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Research Article

The Influence of Recrystallized Caffeine on Water-Swellable Polymethacrylate Mucoadhesive Buccal Films

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Abstract. The aim of this work was to investigate the influence of particles on the properties of polymethacrylate films intended for buccal delivery. A solvent casting method was used with Eudragit RS and RL (ERS and ERL, respectively) as film-forming rate-controlling polymers, with caffeine as a water-soluble model drug. The physicochemical properties of the model films for a series of formulations with increasing concentrations of caffeine were determined in terms of morphology, mechanical and mucoadhesive properties, drug content uniformity, and drug release and associated kinetics. Typically regarded as non-mucoadhesive polymers, ERS and mainly ERL, were found to be good mucoadhesives, with ERL01 exhibiting a work of mucoadhesion (WoA) of 118.9 μJ, which was about five to six times higher than that observed for commonly used mucoadhesives such as Carbopol® 974P (C974P, 23.9 μJ) and polycarbophil (PCP, 17.4 μJ). The mucoadhesive force for ERL01 was found to be significantly lower yet comparable to C974P and PCP films (211.1 *vs.* 329.7 and 301.1 mN, respectively). Inspection of cross-sections of the films indicated that increasing the concentration of caffeine was correlated with the appearance of recrystallized agglomerates. In conclusion, caffeine agglomerates had detrimental effects in terms of mucoadhesion, mechanical properties, uniformity, and drug release at large particle sizes. ERL series of films exhibited very rapid release of caffeine while ERS series showed controlled release. Analysis of release profiles revealed that kinetics changed from a diffusion controlled to a first-order release mechanism.

KEY WORDS: buccal films; caffeine; Eudragit®; mucoadhesive polymer; solvent casting.

INTRODUCTION

The development of films as mucoadhesive dosage forms for buccal delivery of actives is a field that continues to grow due to unique characteristics that are advantageous for drug delivery (1–3). In physical terms, films may be preferred over tablets due to size, flexibility, and comfort (1). As adhesive dosage forms, films can be formulated for a variety of delivery regimens as well providing the opportunity for locally treating diseases by direct application. The buccal route also offers interesting advantages over the oral route mainly for molecules that could be rendered inactive through the gastrointestinal tract, i.e., peptides and proteins. In addition, rapid absorption and peak concentration can be elicited through the venous system that drains from the cheek (4).

Most mucoadhesive films for buccal delivery are manufactured by the solvent casting technique regardless of the growing body of literature describing film manufacture by hot-melt extrusion (5–8). The solvent casting technique is scalable, simple to execute, and cost-effective in the laboratory scale (3). However, this method of manufacture is limited by environmental concerns, due to the use of organic solvents, and additionally long processing times that can impose budget limitations (8). The solvent casting technique involves the solubilization or dispersion of all the ingredients in a suitable solvent system and then controlled drying to yield the drug-containing films. Arising from manufacturing challenges, a recent publication has surveyed the literature regarding drug content uniformity and revealed the lack of reports addressing this issue (9), which is a basic yet an utterly important variable in film manufacture. In the manufacture of films, cast sheets are cut into unit doses which could result in high variability of drug content if this is not addressed adequately during the developmental stages of the formulation. The main concern raised in the literature is the appearance of agglomerates upon drying of films (10). This was attributed to long drying times that allow for attractive forces between molecules to build up and result in the formation of agglomerates and was dealt with the addition of viscosing agents that could prevent agglomeration during drying. In an alternative to this strategy, Perumal *et al.* (9) created casting trays that would allow for the manufacture of unit doses without the need to cut strips from a cast sheet. Even though, this method improves results in terms of content uniformity it does not address uniformity among the

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surface of the single unit, and it could be impractical for scaling up purposes.

Several excipients can be used to control for different properties of the films. Usual materials can include but are not limited to film-forming polymers, mucoadhesive polymers, a backing polymeric layer, plasticizers, taste masking or sweetening ingredients, stabilizers, and rate-controlling polymers (3,11,12). However, the polymer system that controls the release of the active is one of the most prominent areas of development of films. Most recent reports on the use of polymethacrylates as film-forming polymers feature them mainly as a drug-controlling materials in the formulation (13–15). In these studies, Eudragit® polymers have either been part of the drug-containing layer or as part of the release rate-controlling layer. Only a few articles have described the use of Eudragits as a mucoadhesive material (16,17). Eudragit® RS (ERS) and Eudragit® RL (ERL) are polymethacrylates possessing a quaternary ammonium group branching out of their polymer backbone. The presence of these cationic groups allow for water permeability, resulting in swelling of the polymer matrices. In a systematic comparative study, both ERS and ERL were found to be non-mucoadhesive materials with very low adhesion, similar to that determined in the same study for alginic acid and chitosan (18), both of which are normally considered mucoadhesive materials (19). Conversely, a more recent publication by Perumal *et al.* (17) has shown that ERS films can elicit high mucoadhesive properties measured both in terms of maximum detachment force and work of adhesion. Moreover, films containing only ERS exhibited increased mucoadhesive properties compared to those found in ERS-chitosan films. In another study, ERL was found to be the least mucoadhesive material and the polymer that showed the lowest swelling capacity in comparison to HPMC-E15, sodium carboxymethyl cellulose, and Carbopol® 934P (C934P). However, in the same study the *in vitro* residence time was found to be 1.75 h, comparable to that observed for HPMC-E15 (20). One investigation that utilized Eudragit® L100 (EL100) and S100 (ES100) as mucoadhesive materials required prior modification into sodium and potassium salts (16). The modified salt form was used to enhance the mucoadhesive properties of these polymethacrylates by promoting the ionized state of the polymer. As ERL and ERS are cationic polymethacrylates, their mucoadhesive properties could be explained by the positive charge in the polymer structure.

