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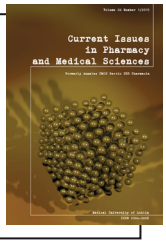

Effect of standard and reversible arrangements of Ph.Eur./USP extraction cells during dissolution tests of calcium dobesilate in hydrogel formulation

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ABSTRACT

The aim of the study was to evaluate, in comparison to the reference product, the effect of the hydrophilic nonionic polymers: methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC), as well as the anionic polymers – copolymers of acrylic acid, on the release kinetics of a calcium dobesilate hydrogel formulation intended for application on the skin. In this work, we used an ointment cell for the release of the active pharmaceutical ingredient (API) from the formulations. This release was performed by employing the paddle method at 100 rpm, with the extraction cells placed in the release vessels in two different positions: with the semipermeable membrane faced to the top, or to the bottom of the vessel. Released API percentage was assessed via the validated spectrophotometric method. In the study with standard placement of the ointment cell, the release rates ranged from 4.45×10^{-3} min^{-1} for a formulation containing polyacrylic acid (PA), to 6.96×10^{-3} min^{-1} for a formulation based on HPMC. In the group of nonionic polymers, the release rate is higher in the case of HPMC, and lower in the case of MC. In the group of anionic polymers, the release rate is higher with the formulation of a modified copolymer of acrylic acid 11 (PC11), while release from a formulation comprising a polymer PA is rather prolonged. We found that the placement of the extraction cell does not affect the alignment of the formulations investigated in terms of the release rates in the group of non-ionic formulations: HPMC > MC, and in the group of preparation of ionic polymers: PC11 > PA.

INTRODUCTION

Calcium dobesilate is of high interest as an active pharmacological substance with multidirectional activity, especially in the terms of the function of blood vessels [12]. The drug affects the capillaries, improves endothelial function and reduces the capillary permeability induced by histamine and bradykinin. This action facilitates the formation of fibrinogen, a_1 globulin and a_2 globulin. Calcium dobesilate also inhibits the breakdown of collagen. Moreover, it improves microcirculation and the plasticity of erythrocytes by inhibiting their tendency to stick. What is more, calcium dobesilate indirectly increases lymphatic drainage, and also improves peripheral circulation, preventing stagnation of venous blood. Due to its characteristics, it has been employed in ophthalmology and angiology.

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The applied forms include: tablets, capsules and gel. Tablets containing calcium dobesilate are used for the treatment of vascular eye diseases induced by long-term hypertension and diabetes, and thus are essential in diabetic retinopathy. In addition, this substance is applied in the treatment of hemorrhoids, phlebitis, leg ulcers and edema of the lower limbs [8]. Tablets and capsules should be used with caution, however, in patients with chronic, recurrent gastritis and peptic ulcers of the stomach and duodenum [5]. Studies have also shown that the intraocular injection of a preparation containing calcium dobesilate is an effective treatment for Stargardt disease [3]. Other research has demonstrated that it can be used in the form of eye drops to inhibit Pterygium [2].

Calcium dobesilate is an interesting active pharmaceutical ingredient (API) with many potential applications, but published data on the kinetics of release of the API from the

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dosage form are scarce. The technology of gel preparations, containing calcium dobesilate within its composition, may result in formulation difficulties, due to the ionic interactions between the API and the functional groups of the polymer. Further studies are required that can help to assess the effectiveness of the new formulations of ointments and gels containing calcium dobesilate. This form of the drug can then be an effective alternative for patients with problems of the stomach and duodenum.

Hydrogels, due to their biocompatibility, biodegradability and their hydrophilic nature, are now recognized as very good carriers of therapeutic substances [10]. They are defined as being preparations consisting of two or more compartments. This means that they are composed of polymer chains, and water that fills the spaces between the macromolecules. Many thermoplastic hydrogels are reversible gels, meaning that they can exist in liquid or gel form, depending on their temperature. This type of gel is intensively studied, due to the possibility of providing controlled and targeted delivery of API to a specific site of action [7]. Methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) are two of the most frequently used substrates hydrogel.

