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Periodontal delivery of ipriflavone: new chitosan/PLGA film delivery system for a lipophilic drug

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Abstract

The aim of the present work was to design a film dosage form for sustained delivery of ipriflavone into the periodontal pocket. For this purpose, monolayer composite systems made of ipriflavone loaded poly(D,L-lactide-co-glycolide) (PLGA) micromatrices in a chitosan film form, were obtained by emulsification/casting/evaporation technique. Multilayer films, made of three layers of polymers (chitosan/PLGA/chitosan), were also prepared and compared to monolayer films for their "in vitro" characteristics. Morphology and physico-chemical properties of the different systems were evaluated. The influence of pH, ionic strength and enzymatic activity on film degradation, was also investigated. Significant differences in swelling, degradation and drug release were highlighted, depending on film structure and composition. In vitro experiments demonstrated that the composite micromatricial films represent a suitable dosage form to prolong ipriflavone release for 20 days.

Keywords: Chitosan; Polymeric films; PLGA; Ipriflavone; Periodontal delivery

1. Introduction

Inflammation of the periodontium, which comprises the periodontal ligament, gingiva, cementum and alveolar bone, usually begins with gengivitis. The gingivae progressively lose their attachment to the teeth, and bone loss begins causing the periodontal pockets to deepen. With progressive bone loss, teeth may loosen and gingivae recede (Merck Manual, 1999).

The treatment of periodontitis usually involves a systemic regimen with antibiotics to alter the presumably pathogenic flora. Furthermore, some tetracyclines, by inhibiting collagenase, seem to diminish bone destruction. Another approach is to surgically eliminate the pocket and recontour the bone to encourage alveolar bone growth.

Recently, some authors investigated the potential application of ipriflavone, a synthetic flavonoid derivative, on the healing process of experimentally injured rat perialveolar bone (Martini et al., 1998). This compound is usually employed in the treatment of post-menopausal and senile osteoporosis by oral administration (Head, 1999). Local application into periodontal pocket could be very advantageous, both in terms of raising drug concentration directly in the action site, and in preventing systemic side effects such as gastrointestinal complaints, depression, and tachycardia.

Conventional drug formulations for the mouth, such as toothpaste and mouthwash, have very low penetration into periodontal pocket. Films appear to be a suitable dosage form to deliver drugs into periodon-

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tal pocket, because the anatomic construction of the pocket allows for relatively easy insertion of such a delivery device (Steinberg and Friedman, 1999). Moreover, the use of biodegradable polymers can increase patient compliance, as the inserted film does not need to be removed.

Commercially speaking, periodontal delivery systems are available on the US market, such as PerioChip®, consisting of a cross-linked gelatin matrix capable to maintain chlorhexidine concentration for up to 7 days (Soskolone and Freidman, 1996).

In the present work, the goal was to prepare chitosan/poly(D,L-lactide-co-glycolide) (PLGA) films containing ipriflavone for periodontal pocket delivery. Chitosan has been recently studied in dental and oral application for its osteoconductive properties as a bioactive bone substitute (Muzzarelli et al., 1994). It has proved itself to be a suitable bioadhesive material to develop as dosage form, such as bioadhesive tablets, films and gels to improve intraoral administration of drugs for systemic or local therapy (Senel et al., 2000a).

The use of chitosan as film matrix is well documented for the delivery of hydrophilic drugs, such chlorhexidine gluconate, because it is very easy to produce homogenous film matrix with drug and polymer dissolved in the same vehicle, whereas few data on lipophilic drug delivery by chitosan films are reported (Senel et al., 2000b; Shu et al., 2001). However, due to the fast dissolution rate in aqueous media, this polymer does not promote prolonged drug release.

PLGA copolymer was selected as the polymer to obtain prolonged drug release, due to its biodegradability and biocompatibility properties. Furthermore, poly(L-lactide) and PLGA polymers have already been used as constituents of biodegradable implant material in dentistry and orthopedics (Gogoleswi and Mainil-Varlet, 1997). Recently, composite porous matrices of chitosan-poly(L-lactide) were evaluated for their bone regenerative potential (Lee et al., 2002).

