The Effect of Methylcellulose on Metronidazole Release from Polyacrylic Acid Hydrogels

Witold MUSIAL

Drug Form Technology Unit, Wroclaw Medical University; Wroclaw, Szewska Str. 38, 50-139 Wroclaw, Poland.
Received January 19, 2007; accepted May 8, 2007

Topical treatment of acne rosacea, a chronic condition characterized by recurrent course for many years, is primarily based on metronidazole preparations. The aim of this study was to evaluate the effect of various acrylic acid polymers, in composition with methylcellulose on metronidazole release rate from hydrogels proposed for the treatment of acne rosacea. Viscosity and release studies using "Paddle over Disk" system with semipermeable membrane of MWCO 3500 were performed. Compositions of Carbopol 971P and methylcellulose revealed an increase in viscosity with increasing concentration of methylcellulose in the range of 17200—26166 mPa·s. In all the examined formulations, the release process was characterized by a two-stage course. Among bipolymeric formulations, the highest first-stage release rate of 9.18×10^{-3} min^{-1} was determined for the gel consisting of 2.00% Carbopol 980NF with 1.00% methylcellulose. The second-stage release rates ranged between 2.88×10^{-3} and 8.00×10^{-3} min^{-1}. Two-stage release course can thus be attributed to metronidazole distribution into two compartments of hydrogel matrix. Proposed gels, with similar rheological properties, may be used for *ex vivo* and *in vivo* studies to obtain a suitable drug activity of metronidazole in the treatment of acne rosacea.

Key words acne rosacea; metronidazole; polyacrylic acid; methylcellulose; release rate; viscosity; hydrogel

Topical treatment of acne rosacea, a chronic condition characterized by recurrent course for many years, is primarily based on metronidazole preparations. This is the first compound authorized by FDA for local acne rosacea therapy.[1)] Majority of patients respond to oral administration of an antibiotic complemented with topical application of metronidazole. The available metronidazole products involve 0.75—2% ointments and gels.[2)] To achieve good compliance, metronidazole products need to be characterized by prolonged release of the compound, which can be achieved, for instance, by binding the drug in a polymer bed, although, the amount of drug released should enable bactericidal activity of metronidazole. Numerous reports have been devoted to the study of interaction between polyacrylic acid and various drugs or polymers, such as polyvinylpyrrolidine or carboxymethylcellulose.[3—7)] The polyacrylates possess an interesting property of water binding in the form of so-called primary bound water, secondary bound water, and bulk water.[8—10)] The use of polyacrylates or their compositions with methylcellulose as metronidazole carriers in the dermal preparations may provide interesting patterns of release at the site of application. The aim of this study was to evaluate the effect of various acrylic acid polymers, in composition with methylcellulose on metronidazole release rate from hydrogels proposed for the treatment of acne rosacea.

Experimental

Materials The following materials were used: metronidazole (Sigma-Aldrich, EU, Poland), acrylic acid polymers: Carbopol 934P, Carbopol 971P, Carbopol 980NF (BF Goodrich, EU, Belgium), and methylcellulose (Methyl Cellulose 4000, Sigma Chemical, EU, Germany), boiled bidistilled water, semipermeable membrane (Membra-Cel®, MWCO 3500, Serva, U.S.A.), and Statistica 6.0 software.

Preparation of Gels All the formulations were prepared using freshly boiled bidistilled water. The composition is listed in Table 1.

Monopolymeric methylcellulose gel (No. 1) and Carbopol gels (No. 2—13) were obtained by sprinkling polymers over the surface of metronidazole 0.75% aqueous solution, and leaving them to swell during the process of sedimentation. All the gels were stored at 8 °C. Low temperature prompted dissolution of methylcellulose. The decreased temperature effect on Carbopol swelling was not evident. Gels with over 1.00% content of the above polymers were stirred after sprinkling the polymer over the surface of water. Time required for the gels to swell was from 24 to 48 h except for the methylcellulose gel, for which the swelling time was almost 72 h. Formulations consisting of bipolymeric compositions (No. 14—25) were prepared by combining previously prepared monopolymeric gels in a defined weight ratio. After swelling, all the gels were centrifuged at 4000 rpm for 15 min to remove air bubbles.

Measurement of Viscosity Viscosity measurements were performed using a Brookfield DV-III+ rotary rheometer and applying 0.5 ml of gel in the measurement chamber. CPE-51 cone was used. Viscosity was investi-

Table 1. Composition of Investigated Aqueous-Gel Formulations

No.	Methylcellulose (%)	Carbopol 934P (%)	Carbopol 971P (%)	Carbopol 980NF (%)	Metronidazole (%)
1	5.00	—	—	—	0.75
2	—	0.50	—	—	0.75
3	—	1.00	—	—	0.75
4	—	1.50	—	—	0.75
5	—	2.00	—	—	0.75
6	—	—	0.50	—	0.75
7	—	—	1.00	—	0.75
8	—	—	1.50	—	0.75
9	—	—	2.00	—	0.75
10	—	—	—	0.50	0.75
11	—	—	—	1.00	0.75
12	—	—	—	1.50	0.75
13	—	—	—	2.00	0.75
14	1.00	2.00	—	—	0.75
15	2.00	2.00	—	—	0.75
16	3.00	2.00	—	—	0.75
17	4.00	2.00	—	—	0.75
18	1.00	—	2.00	—	0.75
19	2.00	—	2.00	—	0.75
20	3.00	—	2.00	—	0.75
21	4.00	—	2.00	—	0.75
22	1.00	—	—	2.00	0.75
23	2.00	—	—	2.00	0.75
24	3.00	—	—	2.00	0.75
25	4.00	—	—	2.00	0.75