The use of hydrogels in the pharmaceutical industry is increasing. Hydrogels are most commonly used as carriers of API in transdermal systems. Hydrogels have a beneficial effect on wound healing, protecting them against infections, as well as shortening the healing time of chronic ulcers, burns and bedsores. Currently, due to the possibilities of modifying the chemical structure and physical properties, they are widely used in the preparation of pharmaceutical forms administered orally, subcutaneously, to the eye or on the mucous membranes. MC and HPMC are among the semisynthetic polymers forming hydrogels of semi-solid consistency under the influence of elevated temperature, i.e. 40-50°C for gels of MC, and 75-90°C in the case of HPMC gels [9]. MC and HPMC are examples of nonionic polymers, while the polyacrylic acid derivatives contain numerous carboxylic groups. Cross-linked polyacrylates play important role in the formulation technology of topical preparations – the most widely utilized being carbopol (PA). Other polyacrylates containing specific co-monomers, i.e. amine groups and sulfo groups are also in use. Polyacrylate crosspolymer 11 (PC11) is mainly employed in cosmetology, has good compatibility with various solvents, high tolerance for the presence of salt in the formulation, and has a stabilizing effect on the gel formulation. It easily dissolves in water at low temperature and has much lower viscosity than MC and HPMC.

API release kinetics studies are carried out in different ways, and appropriate recommendations are included, among others, in the United States Pharmacopoeia (USP) and in the European Pharmacopoeia (Ph.Eur.), and are widely discussed [1,14].

The aim of the study was to evaluate the effect of hydrophilic nonionic polymers and anionic polymers on the release kinetics of calcium dobesilate from a hydrogel formulation intended for application to the skin, as compared to the reference product in the market, using two arrangements of extraction cells applied during the release study.

MATERIALS

Our studies employed preparations that differed on the basis of the different hydrophilic polymers utilized. For the preparation of the gels, we used the following: methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), and a modified polyacrylic acid moiety comprising nitrogen and the sulfo group (Polyacrylate-11 Crosspolymer, PC11). As a reference, a marketed formulation comprising in its composition, calcium dobesilate and modified polyacrylic acid (PA), was applied. The composition of the prepared gels is presented in Table 1.

Table 1. Composition of hydrophilic gels with calcium dobesilate, prepared with different polymers

Type of hydrogel:	A	B	C	D*
CD [g]	2.0	2.0	2.0	reference CD product with PA
MC [g]	1.5	-	-	
HPMC [g]	-	3.0	-	
PC11 [g]	-	-	1.5	
Aqua [g]	ad 100.0	ad 100.0	ad 100.0	

CD – calcium dobesilate, MC – methylcellulose, HPMC – hydroxypropyl methylcellulose, PC11 – polyacrylate-11 crosspolymer, PA – polyacrylic acid, * – reference preparation available on the Polish market

METHODS

The dissolution study was performed by way of the paddle method at 100 rpm, with a gel placed in the extraction cells (Perspex, Pharma Test Apparatenbau, Germany) in a manner compatible with the dissolution test that is undertaken for transdermal patches as stated in Ph. Eur., Chapter 2.9.4. The release experiment was performed by way of the drug dissolution tester Erweka DT706 (Germany). The acceptor fluid was purified water, in accordance with recommendations stated in Ph. Eur., in a volume of 900 ml. The membrane was made from regenerated cellulose (Rothilabo, Germany) with pore diameter of 0.45 mcm. The temperature of the acceptor fluid was 37°C. Measurements were performed in three parallel extraction cells, by sampling the acceptor fluid volume of 2 ml, approximately every 10 minutes over 4.5 hours. The acceptor fluid was not supplemented. Absorbance measurements were made on a spectrophotometer UV-VIS T60 (PG Instruments, USA), at a wavelength of approx. 300 nm, in accordance with the outlined absorption spectrum of calcium dobesilate in an aqueous solution. The percentage of the released API was determined according to the prepared standard curve shown in Figure 1.

In order to obtain a proper absorbance of the sample, we diluted the samples with water two or three times, depending on the value of the initial absorbance. Furthermore, in order to determine the effect of extraction cell arrangement on the rate of release, we conducted another experiment (series: I and II). Herein, the possible arrangements of the extraction cells for testing release of the API are shown in Figure 2. In set I, the donor compartment is arranged in accordance with the pharmacopoeial recommendations, whereas in set II, the release of the drug occurred from the extraction cell arranged with the membrane to the bottom of the vessel.