Through the present work, a new polymeric system was developed, obtained through an emulsification process in which PLGA micromatrices, containing a lipophilic drug, are homogeneously dispersed through a chitosan matrix. These matricial films were compared with PLGA and chitosan composite layer films for their “in vitro” characteristics, in order to establish the most suitable film for periodontal application.

2. Materials and methods

2.1. Materials

Chitosan glutamate salt (G210) M_r 150,000, medical grade, deacetylation degree 85%, was purchased from Pronova Biomedical AS, Oslo, Norway; D,L-lactide-co-glycolide copolymer 50:50 lactide/glycolide molar ratio (Resomer RG 503), M_r 34,000, was purchased from Boerhinger Ingelheim (Ingelheim, Germany); ipriflavone, M_r 280.31, was granted by Chiesi Farmaceutici (Chiesi Farmaceutici S.p.A., Italy); all reagents were of analytical grade.

2.2. Film preparation

Films of fixed area (15 cm^2) were prepared utilizing two different techniques to obtain micromatricial monolayer films and multilayer films:

(1) Emulsification/casting/solvent evaporation process (matricial films (MF)): an o/w emulsion was formed by dropping 5 g of CH_2Cl_2 solution containing ipriflavone (4%, w/w) and PLGA (20%, w/w) (MF_2) into 15 g of chitosan aqueous solution (2%, w/w) with glycerin 0.5% (w/w), stirring at 9500 rpm utilizing an Ika homogenizer at 15 °C. The o/w emulsion was maintained under stirring by a vibromixer, 50 vibration/min, at 37 °C for 15′, then poured into a glass dish and kept at 30 °C to allow solvent evaporation.

MF films containing ipriflavone, but without PLGA, were also prepared with the same method, as control (MF_1)

(2) Casting/solvent evaporation process (multilayer films (MLF)): the first layer was made of chitosan film. A total of 7.5 g of chitosan aqueous solution at 2% (w/w) with glycerin 0.5% (w/w) was poured into a glass dish and kept at 30 °C, for at least 12 h to allow complete solvent evaporation. The second layer, made of 5 g of CH_2Cl_2 solution containing ipriflavone and PLGA (ipriflavone 4%, w/w; PLGA 20%, w/w) (MLF_2),

Table 1
Composition of ipriflavone loaded chitosan/PLGA films (ipriflavone:G210:RG503 1:2:5, w/w/w)

Batch number	Film type	Chitosan solvent	Composition
1	MLF_1	Water	Ipri/G210
2	MLF_1	Citric acid[a]	Ipri/G210
3	MLF_2	Water	Ipri/RG503/G210
4	MLF_2	Citric acid[a]	Ipri/RG503/G210
5	MF_1	Water	Ipri/G210
6	MF_1	Citric acid[a]	Ipri/G210
7	MF_2	Water	Ipri/RG503/G210
8	MF_2	Citric acid[a]	Ipri/RG503/G210

[a] Citric acid aqueous solution (10^{-2} M).

was poured onto the first layer and dried before spreading a third layer, whose composition was the same as the first one. Films were dried until constant weight. This resulted in a sandwich film where the drug was positioned in between the two chitosan layers. MLF films containing ipriflavone, but without PLGA, were also obtained utilizing the same method, as control (MLF_1). Solvent residues were determined by Karl Fisher analysis for water and by gas chromatography for CH_2Cl_2 (Pavanetto et al., 1996).

Table 1 lists the composition of the ipriflavone loaded film batches produced. All types of film were produced dissolving chitosan at 2% (w/w) in citric acid 10^{-2} M (batches 2, 4, 6 and 8). One "blank" preparation (without drug) for each type of film was prepared as control.