e-mail: witold.musial@interia.pl

gated point-wise at 32 °C and the investigation was repeated five times, for every measurement. Prior to the examination, every sample was kept in room temperature for 1 h and then in a closed measurement chamber for 1 min to achieve the desired temperature of 32 °C. The cone rotation rate was 1.0 s^{-1} (0.26 rpm). The rate was selected according to preliminary trials at shearing rates of 1.0, 10.0, and 100.0 s^{-1}. The wide measurement range was obtained at a shearing rate of 1.0 s^{-1}. The measurements were taken 5 s after starting the cone.

Release Rates The process of metronidazole release from hydrogels was carried out according to the method described in USP 28: Apparatus 5—Paddle over Disk. Bidistilled water was used as the acceptor fluid. One thousand milliliters of the acceptor medium were used. The temperature was maintained at 32±0.5 °C. During the release process, beakers with acceptor fluid were covered to minimize evaporation. Teflon disks with a mean release surface of 16.0815 cm^2 (Hanson, U.S.A.) were used as release chambers. Semipermeable hydrophilic Membra-Cel®, MWCO 3500 manufactured by Serva was applied after soaking in bidistilled water for 60 min before use. The volume of the formulation carried on the disk was about 500.0 mg, selected to acquire flat release surface. Any possible air bubbles between the gel and the membrane were eliminated during placement of the semipermeable membrane. The distance between the disk and lower edge of the paddle was 25±2 mm.

The release time was 480 min and 5.00 ml of samples were taken every 10 min, supplemented with equal amount of bidistilled water, so that the dilution effect could be determined when drug concentration was calculated. The samples were received from the space between the surface of the disk and the lower edge of the paddle. The measurements were repeated six times. Formulations of viscosity approximating 5.00% methylcellulose were used to maintain rheological characteristics of the examined preparations. In the case of 2.00% Carbopol 934P, viscosity increased with increase of methylcellulose concentration in formulation; however, it did not achieve viscosity of 5.00% methylcellulose formulation. Thus, a composition of Carbopol 934P with the highest methylcellulose content, *i.e.* 4.00% (No. 17, Table 1), of the viscosity most approximated to the required range, was used.

Spectrophotometric Measurements Absorbance measurements were carried out using CECIL CE 5501 apparatus. Absorbance was measured in aqueous solution. Spectrum for metronidazole in aqueous solution was determined in the wavelength range from 250 to 370 nm. Maximum absorbance was at 319.7 nm.

Statistical Analysis The results were analyzed statistically, the mean values and standard deviations (SDs) were calculated, which enabled the evaluation of differences in groups and between groups for the obtained results—ANOVA. In the case of graphs presenting natural logarithm of the percentage of residual metronidazole in time, regression lines were determined by means of least squares method, and Pearson's correlation coefficient square (r^2) was calculated.

Results

Viscosity of Monopolymeric Gels The viscosity of monopolymeric gels is shown in Fig. 1. In case of Carbopol gels, viscosity was positively correlated with the increase of polymer concentration. Moreover, differences in viscosity were observed among groups of polyacrylic gels with various Carbopol types.

The highest viscosity was observed for gels with Carbopol 980NF, while the lowest viscosity was revealed by gels with Carbopol 971P. Gels with Carbopol 934P revealed intermediary viscosity. The viscosity of 5.00% methylcellulose gel (No. 1) was 19923±718 mPa·s.

Viscosity of Bipolymeric Gels To prepare bipolymeric gels containing compositions of polymers, 2.00% dispersion of Carbopol 937, 971P, or 980NF, and 1.00—4.00% methylcellulose dispersions were used. The viscosities of individual gels are shown in Fig. 2.

Compositions of Carbopol 971P and methylcellulose (No. 18—21) revealed an increase in viscosity with increasing concentration of methylcellulose. The viscosity increased from 17200±415 mPa·s for the composition of 2.00% Carbopol 971P and 1.00% methylcellulose (No. 18) to 26166±1217 mPa·s for the composition with 4.00% methylcellulose (No. 21).

