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Rheological and functional characterization of new antiinflammatory delivery systems designed for buccal administration

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Abstract

The aim of the present paper was to investigate the influence of different formulation parameters on the rheological and functional properties of emulgels (gelified emulsions), intended for the buccal administration of the antiinflammatory drug flurbiprofen. The influence of formulation parameters, such as (a) the amount of gelling polymeric emulsifier (Pemulen® 1621 TR-1) used, (b) the oil to water ratio present in the O/W emulgel and finally (c) the pH of the formulation, was studied by a experimental design (DoE) approach. Formulations were analyzed in term of size and morphology of the internal semi-solid oil droplets as well as in term of rheological properties in the presence or in the absence of flurbiprofen by "shear stress *vs*. shear rate tests" and "frequency sweep tests". Emulgels were also characterized *in vitro* both by bioadhesion tests and release studies. In particular release studies demonstrated that flurbiprofen is released by the emulgels in a controlled manner, the drug release efficacy within the first 100 min was comprised between 50 and 80% of the total amount of the drug. Finally, *in vivo* tests on healthy volunteers have demonstrated that emulgels were able to remain on buccal mucosa for an average period of 1 h, moreover emulgels did not have bad taste and volunteers referred that were agreeable and pleasant.

Keywords: Buccal delivery; Mucoadhesion; Emulgels; Flurbiprofen; Rheology

1. Introduction

In recent years, there has been an increasing interest on the study and treatment of oral cavity diseases. The inflammatory status of oral tissue is in fact not only involved in a number of local pathologies, including gingivitis, periodontitis, oral ulcers and apthous stomatitis (Montagna et al., 2000), but represents also a risk factor for a number of systemic diseases such as: metabolic disorders (*e.g.*, diabetes), blood dyscrasias (*e.g.*, leukemia) and autoimmune disease (*e.g.*, pemphigus). Pregnancy, vitamin shortage and drug side effects are often the causes of a great part of oral mucosa inflammations, that are often treated with non-steroidal antiinflammatory drugs (NSAID) (Palazzo et al., 1996; Heasman et al., 1993). The NSAID therapy of oral diseases was mainly based on two different approaches: extensive local treatment with topical formulations and systemic administration. The topical formulations commonly employed (mouthwash, sprays, gels, lozenge) are often characterized by a limited drug retention time in oral cavity, due to its self-clearing mechanism and various mechanical movements. Therefore numerous and repeated administrations are required in order to obtain effective drug levels.

Mucoadhesive semi-solid formulations could overcome the problem of scarce bioavailability by allowing the application of the drug at the pathological site, thereby increasing the contact time between formulation and mucosa (Bonacucina et al., 2006).

In a recent review on the effects of selective and non-selective NSAIDs on the treatment of periodontal diseases (Salvi and Lang, 2005), the authors have stated that the development of topical NSAIDs formulations with a daily application seems to be of particular interest. This may help to further reduce adverse systemic effects of non-selective NSAIDs in the long-term host modulation of periodontitis-susceptible patients.

In this respect, semi-solid formulations can possess a high biocompatibility and bioadhesivity, allowing adhesion to the mucosa in the dental pocket; finally, they can be rapidly elimi-

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Table 1
Effect of emulgel composition on the size and size distribution of internal oil droplets

Formulation	Polymer (%, w/v)	O/W ratio (w/w)	pH	Mean diameter ± S.D. (μm)
#1	0.3	0.5	5.5	33.8 ± 14.5
#2	0.3	0.5	6.5	8.6 ± 2.6
#3	0.3	1.0	6.0	23.3 ± 9.0
#4	0.3	1.5	5.5	22.5 ± 18.3
#5	0.3	1.5	6.5	33.4 ± 20.3
#6	0.4	0.5	6.0	32.6 ± 14.1
#7	0.4	1.0	5.5	26.1 ± 10.4
#8	0.4	1.0	6.0	19.5 ± 6.3
#9	0.4	1.0	6.5	29.9 ± 8.6
#10	0.4	1.5	6.0	33.7 ± 21.4
#11	0.5	0.5	5.5	16.6 ± 6.6
#12	0.5	0.5	6.5	13.7 ± 6.3
#13	0.5	1.0	6.0	37.5 ± 17.4
#14	0.5	1.5	5.5	21.3 ± 9.0
#15	0.5	1.5	6.5	15.2 ± 9.7

nated through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site.

The aim of this study was to prepare and characterize new mucoadhesive semi-solid formulations (O/W emulsions, emulgels) designed for flurbiprofen (FLUR) administration. This drug is a member of the phenylalkanoic acid derivative family of NSAIDs.

The topical formulations, here described, are based on the polymeric emulsifier Pemulen® 1621 TR-1 (Acrylates/C10-30 alkyl acrylate crosspolymer).

Pemulen® polymeric emulsifiers are high molecular weight, cross-linked copolymers of acrylic acid and a hydrophobic comonomer. This chemical structure allows these compounds to function as primary emulsifiers in oil-in-water emulsions. In fact the lipophilic portions of the polymer adsorb at the oil–water interface, while the hydrophilic portions swell in water forming a gel network around oil droplets to provide exceptional emulsion stability to a broad range of oils.

For the production of the emulgels, as internal phase of the O/W emulsions, was employed the solid, neutral lipid glycerol behenate (Compritol®888ATO) that is widely used as an excipient in many pharmaceutical dosage forms including sustained release tablets and capsules.

In particular, this paper describes: (a) the formulation study of the flurbiprofen-containing semi-solid emulgel formulations, (b) the rheological characterization of emulgels, (c) the *in vitro* release kinetics of flurbiprofen from emulgels and finally (d) the evaluation of their *in vivo* performances in the treatment of oral inflammations.

2. Materials and methods

2.1. Materials

Pemulen® 1621 (TR-1), was a gift from BF Goodrich Company (Ohio, U.S.A.); Compritol®888 ATO was a gift from Gattefossè (Milano, Italy), ultrafiltered water was prepared by QI system (Billerica, U.S.A.); flurbiprofen was provided by Angelini (Ancona, Italia). All other materials were of reagent grade. Froben®, Benactiv®Gola, Alovex®gel, Gengigel®, Daktarin®gel, Corsodyl®dental gel, Dentosan® parodontal gel, present in italian market, were purchased from a pharmacy.