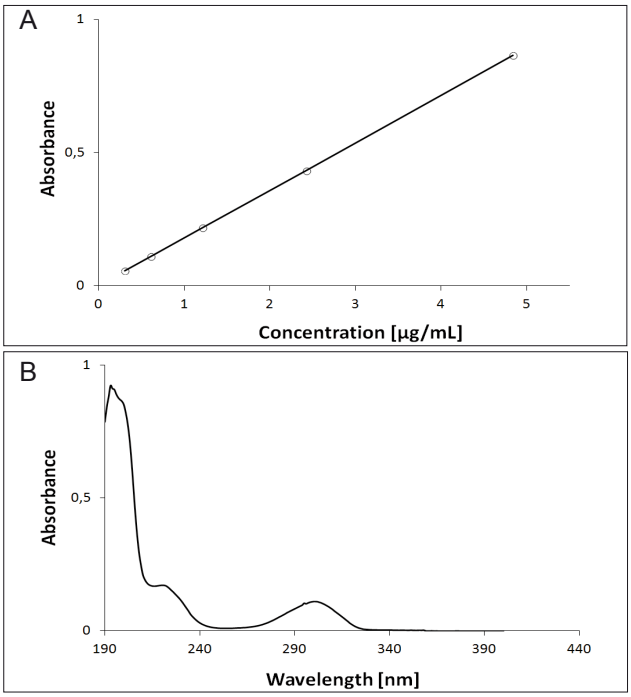
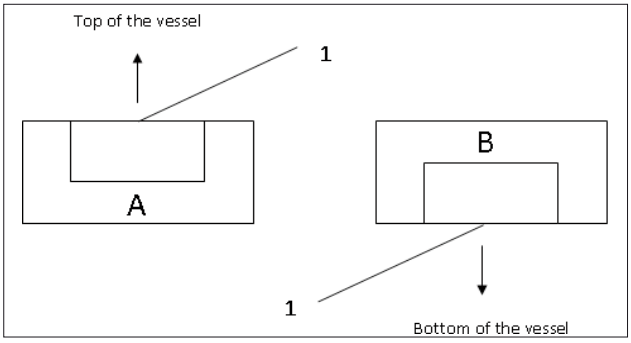


Figure 1. Calibration curve of an aqueous solution of calcium dobesilate (A), and the graph of a continuous spectrum of calcium dobesilate solution (B)



A – standard placement according to the pharmacopoeial monograph (set I of measurements), B – reversible placement (set II measurements), 1 – semipermeable membrane

Figure 2. Schematic representation of the placement of the cell in the assay of the amount of released calcium dobesilate

Statistical evaluation of the results was performed using Statistica, wherein the kinetic curves were analyzed as a first order kinetic equation in accordance with:

$$C = C_0 e^{-kt}$$

where: C – concentration at any point in the process, C_0 – concentration at the beginning of the process, k – release rate, t – time

The viscosity was evaluated by way of a Brookfield TV2 Rheometer, at 20°C, 200 rpm. The error was 20 cps. Standard spindles were used, respectively, No 04 for MC and PC11, No 05 for HPMC and PA.

RESULTS

The courses of the release kinetics of tested calcium dobesilate gels are shown in Figure 3. Using unfilled dots (○), we marked the kinetic release profile in the case of the standard arrangement of the donor extraction cells as set out in a manner recommended by the pharmacopoeial standards. In contrast, the release kinetics assessed from donor extraction cells arranged with the membrane towards the bottom of the vessel are marked by black dots (•).

The kinetics curves for the standard cell arrangement are illustrated in Figure 3 (as bright spots), whereas the kinetic parameters, i.e.: the release rates, half- release periods, and correlation coefficients are listed in Table 2. As revealed in this table, when the standard position of the extraction cell

