ORIGINAL ARTICLE

IN VITRO/IN VIVO PERFORMANCE STUDY OF NEW METRONIDAZOLE PERIODONTAL GEL FORMULATIONS

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Abstract

The aim of this paper is to continue previous studies of gel formulation containing metronidazole and having mucoadhesive properties for the treatment of periodontal diseases by assessing their *in vitro* and *in vivo* characteristics. We started from the premise that the crosslink density and swelling capacity of the polyacrylic acid derivatives greatly affect the swelled mucoadhesive polymer which penetrates into the tissue crevices followed by the interpenetration between the polymer chains and protein chains of the mucus aimed to achieve correlations between the rheological properties of gels and mucoadhesivity. The results showed that the analysed gels F3_3, F3_1 (Ultrez 10) and F2_3 (Carbopol® 974P) have the most appropriate rheological properties. To evaluate the kinetics of drug release from the analysed gels, 9 kinetic models (functions of time) were performed. $R^2_{adjusted}$ (the *adjusted coefficient of determination*), MSC (*model selection criterion*) and AIC criterion (*Akaike informational criterion*) used as goodness of fit parameters indicated that *Korsmeyer-Peppas* is the most appropriate model to describe the release kinetics of the drug release from the 9 gel formulations. Preliminary clinical studies have shown that the topical application of metronidazole gel formulations, in combination with mechanical treatment of smoothening of the root surface appears to be more efficient, improving clinical parameters. Plaque index (PI), bleeding on probing index (BOP), and probing pocket depth (PPD) evaluated at one month post-treatment had better values compared with those of the initial examination values in a greater number of the periodontal examined sites (PI-54.15%, BOP-62.38%, and PPD-28.45%) in the group B of patients receiving scaling and root planning (SRP) and 15% metronidazole gel formulation F3_3.

Rezumat

Lucrarea reprezintă continuarea unor studii anterioare de formulare a unor geluri cu proprietăţi mucoadezive ce conţin metronidazol, destinate tratamentului afecţiunilor parodontale, prin evaluarea *in vitro* şi *in vivo* a caracteristicilor acestora. Ştiind că densitatea de reticulare şi capacitatea de îmbibare a derivaţilor de acizi poliacrilici influenţează în mare măsură interacţiunea cu lanţurile glicoproteice ale mucusului în procesul adeziunii, ne-am propus realizarea unor corelaţii între proprietăţile reologice ale gelurilor şi mucoadezivitate, rezultatele obţinute arătând că, dintre gelurile analizate, gelurile F3_3, F3_1 (Ultrez 10) şi F2_3 (Carbopol® 974P) prezintă proprietăţile reologice cele mai potrivite. Pentru a evalua cinetica proceselor de cedare a substanţei active din gelurile analizate, s-au utilizat 9 modele cinetice (funcţii de timp). Parametrii $R^2_{adjusted}$ (coeficientul de determinare ajustat), MSC (criteriul de selecţie a modelului) şi AIC (criteriul informaţional Akaike) au indicat că cel mai potrivit model pentru descrierea cineticii de cedare a substanţei medicamentoase din cele 9 formulări de geluri analizate este *cinetica Korsmeyer-Peppas*. Studiile clinice preliminare au demonstrat că aplicarea topică a geluriolr cu metronidazol în combinaţie cu tratamentul mecanic de netezire a suprafeţei radiculare este mai eficientă, îmbunătăţind parametrii clinici. Indicele de placă (PI *Plaque Index*), indicele de sângerare papilară (BOP *Bleeding On Probing*) şi adâncimea pungii parodontale (PPD *Probing Pocket Depth*) evaluaţi la o lună după tratament au avut valori îmbunătăţite faţă de examinarea iniţială, într-un număr mai ridicat de situsuri examinate (PI-54.15%, BOP-62.38% şi PPD-28.45%) la grupul B de pacienţi, cărora li s-a aplicat tratament mecanic (SRP) şi gel cu metronidazol 15%, formularea F3_3.

Keywords: metronidazole, mucoadhesive gels, rheology, release kinetics, clinical study

Introduction

Periodontal disease is a multifactorial condition, often affecting the adult population, therefore, its treatment is complex. The main determining role is that of the microbial factor, the others being favouring or predisposing factors. Consequently, periodontal antimicrobial therapy is an important step to be covered regardless of the form or status of the periodontal disease [16].