2.3. Morphology study

Film surface was evaluated optically by a Sony Digital Mavica Camera. Morphological characteristics and film thickness were studied by scanning electron microscopy (SEM), using an electron microscope Jeol JX 840-A (Jeol LTD, Tokyo, J). Film samples were gold sputtered and visualized at 80 kV.

2.4. Water absorption capability

Weighed pieces of film were immersed in NaCl 0.9% (w/v); at predetermined times films were removed from the medium, blotted to remove liquid excess and weighed immediately. The swelling index was calculated from the weight increase, as follows:

$$\text{Swelling index} = \frac{(W_2 - W_1)}{W_1}$$

where W_1 and W_2 are the weight of the film before and after immersion in the medium, respectively.

2.5. Film degradation study

Film pieces were immersed in different media at 37 °C over a period of 3 months. The influence of the pH, ionic strength and enzyme presence was investigated. Film degradation was carried out in water, in saline phosphate buffer pH 7.4 (PBS) or in NaCl 0.9% (w/v) solutions. Lysozyme enzymatic activity in relation to film degradation was also investigated, putting film in water or in saline phosphate buffer pH 7.4 containing lysozyme 0.5 mg/ml.

Film degradation was evaluated by optical inspection. Degradation was considered complete when film was no longer visible to naked eye and only a powdered suspension was evident.

2.6. Chitosan dissolution study

Chitosan dissolution in the films was evaluated by putting films in water and saline phosphate buffer pH 7.4. The amount of chitosan dissolved was detected by means of colorimetric analysis, with the reactive anionic dye Cibacron Brilliant Red, using a spectrophotometer Beckman model DU7500 at 575 nm (Muzzarelli, 1998).

Calibration curves of chitosan, both in water and in saline phosphate buffer between 6 and 40 μg/ml, were employed.

2.7. Ipriflavone content

Films were divided in eight pieces. Each weighed piece was soaked in a mixture of HCl 0.1N:CH_2Cl_2 (1:0.7, v/v) for 48 h at 37 °C to allow film dissolution. After ethanol (HCl 0.1N:CH_2Cl_2: ethanol 1:0.7:2, v/v/v) addition, methylene chloride was removed by rotary evaporator under vacuum and the amount of drug was determined by UV analysis at 298 nm, using a Beckmann DU 7500 spectrophotometer. Analyses were carried out in duplicate on each piece of drug

loaded film, on "blank" films and on physical mixture ipriflavone:chitosan:PLGA, to establish the drug recovery percentage of the extraction method. A calibration curve of ipriflavone in ethanol between 2 and 40 μg/ml was employed.

2.8. "In vitro" ipriflavone release

Ipriflavone release from chitosan films was determined utilizing USP XXV dissolution apparatus (rotating paddle method) using 40:60 water:ethanol mixture as dissolution medium at 37 °C and with agitation speed of 50 rpm. Films with constant exposed area were soaked in a suitable volume of dissolution medium to maintain "sink conditions." The amount of drug released was determined through spectrophotometer analysis at 298 nm, on samples withdrawn at prefixed times.

3. Results

3.1. Film preparation and characterization

All films appeared quite elastic and flexible due to the presence of glycerin as plasticizer. Fig. 1 shows digital photographs of batches 3 and 7: multilayer film (batch 3, Fig. 1a) appeared well formed and no separation among the three layers was evident. Matricial film (batch 7, Fig. 1b) looked homogeneous with a rough surface.

Multilayer films were thicker than matricial films. This result was confirmed by SEM analysis: matricial film thickness was between 140 and 250 μm, whereas multilayer film thickness was about 1 mm (data not reported).

Fig. 2 shows SEM photomicrographs of MF_2 and MLF_2 films. Surface characteristics were very different between the films. In the matricial films PLGA micromatrices containing ipriflavone were evident and homogeneously dispersed (batch 7, Fig. 2a), whereas the chitosan surface of multilayer film appeared homogeneous (batch 3, Fig. 2b).

The structure of matricial films depends on the preparation method in which embryonic PLGA microspheres containing ipriflavone have been formed and entrapped in the chitosan matrix.

