexidine-to-chitosan ratio 1:2, and 18% for the particles with the chlorhexidine-to-chitosan ratio 1:4. This corresponds to encapsulation efficiencies of 99 and 90%, respectively. A typical SEM micrograph of drug-loaded microspheres (chlorhexidine-to-chitosan ratio 1:2) is presented in Fig. 1. The microparticles have a spherical shape and a smooth surface and no free drug crystals appear. As shown by particle size analyses, loaded particles (batches A and B) showed $d_{vs} = 4.4$ and 6.1 μm, respectively, and blank microspheres of 4.2 μm.

Fig. 2 shows the in vitro release profiles obtained from the

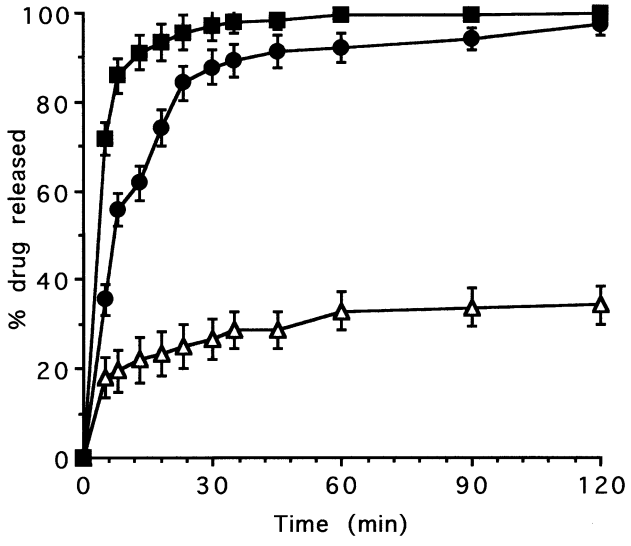

Fig. 2. In vitro release profiles (USP phosphate buffer pH 7.0) of: chlorhexidine (drug as powder) (△); microspheres with a chlorhexidine-to-chitosan ratio of 1:2 (●); microspheres with a chlorhexidine-to-chitosan ratio of 1:4 (■). Each point represents the mean ± SD ($n = 6$).

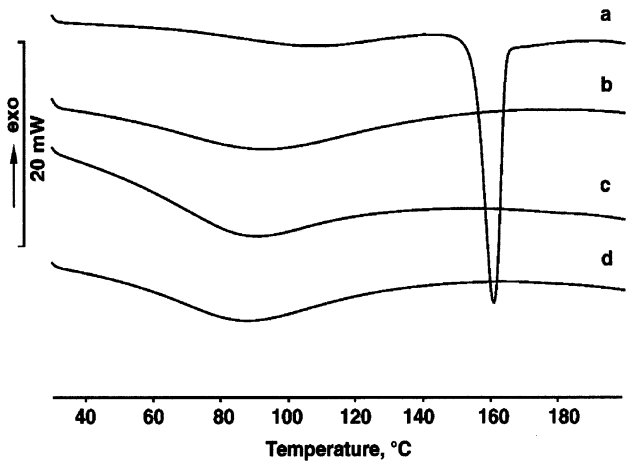

Fig. 3. Thermal profiles ($n = 3$) of chlorhexidine diacetate (a), chitosan (b), chlorhexidine-to-chitosan ratio 1:2 (c) and chlorhexidine-to-chitosan ratio 1:4 (d).

drug-loaded microparticles, compared to the dissolution profile of the drug alone. The rate of dissolution of chlorhexidine powder is significantly slow (approximately 40% of the drug dissolved in 2 h). Loading of chlorhexidine into chitosan microparticles leads to a remarkable improvement of its dissolution rate. The increase in the rate of drug dissolution seems to depend on the amount of chitosan present in the formulations, the highest rate corresponding to the chlorhexidine-to-chitosan weight ratio of 1:4 (approximately 90% of drug dissolved in 10 min). Chitosan is a polymeric material which is already known for its properties of dissolution rate enhancer of drugs that are poorly soluble in water. For example, previous studies [20] demonstrated that prednisolone when ground and mixed with chitosan achieved an enhancement of its dissolution properties. A similar result was also observed upon loading dexamethasone into spray-dried chitosan microparticles [21].

Fig. 3 reports the thermal profiles of chlorhexidine as pure drug (curve a) and of blank (b) and of drug-loaded microparticles (c and d). Chlorhexidine (a) is present in a crystalline form ($T_{onset} = 155.0 \pm 0.3$ °C, $T_{peak} = 160.3 \pm 0.2$ °C, $\Delta H = 94 \pm 2$ J g^{-1}), while the loading into chitosan microparticles (c and d) leads to its complete amorphization: the endothermal effect due to the fusion of the crystalline drug disappears.

