STUDIA UBB CHEMIA, LX, 1, 2015 (p. 177-185)
(RECOMMENDED CITATION)

RHEOLOGICAL BEHAVIOR AND MICROBIOLOGICAL STUDIES OF CARBOPOL® HYDROGELS

EMESE SIPOS[a], ZOLTÁN – ISTVÁN SZABÓ[a*], LILLA LŐRINCZI[b], ADRIANA CIURBA[c]

ABSTRACT. Rheological and microbiological studies were performed for hydrogels prepared with different types of crosslinked polyacrylate polymers (Carbopol® 940, 980 and Ultrez® 10). All hydrogels exhibited typical viscoelastic properties, with no Newtonian flow regimen. Linearizing viscosity profiles, excellent correlations were obtained between logarithmic values of viscosity and shear rate. The study also revealed that Carbopol® 980 and Ultrez® 10 based hydrogels showed higher viscosity than the Carbopol® 940 based. Microbiological studies were performed in order to find a suitable preservative for the prepared hydrogels. Both the diffusion based method and challenge test indicated that phenylmercuric borate was the most effective preservative, regardless of the microorganism tested and can be suitable for preventing antimicrobial growth in the prepared hydrogels.

Keywords: *Carbopol®, hydrogel, pseudoplastic, challenge test*

INTRODUCTION

Hydrogels are three-dimensional, hydrophilic networks of cross-linked water soluble polymers. They are viscoelastic, solid-like materials, owing their three-dimensional properties to the cross-linking process of the polymer strands of molecules as a result of physical or chemical forces[1,2].

[a] *University of Medicine and Pharmacy Tîrgu Mureș, Faculty of Pharmacy, Department of Drugs Industry and Pharmaceutical Management, Gh. Marinescu 38, RO-540139, Tîrgu Mureș, Romania*
[b] *University of Medicine and Pharmacy Tîrgu Mureș, Faculty of Medicine, Department of Microbiology, Gh. Marinescu 38, RO-540139, Tîrgu Mureș, Romania*
[c] *University of Medicine and Pharmacy Tîrgu Mureș, Faculty of Pharmacy, Department of Pharmaceutical Technology, Gh. Marinescu 38, RO-540139, Tîrgu Mureș, Romania*
** Corresponding author szabo.zoltan@umftgm.ro*

In recent years, hydrogels have attracted a wide interest due to their responsiveness to environmental stimulants (pH, ionic strength, solvent properties, electrical field etc), hydrophilic nature and biocompatibility. There are several biomedical fields, where hydrogels were successfully applied, such as wound dressing[3–5], tissue engineering[6], contact lens development[7,8] etc. A particular interest for hydrogels represents their use as drug delivery systems for various active pharmaceutical ingredients, being beneficial in altering pharmacokinetic properties of incorporated drugs[1,2].

Carbopols are a family of synthetic microgels formed by crosslinking linear polyacrylates with various other chemical compounds. They are soluble in polar solvents and due to the hydration of the individual polymer molecules; they partially uncoil and begin to swell. Upon titration with a basic solution (ex. sodium hydroxide), the carboxylic groups in its structure ionize and uncompensated sodium cations lead to an increase in the osmotic pressure, which causes individual polymer strands to swell drastically. The formed system, maintains its gel-like characteristics until local deformations exceed a certain level, upon which the gel network breaks apart and material flow occurs[9,10].

Due to their rheological properties, low thixotropy and optical transparency, until recently, Carbopol® gels have been considered *model yield stress fluids.* However, recently, it has been shown that at certain experimental conditions, thixotropic effects and irreversible deformations can occur[10–12].

As it can be observed proper knowledge of the flow behaviour of Carbopol® gels is necessary for their further use in pharmaceutical formulations. Our aim was to investigate the rheological properties of gels of three types of Carbopol® (Carbopol® 940, 980 and Ultrez® 10) in order to find a suitable candidate for a mucoadhesive pharmaceutical form.

Another important aspect of the formed hydrogels is their microbial purity. During formulation studies, it is essential to prove that the future pharmaceutical formulation will provide the needed protection against damages caused by bacterial multiplication or by microbiologic contamination during the period of validity. If a pharmaceutical formulation – especially those obtained with water – does not possess antimicrobial effect, it requires the use of preservatives, for microorganism multiplication prevention or for microorganism contamination restriction. These types of damages – characteristic for multidose forms – can appear both at usual storage conditions and during usage. The use of microbiological preservatives prevents the danger of a possible infection and that of microbial disintegration.