In the periodontal disease, destructive inflammatory phenomena occur, these having an effect on the supporting tissues of the teeth. Consequences are

loss of attachment level, and bone resorption, the formation of periodontal pockets and/ or gingival retraction, changes which over time can lead to the loss of teeth in the dental arches. The most important tissue involved in maintaining tooth arch is the alveolar bone, which in periodontal conditions undergoes demineralization. Tooth support is diminished as the supporting structures are destroyed, this causing changes in resorption and the occurrence of periodontal pockets. These pockets which produce severe exudate are a favourable environment for the development and proliferation of pathogenic anaerobic bacteria. Expanding of the inflammation in the tooth-supporting structures causes tissue destruction in the arch and leads to tooth loss [15, 16].

The conventional treatment of periodontal disease, which consists largely of mechanical debridement of root surfaces, has a limited effect in some clinical cases with deep pockets or furcations, areas that may remain untreated. Due to these drawbacks, the use of antimicrobial agents is required as adjuvant of SRP (scaling and root planning [15, 22, 24]. Metronidazole (MTZ) is the most widely used medicine in these cases, because it is active against periodontal pathogens. Metronidazole acts preferentially on anaerobic germs; it prevents hydrogen production, exercising its toxic action by depriving anaerobic microorganisms of reducing equivalents essential for certain anabolic processes. In addition, the metabolite resulting from the reduction of the nitro group of metronidazole molecule damages the DNA chain. This results in DNA damage in the form of loss of helical structure, probably acting as a nuclease [10, 14, 21].

Mucoadhesive gels for periodontal diseases offer many advantages over other conventional preparations used in this treatment. Compared with antimicrobial mouthwash solutions, mucoadhesive gels have greater efficacy for a longer period because they remain at the application site and are more difficult to be removed by saliva. They release the drug exactly where the affected mucosal absorption is rapid, thus the drug concentration is higher at the site of absorption due to the well-vascularized mucosa, they can be easily applied, and have a prolonged effect [3, 13].

Acrylic acid polymers, known as Carbopols, are composed of long chains of carbon atoms, based on the acrylic acid polymer with a high degree of polymerization. Chains are cross-linked by allyl sucrose or allyl pentaerythritol as crosslink formers. This class of polymers is enlarged by the emergence of new compounds with properties best obtained by modifying the cross-links and hydrophobic nature of the co-monomers [2, 6].

In our study, we used Carbopol® 940 NF (C-940), Carbopol® 974P NF (C-974P), and Carbopol® Ultrez 10 NF polymer (U-10) as agents for forming hydrogels for the following reasons: Carbopol® 940 NF has the advantage of providing high viscosities ensuring prolonged contact in the mouth; Carbopol® 974P requires less dispersing time compared with C-940 and is recommended especially for bioadhesive oral preparations; Carbopol® Ultrez 10 NF polymer allows a greater versatility in formulating and processing because it is easy to disperse in water. The unique dispersion performance of Carbopol® Ultrez 10 NF polymer allows it to wet quickly, yet hydrate slowly. This property helps minimize lumping, which can be problematic when turbulent mixing is not available during dispersion [4, 5].

Carbopols manifest their properties of thickening and gelling in water only after the neutralization of their dispersion with various inorganic or organic bases. The viscosity and properties of the polymer are dependent on the degree of neutralization (pH) and the ionic forces present in the dispersion. Compared with traditional Carbopol polymers, Carbopol® Ultrez 10 NF polymer provides dispersions in water that are much lower in viscosity prior to neutralization. The lower un-neutralized dispersion viscosity enables easier handling in mixing tanks and process lines.

The bioadhesive effect of the acrylic polymers and their derivatives is explained by the fact that those with higher molecular weight and high moisture capacity have a large adhesion surface and are able - due to hydrogen bonding - to exhibit a greater interaction with the mucin glycoproteins. Once adhered, Carbopol gels show a high resistance to "washing" and thus a longer residence time of drugs at the sites of their therapeutic action, and ensure intimate contact between the formulation and the mucosal surface [2, 6, 8].