2.2. Preparation and experimental design analysis of emulgels

Emulgel formulations were prepared by a three step method: (i) polymer dispersion in water, (ii) neutralization of the polymeric aqueous dispersion, and (iii) emulsification of the oil phase. With respect to the first step, three different TR-1 percentages, namely 0.3, 0.4 and 0.5%, w/v, were used. The polymer was suspended in deionized water under stirring at 900 rpm, for 20 min, at room temperature using a mechanical stirrer equipped with a three blade helical impellers (DLS VELP® Scientifica). The resulting slurry was neutralized by a NaOH solution (18%, w/v) to a final pH value of 5.5, 6.0 and 6.5. The neutralization process caused the distension of polymer chains resulting in a clear stable gel. In order to obtain a complete polymer hydration, the gels were stored at 4 °C for 24 h before the addition of the oil phase. Successively different amounts of oil phase (O), constituted by Compritol®888ato, were slowly added to the gels (W, water phase), at three O/W ratios (w/w): 0.5, 1.0 and 1.5, respectively. The addition was performed under stirring at 800 rpm at 80 °C. After cooling at room temperature, the pH value was measured (Table 1).

The effect of the main experimental parameters (concentration of polymer, O/W ratio and pH) on the dimensional characteristics of the internal phase of the emulgels, was studied by DoE approach, based on a randomized central composite face-centered design (CCF), consisting of 17 runs. The parameters were varied as reported in the experimental matrix (see Table 1). The experimental design and the evaluation of the experiments were performed by a PC software (MODDE 8.0, Umetrics AB, Sweden), followed by multiple linear regression (MLR) algorithms.

2.3. Emulgel morphological characterization

The morphology, size and size distribution of the internal phase of emulgel (oil phase droplets) were evaluated by optical stereomicroscopy, using a Nikon SMZ 1500 Stereomicroscopy (Nikon, U.S.A.) equipped with a digital camera. The size and size distribution of the oil droplets were determined by photomicrographs analysis (Eclipsnet 1.20.0), considering at least 300 microdroplet/samples.

2.4. Rheological studies

Rheological measurements were carried out by means of a controlled stress rheometer Stresstech HR (Reologica Instruments, AB Milano, Italy) equipped with a cone-plate geometry (diameter of 40 mm and angle 1°). Formulations were characterized by viscometry and oscillation measurements (viscosity at 25 and 37 °C, yield stress at 25 °C and oscillation stress sweep at 37 °C). The samples were applied to the lower plate using a spatula to ensure that formulation shearing did not occur.

As preliminary experiment, the linear viscoelastic region of the formulations, was determined, using a frequency of 1 Hz and at stress value comprised between 0.1 and 100 Pa. The frequency sweep measurements were performed using the frequency range 0.1–10 Hz and the stress value previously determined in the linear viscoelastic region.

2.5. Mucoadhesion studies

The *ex vivo* adhesion strength was measured by determining the force needed to pull out the emulgels from mucosa. Porcine oral mucosa was obtained from Large White pigs (furnished by veterinary service of USL N 1 Città di Castello, Perugia-Italy). Before measurements, the mucosa was washed with physiological solution, stored at 4 °C, and used within 12 h from pig death. Bioadhesion strength was assessed by a dynamometer (Perioli et al., 2007) (Lehrmittelbau, Bonn, Germany) after fixing the porcine oral mucosa to the upper support connected to the dynamometer by cyanoacrylate glue. The formulation was kept in a vessel placed in a thermostatic bath at 37 °C (±0.1). The free surface of oral mucosa was firstly wetted with 100 μl of simulated salivary fluid (pH 6.75) and then was put in contact with the formulation for 30 s. Simulated saliva solution was prepared by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4, 8.00 g of NaCl and H_3PO_4 for adjusting the pH at 6.75) in 1000 ml of distilled water (Peh and Wong, 1999). The adhesive force data represent the average of three experiments (on three emulgel independent batches) performed in triplicate.

2.6. In vitro release studies

In vitro FLUR release was assessed by Franz diffusion cells, (PermeGear, Inc., Bethlehem, PA, diameter 20 mm) constituted by a water jacketed receptor chamber (15 ml) and a donor chamber. The receptor phase (simulated salivary fluid) was maintained at 37 °C and constantly magnetically stirred at 600 rpm. The two chambers were separated by a cellulose membrane (Filter paper Whatman 41, 20–25 μm, Whatman GmbH, Dassel, Germany). Each formulation (200 mg) was loaded into the upper donor chamber and that was successively sealed with parafilm®. At regular time intervals, samples of the receiving phase were withdrawn, and the drug content was spectrophotometrically determined at λ_{max} of 245.0 nm by a UV–vis spectrophotometer (UV-Visible Agilent model 8453) by a previously constructed FLUR calibration curve ($r = 0.9894$). The drug percentage released at each time point was expressed as a fraction of the total amount of the formulation FLUR. The experiments, in triplicate, were carried out in sink conditions.

2.7. In vivo evaluation of organoleptic properties and acceptability

In vivo studies were performed upon achievement of the approval of the Ethic Committee of the Aziende Sanitarie dell'Umbria (CEAS) on 18 healthy volunteers and upon their written consent. The procedures followed were in accordance with the ethical standards of the responsible Committee on human experimentation (regional) and with the Helsinki Declaration. Volunteers were divided into six groups and instructed to use the preparations. The used formulations were the drug loaded emulgels #6, #7, #11, #12 and two comprovate effectiveness market formulations, containing FLUR: Benactiv®Gola 8.75 mg lozenges and Froben® 0.25% mouthwash.