Although the Carbopol® types selected do not constitute an ideal medium for microorganism or fungi, they do not prevent their growth and some microorganisms may develop without causing polymer disintegration.

Therefore, apart from the rheological studies, four different preservatives (combination of methylparaben and propylparaben, phenylmercuric borate, benzoic acid and sodium benzoate) were employed and tested for their efficacy in preventing microbial growth in the prepared hydrogels.

RESULTS AND DISCUSSION

Rheological studies

Flow and viscosity curves were recorded for each of the prepared gels (Fig. 1 and 2).

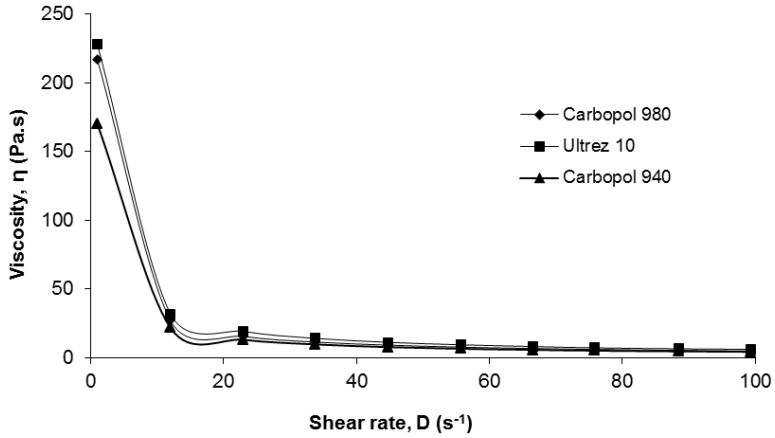

Figure 1. Viscosity curves of the studied hydrogels

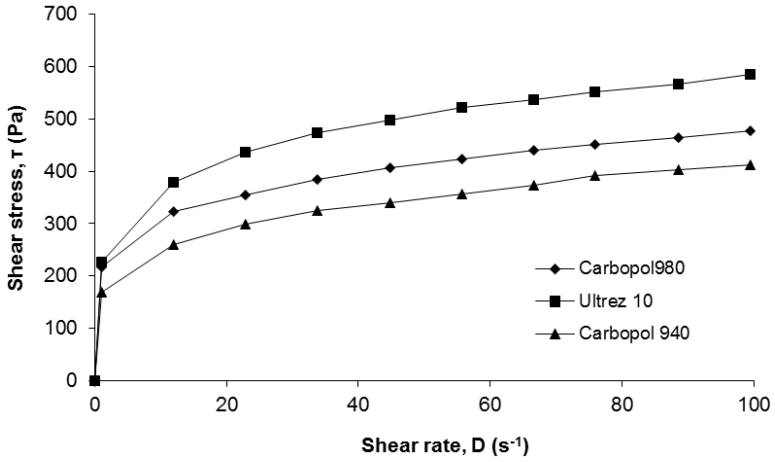

Figure 2. Flow curves of the studied hydrogels

No Newtonian flow regimen has been observed in the selected shear rate region. Viscosity values obtained at low shear rate were around 200 Pa.s for all hydrogels, which decreased significantly, when higher shear rates were applied. When a shear rate of 12 s^{-1} has been used, viscosity values decreased almost to one tenth of that obtained at a shear rate of about 0.1 s^{-1}. The hydrogel obtained with Ultrez® 10 had the highest viscosity values, followed by Carbopol® 980. The lowest viscosity values were obtained with the hydrogel obtained from Carbopol® 940.

Each viscosity profile exhibited a power law relationship between viscosity and shear rate. Using a simple logarithmic transformation, we can obtain a linear relationship between shear viscosity and shear rate:

$$\log \eta = \log \eta^{+} - m \log D$$

where, η - shear viscosity (Pa.s)
D - the shear rate (s^{-1})
η^{+}- the extrapolate viscosity
m - the tangent of the line

Results obtained after linearizing the viscosity curve are shown in Table 1.