The current study is a continuation of previous ones on mucoadhesive gel formulations with metronidazole [7], by making correlations between rheological properties and *ex-vivo* bioadhesion of the nine proposed formulations, determining the *in vitro* rate and kinetic mechanisms of MTZ release, and then evaluating the clinical efficacy of metronidazole gels used as an adjunct in the SRP of periodontal diseases [19, 21]. MTZ release mechanism from gels was evaluated by modelling the release profiles to nine mathematical equations: zero-order, first-order, Higuchi, Korsmeyer-Peppas, Hixon-Crowell, Hopfenberg, Peppas-Sahlin-1, Peppas-Sahlin-2, and Weibull-1 [27].

To demonstrate the therapeutic efficacy of gels, the preliminary clinical study was conducted on patients diagnosed with generalized chronic parodontopathy by assessing the following clinical

parameters: plaque index (PI), bleeding on probing (BOP), and probing pocket depth (PPD) [1, 12, 17].

Materials and Methods

Materials: metronidazole (BASF, Germany), Carbopol® 940 NF, Carbopol® 974P NF, Carbopol® Ultrez 10 (Lubrizol Corporation, Cleveland, USA), sodium hydroxide (Chemapol Prague, Czech Republic), sodium carbonate (Reactiv Bucharest, Romania) and triethanolamine (Merck, Germany).

Metronidazole gels preparation

We formulated and prepared three types of mucoadhesive gels containing 15% metronidazole, based on C-940, C-974P and 1% U-10 hydrogels [7]. The hydrogels were prepared at concentrations of 1%, by direct dispersion of Carbopol in water. For each type of Carbopol, aqueous dispersions were neutralized to pH = 7 using three kinds of bases: sodium hydroxide-NaOH (A), sodium carbonate-Na_2CO_3 (B), and triethanolamine-TEA (C). Finely powdered metronidazole (80 μm) was gradually incorporated into the hydrogels prepared in advance. Nine gel formulations were obtained and they were coded according to the data in Table I.

Table I

Composition and codification of metronidazole gel formulations

Hydrogel forming agent (1%)	Neutralizing agent		Code of metronidazole gel (15% MTZ)
	Type	Quantity (g)	
Carbopol® 940 NF	A	0.442	F1_1
	B	1.676	F1_2
	C	1.262	F1_3
Carbopol® 974P NF	A	0.464	F2_1
	B	1.288	F2_2
	C	1.142	F2_3
Carbopol® Ultrez 10	A	0.466	F3_1
	B	1.468	F3_2
	C	1.147	F3_3

Evaluation of in vitro rheological characteristics

Rheological studies were performed with a rotational viscometer Rheotest RV-MLV, Germany, at 37±0.5°C, using the inner cylinder H. The structural viscosity of the metronidazole gels was determined and flow and viscosity curves were plotted.

To establish the bioadhesive properties of *in vitro* Carbopol gels we determined the necessary detachment force of the gel from a synthetic semipermeable membrane. We used an adjusted balance according to the methods described in the literature in order to perform the measurements [8, 11, 26].

In vitro release studies

We studied the drug release from the nine gels with 15% metronidazole according to the paddle method of the USP dissolution test apparatus, using the Pharma Test PTWS dissolution apparatus.

We weighed a given amount of sample (containing 250 mg of MTZ) in the diffusion cell which was carefully placed at the bottom of the dissolution vessel in a thermostat device of dialysis with continuous mixing (37°C). The donor phase was represented by the metronidazole gel and the acceptor phase was 100 mL phosphate buffer (pH 6.8). Samples were taken at 60 min time intervals, the determining time being correlated with the residence time of the gel on the oral mucosa. The absorbance of samples was measured spectrophotometrically at 320 nm using a Shimadzu Hyper UV Spectrophotometer [7, 9, 25].

Drug release kinetics

Nine profiles of metronidazole release from Carbopol gels were obtained by plotting the experimental results [18, 23]. The obtained curves were fitted with nine kinetic equations - time functions (Table II) using DDSolver software [27].