The 18 volunteers were divided into six groups. Four groups were instructed to apply on the lower gums emulgel 1.75 g (corresponding to 8.75 mg of FLUR) through a polypropylene syringe, without needle, with central luer 23G. Benactiv®Gola (containing 8.75 mg of FLUR) was administered to the fifth group and in this case lozenges were simply dissolved in the buccal cavity and not applied on the gum. Froben® 0.25% mouthwash (3.5 ml, containing 8.75 mg of FLUR) was administered to the sixth group. These last volunteers were instructed to maintain this amount in the buccal cavity (oral wash) for 2 min and then to eliminate it. Volunteers were invited to refer about possible irritation, bad taste, dry mouth or excessive salivation and all other parameters able to influence patient usability. Volunteers did not consume food and water from 30 min before the study and fasting was strictly observed during all the experiment (Ali et al., 2002).

3. Results and discussion

New FLUR emulgel formulations, suitable to prolong the contact between drug and buccal mucosa, were designed, produced, characterized and compared to commercial products. Emulgels were chosen since they resume the favourable characteristics of both O/W emulsions (in term of viscosity, consistency and drug release) and those of hydrophilic gels (manageability and palatability).

3.1. Production of emulgels: DoE approach

In a preliminary phase of the study, DoE optimisation and screening of the experimental parameters were performed.

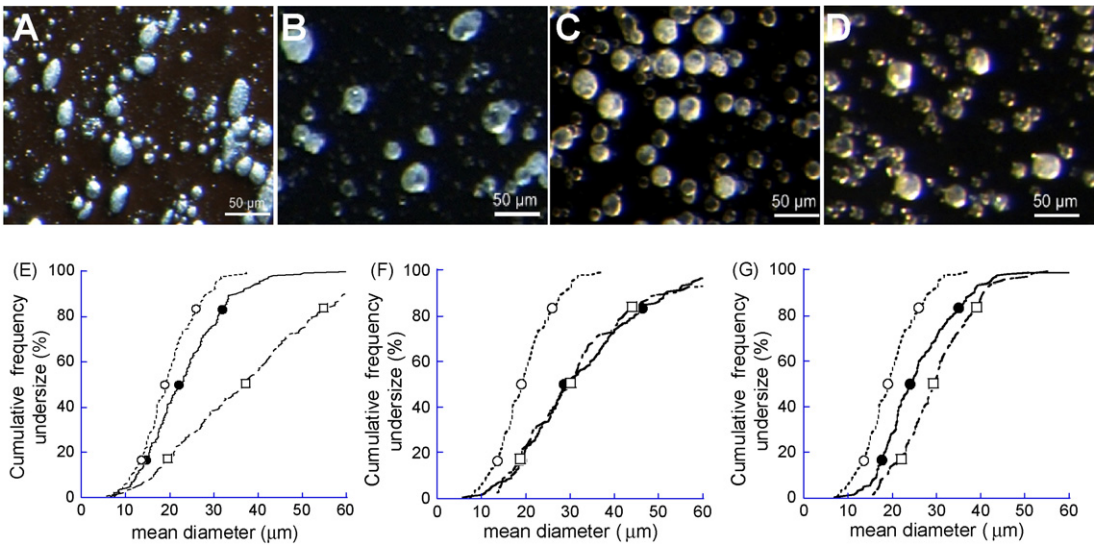

Fig. 1. Morphological and dimensional characterization of emulgel semi-solid formulations. Panels A–D report the stereo photomicrographs showing the morphology of the internal oil phase droplets; formulation #6 (A), #7 (B), #11 (C), #12 (D). In panels E–G are reported the cumulative undersize distribution plots for the emulgel formulations prepared respectively varying: the content of the polymeric emulsifier Pemulen® 1621 TR-1 (E): 0.3 (filled circles), 0.4 (open circles) and 0.5% (w/v) (open squares), the solid, neutral lipid glycerol behenate (Compritol®888ATO) (F): 0.5 (filled circles), 1.0 (open circles) and 1.5 O/W ratio, w/w (open squares) and pH (G): 5.5 (filled circles), 6.0 (open circles) and 6.5% (w/v) (open squares). For the complete formulation composition see Table 1. Size distribution data represent the average of three independent determinations made on two different batches for each formulations.

The DoE approach aimed to yield the maximum amount of information from the minimum number of experiments, in a rational, statistical fashion. To perform the factorial design test, the parameters "polymer concentration", "O/W ratio" and "pH" were chosen as variables and tested at three levels (Table 1).

Since three variables for each three levels require 27 possible combinations for a full factorial design, we selected 14 experiments for a randomized CCF which requires fewer trials. We also added three center points in order to have an estimation of the experimental error. The experimental runs were carried out in random order and the response surface function was estimated from the experimental results. As it is clearly evident from the microphotographs of emulgels (formulations #6, #7, #11 and #12) reported in Fig. 1, the size of the internal droplet phase, shows a relatively wide variation in response, ranging from a minimum mean diameter of 8.69 to a maximum of 33.87 μm.

This variability in droplet size is also confirmed by the complete cumulative size distribution analysis reported in Fig. 1E–G.

Data reported in Table 1 show the significant effect of the formulation variables, and their interactions, on the oil droplet size of emulgels. By examining the results, the main observation was that a change in pH value from low to high level (5.0–6.5) results in an substantial decrease of the droplets size (Fig. 2B and C). The interaction between the two variables "pH" and "O/W ratio" shows a positive effect on the droplet size, leading, as expected, to a decrease of the mean size when the pH is high and the O/W ratio is low.

3.2. Viscometry studies

3.2.1. Viscosity

Viscometry studies were carried out in order to investigate formulation flow properties and qualitative/quantitative polymer influences. The measurements (shear stress *vs*. shear rate) were performed at 25 °C, to simulate storage conditions, and at 37 °C in order to reproduce the mouth application environment. Viscosity curves, reported in Fig. 3, show the influence of pH, polymer (TR-1) and oil phase (Compritol®) percentages, on the emulgel flow properties.

The data suggest that the formulation viscosity is mainly influenced by the amount of the internal oil phase. Increasing the Compritol® content increases the viscosity: in fact formulation #2 showed a much lower viscosity than formulation #5 (Fig. 3C). This trend is confirmed by observation of viscous flows of other formulations containing different Compritol® percentage.