In this investigation we sought to evaluate systematically the performance of ERS and ERL as mucoadhesive polymers to be suitable for the delivery of the water-soluble model drug caffeine. A series of films containing increasing quantities of caffeine revealed the appearance of agglomerates and the effect of these was evaluated in terms of mucoadhesion as well as content uniformity, mechanical properties, drug release, and morphology.

MATERIALS AND METHODS

Materials

Eudragit® RSPO and RLPO (ERS and ERL) were kindly donated by Evonik Industries (Essen, Germany). Carbopol® 974P (C974P) and Noveon® AA-1 Polycarbophil (PCP) were donated by Lubrizol Advanced Materials (Cleveland, OH). Triethyl citrate (TEC; Morflex Inc., Greensboro, NC), mucin (Spectrum Chemical, New Brunswick, NJ), and caffeine (CAF; Sigma-Aldrich, St. Louis, MO) were purchased and used as received. All other chemicals used were of analytical or reagent grade.

Methods

Preparation of Films

For ERS and ERL series of films, polymers were firstly dissolved in an acetone/isopropanol (4:6 ratio) solvent system and then 10% *w*/*w* TEC was added as plasticizer. Increasing quantities of caffeine were added to yield solutions containing 1, 2, 3, 4, or 5% *w*/*w* caffeine. Films made of both ERS and ERL polymers were obtained for each concentration. These solutions were casted on PTFE plates and let to dry overnight at 40°C to yield the final product. Films were peeled off and stored in aluminum foil sachets in a dessicator until characterization. To compare with conventional mucoadhesive materials, films containing C974P and PCP were manufactured similarly. Adequate amounts of the polymers were dissolved in ethanol and then cast in the same fashion as described above.

Morphology of Films

To observe the ultrastructure of films, scanning electron microscopy (SEM) was performed on the surface and cross-sections of films. Samples were obtained by a freeze fracture method to ensure clean-cut edges and to avoid plastic deformation (often resulting from mechanical cutting). Fragments of the surface of the film were frozen by submerging in liquid nitrogen and thus cracked by freezing. Pieces of the films were fixed on aluminum stubs by means of conductive carbon tape. A Cressington 208 HR sputter coater (Cressington Scientific Instruments Ltd, Watford, UK) was used to coat samples with Pt/Pd to a thickness of 10–15 nm in a high vacuum evaporator. A Hitachi S-5500 field emission scanning electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan) was operated for imaging of coated particles. The electron beam voltage was kept at 2–5 kV to avoid structural deformation during imaging (21).

A Bruker 175 EDS Quantax 4010 energy-dispersive spectroscopy (EDS) detector (Bruker Nano, Ewing, NJ) combined with the SEM was used to analyze elemental distribution and two-dimensional mapping of selected elements. Although caffeine and both Eudragit possess the same elements, the concentration of nitrogen in caffeine was known to be higher and was used to elucidate caffeine-rich domains in cross-sections of films.

Mechanical Properties of Films

Using a TA.XTPlus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a 5-Kg load cell, stress *versus* strain curves were obtained and the mechanical properties of film strips were determined. Briefly, rectangular strips of 1×5 cm^2 were cut and 1 cm on each end was held between clamps attached to the texture analyzer, leaving a testing area of 1×3 cm^2 for determination of mechanical properties. The upper clamp (connected to the mobile arm of the texture

analyzer) was moved upwards at a rate of 0.5 mm/s until film failure. Stress is obtained from the force measurements obtained from the instrument divided by the cross-sectional area of the film, while strain is computed by dividing the increase in length by the initial film length. From the plot, the tensile strength (TS) and the elongation at break (EB) are obtained from the peak stress and the maximum strain, respectively, also represented by the following equations (3):

$$\text{Tensile strength (TS)} = \frac{\text{Peak stress}}{\text{Cross-sectional area of film}}$$

$$\text{Elongation at break (EB)} = \frac{\text{Increase in length at break}}{\text{Initial film length}} \times 100$$

Additionally, the elastic modulus (EM) was obtained from the initial elastic deformation region in the stress *vs.* strain plot (22). Since the rate of the mobile arm was constant during the test as well as for all different experiments, direct comparison of the slope in this region can be done. To further evaluate mechanical properties three additional parameters were computed from the conventional mechanical parameters obtained from the plot as follows (23):

$$\text{Tensile strength to modulus ratio} = \frac{\text{TS}}{\text{EM}}$$

$$\text{Relative surface energy (RSE)} = \frac{\text{TS}^2}{2 \times \text{EM}}$$

$$\text{Toughness index (TI)} = \frac{2}{3} \times \text{TS} \times \text{EB}$$

Mucoadhesion of Films

Mucoadhesion tests were conducted on the texture analyzer equipped with a 5-Kg load cell. Briefly, films were held in the horizontal position and 5 μL of model mucus (a freshly made 2% *w/v* mucin solution) was placed on top of the film. This amount is sufficient to mimic the thickness of the average saliva thickness (24). A 7-mm diameter stainless steel cylindrical probe was attached to the mobile arm of the texture analyzer and it was brought in contact with the film and mucin solution, held at an applied force of 50 mN for 15 s and then withdrawn at a 0.5-mm/s rate. Mucoadhesive force (MAF) and work of adhesion (WoA) are obtained from the peak and the area under the curve in the force *versus* distance profile, respectively.

Caffeine Assay

Caffeine concentration in samples obtained above was determined by UV spectroscopy using a μQuant microplate reader (Bio-Tek Instruments, Inc, Winooski, VT). Briefly, 300 μL aliquots were added in each well in the microplate in triplicates. UV absorbance was measured at 273 nm and the concentration was calculated from a calibration curve of a stock solution of caffeine.