Clinical evaluation

To assess the clinical status, we studied the gels with the most suitable *in vitro* characteristics (F2_3, F3_3). We selected 40 patients who presented at the Department of Periodontology, Faculty of Dentistry, University of Medicine and Pharmacy of Tîrgu Mureș, with various forms of periodontal disease. The inclusion criteria were as follows: the diagnosis of generalized chronic moderate periodontitis, the presence of at least 16 teeth, and four non adjacent sites with periodontal pocket depth greater than or equal to 5 mm, age between 20-45 years old, irrespective of sex, proper cooperation. The exclusion criteria were as follows: presence of systemic disease, poor oral hygiene after oral hygiene instruction, smoking, pregnant or lactating women, and periodontal treatment in the previous six months, and antibiotic therapy over the last year.

After patients were explained the objective of the study and after the written consent was signed, the mouth quadrants of each patient were randomly

divided into four groups: Group A (scaling and root planning-SRP), Group B (SRP and Carbopol® 974P gel-F2_3), Group C (SRP and Ultrez 10 gel-F3_3) and Group D (no treatment, control group).

All patients were subjected to initial periodontal therapy, including motivational, and were instructed on oral hygiene. In the groups treated with 15% metronidazole gel, the periodontal pockets were filled up to the surface, so that a part of the gel was visible in the oral cavity. Patients were asked not to drink or eat for an hour and to perform the routine recommended oral hygiene throughout the entire study period. For the clinical evaluation of the periodontal status we recorded the following parameters: plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD) which were assessed at six sites (distofacial/buccal, midfacial/buccal, mesiofacial/buccal, distolingual/palatal, midlingual/palatal, and mesiolingual/ palatal) of each tooth included in the study at the beginning and one month after treatment [12, 17, 20]. Each patient signed a written statement of informed consent prior to receiving the study medication.

The study protocol was approved by the Ethics Committee for scientific research of the University of Medicine and Pharmacy of Tîrgu Mureş.

Table II

Kinetic models used for the analysis of release profiles

No.	**Kinetic model/ Equation/ *In vitro* parameters** [27] (F= % of drug released in time *t*)	
1.	**Zero-order:** $F = k_0 \cdot t$	**-k_0, k_1, k_H** = the released constant in the applied model **-k_{KP}** = the release constant incorporating structural and geometric characteristics of the drug-dosage form **-n** = the diffusional exponent indicating the drug-release mechanism
2.	**First-order:** $F = 100 \cdot [1 - Exp(-k_1 \cdot t)]$	
3.	**Higuchi:** $F = k_H \cdot t^{0.5}$	
4.	**Korsmeyer-Peppas:** $F = k_{KP} \cdot t^n$	
5.	**Hixon-Crowell:** $F = 100 \cdot [1 - (1 - k_{HC} \cdot t)^3]$	**-k_{HC}** = the release constant in Hixon-Crowell model
6.	**Hopfenberg:** $F = 100 \cdot [1 - (1 - k_{HB} \cdot t)^n]$	**k_{HB}** = the combined constant, $k_{HB} = {}_0/(C_0 \times a_0)$; where **$k_0$** is the erosion rate constant, **C_0** is the initial concentration of drug in the matrix, **a_0** is the initial radius for a sphere or cylinder or the half-thickness for a slab; **n** is 1, 2, and 3 for a slab, cylinder, and sphere, respectively
7.	**Peppas-Sahlin-1:** $F = k_1 \cdot t^m + k_2 \cdot t^{(2 \cdot m)}$	**k_1** = the constant related to the Fickian kinetics **k_2** = the constant related to Case-II relaxation kinetics **m** = the diffusional exponent for a device or any geometric shape which inhibits controlled release
8.	**Peppas-Sahlin-2:** $F = k_1 \cdot t^{0.5} + k_2 \cdot t$	
9.	**Weibull-1:** $F = 100 \cdot \{1 - Exp[-((t - T_i)^\beta)/\alpha]\}$	**Ti** = the location parameter which represents the lag time before the onset of the dissolution or release process **α** = the scale parameter which defines the time scale of the process **β** = the shape parameter which characterizes the curve

Statistical analysis

Statistical analysis was performed using SPSS software version 15.0.

The experimental values are expressed as mean ± SD (standard deviation, *n* =3), or as mean ± SEM (standard error of mean). For both *in vitro* and *in vivo* experimental results, unpaired *t*-tests were used to assess the statistical significance of variance between the groups. Results were considered statistically significant if $p < 0.05$.