As a general consideration, the changes in formulation viscosity were mainly attributed to the size of the internal droplet phase. It was possible to note that viscosity increased when diameter value decreased, perhaps because of the higher contacts between the two phases.

As expected, also the polymer concentration plays a fundamental role in influencing the emulgel viscosity. Rheogram in Fig. 3B showed that by increasing Pemulen® content the viscosity grows (Brisaert and Plaizer-Vercammen, 1997), *e.g.* #1 viscosity was lower than #11. This general trend is easily confirmed by comparing other formulations varying in polymer content.

The pH too, even though to a lower degree, affects the rheological properties of emulgels. Increasing pH value, the

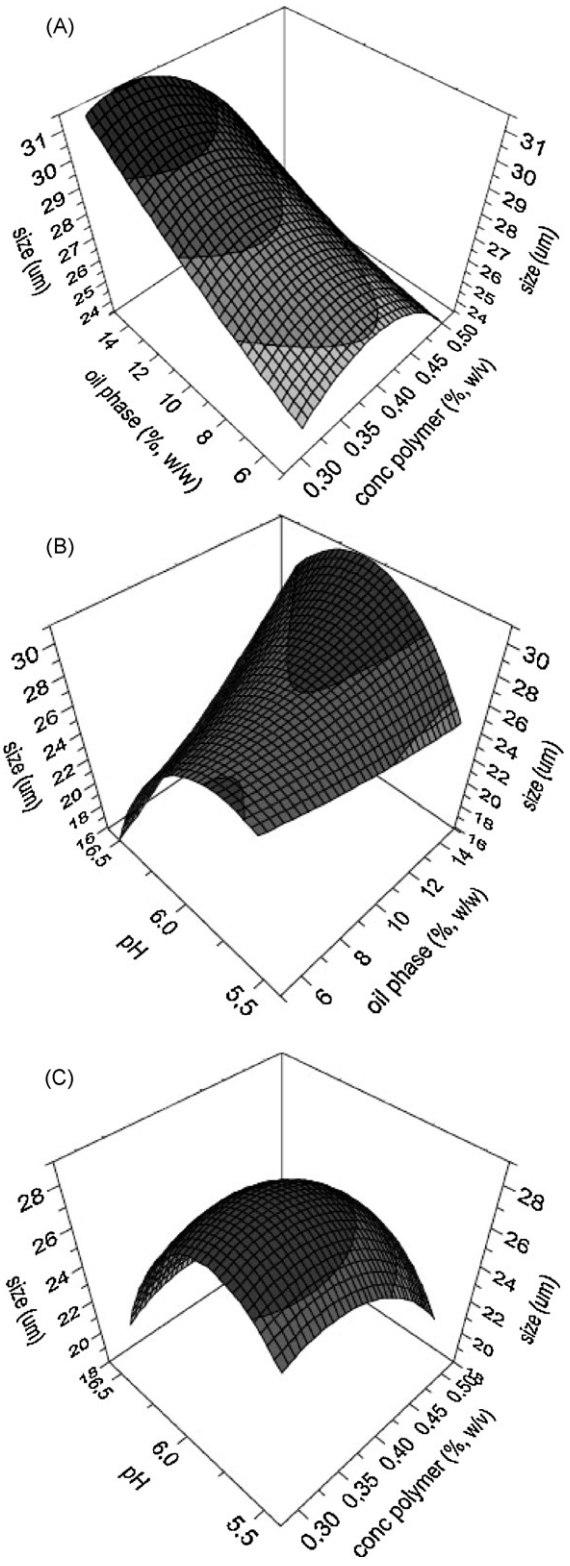

Fig. 2. DoE analysis for emulgel semi-solid formulations. Response surface plots of the response "mean diameter" for the interaction between polymer (%, w/v) and oil phase (O/W ratio, w/w) (A), between pH and oil phase (O/W ratio, w/w) (B) and between pH and polymer (%, w/v) (C).

neutralization of a greater number of TR-1 carboxylic groups takes place. This causes a more pronounced distension of the polymer chains that breaks the intramolecular bonds, forming concomitantly a series of intermolecular bonds (Burruano et al., 2004). This behaviour is confirmed by comparing the viscosity of emulgels #11 (pH 5.5) and #12 (pH 6.0) (Fig. 3B).

The rheological studies performed on the same emulgels at 37 °C (data not shown) confirmed what was observed at 25 °C with only a slight reduction of the formulation viscosity. As general rule, all rheograms, obtained both at 25 or 37 °C indicated that all emulgels exhibit a plastic flow behaviour, with high yield of stress value.

3.2.2. Yield stress studies

Yield stress studies were carried out since this property is very important for many issues related to the use of the formulation and its compliance. Extrusion from packaging, squeezing, spreading and, in this specific case, application to the periodontal pocket (by pre-filled-disposable syringe) are aspects that are strictly related with the rheological characteristics of the formulation. Shear stress variations *vs*. viscosity plots for each emulgel formulation are reported (Fig. 4). The rheograms of the more viscous emulgels, namely #8, #9, #13, #4, #5, #10, #14, #15 (Fig. 4A), were obtained setting the yield stress value at high values (600.000 Pa, minimum value). This analysis demonstrated that emulgels containing a high oil phase content (Compritol® comprised between 1.0 and 1.5 O/W ratio, w/w) are scarcely suitable for buccal/periodontal application because of their high viscosity and very high yield stress value. This negative feature can indeed be responsible for the difficulty on syringe (or other packaging) extrudability.

On the contrary, formulations #1, #2, #3, #6, #7, #8, #9, #11, #12 (Fig. 4B), present much lower yield stress values. These emulgels, containing Compritol® in low and medium percentages (0.5 and 1.0 O/W ratio, w/w), can be more easily extruded/squeezed from the packaging, guaranteeing at the same time a much better manageability.