Drug Content Uniformity

To measure the average amount of drug loading in the films and to determine homogeneity among the cast surface, film samples were analyzed for caffeine content uniformity. Samples were cut to yield 1×1 cm^2 squares and allowed to release caffeine for 24 h in 15 mL phosphate buffer pH 6.8 in an orbital shaker at 20°C. Aliquots from these vials were analyzed for caffeine content using the UV spectroscopy method described above.

In Vitro Drug Release

Dissolution tests were conducted to determine drug release profiles from Eudragit films. A small vessel USP apparatus I (basket) was used for this purpose and 150 mL phosphate buffer pH 6.8 was used as dissolution media. Film were cut into 1×1 cm^2 samples and dissolved into each vessel with a rotating speed of 25 rpm at 37°C. At intervals of 0, 0.25, 0.5, 1, 2, 3, and 4 h 1 mL samples were withdrawn and replaced with 1 mL of fresh warm media. Caffeine concentration was determined as depicted above using a UV spectroscopy method of quantification. Comparison of the release profiles was performed using the similarity factor, f_2 (25).

Kinetic Analysis of Release Profiles

Kinetic models were used to compare the release mechanisms from the various caffeine-containing films. The Higuchi (26), Korsmeyer-Peppas (27), and first-order kinetic models were used to fit the data and were compared on the basis of r^2 adjusted (28) and the Akaike information criterion (AIC) (29). The evaluation of the drug transport mechanism was addressed in accordance with the Korsmeyer-Peppas model.

Statistical Analysis

All statistical analyses were performed with the software Minitab® Release 14 (Minitab Inc., State college, PA). One-way ANOVAs were used for multiple comparisons and Tukey's post-hoc pairwise comparisons were performed to compare which results led to significant differences. All values are reported as the mean and standard deviation of the mean in parenthesis. For the evaluation of the kinetics models and calculation of adjusted R^2 values the software Origin® 8.0 (OriginLab Corporation, Northampton, MA) was used to perform the non-linear regressions for each equation.

RESULTS AND DISCUSSION

Morphology of Films

SEM images shown in Figs. 1 and 2 reveal that increasing the concentration of caffeine in both ERS and ERL films leads to an increasing appearance of agglomerates in cross-sections of films obtained by freeze fracture. A survey of cross-sections reveals that the use of ERS leads to a higher quantity and larger size of these agglomerates at similar concentrations of caffeine compared to those seen in ERL films. For example, ERS03 reveals a larger number of the needle-like agglomerates compared to ERL03 (Fig. 2). In addition, ERS04 reveals the appearance of larger agglomerates possibly composed of aggregation of the needle-like caffeine crystals observed at lower concentrations, while ERL04 still shows only needle-like agglomerates.

EDS mapping of nitrogen (Fig. 3) on SEM scan fields revealed that the agglomerates consist of caffeine and appear to have an organized crystalline structure, which is also