Results and Discussion

In vitro rheological behaviour

The analysed gels present pseudoplastic type rheological behaviour which can be explained by the gradual reorientation of asymmetric particles, and therefore the decreasing of viscosity (η), under the destructuring action of the gradual increased shear stress exerted by changing the shear rate (D) of inner cylinder from 1 to 12.

The type of Carbopol and the neutralizing agent influenced the structural viscosity (Figure 1) and the adhesion of the gels (Figure 2), but the influences are statistically insignificant.

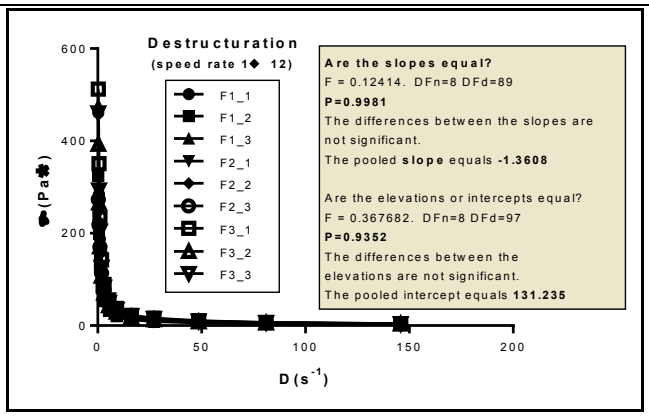

Figure 1.
Viscosity curves and the statistical mean of differences expressed by the regression lines

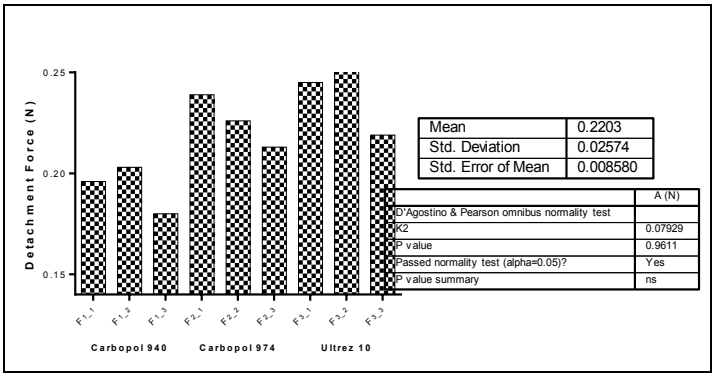

Figure 2.
In vitro adhesion of the gels and the statistical mean of differences analysed by a normality test

When analysing the flow behaviour of gels during the destructuration determined by the increased share rate action, some statistically significant differences were found between the values calculated for the area under the rheological curves (AUR_C) (Figure 3).

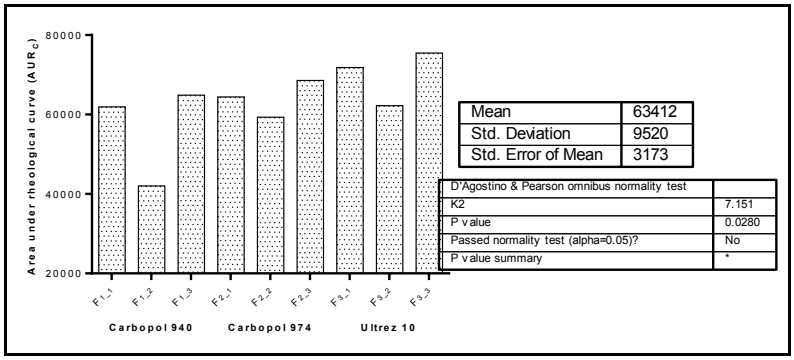

Figure 3.
Rheological behaviour of gels under the increased shear stress, expressed by area under the rheological curves (AUR_C), and the statistical mean of differences

Assuming that a higher value of the area under the flow curve could be an indicator of the extension ability of the gel during application on the biological substrate, it appeared that the following gels were in these respects: F3_3, F3_1 and F2_3.