3.3. Oscillatory measurements

Non-destructive oscillatory measurements were performed in order to obtain information about the chemical and physical stability of formulations. Viscoelastic materials (as emulgels) show, after mechanical stress, contemporaneously viscous and elastic flow. In this respect, oscillation measurements furnish data regarding energy stored (storage modulus G'), dissipated energy (loss modulus G'') and loss tangent (tan δ). The last term, representing the ratio of the loss modulus to the storage modulus, it offers information about the structure of the polymeric system. As the loss tangent approaches zero, the elastic structure of the system predominates, whereas, if the loss tangent exceeds the unity, the system is considered to be viscous.

Emulgel measurements were performed in a two step fashion. Firstly the sample was strained by an increasing stress from 0.1 to 100 Pa, working at constant frequency of 1 Hz (Madsen et al., 1998). This test permitted to individuate the linear viscoelastic region in which it is possible to recognize the stress value

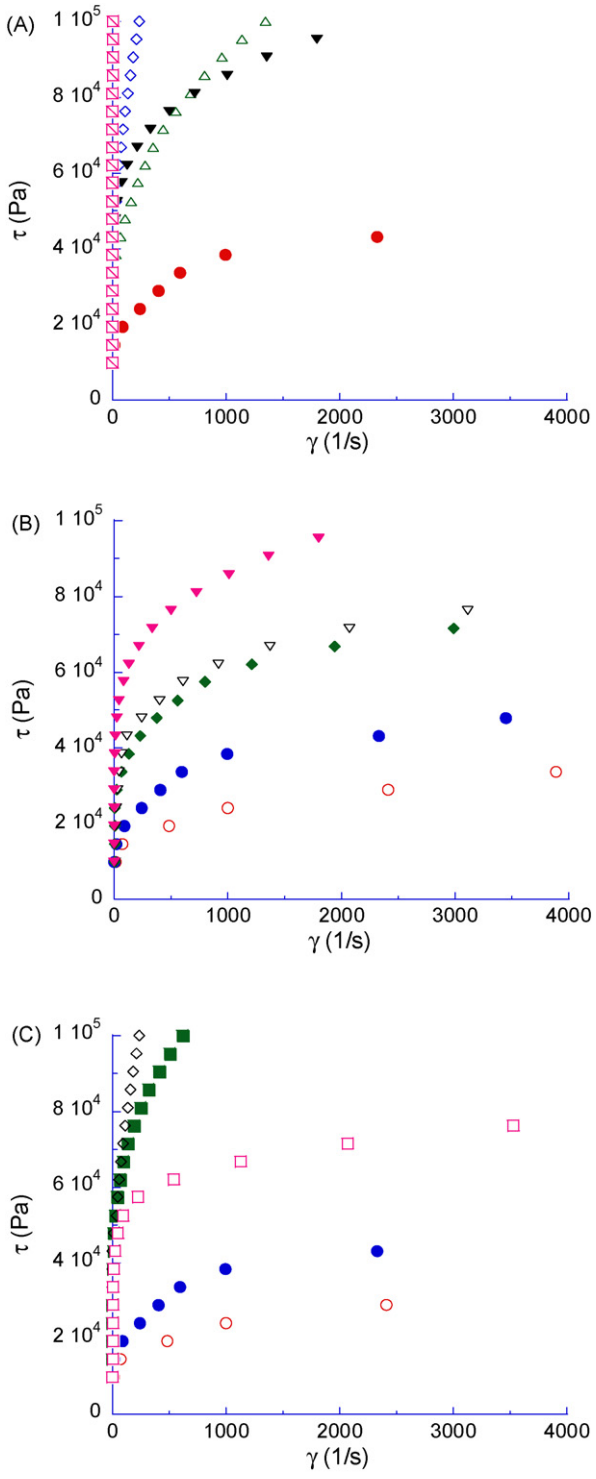

Fig. 3. Plots of the shear stress (τ) as a function of the shear rate (γ) for the different emulgels measured at 25 °C (panels A–C). Effect of the content of polymeric emulsifier Pemulen® 1621 TR-1 and of solid, neutral lipid glycerol behenate (Compritol®888ATO) at fixed pH (6.5); formulations #2 (●), #5 (◊), #9 (△), #12 (▼) and #15 (◹) (panel A). Effect of the content of polymeric emulsifier Pemulen® 1621 TR-1 and pH at fixed solid, neutral lipid glycerol behenate (Compritol®888ATO) content (0.5); formulations #1 (◯), #2 (●), #6 (⧫), #11 (▽) and #12 (▼) (panel B). Effect of solid, neutral lipid glycerol behenate (Compritol®888ATO) and pH at fixed polymeric emulsifier (0.3%, w/v); formulations #1 (◯), #2 (●), #3 (□), #4 (■) and #5 (◊) (panel C). Data represent the average of three independent determinations.

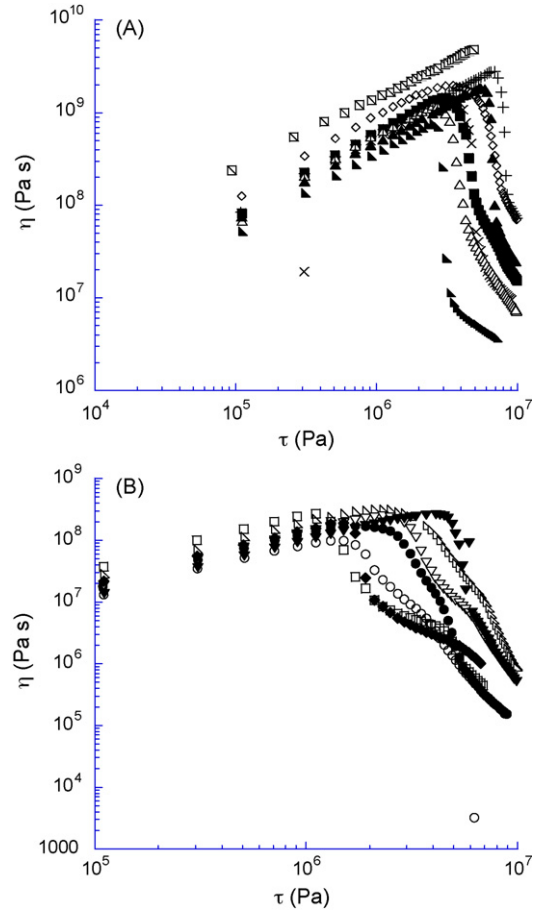

Fig. 4. Plots of viscosity (η) as a function of the shear stress (τ) for the different emulgel semi-solid formulations as indicators of the formulation extrudability. Panel A: formulations #4 (■), #5 (◊), #8 (◣), #9 (△), #10 (▲), #13 (×), #14 (+) and #15 (◹). Panel B: formulations #1 (◯), #2 (●), #3 (□), #6 (⧫), #7 (◺), #11 (▽) and #12 (▼). Data represent the average of three independent determinations.

useful to ensure instantaneous recovery after the removal of the applied force. Successively, oscillatory measurements were performed over a frequency range of 0.1–10 Pa and at constant stress, identified in the linear viscoelastic region.