Table 1. Results of the linearization of viscosity curves

Carbopol type	Regression equation and coefficient of determination
Carbopol® 980	$y = 2.3286 - 0,8293x$; $r^2=0,9998$
Ultrez® 10	$y = 2.3591 - 0,7964x$; $r^2=0,9999$
Carbopol® 940	$y = 2.2185 - 0,8066x$; $r^2=0,9996$

In all cases, excellent linear correlations were found between logarithmic values of shear viscosity and shear rate. The negative *m* value confirms the pseudoplastic behaviour of gels *i.e* a decrease in viscosity with increase of shear rate. It indirectly characterizes the binding energy and the number of the bonds from the structure of the formed hydrogels. The bigger the *m* value, the decrease in viscosity is also bigger, meaning that the breakage of bonds in the coherent structure appears more easily.

Microbiological studies

The total microbial load is an important parameter in evaluating excipients' or drug carriers' suitability in pharmaceutical formulations. Hydrogels prepared from Carbopol® lack intrinsic antimicrobial activity, thus, in order to achieve

acceptable microbiological purity of the drug carrier, four different preservatives were tested for their capacity to prevent microbial growth in the hydrogels prepared (combination of methylparaben and propylparaben, phenylmercuric borate, benzoic acid and sodium benzoate).

Two separate methods were involved in the evaluation, a diffusion-based method and a challenge test for the following microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*.

Results of the diffusion-based methods are presented in Table 2, based on the exerted inhibition zones on the selected microorganisms.

Table 2. Results of the microbiological studies obtained with the diffusion-based method

Polymer used	Preservative	Inhibition zones for tested microorganism			
		Staphylococcus aureus	*Candida albicans*	*Escherichia coli*	*Pseudomonas aeruginosa*
Carbopol® 940	1	14.8 ± 0.8	-	9.4 ± 0.5	-
	2	39.2 ± 2.8	24.4 ± 1.5	42.2 ± 0.8	9.6 ± 0.9
	3	22.2 ± 3.0	-	26.6 ± 1.1	-
	4	15.6 ± 1.0	-	20.8 ± 1.9	-
Carbopol® 980	1	20.2 ± 0.8	-	30.0 ± 1.6	-
	2	39.6 ± 1.1	31.2 ± 3.1	41.6 ± 2.7	13.4 ± 1.1
	3	13.2 ± 1.5	-	25.4 ± 1.1	-
	4	14.0 ± 1.6	-	7.0 ± 2.1	-
Ultrez® 10	1	17.2 ± 1.9	-	14.8 ± 1.5	-
	2	39.8 ± 1.5	31.8 ± 2.3	42.2 ± 1.5	10.8 ± 2.2
	3	21.2 ± 3.1	-	25.8 ± 1.3	-
	4	14.0 ± 1.6	-	11.0 ± 1.6	-

Values represent mean inhibition zones ± standard deviations in millimeters
1 – Methylparaben/propylparaben; 2 – Phenylmercuric borate; 3 – Benzoic acid;
4 – Sodium benzoate

Comparing the inhibition zones for the hydrogels prepared, we can observe similar results. The biggest inhibition is observed in the case of phenylmercuric borate solution 0,007 %, both for Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and fungi (*Candida albicans*). Moreover, only this preservative was effective in the case of *Candida albicans* and *Pseudomonas aeruginosa*, the other preservatives failing to present any inhibition zone for the mentioned microorganisms. Phenylmercuric borate showed similar inhibition zones, regardless of the Carbopol® type used for the preparation of hydrogels.

Microbial challenge testing was also performed for the prepared hydrogels in order to determine the ability of the preservatives to inhibit the growth of pathogens. The same microorganisms were used for contamination as in the previous study. The duration of the challenge test was 7 days, with periodic sample removals at the start of the test, 6 hours, 24 hours, 48 hours, 168 hours (7 days) and at the end of the challenge test. As expected from the diffusion based test results, out of the four antimicrobial preservatives studied phenylmercuric borate proved to be the most efficient for the microorganisms tested. Formulations containing this preservative, showed no contamination at the 6 hour sampling. Apart from phenylmercuric borate, the combination of methylparaben and propylparaben was also efficiently on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* and eliminated completely *Escherichia coli* after 6 hours from Carbopol® 940 and after 24 hours from Carbopol® 980 based hydrogels.