In vitro release studies

After conducting dissolution studies, we noted that after 60 minutes a release of metronidazole between 7.83% and 19.3% was obtained (Figure 4). In the case of Carbopol® 940 and Ultrez 10 the release was more pronounced in those neutralized with sodium carbonate, whereas there was a very similar release in Carbopol® 974P gels, around 17%, regardless of the neutralizer used.

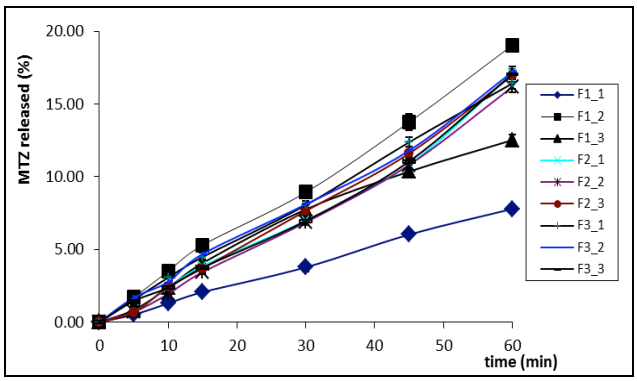

Figure 4.
Cumulative release profiles of metronidazole from 15% MTZ gels

Drug release kinetics

In order to choose from the nine mathematical models used to analyse the release mechanisms of metronidazole from the gels, we used the following selection criteria: adjusted coefficient of determination ($R^2_{adjusted}$), *Akaike informational criterion* (AIC) and model selection criterion (MSC). The mean values calculated from the individual values determined for the 9 analysed profiles are shown individually in figures 5, 6, and 7, in comparison for the 9 used fitting models

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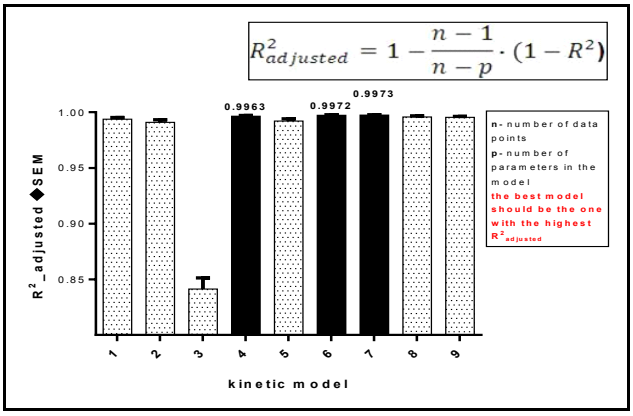

Figure 5.
Adjusted coefficient of determination (R^2_adj) as goodness of fit parameter