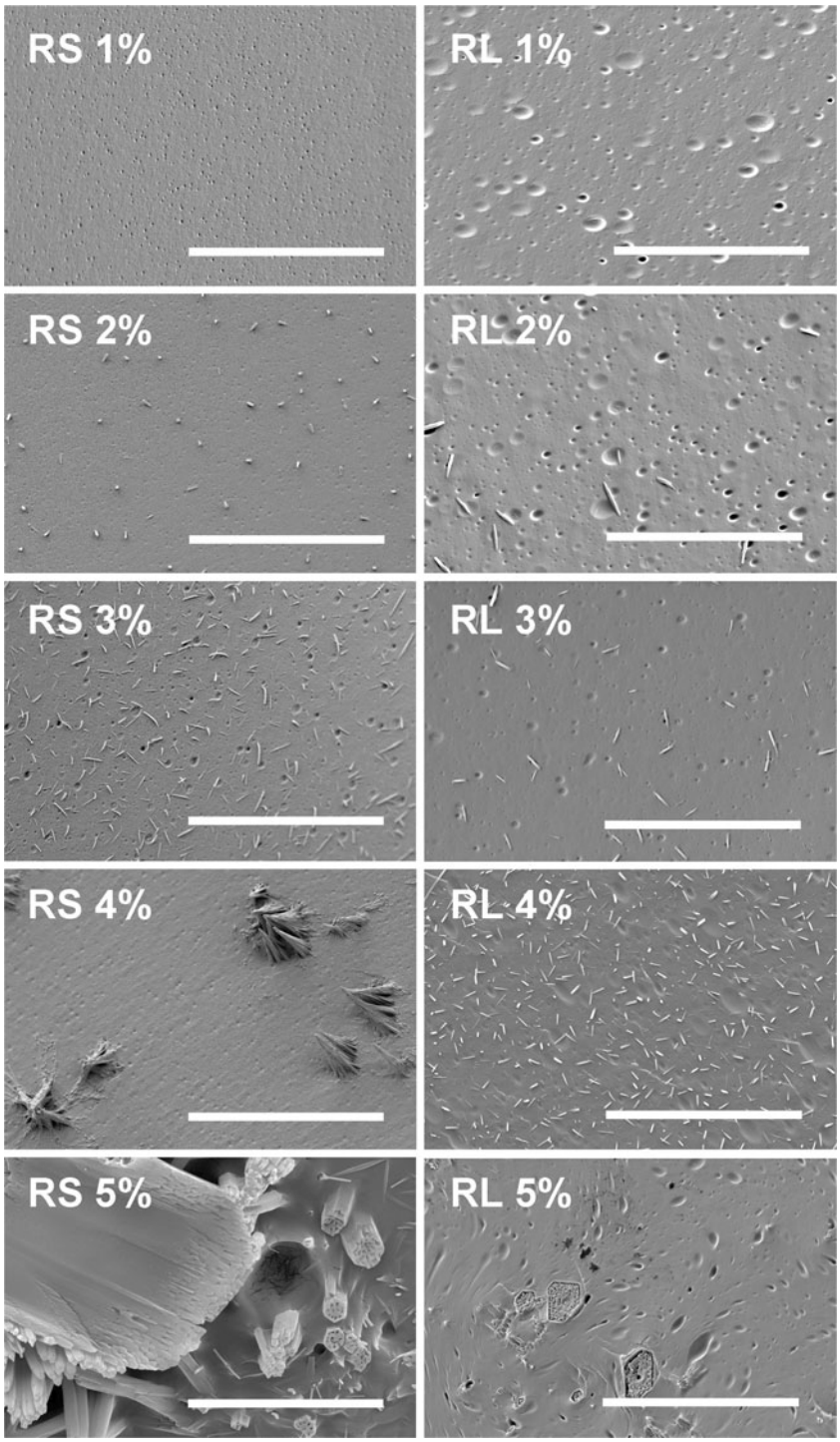

Fig. 1. SEM images of ERS and ERL films at various concentrations of caffeine. *Scale bar* represents 30 μm

appreciated at higher magnification micrographs obtained for formulations with higher content of caffeine (Fig. 2). Even though, the polymer structure possess nitrogen atoms branching out of the backbone, the density of nitrogen atoms is higher in the caffeine molecule than the polymer, thus for the same time of detection of X-rays emitted from the field of view of the sample, the bulk of the signal can be attributed to caffeine (30). The shape of the agglomerates observed in cross-sections of the films is also consistent with caffeine crystals shapes reported in the literature. It has been reported in the literature that when recrystallized from organic solvents, anhydrous caffeine crystals can adopt different space groups in a rhombohedral lattice system including but not limited to R3c and R3 (31,32). These space groups result in hexagonal

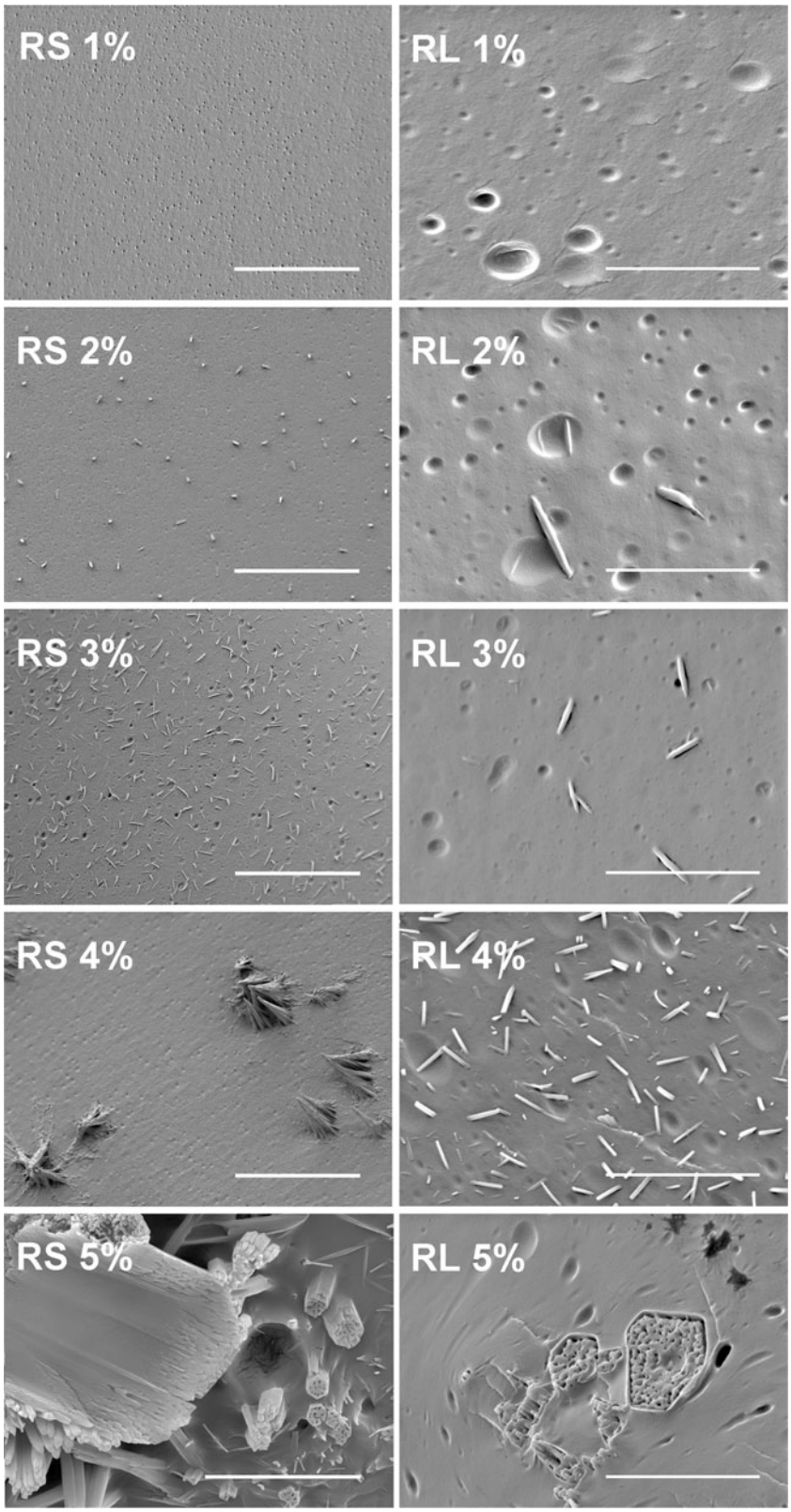

Fig. 2. SEM images of ERS and ERL films at various concentrations of caffeine. *Bar* represents 10 μm

prisms, which concur with the SEM observations. The difference in the extent of caffeine recrystallization and size and number of agglomerates can be attributed to the differences in hydrophillicity elicited by both ERS and ERL (33). Both