The drug amorphization is due to the process of microparticle preparation, which leads to the dispersion of the drug into the polymeric network. The improvement of the drug dissolution rate shown by the in vitro release test can be explained by its amorphous state.

The results of microbiological tests, expressed as MIC and MBC, are reported in Table 2. Against the microbial strains, chlorhexidine as powder exhibits MIC values that range between 0.9 and 21.9 μg ml^{-1} and MBC values that range between 3.9 and 125 μg ml^{-1}. Against *C. albicans* MIC and MBC values are superimposed (7.8 μg ml^{-1} in both cases). Drug-empty microspheres (spray-dried chitosan) have an anti-microbial activity, due to the polymer itself. This confirms previous studies [22]. Drug-loaded microparticles show higher MIC and MBC values with respect to chlorhexidine powder.

However, considering the actual drug content (approximately 18 and 33%), the chlorhexidine activity in the loaded microparticle was maintained or improved. For example, chlorhexidine powder (pure drug) has a MBC of 3.9 μg ml^{-1} against *S. aureus* while microspheres with a chlorhexidine-to-chitosan ratio of 1:4 have a MBC of 7.8 μg ml^{-1} (corresponding to 1.4 μg ml^{-1} of drug). MBC values against *P. aeruginosa* are an exception because lower drug activity is present in both batches with respect to the drug alone. The anti-*candidal* activity is improved in both formulations of microparticles and MIC and MBC values are superimposed (as in the case of the drug alone): 15.6 μg ml^{-1} (corresponding to 5.1 μg ml^{-1} of drug) for the microspheres with a chlorhexidine-to-chitosan ratio of 1:2, and 31.3 μg ml^{-1} (corresponding to 5.6 μg ml^{-1} of chlorhexidine) for the microspheres with a 1:4 ratio. The improvement of drug activity against *C. albicans* is important as *Candida* is responsible for a mycosis frequent in the buccal region.

Killing times (min) of drug-loaded microparticles are reported in Fig. 4, compared to the drug alone. The test shows that the activity of microparticles with a chlorhexidine-to-chitosan ratio of 1:4 is quicker than microparticles with a 1:2 ratio. This result is due to the anti-microbial effect of the polymer itself. Analogous tests have been carried out on blank microspheres; they confirm that the highest concentration of polymer results in the quickest killing effect (data not shown). The fast killing effect of drug

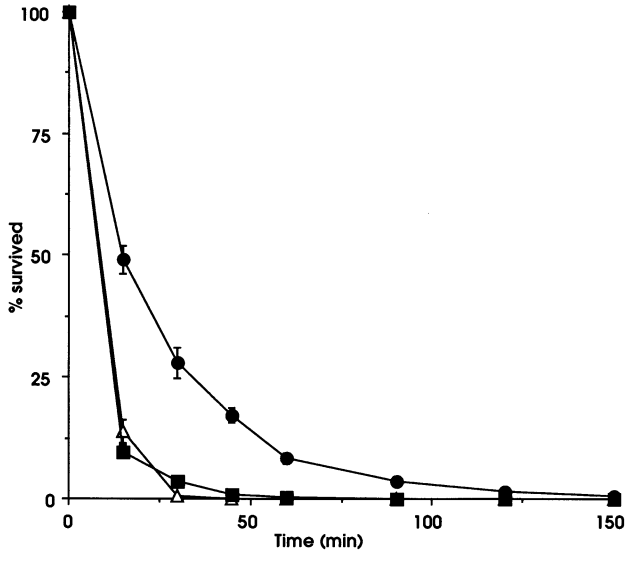

Fig. 4. Killing times (min) of: chlorhexidine (15.6 μg ml^{-1} concentration) (△); microspheres with a chlorhexidine-to-chitosan ratio of 1:2 (47.2 μg ml^{-1} concentration) (●); microspheres with a chlorhexidine-to-chitosan ratio of 1:4 (87.6 μg ml^{-1} concentration) (■). Each point represents the mean ± SD ($n = 3$).