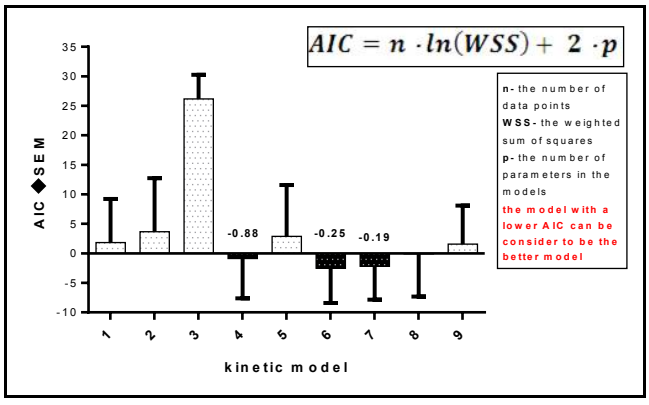

Figure 6.
Akaike informational criterion (AIC) as goodness of fit parameter

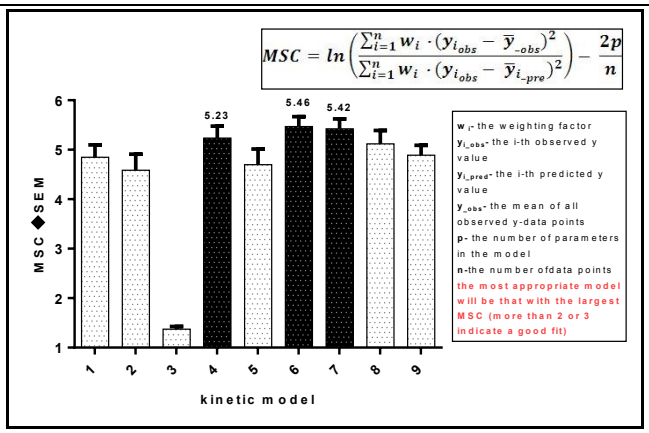

Figure 7.
Model selection criterion (MSC) as goodness of fit parameter

Table III
Best fit kinetic models and the calculated kinetic parameters

F	Kinetic model/ Calculated parameters						
	Korsmeyer-Peppas		Hopfenberg		Peppas-Sahlin-1		
	k_{KP}	n	k_{HB}	n	k_1	k_2	n
F1_1	0.1451	**0.97**	0.0000	36.55	-0.2949	0.3064	0.41
F1_2	0.3446	**0.97**	0.0032	0.98	0.5084	0.0186	0.74
F1_3	0.3446	**0.97**	0.0032	0.98	0.5084	0.0186	0.74
F2_1	0.1391	**1.16**	0.0092	0.22	0.2363	0.0038	0.91
F2_2	0.1198	**1.19**	0.0093	0.21	0.1777	0.0009	1.02
F2_3	0.1746	**1.11**	0.0072	0.32	0.0582	0.1481	0.57
F3_1	0.3303	**0.95**	0.0008	3.43	0.4426	0.0773	0.60
F3_2	0.2783	**1.00**	0.0045	0.59	0.4194	0.0150	0.75
F3_3	0.1461	**1.15**	0.0094	0.22	0.2593	0.0073	0.85
Mean ± SD	**0.2247 ± 0.0976**	**1.05 ± 0.09**	**0.0052 ± 0.0037**	**4.83 ± 11.93**	**0.2573 ± 0.2594**	**0.0662 ± 0.1020**	**0.73 ± 0.18**

The results indicate three suitable mathematical models to describe the analysed profiles, namely: model 4 (Korsmeyer-Peppas), model 6 (Hopfenberg) and model 7 (Peppas-Sahlin-1). When comparing the individual values of kinetic parameters calculated by fitting the release profiles with the equations of these kinetic models (Table III), we can note that the parameters calculated by Korsmeyer-Peppas kinetic have values with much lower dispersion within each group of gels, in comparison with the parameters calculated by the other two models. Therefore, we can conclude that Korsmeyer-Peppas kinetics can be used both for the comparison of release kinetics of metronidazole from the three categories of Carbopol gels, and for comparing, within each group, the release kinetics depending on the type of the used neutralizer.

Clinical evaluation

Following the clinical evaluation of metronidazole gels used as adjuvants in SRP therapy, we obtained the results listed in Table IV.

Table IV
Comparison of proportional changes (in percentage %) of periodontal parameters in groups between the initial results and after one month

Parameter / Group	PI ± SD	BOP ± SD	PPD ± SD
Group A	36.31 ± 7.12	51.34 ± 9.52	21.35 ± 2.75
Group B	54.15 ± 7.14	62.38 ± 4.63	28.45 ± 3.47
Group C	52.34 ± 6.67	61.64 ± 5.48	26.37 ± 4.54
Group D	1.32 ± 0.65	2.11 ± 0.27	0

The topical application of metronidazole gel associated with SRP (groups B and C) improved periodontal health, the therapeutic effects were better than those of SRP. From a clinical point of view, there were no significant differences in the use of the two types of metronidazole gel.

We also noted that the topical application of metronidazole in combination with SRP appears to

be more efficient, improving the clinical and microbiological parameters.

Conclusions

The type of carboxivinil polymers affected the rheological behaviour of the studied mucoadhesive gel systems containing 15% metronidazole in form of fine suspensions of particles. Ultrez 10 gels had proper adhesion and capacity of extension after application. The bases used for neutralization of the hydrogels had lower influence on the rheological characteristics.

After one hour, the quantity of metronidazole released from the nine studied gels ranged between 7.83 and 19.30% of the initial content. In the case of gels with Carbopol® 974P and Ultrez 10, the neutralizing agent used had no influence on drug release, about 17% of the metronidazole being released.

The combination of metronidazole gel with SRP resulted in improved periodontal status, proving its efficiency in the treatment of periodontal disease.

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