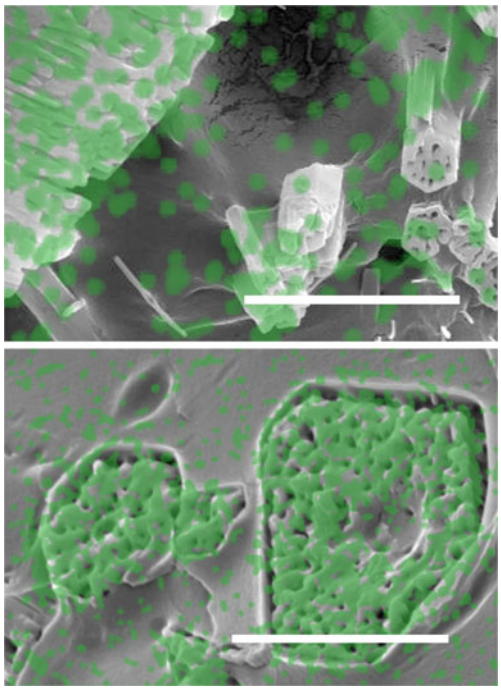

Fig. 3. SEM images merged with EDS mapping for nitrogen (in *green*) showing that caffeine is highly concentrated in the crystalline agglomerates found in ERS05 and ERL05. *Bar* represents 10 μm

polymers, ERS and ERL, are pH-independent and insoluble but swellable in water. This is due to the quaternary ammonium groups that branch out of the polymethacrylate backbone of the polymer structure. The ammonium groups are present as salts and allow for swelling of the polymer. ERL is the more permeable polymer due to its ionic functional group content of about 10%, while the content for ERS is approximately 5% (34). Therefore, ERL can solubilize to a higher extent than ERS, and caffeine contained within the polymer matrix increasingly retards the appearance of large agglomerates with increasing concentrations. A similar effect has been observed by Omari *et al.* (35) where the interaction between lactic acid and ERL and ERS were compared. Lactic acid-containing ERL films revealed a higher extent of interaction by differential scanning calorimetry and nuclear magnetic resonance studies. This effect was attributed to the higher hydrophillicity featured by ERL compared to ERS allowing for a further ionic interaction with the acid. This effect also accounted for an increase in drug permeation when release of paracetamol was studied. It was found that lactic acid clearly modified the release in ERL due to the higher extent of interaction as opposed to ERS films in which the modification of permeation was less pronounced.

Table II. Derived Mechanical Parameters Calculated from Conventional Mechanical Properties Derived from a Stress *Vs.* Strain Plot

Formulation	TS/EM	Relative surface energy	Toughness index
ERS01	4.98 (0.84)a	13.90 (3.23)abcd	515.88 (38.39)abc
ERS02	3.23 (0.53)a	6.25 (1.95)a	391.16 (116.40)def
ERS03	3.14 (0.46)	6.60 (1.97)b	222.07 (5.69)adg
ERS04	3.35 (1.14)	6.91 (3.27)c	96.82 (34.12)be
ERS05	3.16 (0.54)	3.95 (0.91)d	58.53 (28.48)cfg
ERL01	3.31 (0.65)	2.38 (0.71)	233.04 (27.10)ab
ERL02	3.29 (0.23)	1.80 (0.07)	204.80 (33.14)cd
ERL03	3.14 (0.71)	1.17 (0.37)	136.80 (8.07)ac
ERL04	2.94 (0.57)	1.88 (0.63)	186.94 (41.36)e
ERL05	2.44 (0.39)	1.86 (0.45)	94.53 (15.45)bde

Values are represented as average and standard deviation in parenthesis. Among parameters and between series of formulations, statistically significant differences are paired by the same letters ($p<0.01$)

Mechanical and Mucoadhesive Properties

The mechanical properties of films as solid dosage forms are of great importance since they account for the ability of a film to withstand various sources of stress. Initially, films need to withstand the stress imposed by the manufacturing, handling, and administration (17). Additionally, films for buccal delivery need to be able to remain in contact with the mucosa for as long as the delivery of the active is ongoing (36). This involves mechanical stress originating from various mouth activities. Therefore, films are preferred to exhibit a relatively high TS, EB, and a low EM (36). In addition, regarding derived mechanical parameters, a relatively high TS/EM, RSE, and TI are desired (23,35).