Results indicate that maximum 48 hours after inoculation benzoic acid ensures sterility regardless of the inoculated microorganisms in all hydrogels studied. The highest efficacies were found for the Gram positive microorganism, *Staphylococcus aureus*, which were completely destroyed 6 hours after the inoculation (Table 3).

Sodium benzoate reduces colony count by more than 100 units after two days, regardless of the microorganisms used (Table 4). Furthermore, microorganism count did not increase after this point, thus, meeting regulatory criteria[13].

CONCLUSIONS

Results of the rheological studies indicate that all hydrogels examined, exhibit a pseudoplastic flow, characteristic of macromolecular dispersions of linear molecules as in the case of carboxyvinyl polymers. Rheological characterization of the obtain hydrogel revealed greater viscosity values for Carbopol® 980 and Ultrez® 10, while the smallest values were obtained for Carbopol® 940.

Microbiological study concludes that all preservatives were able to confer adequate microbiological quality to the prepared hydrogels, as amended by the European Pharmacopoeia. Comparing the different preservatives employed, phenylmercuric borate proved to be the most efficient, regardless of the testing method.

Table 3. Efficacy of benzoic acid as a preservative during the challenge test

Time	Carbopol® 980				Ultrez® 10				Carbopol® 940			
Microrganism*	1	2	3	4	1	2	3	4	1	2	3	4
At inoculation	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6 h	-	-	1.69	1.90	1.47	-	1.47	1.30	1.3	-	1.69	1.90
24 h	-	-	1.47	1.69	1.39	-	-	-	-	-	1.30	1.69
48 h	-	-	-	-	-	-	-	-	-	-	-	-

Values indicate logarithmic microorganism colony count
***** 1 – *Escherichia coli;* 2 – *Staphylococcus aureus;* 3 – *Pseudomonas aeruginosa;* 4 – *Candida albicans*

Table 4. Efficacy of sodium benzoate as a preservative during the challenge test
Values indicate logarithmic microorganism colony count

Time	Carbopol® 980				Ultrez® 10				Carbopol® 940			
Microorganism*	1	2	3	4	1	2	3	4	1	2	3	4
At inoculation	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
6 h	2.17	-	2.00	2.17	2.17	1.60	2.17	2.00	2.30	1.77	1.69	2.00
24 h	1.90	-	1.90	1.90	2.00	-	1.90	1.30	2.00	1.69	1.60	-
48 h	1.30	-	-	-	1.90	-	0.84	-	1.69	-	-	-
168 h	1.17	-	-	-	1.84	-	-	-	1.60	-	-	-
336 h	-	-	-	-	-	-	-	-	-	-	-	-

***** 1 – *Escherichia coli;* 2 – *Staphylococcus aureus*; 3 – *Pseudomonas aeruginosa*; 4 – *Candida albicans*

EXPERIMENTAL SECTION

Materials

Carbopol® 980, Carbopol® 940, Ultrez™ 10 were from Lubrizol. Sodium hydroxide, methylparaben, propylparaben, phenylhydrargiri boras were purchased form Merck, while sodium benzoate was obtained from Reactiv Bucharest. Agar and Sabouraud medium were obtained from Cantacuzino Institute, Romania. The bacterial strains used in the microbiological studies were the following: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231)*, Escherichia coli* (ATCC 8739).

Gel preparation

Composition of the prepared hydrogels, used in the microbiological studies is described in Table 5. The gels for the rheological studies were prepared with deionized water, instead of preservative solution.

Carbopol polymers were added to the preservative solution and were allowed it to hydrate. After hydration (in case of Carbopol® 940 and Carpopol® 980 polymer - 30 minutes and in case of Ultrez® 10 polymer - 2 minutes) the dispersion was mixed at a low agitation rate of 500 rpm. The obtained samples were neutralized with sodium hydroxide solution until pH 7 was reached. Thereafter, the remaining aqueous preservative solution was added. The mixture was agitated thoroughly to obtain a clear, viscous gel.