Metronidazole Release Assay The investigations of the kinetics of metronidazole release were performed using formulations characterized by impending rheological properties in terms of dynamic viscosity at about 19310±8%. The following formulations were characterized by viscosity values similar to those determined for 5.00% methylcellulose gel with 0.75% of metronidazole (No. 1, Table 1): No. 4, 9, 10,

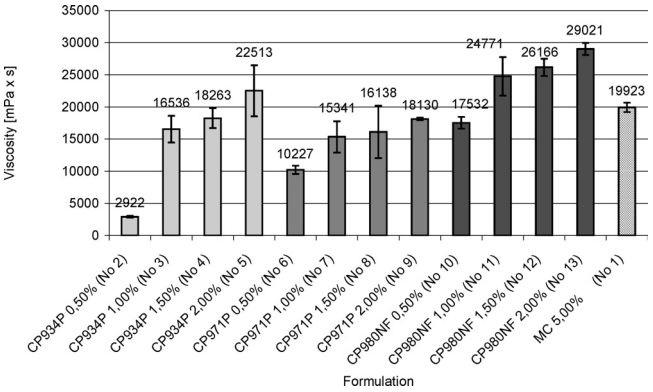

Fig. 1. Comparison of Dynamic Viscosities of Monopolymeric Metronidazole Formulations and Methylcellulose Gel with Metronidazole, CP, Carbopol; MC, Methylcellulose

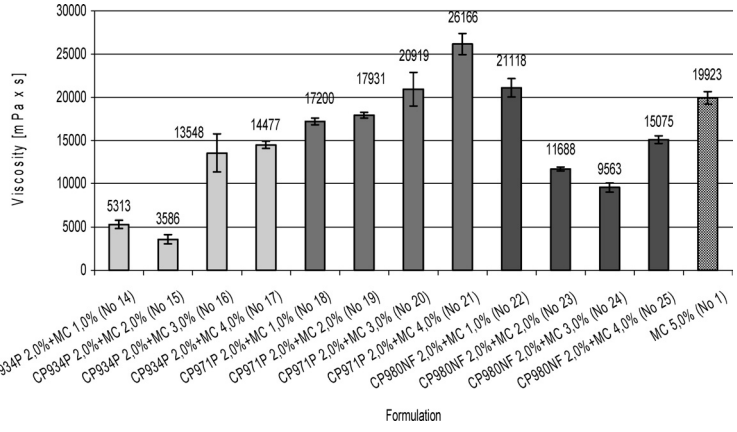

Fig. 2. Comparison of Dynamic Viscosities of Bipolymeric Metronidazole Formulations and Methylcellulose Gel, CP, Carbopol; MC, Methylcellulose

20, and 22. Formulation No. 17 was included, representing composition of Carbopol 934P and methylcellulose, with lower viscosity values than the remaining ones. The percentage of residual active substance was marked on the graphs against the function of time for better depiction of the residual metronidazole in drug form.

In all the examined formulations, the release process was characterized by a two-stage course. This tendency was evident on a semilogarithmic graph, in which the natural logarithm of the percentage of residual active substance was marked against the function of time. The relationships for selected sample gels No. 1, 9, and 17 are illustrated in Figs. 3—5, respectively.

The transition time between the stages was determined using the method of first derivative. To investigate the difference between two successive values of the percentage of metronidazole residue, a measurement point indicating a significant decrease of the difference was determined. To avoid any errors resulting from overlapping of two different processes, Gran's method of titration end-point determination was adapted. Measurements relatively far from the end point were used for linearization of the curved line. Thus, the point determined by means of first derivative method was excluded together with two neighboring values on both sides.

The remaining values of the natural logarithm from the percentage of active substance residue were used to determine two straight lines describing two stages of the process by means of a linear regression method. The transition point,

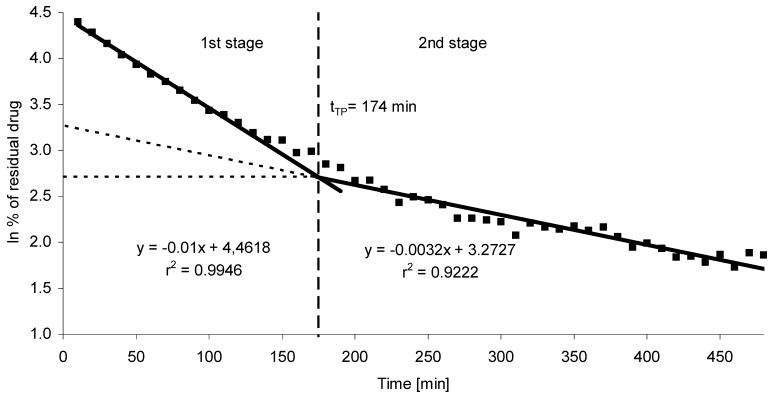

Fig. 3. Release Kinetics of Metronidazole from Methylcellulose Gel (No. 1)

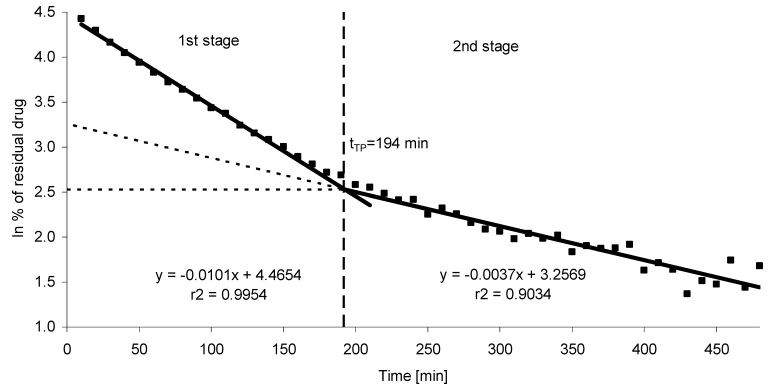

Fig. 4. Release Kinetics of Metronidazole from 2.00% Carbopol 971 Gel (No. 9)