From stress *vs.* strain curves, TS, EB, and EM were obtained and the derived magnitudes of TS/EM, RSE, and TI were computed for each sample and are summarized in Tables I and II. TS/EM is a measure of the level of internal

Table I. Mechanical Properties of Formulations from ERS and ERL Series

Formulation	Tensile strength/N/mm^2	Elongation at break/%	Elastic modulus/N/mm^2/%
ERS01	5.71 (1.72)a	142.19 (35.46)ab	1.19 (0.46)
ERS02	3.62 (0.61)	162.40 (44.06)cde	1.18 (0.04)
ERS03	4.16 (0.86)	82.88 (20.74)c	1.33 (0.26)
ERS04	4.04 (0.83)	35.30 (6.24)ad	1.27 (0.36)
ERS05	2.48 (0.14)a	35.82 (17.99)be	0.80 (0.09)
ERL01	1.51 (0.19)ab	233.04 (23.85)a	0.43 (0.05)ab
ERL02	1.17 (0.13)acd	262.21 (34.06)b	0.34 (0.05)
ERL03	0.75 (0.06)bcef	275.23 (35.84)cd	0.24 (0.03)a
ERL04	1.26 (0.17)e	221.83 (30.38)ce	0.43 (0.04)
ERL05	1.51 (0.14)df	93.41 (9.65)abde	0.63 (0.07)b

Values are represented as average and standard deviation in parenthesis. Among parameters and between series of formulations, statistically significant differences are paired by the same letters ($p<0.01$)

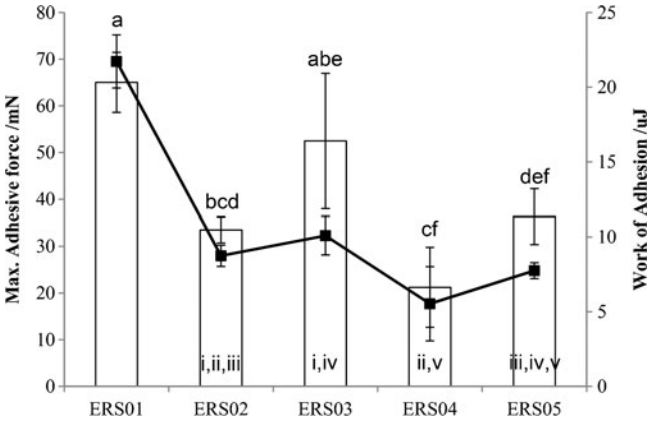

Fig. 4. Mucoadhesive properties of ERS films: maximum adhesive force (*white square*) MAF, with non-significant differences indicated in pairs of letters (*a–f*); and work of adhesion (*black square*) WoA, with non-significant differences indicated in pairs of roman numerals (*i–v*)

stress in a film. The larger its value the higher the film crack resistance. RSE is also utilized to estimate crack resistance and is approximated from the surface energy of the film. Finally, toughness index (TI) is an estimation of energy absorbed per unit volume of film under stress (23). In Table I, it can be evidenced that films from the ERL series have a significantly lower TS and EM, but a higher EB than each of the corresponding ERS film, indicating that ERL is a softer and more elastic material than ERS. However, when both TS and EB are taken into account as TI we can observe that the increase in EB for ERL compensates the decrease in TS yielding tough films at all concentrations of caffeine except for ERL05. Additionally, analysis of TI also reveals that ERS04 and ERS05 are less tough films, which is not evident by a direct analysis of conventional mechanical parameters (35). Results of TS, EB, and EM indicated a significant difference on both ERS05 and ERL05, as well as ERS04 with respect of EB. As discussed above, as concentration of caffeine increases the capacity of the polymer to dissolve the drug content reaches a saturation point; allowing for recrystallization. It has been suggested in the literature that unsolubilized drug, which in our case would result in recrystallization, can physically interrupt the polymer matrix resulting in hard and brittle films (37). This is also consistent with inspection of ERS04 micrographs in which we can observe large agglomerates, similar to those found in ERL05.

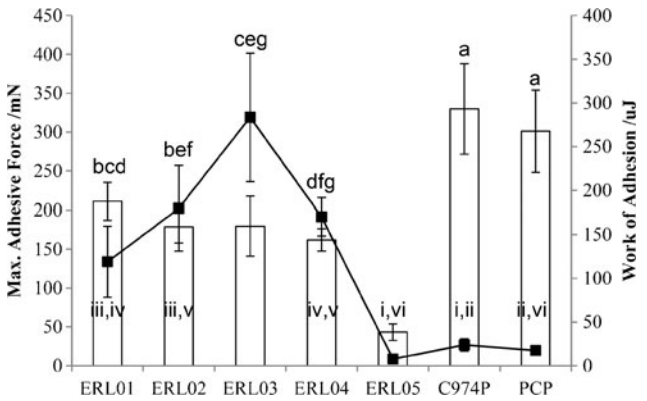

Fig. 5. Mucoadhesive properties of ERL films and C974P and PCP as conventional mucoadhesive polymers: maximum adhesive force (*white square*) MAF, with non-significant differences indicated in pairs of letters (*a–g*); and work of adhesion (*black squares*) WoA, with non-significant differences indicated in pairs of roman numerals (*i–vi*)

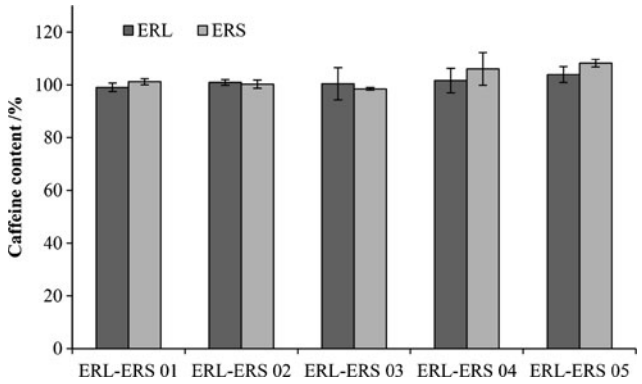

Fig. 6. Caffeine content uniformity for ERL and ERS series. *Darker grey columns* represent the ERL series, while the *lighter grey columns* represent the ERS series. Values (mean ± standard deviation, n=4–6) are reported as percentages of the theoretical amount of caffeine in each sample studied. Differences among all ten formulations are not statistically significant ($p>0.05$)