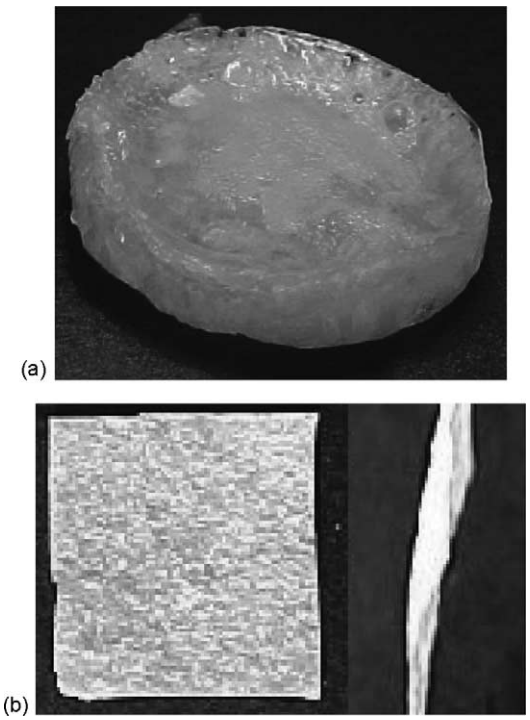

Fig. 1. Digital photographs of ipriflavone loaded chitosan/PLGA films: (a) batch 3; (b) batch 7.

The amount of water residual into films, determined by Karl Fisher method, was always about 5% (S.D. = 0.5) and the amount of residual CH_2Cl_2 was always acceptable (<500 ppm).

3.2. Water absorption capability

Swelling behaviour was mainly dependent on film structure and composition. Fig. 3 shows the swelling index of matricial films.

Films made without PLGA showed higher swelling index (batches 5 and 6). Batch 6, in which chitosan has been dissolved in citric acid solution, showed a lower swelling index. In composite matricial films, the swelling capability of chitosan decreases due to the hydrophobic effect of the PLGA micromatrices (MF_2, batches 7 and 8). Multilayer films showed the same behaviour however, after 20 min of soaking film integrity was lost, with evident separation of the three layers.

Multilayer films were not investigated further due to their physical instability in aqueous medium.

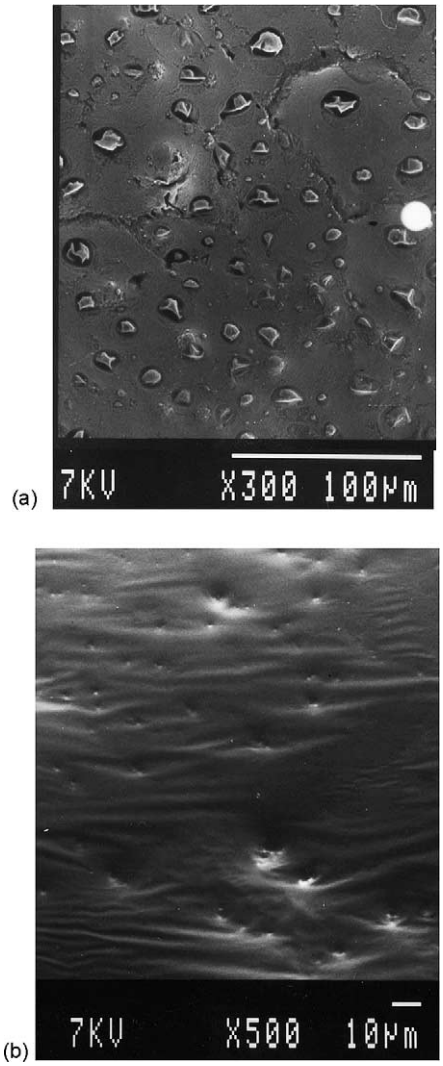

Fig. 2. Scanning electron micrographs of ipriflavone loaded chitosan/PLGA films: (a) batch 7; (b) batch 3.