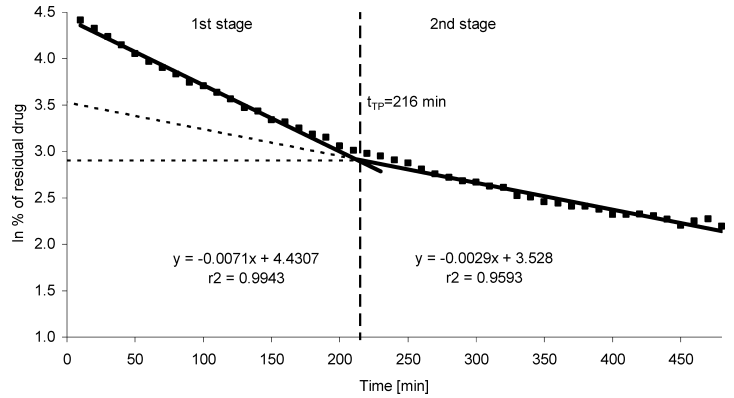

Fig. 5. Release Kinetics of Metronidazole from Mixed 2.00% Carbopol 934 and 4.00% Methylcellulose Gel (No. 17)

Table 2. Metronidazole Release Equations, Release Rates, Half-Release Times, Transition Points and pH for Selected Gels

Formulation	MC 5.00% (No. 1)	CP934P 1.50% (No. 4)	CP971P 2.00% (No. 9)	CP980NF 0.50% (No. 10)	CP934P 2.00% +MC 4.00% (No. 17)	CP971P 2.00% +MC 3.00% (No. 20)	CP980NF 2.00% +MC 1.00% (No. 22)
1st stage equation	$-0.0102x$ $+4.4618$	$-0.0109x$ $+4.4397$	$-0.0101x$ $+4.4654$	$-0.0142x$ $+4.4334$	$-0.0071x$ $+4.4307$	$-0.0068x$ $+4.4118$	$-0.0092x$ $+4.4674$
Pearson's correlation coefficient	0.9946	0.9952	0.9954	0.9941	0.9943	0.9948	0.9945
Release rate of 1st stage (K_1)	1.02×10^{-2} min^{-1} $\pm2.65\times10^{-4}$ S.D.	1.09×10^{-2} min^{-1} $\pm3.38\times0^{-4}$ S.D.	1.01×10^{-2} min^{-1} $\pm3.60\times10^{-4}$ S.D.	1.42×10^{-2} min^{-1} $\pm4.23\times10^{-4}$ S.D.	7.05×10^{-3} min^{-1} $\pm2.13\times10^{-4}$ S.D.	6.86×10^{-3} min^{-1} $\pm1.21\times10^{-4}$ S.D.	9.18×10^{-3} min^{-1} $\pm3.07\times10^{-4}$ S.D.
Half release time of 1st stage	68.24 min ±1.79 S.D.	63.91 min ±1.99 S.D.	69.00 min ±2.42 S.D.	48.96 min ±1.48 S.D.	98.33 min. ±2.94 S.D	101.10 min ±1.80 S.D.	75.55 min ±2.51 S.D.
Transition point	174.4 min ±2.5 S.D.	173.3 min ±3.1 S.D.	194.2 min ±2.6 S.D.	162.3 min ±2.7 S.D.	216.1 min ±1.8 S.D.	215.3 min ±3.3 S.D.	202.3 min ±2.9 S.D.
2nd stage equation	$-0.0032x$ $+3.2727$	$-0.0080x$ $+3.9316$	$-0.0037x$ $+3.2569$	$-0.0076x$ $+3.3899$	$-0.0029x$ $+3.528$	$-0.0034x$ $+3.6194$	$-0.0054x$ $+3.705$
Pearson's correlation coefficient	0.9222	0.9405	0.9034	0.9053	0.9593	0.9624	0.9669
Release rate of 2nd stage (K_2)	3.19×10^{-3} min^{-1} $\pm1.24\times10^{-4}$ S.D.	8.00×10^{-3} min^{-1} $\pm2.48\times10^{-4}$ S.D.	3.68×10^{-3} min^{-1} $\pm5.83\times10^{-5}$ S.D.	7.64×10^{-3} min^{-1} $\pm1.50\times10^{-4}$ S.D.	2.88×10^{-3} min^{-1} $\pm8.52\times10^{-5}$ S.D.	3.34×10^{-3} min^{-1} $\pm1.09\times10^{-4}$ S.D.	5.36×10^{-3} min^{-1} $\pm1.59\times10^{-4}$ S.D.
Half release time of 2nd stage	217.25 min ±8.42 S.D.	86.68 min ±2.71 S.D.	188.61 min ±2.93 S.D.	90.81 min ±1.77 S.D.	240.90 min ±7.02 S.D.	208.02 min ±6.95 S.D.	129.39 min ±3.78 S.D.
pH	5.57	3.35	3.33	3.80	3.46	3.39	3.33

CP, Carbopol; MC, methylcellulose; S.D., standard deviation.

separating both stages, was determined on the semilogarithmic graph by extrapolation of the straight lines at the point of their intersection.