Storage modulus (G') predominated in all emulgels demonstrating that these formulations were able to keep the furnished energy (applied stress), maintaining their primitive structure. A slightly different behaviour was observed for formulation #1, in which the viscous modulus prevailed ($G''/G' = 1.99$). This behaviour was tentatively attributed to the particular composition of this formulation (low TR-1 and Compritol® content and minimum pH value), that does not permit a great polymer relaxation and distension making the formulation scarcely suitable for the planned use.

Storage modulus (data not shown) increased with Compritol® and TR-1 content. Particularly, increasing the polymer percentages causes a progressive improvement of the formulation elastic properties. This characteristic is probably attributable to a more consistent polymer chain network able to slow down the coalescence process of internal droplets phase (O), resulting in more stable emulgels.

With respect to the pH influence on the emulgels rheological properties, it must be underlined that at pH 6.0 and 6.5, the polymeric chains are more stretched than at 5.5; so the higher pH values contribute to enhance the elastic properties of the emulgels.

3.4. Ex vivo mucoadhesive study

In order to evaluate the mucoadhesive force of emulgels, two kinds of oral tissue were considered: (i) the specialized mucosa, from porcine tongue and (ii) the non-specialized mucosa from porcine cheek (Winning and Townsend, 2000). Mucoadhesion force values were reported in Table 2. It is noteworthy to point out that all formulations showed good bioadhesion properties, particularly on specialized mucosa. The differences found from specialized and non-specialized tissues probably can be attributed to the particular epithelium of the tongue that is able to bind (adhere) to formulations in a much stronger fashion thanks to its partially keratinisation and its irregular surface due to the presence of indentation of connective tissue (papillae) (Hoogstraate and Wertz, 1998).

Formulations with low TR-1 percentage and/or low pH value showed lower mucoadhesion forces probably due to a reduced amount of interactions between the polymeric carboxylic groups and mucin.

A medium/high TR-1 percentage can assure a stronger mucoadhesion, especially when polymeric chains are completely stretched at the higher pH values. It is also important to take into account the oil phase (Compritol®) content, in fact, a high internal phase content (1.0 and 1.5 O/W ratio, w/w) decreases the bioadhesion by two reasons: (i) the increased number of interactions between the lipophilic monomers of the TR-1 polymer and the Compritol® droplets, that reduces the polymer TR-1 group disposability to link mucin and (ii) the increased viscosity of formulation that is responsible for a reduced adaptability of the formulation to the oral mucosa surface, lowering consequently the bioadhesive properties of the emulgels.

According to these considerations the most adhesive emulgel formulations were #6, #7, #11 and #12.

3.5. Rheological characterization of FLUR loaded emulgels

On the basis of the above described results, emulgel formulations #6, #7, #11 and #12 were selected and employed for further studies. These formulations were prepared in the presence of FLUR (see details in the experimental section) and successively their rheological and mucoadhesive characteristics were studied and compared to those of plain emulgels. All the drug containing formulations were prepared using the sodium salt of FLUR (resulting in a 0.5%, w/w drug content). The drug salt form was selected in order to maximize its water solubility with the final aim to achieve an immediate drug release from the external aqueous phase of the emulgels.

Viscous flows at 25 and 37 °C were determined and compared with those of the corresponding plain emulgels. The drug loaded emulgels, particularly formulations #6 and #7, showed an increase in viscosity at 25 °C (data not shown). It is possible to hypothesize that the addition of ions ($FLUR^-Na^+$) can increase polymer chain distension determining the viscosity growth. Probably ionized $FLUR^-$ carboxylic groups establish hydrogen bonds with polymeric chains making them more stretched. This effect is more evident in the case of emulgel #7 at pH 5.5.

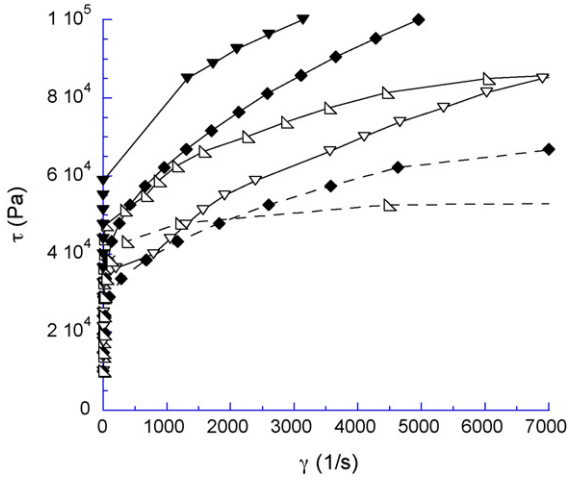

Fig. 5. Effect of drug loading on the rheological properties of emulgel semi-solid formulations. The following plain (solid lines) and flurbiprofen loaded formulations (dashed lines) were considered. Formulations #6 (♦), #7 (◺), #11 (▽) and #12 (▼). Data represent the average of three independent determinations.

Viscosity studies, performed at 37 °C (reported in Fig. 5), confirmed the same trend of those conducted at 25 °C, with the only exception of formulation #11 that showed a slight reduction in viscosity (again compared to the plain formulation). This behaviour was attributed to the contemporary presence of low Compritol® content and low pH value.