3.3. Film degradation study

Degradation behaviour of chitosan films depended on film structure and on degradation media used. Table 2 shows the degradation time, determined by visual inspection, of all batches of ipriflavone loaded chitosan/PLGA matricial films kept at 37 °C.

Results showed that the presence of PLGA micromatrices greatly influenced film degradation times; in fact, batches 7 and 8 showed longer degradation times than batches 5 and 6, respectively, in all media tested.

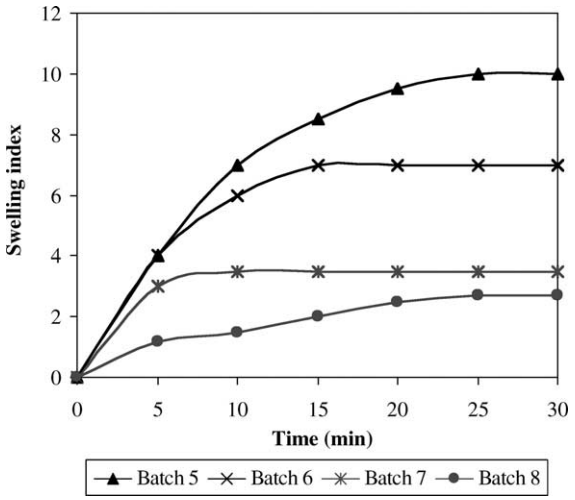

Fig. 3. Swelling index of matricial films vs. time ($n = 3$; S.D. < 5%).

Batches 6 and 8, produced with citric acid as chitosan solvent, showed degradation rates, in all media, slower than corresponding films made of chitosan dissolved in water (batches 5 and 7). In particular, in water batch 6 maintained its physical integrity up to 10 days and batch 8 up to 90 days, in comparison to 24 h and 35 days of batches 5 and 7, respectively.

Lysozyme increased degradation of chitosan film given its ability to depolimerize chitins and chitosans (Muzzarelli, 1997). This effect was more evident for all batches of films when soaked in PBS medium, than when soaked in water (Table 2). Enzymatic activity of lysozyme is increased by the ionic strength of the buffer used (Moss et al., 1997).

3.4. Chitosan dissolution study

Chitosan solubility in water and PBS from the matricial films was evaluated analyzing the amount of chitosan versus time by colorimetric assay, using an anionic dye that binds selectively free NH_2 groups of chitosan.

Results from this study indicated that the chitosan recovery percentage in solution differed in relation to film structure, composition and degradation medium used. Fig. 4 shows chitosan dissolution profiles both from chitosan and chitosan/PLGA films in (a) water and (b) saline phosphate buffer pH 7.4.

Table 2
Degradation times of ipriflavone loaded chitosan/PLGA matricial films

Batch number	Storage conditions				
	PBS	Water	NaCl	PBS + Lys	Water + Lys
5	48 h	24 h	48 h	24 h	24 h
6	8 days	10 days	15 days	3 days	10 days
7	12 days	35 days	30 days	8 days	15 days
8	30 days	90 days	30 days	12 days	60 days

The times were taken when the film was no longer visible to naked eye and only a powdered suspension was evident. PBS, saline phosphate buffer pH 7.4; Lys, lysozyme solution 0.5 mg/ml.