The values of the transition point (t_{TP}), marked on sample graphs in Figs. 3—5 and listed in Table 2, for monopolymeric formulation ranged from 162.3 min in the case of 0.50% Carbopol 980NF (No. 10) to 194.2 min for 2.00% Carbopol 971P (No. 9). Samples with 5.00% methylcellulose (No. 1) or 1.50% Carbopol 934P (No. 4) revealed an intermediary value of 174.4 min. In all bipolymeric formulations, the transition point occurred over 200 min. The value of the transition point for composition of 2.00% Carbopol 980NF with 1.00% methylcellulose (No. 22) was 202.3 min, while for the composition of 2.00% Carbopol 934P with 4.00% methylcellulose (No. 17) was 216.1 min, and for 2.00% Carbopol 971P with 3.00% methylcellulose (No. 20) was 215.3 min.

In all the investigated gels, release rates (Table 2) describing the first stage of the process (K_1) exceeded the release rates in the second stage (K_2).

The K_1 rates for monopolymeric formulations ranged from 1.02×10^{-2} min^{-1} for gel No. 1 with 5.00% methylcellulose, to 1.42×10^{-2} min^{-1} for preparation No. 10 on the base of 0.50% Carbopol 980NF (Table 2). The K_2 rates of all monopolymeric formulations can be grouped into two ranges. In case of 0.50% Carbopol 980NF (No. 10) and 1.50% Carbopol 934P (No. 4), K_2 rate assumed values of 7.64×10^{-3} and 8×10^{-3} min^{-1}, respectively. The values for gels No. 1 and 9 were twofold lower and ranged from 3.19×10^{-3} min^{-1} for 5.00% methylcellulose, to 3.68×10^{-3} min^{-1} for 2.00% Carbopol 971P.

Among bipolymeric formulations, the highest K_1 of 9.18×10^{-3} min^{-1} was determined for gel No. 22, *i.e.*, 2.00% Carbopol 980NF with 1.00% methylcellulose. The remaining values of K_1 were 6.86×10^{-3} min^{-1} for the formulation of 2.00% Carbopol 971P with 3.00% methylcellulose (No. 20), and 7.05×10^{-3} min^{-1} for formulation of 2.00% Carbopol 934P with 4.00% methylcellulose (No. 17). The K_2 values of the investigated bipolymeric gels were 2.88×10^{-3}, 3.34×10^{-3}, and 5.36×10^{-3} min^{-1}, for formulations 17, 20, and 22, respectively. Half-release times corresponding to the above release rates are listed in Table 2.

The mean percentage of metronidazole residue in formulation in the last hour of the release process was the highest in the bipolymeric formulations; about 9.48%±0.41 for the composition of 2.00% Carbopol 934P with 4.00% methylcellulose (No. 17), and 8.49%±0.30 for 2.00% Carbopol 971P and 3.00% methylcellulose gel (No. 20). The remaining values of mean percentage of metronidazole residue in the formulation after finalizing the release process are listed in Table 3.

The mean values of the residue percentage in the last 60 min of the release process were used, with respect to the high amplitude of values in the final stage of the investigation. The values obtained from the last six measurements were impending and gave more precise presentation than the last survey.

Discussion

The metronidazole release process from the formulations consisted of two stages in all the investigated gels. This phenomenon can be attributed to two parallel processes, depending on the metronidazole distribution in polymeric bed. The active substance embedded in the hydrogel matrix becomes distributed between two compartments.[11,12] A similar occurrence was observed in the case of water bound in hydrogel matrix.[8,13] An impending model was proposed for hydrocortisone release from methylcellulose gels with polysorbates.[14] In the case of water, its free and bound fractions are differentiated.

Binding of polar molecules of water may occur because of interaction with hydrophilic regions of the polymer network—the layer of first-order bound water, and because of hydrophobic interactions with hydrocarbon parts of the poly-

Table 3. Comparison of the Percentage of Metronidazole Residue after 8 h and the Theoretically Calculated Irreversibly Bound Amount in Selected Formulations

Formulation	Metronidazole residue after 8 h (%)	±S.D.	Metronidazole irreversibly bound to polymer
Methylcellulose 5.00% (No. 1)	6.26	0.37	—
Carbopol 934P 1.50% (No. 4)	1.56	0.55	0.99
Carbopol 971P 2.00% (No. 9)	4.70	0.69	1.26
Carbopol 980NF 0.50% (No. 10)	0.94	0.38	0.93
Carbopol 934P 2.00%+methylcellulose 4.00% (No. 17)	9.48	0.41	1.68
Carbopol 971P 2.00%+methylcellulose 3.00% (No. 20)	8.49	0.30	1.44
Carbopol 980NF 2.00%+methylcellulose 1.00% (No. 22)	3.70	0.31	1.26

S.D., standard deviation.

mer macromolecule chain—secondary bound water. Metronidazole fraction corresponding to free water forms the external compartment and its release rate is determined by the rate of diffusion. Diffusion rate may be modified by free volume of the hydrogel system, which confirms the investigations carried out by Fujita,[15] Yasuda and Lamaze,[16] Peppas and Reinhart,[17] Favre and Girard,[18] as well as Barreiro-Iglesias *et al.*,[19] and by the diameter and distribution of the polymer bed pores described by Hoffman[8] as the environment tortuosity coefficient. Free volume of the matrix as well as the environment tortuosity coefficient depend on the polymer concentration and its cross-linking.