Oscillatory measurements (Fig. 6) showed that also in drug loaded formulations the storage modulus (G') is prevalent. On the basis of previous considerations, the ion presence resulted in a more pronounced polymer chain distension that increased the elastic properties of the emulgels. This feature was confirmed by the reduction of the loss tangent ($\tan\delta$) value.

3.5.1. Ex vivo mucoadhesion force studies

Mucoadhesive FLUR containing emulgel formulation properties were studied and were compared to those of targeted buccal diseases commercial gel formulations, containing different active pharmaceutical ingredients, since so far, to our knowledge, FLUR-containing gel based formulations are not in the market. From the data reported in Table 2, it is possible to appreciate that FLUR loaded emulgels showed, in general, good mucoadhesive forces, greater than both corresponding empty formulations and the tested commercial products. From the comparison of plain and FLUR loaded emulgels resulted that formulations #6 and #12 showed a marked bioadhesion enhancement towards both specialized and non-specialized mucosa; while formulation #11 only towards non-specialized mucosa. Finally, in the case of formulation #7 no significant

Table 2
Emulgel adhesion force to oral porcine mucosa ($n = 3$)

Formulation	Mucoadhesion force (mN)			
	Empty formulations		Drug loaded formulations	
	Non-specialized mucosa (cheeks)	Specialized mucosa (tongue)	Non-specialized mucosa (cheeks)	Specialized mucosa (tongue)
#1	270 ± 11	303 ± 40	–	–
#2	260 ± 11	286 ± 10	–	–
#3	280 ± 11	333 ± 3	–	–
#4	283 ± 17	276 ± 2	–	–
#5	266 ± 6	326 ± 5	–	–
#6	320 ± 23	400 ± 41	446 ± 19	716 ± 28
#7	436 ± 11	0.456 ± 0.176	343 ± 13	500 ± 20
#8	263 ± 26	0.286 ± 0.028	–	–
#9	243 ± 26	353 ± 13	–	–
#10	266 ± 17	13 ± 9	–	–
#11	306 ± 17	413 ± 5	500 ± 31	400 ± 5
#12	366 ± 34	350 ± 71	543 ± 24	633 ± 61
#13	263 ± 47	303 ± 28	–	–
#14	273 ± 46	420 ± 85	–	–
#15	243 ± 23	290 ± 52	–	–
Alovex® gel	–	–	416 ± 86	450 ± 12
Dentosan® parodontal gel	–	–	400 ± 22	750 ± 46
Gengigel®	–	–	396 ± 64	583 ± 64
Daktarin® oral gel	–	–	0.370 ± 17	520 ± 89
Corsodyl® dental gel	–	–	386 ± 6	666 ± 32

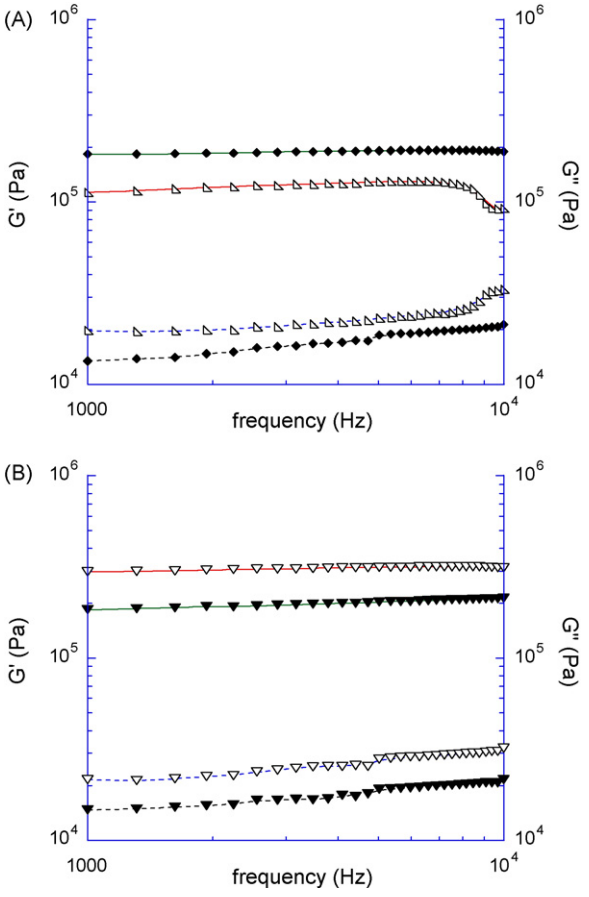

Fig. 6. Frequency sweep tests showing the frequency dependence of the elastic G' (solid lines) and loss G'' moduli of emulgel semi-solid formulations. Panel A: formulations #6 (♦) and #7 (◺); panel B: formulations #11 (▽) and #12 (▼). Data represent the average of three independent determinations.

variations were observed. Probably, the sodium salt increases polymer–mucin interaction by improving the mucoadhesion characteristics. This behaviour is more evident when the pH value and TR-1 percentage are high and the amount of internal phase is low.

It is interesting to note that FLUR emulgels showed mucoadhesion forces very similar or superior (#6 and #12) to commercial buccal formulations. This result is particularly noteworthy, if it is taken into account that the emulgels have a very simple formulation and contain a low amount of polymer if compared to the complex formula composition of the commercial products.

3.5.2. *In vitro release studies*

In order to have a complete characterization of emulgels, *in vitro* drug release studies were performed using a Franz cell experimental model.

As demonstrated by the drug release profiles (Fig. 7), all emulgels showed a rather similar behaviour. FLUR release was quite rapid during the first 15–30 min and reaches a plateau in about 240 min. Formulations #11 and #7 showed similar drug release profiles. From these formulations the drug was released in a slightly slower fashion with respect to formulations #12 and #6 that released (in the timeframe of 8 h, used for the release experiment) a higher proportion of drug, 69.99 and 78.63%, respectively. Considering these data with respect to emulgel composition, it is possible to hypothesize that a low TR-1 content forms a loose polymer network through which FLUR diffuses quite easily. This effect is more evident when a low TR-1 content is associated to a high pH value (6.0 and 6.5) that assures a good polymeric distension.