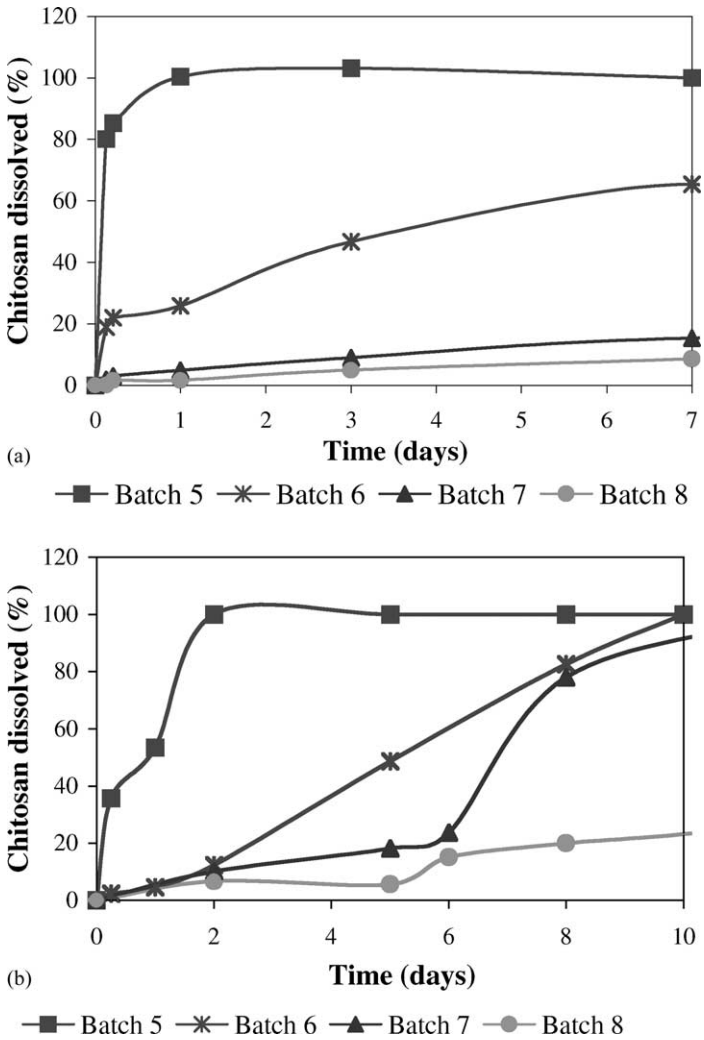

Fig. 4. Chitosan dissolution profiles from matricial films in: (a) water; (b) saline phosphate buffer pH 7.4 ($n = 3$; S.D. < 5%).

Batch 5, produced dissolving chitosan in water, represents the only batch in which chitosan recovery was approximately 100% within 2 days (Fig. 4a). In the case of batch 6, produced with citric acid as chitosan solvent, 100% of chitosan was recovered in PBS (Fig. 4b) and approximately 60% in water. This result is probably due to an ionic effect of phosphate salts on displacement reaction of the chitosan citrate, improving chitosan dissolution.

Chitosan dissolution studies carried out in water showed that the presence of PLGA micromatrices greatly reduced chitosan dissolution (Fig. 4a). In fact, the presence of a hydrophobic polymer such as PLGA, reduces the ability to absorb water and consequently level of film solubility.

However, PBS increases PLGA/chitosan film dissolution, as shown in the Fig. 4b. In fact, 90% of chitosan was recovered in approximately 10 days when batch 7 was soaked in phosphate saline buffer, compared to the same film soaked in water (Fig. 4a) in which only 15% of chitosan dissolved in 10 days.

3.5. Ipriflavone content in film and "in vitro" release

The yield of ipriflavone loading into matricial films was very high in all batches produced (>70%). The most important result was the low standard deviations achieved (between 0.13 and 0.28%) that highlighted the homogeneous dispersion of the drug into film matrix.

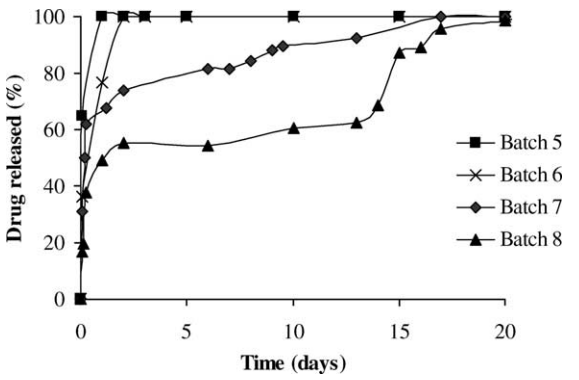

Fig. 5. Ipriflavone release profiles from chitosan/PLGA matricial films ($n = 3$; S.D. < 5%).