Moreover, metronidazole is bound by the polymer network. There may be two kinds of interactions of the drug with the macromolecule. These are weak hydrogen bonds between the polar groups of metronidazole and hydrophilic groups of the polymers as well as strong ionic interactions between nitrogen in the molecule's ring or polyacrylates carboxyl groups. The total amount of the drug bound, because of these interactions, forms the internal compartment, while the rate of drug–polymer compartment dissociation is the factor limiting the rate of release. According to some studies, ion-bound molecules of metronidazole are not released, or the process is very slow.[12] Figure 6 shows the above-described model, adapted for metronidazole release from polymeric bed.

In the first stage of the release process, represented in the semilogarithmic graphs (Figs. 3—5) by the segment t_0—t_{TP}, a significant predominance of first-order release from external compartment is observed. In t_{TP} (time corresponding to the so-called transition point), according to the chemical kinetics, the external compartment becomes almost completely drained. Evidence of changes in the residue percentage are interpreted in the graph as a result of a decreased release rate from the internal compartment. It also occurs according to first-order kinetics. In the time t_0—t_{TP}, the release course from the internal compartment has a nonlinear character, which is difficult to classify as far as order of the reaction is concerned. This nonlinearity is related to gradual increase of the concentration gradient between constantly drained external compartment and the internal compartment. The decrease of metronidazole concentration in the region of the drug–polymer complex induces a shift of the dissociation equilibrium (R in Fig. 6) of the complex to the right according to Le Chatelier's and Brown's law. This results in a gradual increase in the rate of drug release from the internal compartment. The rate reaches its maximum value when the external compartment is almost completely empty and remains constant—the first-order process in the second stage. Thus, dissociation of the drug–polymer complex becomes the element limiting the drug release rate from the internal compartment. The dissociated metronidazole molecules pass through the external compartment, which is characterized by a higher release rate and does not inhibit their kinetics.

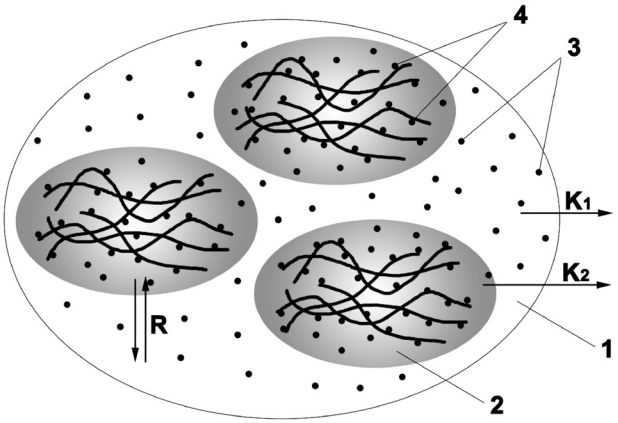

Fig. 6. Two-Compartment Model

1, external compartment; 2, internal compartment; 3, metronidazole molecules in the external compartment; 4, metronidazole molecules in the internal compartment (bound with polymer); R, metronidazole–polymer complex dissociation equilibrium rate; K_1, metronidazole release rate from the external compartment; K_2, metronidazole release rate from the internal compartment.

The linearity of the process is disturbed in the close nearness of TP. This transition can be explained by overlapping of the already finalizing first-order metronidazole release from the external compartment and an increasing drug release from the internal compartment. Consequently, none of the described phenomena predominate and the resultant process deviates from a linear course.

The baseline content of metronidazole in the internal compartment could be another parameter useful in the description of the investigated gels. The extrapolation of a straight line describing the second stage of the release process, as far as the site of its intersection with the axis of ordinates on a semilogarithmic graph assumes a nonlinear course of metronidazole release from the internal compartment in time t_0—t_{TP}. However it does not indicate the exact initial amount of the drug present in the internal compartment at time t_0.

An approximation is possible, determining the range of the percentage of drug residue corresponding to two extreme cases. Extrapolation of a straight line describing the second stage of the release process gives an extreme, maximum

Table 4. Calculated Maximum and Minimum Values of the Initial Content of Metronidazole in the Internal Compartment of Selected Formulations at Time t_0

Formulation	MC 5.00% (No. 1) (%)	CP934P 1.50% (No. 4) (%)	CP971P 2.00% (No. 9) (%)	CP980NF 0.50% (No. 10) (%)	CP934P 2.00% +MC 4.00% (No. 17) (%)	CP971P 2.00% +MC 3.00% (No. 20) (%)	CP980NF 2.00% +MC 1.00% (No. 22) (%)
Minimum	15.08	12.55	12.53	8.59	18.26	18.41	13.76
Maximum	26.38	50.99	25.97	29.66	34.06	37.32	40.65

CP, Carbopol; MC, methylcellulose.

value of the drug-residue percentage in the internal compartment at time t_0 for a situation, in which the process would occur according to the first-order kinetics since the beginning. To obtain the minimum value of the range, the natural logarithm of the residue percentage corresponding to TP should be read on Y axis. The minimum value of the range corresponds to the situation in which the process of release from the internal compartment begins at time t_{TP}. The value corresponding to the initial amount of the drug in the internal compartment assuming the nonlinear character of the process in time from t_0 to t_{TP}, is found between the minimum and the maximum of the range. Calculated theoretical maximum and minimum values of the initial content of metronidazole in the internal compartment are listed in Table 4.