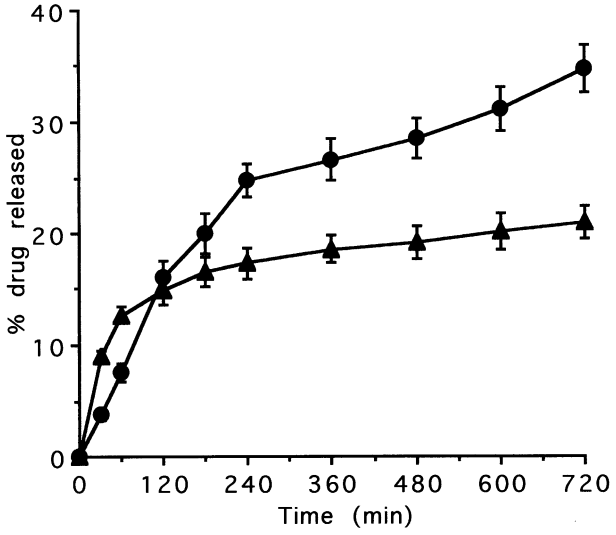

Fig. 5. In vitro release profiles (USP phosphate buffer pH 7.0) of: TAB1 (●) and TAB2 (▲) formulations. Each point represents the mean ± SD ($n = 6$).

alone is due to the conditions in which the test has been performed and which involve the use of a solution of drug.

Buccal tablets were prepared by mixing and tabletting (by direct compression) drug-loaded microspheres belonging to batch A (actual drug content 33.1%) with mannitol and saccharine (TAB1), or with mannitol, saccharine and sodium alginate (TAB2). The compositions of the formulations are reported in Table 3. Fig. 5 shows the corresponding in vitro release profiles. Their release behaviour is quite different: at the beginning of the test TAB1 is characterized by a lower release rate with respect to TAB2. After 2 h the drug release rate from TAB2 slows down, probably owing to the presence of the anionic polymer (sodium alginate) mixed with the cationic polymer (chitosan) and a consequent ionic interaction between them [23,24].

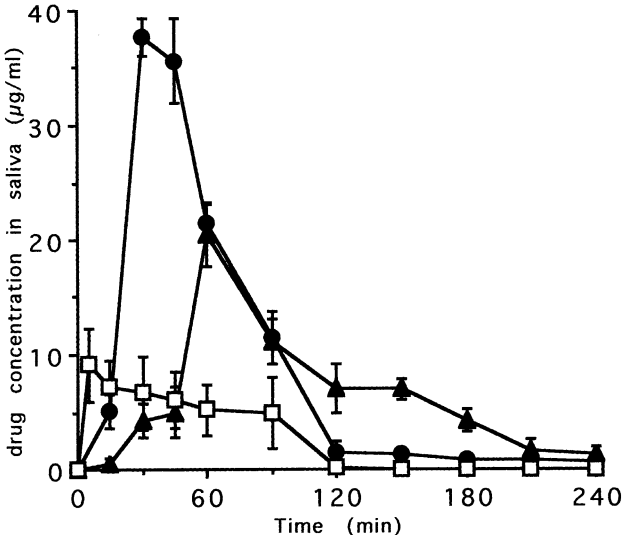

Fig. 6. In vivo concentrations (μg ml^{-1}) of chlorhexidine after the administration of TAB1 (●), TAB2 (▲) formulations and of mouthwash (□). Each point represents the mean ± SD ($n = 3$).

Fig. 6 shows the in vivo chlorhexidine concentrations (μg ml^{-1}) in saliva determined after the buccal administration of TAB1 and TAB2, compared to the concentrations obtained after the administration of a commercial mouthwash (Dentosan®, Warner Lambert, Lainate, Mi, Italy), which is a 0.20% chlorhexidine solution. A total of 20 ml of solution, corresponding to 40 mg of drug, was administered by washing the buccal cavity for 30 s. The tablets were placed into contact with the cheek mucosa and allowed for all the duration of the experiments (4 h) a normal life (the person was able to speak and drink). The administration of the tablets leads to an enhancement of production of saliva, which determines the rapid gelation of both formulations (TAB1 and TAB2) in the buccal cavity. After about 90 min TAB1 partially disintegrates in the buccal cavity, while TAB2 remains almost intact for about 3 h. This different behaviour could be due to the ionic interaction between the two polymers constituting TAB2. As shown by chlorhexidine concentrations in saliva, mouthwashes determine the highest concentration at the beginning of the test (5 min), but the drug level slows down very quickly and after 2 h no detectable level of drug is present in saliva. TAB1 is characterized by the highest peak (after about 30 min). TAB2 tablets are able to maintain the presence of the drug in saliva for the longest period of time (more than 3 h). The high capacity of TAB2 to give prolonged concentrations of drug in the buccal cavity could be due to the combination of chitosan with sodium alginate.

The in vivo results of this study suggest that such tablets are potential candidates for intraoral drug delivery.

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