Fig. 5 shows profiles of drug released from matricial films. Batches 5 and 6 released 100% drug in 48 h. The presence of PLGA permitted prolonging of ipriflavone release up to 20 days. Between batches 7 and 8, the latter showed the slowest drug release.

4. Discussion

The emulsification process investigated in this study allowed us to produce films composed of a homogeneous dispersion of a lipophilic drug into a hydrophilic polymer such as chitosan.

Using this process we have successfully produced a new concept delivery system composed of ipriflavone PLGA micromatrices in a chitosan film.

The films prepared with this emulsification process were compared in terms of physical properties and behaviour to composite films produced with polymeric multilayer stratifications.

Morphologic characteristics were very different between the two types of film. In fact, matricial films were thinner than multilayer films and therefore, more suitable for utilization in the periodontal cavity.

Results concerning film water absorption capability, led to the conclusion that multilayer films were unsuitable due to their low physical stability in aqueous medium. With regards to topical delivery to oral mucosa into periodontal pocket, bioadhesion of delivery vehicles might be one strategy to obtain prolonged drug delivery and to improve localization of drug therapy.

Needleman and coworkers carried out "in vitro" and "in vivo" experiments to assess the bioadhesion of a wide variety of polymers on oral mucosa (Needleman and Smales, 1995; Needleman et al., 1997). They found a direct relationship between the degree of polymer hydration "in vitro" and the duration of bioadhesion "in vivo," therefore, a good degree of "in vitro" swelling would predict satisfactory bioadhesion. Adhesion failure occurs when hydration converts the gel network to slippery mucilage at maximum swelling.

This study has shown that the swelling index of emulsified chitosan films varies greatly, between 2.7 and 10, depending on film composition. The latter

represents maximum swelling before dissolution of film and is reached with films containing chitosan only, thus demonstrating that this polymer alone is not able to give long term adhesion. Interesting results were obtained from film degradation studies showing the influence of natural components of biological fluid washing the periodontal pocket, such as gingival crevicular fluid, on film degradation behaviour. In fact, phosphate salts, NaCl and enzyme presence, greatly effect physical behaviour of chitosan emulsified films. Phosphate ions greatly effected PLGA degradation, reducing its hydrophobicity. This effect appeared evident in chitosan dissolution studies, in which films soaked in phosphate buffer led to higher amounts of dissolved chitosan, compared to the same films soaked in water (Fig. 4).

Chitosan degradation is greatly influenced by the presence of lysozyme, whose ability to depolymerize chitosan is well assessed in literature (Muzzarelli, 1997). All batches of chitosan/PLGA films showed "in vitro" drug release profiles suitable to a prolonged therapy (Fig. 5). Between batches 7 and 8, the latter shows the slowest drug release. This indicates that ipriflavone release depends not only on diffusion through PLGA micromatrices, but also on diffusion through chitosan matrix, which results as being more compact when composed of chitosan citrate.

Significant differences in swelling, degradation and drug release were highlighted depending on the presence of citrate ions in the polymeric film.

Ionic interaction between chitosan and citrate ions has already been reported in literature as a method to improve the controlled drug release properties of chitosan films (Shu et al., 2001).

In conclusion, the emulsifying method to produce films made of the combination of a hydrophilic and a lipophilic polymer proposed in this work, is suitable in order to obtain matricial films made by a homogeneous dispersion of a lipophilic drug into a hydrophilic polymer.

The micromatricial structure of films studied in this work offer the possibility of obtaining polymeric systems with good morphological characteristics, such as thickness and flexibility useful for periodontal pocket delivery. Furthermore, these systems offer the advantage of combining the mucoadhesive properties of chitosan with the prolonged release properties of PLGA.

In addition, the ionic interaction between chitosan and citrate, should give further opportunity to modulate film characteristics, in order to make chitosan/PLGA film suitable for several therapeutic treatments.

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