As shown by Sutani *et al.*,[12] the ionic bound fraction of the drug does not dissociate at the site of action. The theoretical amount of metronidazole bound with polyacrylate corresponds to the molar content of the carboxyl group, corrected by the degree of dissociation. The amount of metronidazole in an ion-bound form with Carbopol was determined by means of averaged molar conversion factor for Carbopols, which enabled to compute the number of carboxyl groups in a weighed portion of the polymer, and by the formula determining the degree of dissociation.[12] Theoretical percentage of metronidazole residue ion bound with Carbopol 934P No. 4 was 0.99%. In Carbopol 971P—formulation No. 9, and Carbopol 980NF—formulation No. 10, the percentages were 1.26% and 0.93%, respectively. The percentage of metronidazole residue after 8 h in formulation No. 10 approximated the theoretically calculated irreversibly bound amount (Table 3, column 3). In formulations No. 4 and 9, the percentage of metronidazole residue, determined during investigation of the release kinetics was higher than the one theoretically determined and irreversibly bound by the polymer network volume (Table 3). Thus, it may be assumed that in Carbopol 980NF, practically the whole available for the acceptor fluid amount of the drug was released, while in the case of formulations No. 4 and 9, the release process may continue. Similar calculations were performed for compositions of polymers (Table 3). The accuracy of theoretically calculated amounts of metronidazole irreversibly bound in the formulation may be lower due to visible interaction between polyacrylates and methylcellulose. Figure 7 illustrates a sample interaction between Carbopol 971P and methylcellulose. Nevertheless, the percentage of metronidazole residue in all the bipolymeric preparations, determined in the course of the release-assay exceeded the theoretical values for the drug irreversibly bound by ionic interactions.

The visible interaction between acrylic acid polymers and methylcellulose may significantly affect the structure of overlapping mutual networks of both polymers as well as the rheological properties of the gels. The above interaction was confirmed by measurements of the dynamic viscosity. The viscosity of pure 2.00% Carbopol gels with the addition of metronidazole is higher than the viscosity of corresponding compositions of 2.00% Carbopol with the addition of methylcellulose (Fig. 2). Collapse of the three-dimensional structure of the network because of decreased hydratation of Carbopol chain in the presence of strongly hydrophilic methylcellulose polymer may be involved in this case.

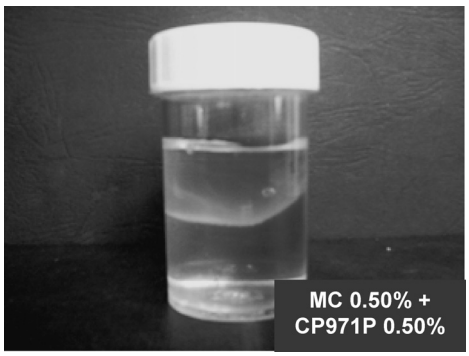

Fig. 7. The Sample Interaction between Phases of Carbopol 971P 0.50% and Methylcellulose 0.50% in Aqueous Dispersion after 24 h

As indicated by the investigations, metronidazole release rate from external compartments (K_1) assumed for 5.00% methylcellulose and for 2.00% Carbopol 971P gels are almost identical and reach about $1\times10^{-2}\,\mathrm{min}^{-1}$ (Table 2). Transverse cross-linking in a macromolecule significantly affects the rate of diffusion in the external compartment.

Release rates K_1 for gels on Carbopol base with different degrees of cross-linking and different concentrations (No. 4, 9, 10, Table 1) were compared. As indicated by data listed in Table 2, the rate of metronidazole release in the first stage is affected by concentration of the polymer. The differences in the degree of cross-linking of individual Carbopols also play a role. The aproximate molecular weights between adjacent crosslinks (Mc) of the analysed polyacrylates are between 104400 g/M for Carbopol 980, and 237600 g/M for Carbopol 971, whereas the Carbopol 934 has an intermediate molecular mass. The polymers of increased Mc are characterised with decreased crosslinker density, which inlfuenced the release process in lesser degree than the concentration (Table 3—the percentage of metronidazole residue after 8 h).

The transition point in monopolymeric formulations was about 200 min, while in the case of all two-polymer formulations, this value exceeded (Table 2). The amount of metronidazole, which remained in the hydrogel bed, was in all the cases, proportional to the release rate constant in the second stage (K_2)—Tables 2 and 3. Metronidazole release rates from both compartments were compared in monopolymeric

methylcellulose gel and Carbopol gels (Table 2). The analysis showed that both 5.00% methylcellulose (No. 1) and 2.00% Carbopol 971P (No. 9) are characterized by a similar release kinetics. In case of the two remaining preparations (No. 4, 10), the active substance release occurred more rapidly. This fact may be reflected by lower percentage of metronidazole residue in formulations No. 4 and 10 in comparison with the similar values for 5.00% methylcellulose and 2.00% Carbopol 971P. In the case of formulation of 2.00% Carbopol with 3.00% methylcellulose (No. 20), the slower kinetics of metronidazole release was obtained, compared with the 5.00% methylcellulose formulation No. 1 (Table 2).