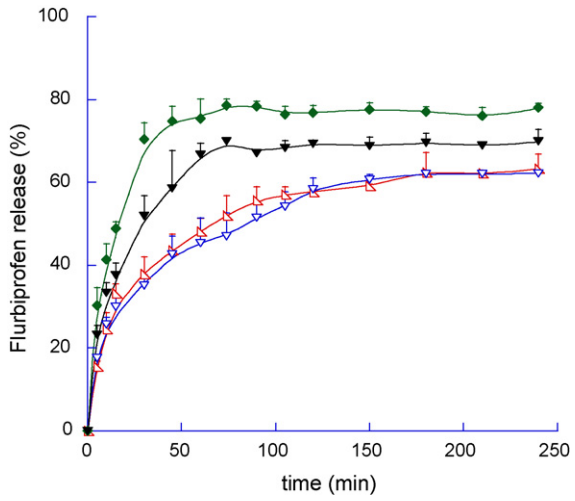

Fig. 7. Release profiles of flurbiprofen from different emulgel semi-solid formulations: #6 (♦), #7 (◺), #11 (▽), #12 (▼). Data represent the average of three independent determinations ± S.D.

Formulations #6 and #12 showed a superior drug release because of the contemporary presence of a medium/high TR-1 percentage (0.4 or 0.5%, w/v) and an high pH value (6.0 or 6.5). In these conditions, the polymeric network would be enough stretched to allow an easy drug release. As regards to formulation #7, the presence of high Compritol® (1.0 O/W, w/w) content, associated to low pH value (5.5), hinders drug diffusion. Finally formulation #11 showed a low release probably due to the high TR-1 percentage and low pH value that do not produce stretched chains.

3.5.3. Statistical and kinetic analysis

The *in vitro* release profile data were used in order to investigate which mechanism could be possibly involved in FLUR release from the emulgels. Data were analyzed using the following general equation (Ritger and Peppas, 1987):

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t/M_∞ is the drug fraction released at time t, k is a constant, depending on structural and geometric characteristics of the system, n is the diffusional coefficient related to the release mechanism.

Depending on the strength of the gel layer network (external phase) formed, drug release can be controlled by different mechanism with different kinetics. Using gelling agent of low viscosity grades, erosion of the swollen polymer represents the release mechanism and generally leads to a zero-order release kinetic. When the employed polymer is able to determine the gel viscosity, as TR-1, a stable gel is formed and polymer dissolution is negligible. Drug is released from the swollen polymeric network principally through a diffusion-controlled mechanism, described by the well-known Fick's law. Often, both diffusion and erosion contribute to the release of the drug. This transition, between the two limit mechanisms, results in a kinetics in-between square root time dependence and zero-order generally described as 'anomalous transport', used when contribution of both diffusion and relaxation, happens (Zulenger and Lippold, 2001).

For all the analyzed emulgel formulations, the best correlation was obtained with $n = 0.5$, suggesting that passive diffusion (Fickian diffusion) was the drug release controlling mechanism; it can be interpreted as a Higuchi-like behaviour. This means that FLUR release rate is not connected to polymer chain relaxation but the drug is released by diffusion through the polymer chains that form the firm gel structure.

3.5.4. In vivo evaluation of organoleptic properties and acceptability

The formulations #6, #7, #11 and #12 were preliminary tested *in vivo*, in order to obtain information on their organoleptic properties and acceptability. The selected emulgels adhered very well to the oral mucosa, showing a good residence time (about 1 h), when compared with two products available on the market, namely: Benactiv®Gola 8.75 mg lozenges and Froben® 0.25% mouthwash. The obtained results appear very promising since: (i) lozenges were completely dissolved in the mouth, after 10 min and (ii) mouthwash was rapidly eliminated after only 2 min from application. After application and along with the entire residence, the emulgels did not cause any appreciable alteration, without producing dry mouth or salivation excess. In addition, emulgels did not cause irritation, burning sensation, smart or itch that were often referred by volunteers treated with mouthwash and lozenges (see Table 3). Particularly one volunteer, treated with mouthwash, referred gingival bleeding. All volunteers referred that emulgels were agreeable, pleasant and tasty notwithstanding flavours and sweeteners were not added to the formulation as found in market products (*e.g.* mint essence and sodium saccharinate for Froben®, cherry essence, saccharose and glucose for Benactiv®Gola).

Table 3
In vivo evaluation of organoleptic properties and acceptability

Formulation	Irritation	Pain	Taste[a]	Dry mouth	Excessive salivation	Other
#6	No	No	+++	No	No	–
#7	No	No	+++	No	No	–
#11	No	No	+++	No	No	–
#12	No	No	+++	No	No	–
Benactiv®Gola	No	No	++	No	Yes	–
Froben®collutorio	Yes	Yes	+	Yes	No	Gum bleeding

[a] Taste: good +++, acceptable ++, bad +.

4. Conclusions

The rheological and pharmaceutical analysis of emulgels based on the use of a mucoadhesive polymer have demonstrated that formulations possess better retention time in comparison to market products for local treatment of oral cavity inflammations. In order to study the formulation response to shearing stresses induced by normal buccal activities, emulgels were characterized by destructive (flow rheometry) and non-destructive (oscillatory rheometry) techniques. The studies showed that the most effective formulations were those characterized by a low to medium oil phase content (oil to water weight ratio of 0.3–0.4, w/w). The formulations appear to have an appropriate viscosity compatible with an administration procedure through pre-filled syringe. The presence of the polymeric acrylic emulsifier gives to the emulgel consistency, stability and mucoadhesivity (Brisaert and Plaizer-Vercammen, 1997; Madsen et al., 1998). This latest property is particularly important in order to obtain formulations characterized by a prolonged retention time, possibly reducing the number of daily administrations. *In vivo* tests have demonstrated that emulgels are well tolerable, comfortable and non-irritant. All healthy volunteers have reported that the formulations were associated with a good palatability and a prolonged drug-tissue contact. These final data appear particularly encouraging, especially when compared with those of the market, mainly employed for the same type of treatment (therapeutic applications), such as collutories, lozenges and aqueous gels.

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