Since ERS and ERL are both water-insoluble polymers and they are normally regarded in the literature as non-mucoadhesive materials (16,18). The results observed in Figs. 4 and 5 reveal that the mucoadhesive properties of ERS are very limited both in terms of MAF and WoA and comparatively always lower than their ERL counterparts. Only when caffeine is in a solid solution with the polymer (ERS01) a significantly higher MAF of 65.04±6.44 mN is found compared to other ERS formulations ($p<0.05$), although in comparison with the more hydrophilic ERL, MAF is much lower (211.11±24.29 mN for ERL01). Conversely, ERL is highly mucoadhesive under the test conditions utilized here. This is not surprising when we consider that even though the polymers are water-insoluble they are swellable in water due to the presence of the quaternary nitrogen groups. The ability of hydrophilic polymers to swell in water is a common characteristic in materials that are generally recognized as mucoadhesive, and is consistent with several of the theories of mucoadhesion (38–40). In saliva, the most relevant component to mucoadhesive interactions is mucin which is the main

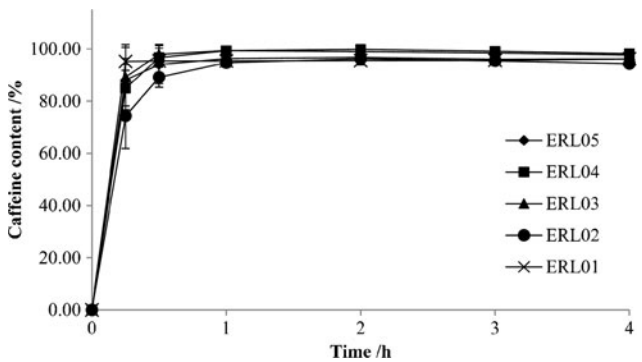

Fig. 7. Drug release profiles for ERL series in phosphate buffer pH 6.8 at 37°C, showing (*diamonds*) ERL05, (*black squares*) ERL04, (*triangles*) ERL03, (*circles*) ERL02, and (*crosses*) ERL01. Values are presented as mean ± standard deviation, n=6

Table III. Differences Among Formulations of ERS and ERL Series Based on the Similarity Factor, f_2

f_2	ERS05	ERS04	ERS03	ERS02	ERS01
ERS01	21.0	41.2	48.0	37.7	–
ERS02	12.9	25.0	54.3	–	
ERS03	16.1	30.4	–		
ERS04	26.8	–			
ERS05	–				
f_2	ERL05	ERL04	ERL03	ERL02	ERL01
ERL01	73.5	56.9	61.9	51.1	–
ERL02	58.6	52.0	50.3	–	
ERL03	66.9	81.2	–		
ERL04	64.0	–			
ERL05	–				

Release profiles are similar if $f_2 \geq 50$

component in our saliva model. Mucins are composed of a protein core and carbohydrate side chains, which are responsible for the non-covalent bonding that occurs when a mucoadhesive material is brought in contact with mucosa (41,42). According to the diffusion theory (38) interpenetration and entanglement between polymer chains (mucin and mucoadhesive material) is believed to be the main reason for mucoadhesive bonding. Control experiments utilizing only the mucus model and the stainless steel probe revealed very little contribution of the mucus–steel interface to the measured force (MAF equals 12.96±1.95 mN and WoA equals 2.70±0.28 μJ). Use of the same experimental set up revealed that the extent of mucoadhesion found with ERL is comparable to that of typical mucoadhesive materials, namely C974P and PCP (Fig. 5) (19,43). Particularly, the formulation exhibiting the highest MAF (ERL01) is about 30% significantly lower than both C974P and PCP (211.1 *vs.* 329.7 and 301.1 mN, respectively). It was found however that the WoA was about 80% significantly higher than conventional mucoadhesive materials (118.9 *vs.* 23.9 and 17.4 μJ), demonstrating that a highly swellable polymer, such as ERL, regardless of being water-insoluble, can elicit strong mucoadhesiveness based on its capacity for entanglement. The various films in the ERL series exhibit high WoA and high MAF when the drug is solubilized in the polymer or small micron size agglomerates are found (ERL01–ERL04); however, the highest concentration of caffeine that renders large recrystallized agglomerates results in a significant decrease of both mucoadhesive variables. This is also in agreement with findings discussed above in terms of morphology and mechanical properties.

The consistent decrease of mucoadhesive and mechanical properties as concentration of caffeine increased led us to investigate the existence of a correlation between the two. After a linear regression analysis, the data shows a strong positive correlation between EB and MAF regardless of the polymer type (r=0.9). Although further investigation would be required on this topic, particularly in isolating variables to allow for a more accurate evaluation, there could be a connection between elasticity of films and measurement of mucoadhesion by the method utilized here. This could be explained as follows: stiff films will not be able to deform enough to allow for a prolonged contact during detachment; this therefore, results in less force needed to break the detachment. More ductile films will be able to support the mucoadhesive bond for longer and will require larger inputs of energy for detachment. This is further corroborated by a strong correlation between EB and WoA for ERL (r=0.9) indicating the possibility for such interaction between mechanical and mucoadhesive properties for films as dosage forms.