The higher the release rate, the higher is the activity of the drug in the place of application. The highest release rate in monopolymeric gels, both in the first as well as in the second stage, was revealed by preparation with 1.5% of Carbopol 934P (Gel No. 4), and similarly observed in gel with 0.5% of Carbopol 980P (No. 10). In the case of bipolymeric formulations, the highest release rate of metronidazole from the 2.00% Carbopol 980P with 1.00% methylcellulose was obtained in comparison with the 5.00% methylcellulose gel (No. 22).

The analyzed gels maintained similar rheological properties of *ca.* 20000 mPa·s (Fig. 2), except gel No. 17. The reference dynamic viscosity was taken as *ca.* 20000 mPa·s, according to the viscosity of 5.00% methylcellulose metronidazole gel. This methylcellulose concentration facilitates suitable gel application to the skin, as found in commercial product with methylcellulose.

The process of methylcellulose release from hydrogel matrices containing acrylic acid, methylcellulose, or their compositions occurs in two stages, differing in intensity. The course of the investigated release-processes may be interpreted as release from a two-compartment system—the amount of metronidazole remaining in the hydrogel matrix after 8 h of the release process is reversibly proportional to the release rate from the internal compartment. The higher the release rate, the higher is the activity of the drug in the place of application. The highest release rate in monopolymeric gels, both in the first as well as in the second stage, was revealed by preparation with 1.5% of Carbopol 934P. In the case of bipolymeric formulations, the highest release rate of metronidazole from the 2.00% Carbopol 980P with 1.00% methylcellulose was obtained. The rheological properties of the investigated bipolymeric formulations were similar in relation to pure methylcellulose or monopolymer Carbopol gels. The proper viscosity, which is important for drug to remain on the skin, and for suitable application was developed in three monopolymeric and two bipolymeric gels. Proposed gels can be used for further *ex vivo* and *in vivo* studies to obtain a suitable drug activity of metronidazole in the treatment of acne rosacea.

Acknowledgments The research was supported by grant no. 1313 of Wroclaw Medical University and Polish Ministry of Science. The author thanks Prof. Eugeniusz Baran from the Department of Dermatology in Wroclaw Medical University for the discussion on acne rosacea etiology, and Mr. Lukasz Beczkowski for his cooperation in preparative works.

References

1) Del Rosso J. Q., Bikowski J., *J. Drugs Dermatol.*, **4**, 473—480 (2005).
2) Gupta A. K., Chaudhry M. M., *J. Eur. Acad. Dermatol. Venereol.*, **19**, 273—285 (2005).
3) Oechsner M., Keipert S., *Eur. J. Pharm. Biopharm.*, **47**, 113—118 (1999).
4) Weuts I., Kempen D., Verreck G., Peeters J., Brewster M., Blaton N., *Eur. J. Pharm. Sci.*, **25**, 387—393 (2005).
5) Bozkir A., Aydinli A., *Acta Pharm.*, **47**, 21—30 (1997).
6) Rossi S., Bonferoni M. C., Ferrari F., Caramella C., *Pharm. Dev. Technol.*, **4**, 55—63 (1999).
7) Gupta A., Garg S., Khar R. K., *Drug Dev. Ind. Pharm.*, **20**, 315—325 (1994).
8) Hoffman A. S., *Adv. Drug Deliv. Rev.*, **54**, 3—12 (2002).
9) Rosiak J. M., Yoshii F., *Nucl. Instr. Meth. Phys. Res. B*, **151**, 56—64 (1999).
10) Roy I., Gupta M. N., *Chem. Biol.*, **10**, 1161—1171 (2003).
11) Musial W., Kubis A. A., *Polim. Med.*, **35**, 39—46 (2005).
12) Sutani K., Kaetsu I., Uchida K., Matsubaraa Y., *Rad. Phys. Chem.*, **64**, 331—336 (2002).
13) Kashyap N., Kumar N., Kumar M. N., *Crit. Rev. Ther. Drug. Carrier Syst.*, **22**, 107—149 (2005).
14) Kubis A. A., Musial W., Szczesniak M., *Pharmazie*, **57**, 479—481 (2002).
15) Fujita H., *Fortschr. Hoch. Polym. Forsch.*, **3**, 1—4 (1961).
16) Yasuda H., Lamaze C. E., *J. Macromol. Sci.-Phys. B*, **5**, 111—134 (1971).
17) Peppas N. A., Reinhart C. T., *J. Membr. Sci.*, **15**, 275—287 (1983).
18) Favre E., Girard S., *Eur. Polym. J.*, **37**, 1527—1532 (2001).
19) Barreiro-Iglesias R., Alvarez-Lorenzo C., Concheiro A., *J. Contr. Rel.*, **77**, 59—75 (2001).