Table 5. Quantitative composition of the prepared hydrogels

Polymer type	Carbopol® 980	Ultrez® 10	Carbopol® 940
Polymer quantity (g)	1.00	1.00	1.00
Sodium hydroxide solution 10.0%(g)	q.s.	q.s.	q.s.
Preservative, aqueous solution* (g)	ad. 100.00	ad. 100.00	ad. 100.00

* The following preservative solutions were used:
Methylparaben/propylparaben 0.18 %/0.02 % (w/w)
Phenylmercuric borate 0.007 % (w/w)
Benzoic acid 0.2 % (w/w)
Sodium benzoate 0.01 % (w/w)

Methods

For rheological studies, a Rheometer MC1 (Paar Physica) was used. Obtained data was analysed with Rheocalc software 1.01. Viscosity determinations had been recorded for ten sequentially increasing and decreasing values of shear rate in the range of 0.995- 99.4 sec^{-1}, at a constant temperature of 25 ± 0.1 °C.

For the microbiological studies, two separate methods were used: one diffusion-based method and a separate, challenge-test.

In the case of the diffusion-based method, a few cavities have been made with small diameter cylinders into the medium, and in each cavity one gram gel had been introduced. Mediums were inoculated on the surface with microbe containing suspensions (3.10^8 microorganisms in one mL^{-1}). The strains were incubated for 24 hours at 30-35 °C, and then the inhibition zone diameter had been measured.

The challenge test was effectuated according to the Romanian Pharmacopoeia ed. X^{14}. The hydrogels have been inoculated with microbe containing suspensions (0.1 mL suspension for every 20 grams of hydrogel so as to obtain a concentration of 10^{-5} microorganism/gram). The artificially infected hydrogels had been stored at 20-25 °C, and at periodic intervals (at inoculation, 6 h, 24 h, 48 h, 7 days – 168 h and 14 days – 336 h) microorganism number had been determined and logarithmic transformations were made.

ACKNOWLEDGMENTS

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893. The first author would like to thank Collegium Talentum for their financial support.

REFERENCES

[1]. T.R. Hoare and D.S. Kohane, *Polymer (Guildf).,* **2008**, *49*, 1993–2007.
[2]. W.A. Laftah, S. Hashim, and A.N. Ibrahim, *Polym. Plast. Technol. Eng.,* **2011**, *50*, 1475.
[3]. S.P. Tan, P. McLoughlin, L. O'Sullivan, M.L. Prieto, G.E. Gardiner, P.G. Lawlor, and H. Hughes, *Int. J. Pharm.,* **2013**, *456*, 10.
[4]. P.T.S. Kumar, V.-K. Lakshmanan, T.V Anilkumar, C. Ramya, P. Reshmi, A.G. Unnikrishnan, S.V Nair, and R. Jayakumar, *ACS Appl. Mater. Interfaces,* **2012**, *4*, 2618.
[5]. R. Jayakumar, M. Prabaharan, P.T. Sudheesh Kumar, S.V Nair, and H. Tamura, *Biotechnol. Adv.*, **2011**, *29*, 322.
[6]. S. Van Vlierberghe, P. Dubruel, and E. Schacht, *Biomacromolecules*, **2011**, *12*, 1387.
[7]. X. Gu, J.D. Jennings, J. Snarr, V. Chaudhary, J.E. Pollard, and P.B. Savage, *Invest. Ophthalmol. Vis. Sci.*, **2013**, *54*, 6217.
[8]. J. Diec, V.E. Evans, D. Tilia, T. Naduvilath, B.A. Holden, and P.L. de la Jara, *Eye Contact Lens*, **2012**, *38*, 2.
[9]. A. Poumaere, M. Moyers-González, C. Castelain, and T. Burghelea, J. Nonnewton. *Fluid Mech.,* **2014**, *205*, 28.
[10]. E. Weber, M. Moyers-González, and T.I. Burghelea, J. Nonnewton. *Fluid Mech.,* **2012**, *183-184*, 14–24.
[11]. A.M.V. Putz and T.I. Burghelea, *Rheol. Acta,* **2009**, *48*, 673.
[12]. T. Divoux, D. Tamarii, C. Barentin, and S. Manneville, *Phys. Rev. Lett.*, **2010**, *104*, 208.
[13]. European Pharmacopoeia, 8th edition, Council of Europe, Strasbourg, **2014**.
[14]. Farmacopeea Română, Ediția a X-a, Editura Medicală Bucuresti, **1993**.