Drug Content Uniformity, Drug Release, and Kinetics

The increase of caffeine in films was correlated with an increase in heterogeneity of drug distribution in the casting surface of films as can be depicted in Fig. 6. Up to a content of 2% *w/w* caffeine, films exhibit very high drug content uniformity (relative standard deviation ≤1.7%), while at higher concentrations heterogeneity is evident. This is in accordance with the ultrastructure of films obtained by SEM. Both ERS03 and ERL03 have more numerous agglomerates of caffeine which are not seen to be uniformly distributed when panning with the microscope is performed across larger areas (Fig. 1). A similar situation is found with higher concentrations of caffeine in addition to the appearance of larger recrystallized agglomerates of caffeine, which contributes to the loss of homogeneity. As hinted above, the extent of the drying times as been acknowledged in the literature as one factor that will allow for particle agglomeration (9,10,44). Strategies such as the addition of gelling and viscosing agents, increasing the rate of drying, and/or casting in unitary wells have all been addressed in the literature as means to increase uniformity and could allow us to improve uniformity at higher concentrations of caffeine.

Due to the high permeability to water of ERL no differences could be evidenced in release profiles, and almost complete release of the drug, regardless of the concentration, was achieved after 30 min (Fig. 7). Using the similarity factor, f_2 (25), it was determined that all of the release profiles were similar (f_2 >50%, Table III). Conversely, all the release profiles for the ERS series, except between ERS02 and ERS04, were different between each other per f_2 (data not shown). ERS behaved as expected from the literature allowing for controlled release of caffeine at every concentration studied as depicted in Fig. 8 (45). As the concentration of caffeine increased the rate of drug release increased as well. This

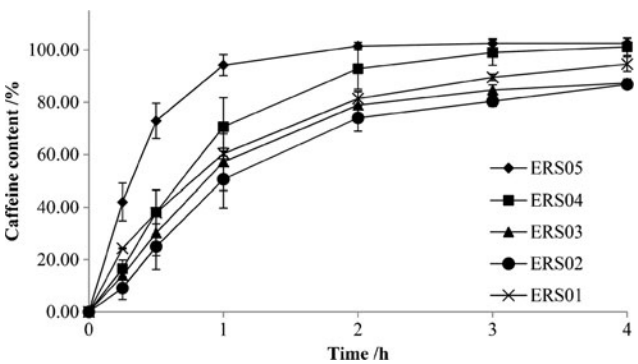

Fig. 8. Drug release profiles for ERS series in phosphate buffer pH 6.8 at 37°C, showing (*diamonds*) ERS05, (*black squares*) ERS04, (*triangles*) ERS03, (*circles*) ERS02, and (*crosses*) ERS01. Values are presented as mean ± standard deviation, n=6

Table IV. Model Parameters, Adjusted R^2, and Akaike Information Criteria (AIC) Values for ERS Series

	Korsmeyer-Peppas $Q = k \times t^n$				Higuchi $Q = k \times t^{0.5}$			First order $Q = k \times (1 - e^{-nt})$			
Formulation	K	n	Adj R^2	AIC	k	Adj R^2	AIC	k	n	Adj R^2	AIC
ERS01	0.637	0.660	0.9998	10.09	0.604	0.9912	10.15	0.876	1.288	0.9966	9.29
ERS02	0.586	1.119	0.9876	10.77	0.540	0.9391	37.07	1.090	0.693	0.9849	29.36
ERS03	0.657	0.973	0.9957	6.86	0.602	0.9409	29.45	5.993	0.116	0.9986	6.10
ERS04	0.703	0.974	0.9896	12.15	0.625	0.9421	31.10	5.854	0.127	0.9964	11.66
ERS05	0.940	0.528	0.9125	20.40	0.929	0.9873	18.61	1.055	2.106	0.9964	13.92

can be attributed to a faster penetration of the water front through the polymer by dissolving agglomerates rather than displacing caffeine molecules from the polymer matrix (ERS01) (46).

In Table IV, it is interesting to note that as the concentration of caffeine increases the release mechanism model that best explains the data (by comparison of the adjusted R^2 and AIC) changes from a diffusion-controlled mechanism (Korsmeyer–Peppas kinetics model) to a first-order mass balance (first-order model). In the Kormeyer–Peppas release kinetics model, n is the release exponent, and is an indicative of the drug release mechanism (27). In the particular case of n equal to 0.5 the drug release mechanism is purely Fickian diffusion (the particular solution that constitutes the Higuchi model equation). When n equals 1 the equation describes a zero-order release mechanism, and the region ranging from $0.5<n<1$ represents the so-called anomalous transport. The first-order kinetics applies to dosage forms that normally contain water-soluble drugs and porous polymer matrices. In said systems, drug release is proportional to the amount of drug remaining inside; therefore, the rate of drug release decreases with time. In accordance with the Korsmeyer–Peppas model, all except for ERS02 follow an anomalous transport implying that drug is transported by a combination of diffusion and case-II transport, characteristic of systems swelling in water (Table IV). ERS02 follows what has been described as a super case-II transport mechanism (28) and has been attributed to the result of an increased plasticization at the relaxing boundary (gel layer) (47,48).

CONCLUSION

In contrast with what has been previously reported in the literature, we have found that ERS and more noticeably ERL have substantial mucoadhesive properties. This was further corroborated by direct comparison with materials typically regarded in the literature as being good adhesives, namely Carbopol 974P and Polycarbophil. In accordance with the diffusion theory of mucoadhesion, this was attributed to the swelling capacity of these polymers due to the presence of quaternary ammonium groups that increase hydrophilicity. Additionally, we have found through direct observations under the microscope that increasing concentrations of caffeine in ERS and ERL matrices yielded recrystallized agglomerates. These agglomerates increase in number and size due to solubility saturation as the concentration of caffeine was increased, which translated not only in a detriment of the mucoadhesive properties, but also in reduced mechanical and uniformity properties in the film. Finally, it was shown that the presence of these agglomerates changes the release kinetics of the films from a diffusion-controlled mechanism to a first-order mass balance with the increased caffeine loading.

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