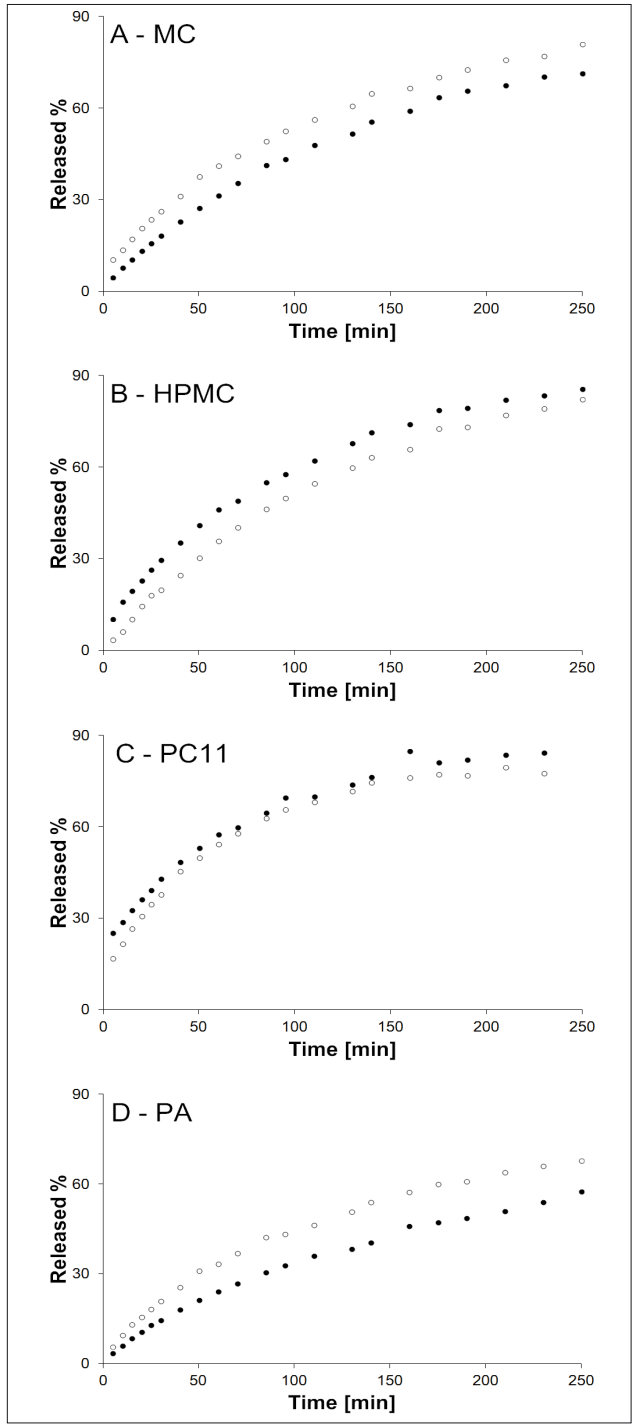


Figure 3. The cumulative percentage of released calcium dobesilate as a function of time, the white dots represent the assay in the standard position of the extraction cell, in accordance with the pharmacopoeal standard (○). The reverse arrangement of the extraction cell is represented as black dots (•), A – MC gel, B – HPMC gel, C –PC11 gel, D – PA gel

Table 2. The parameters determined in the course of obtained release kinetics of calcium dobesilate gel formulations A, B, C and D, when placed in the extraction cell with the membrane directed towards the top of the release vessel (I) and with membrane directed towards the bottom of the vessel (II)

Cell placement	Polymer type	Formulation	Parameter					
			K (min^{-1})		$t_{0,5}$ (min)		r^2	
			Mean	SD	Mean	SD	Mean	SD
Standard (I)	Nonionic	A (MC)	$6,19\times10^{-3}$	$7,64\times10^{-5}$	111,93	1,39	0,9973	0,0009
		B (HPMC)	$6,96\times10^{-3}$	$6,88\times10^{-4}$	100,19	9,40	0,9981	0,0018
	Anionic	C (PC11)	$6,18\times10^{-3}$	$2,97\times10^{-4}$	112,38	5,53	0,9579	0,0205
		D (PA)	$4,45\times10^{-3}$	$3,07\times10^{-4}$	156,38	10,37	0,9911	0,0011
Atypical (II)	Nonionic	A (MC)	$5,18\times10^{-3}$	$9,53\times10^{-5}$	133,93	2,49	0,9955	0,0005
		B (HPMC)	$7,57\times10^{-3}$	$1,30\times10^{-4}$	91,58	1,59	0,9971	0,0008
	Anionic	C (PC11)	$7,53\times10^{-3}$	$2,93\times10^{-5}$	92,08	0,36	0,9793	0,0064
		D (PA)	$3,26\times10^{-3}$	$1,52\times10^{-4}$	212,77	9,85	0,9942	0,0017

K – release rate, $t_{0,5}$ – half- release period, r^2 – correlation coefficient for the regression fit describing the course of the release as a first order process

was applied, the average release rates ranged from 4.45×10^{-3} min^{-1} for a formulation containing PA, to 6.96×10^{-3} min^{-1} for a formulation based on HPMC. As it is apparent from the graphs, after more than four hours from start of experiment, regarding HPMC, approx. 18% of the initial active ingredient content remained. As to formulations of MC and PC-11, the percentage was slightly higher, i.e. ca. 19%. In the case of release tests conducted with the semipermeable membrane directed towards the bottom of the vessel, the values were generally higher. Average release rates ranged from 3.26×10^{-3} min^{-1} for the PA preparation, to 7.57×10^{-3} min^{-1} for the HPMC preparation. After more than four hours subsequent to the start of the release process, in the case of the custom arrangement of the extraction cell, the HPMC formulation with HPMC held only approx. 15% of the initial API content, while PC11 and MC formulations held, respectively, 16%, and 29%.

What is more, the viscosity of the nonionic polymers was between 914 cps and 2564 cps, whereas the anionic polymers were characterized by displaying viscosity between 386 cps and 2272 cps. A graphed comparison of the viscosities is presented in Figure 4.

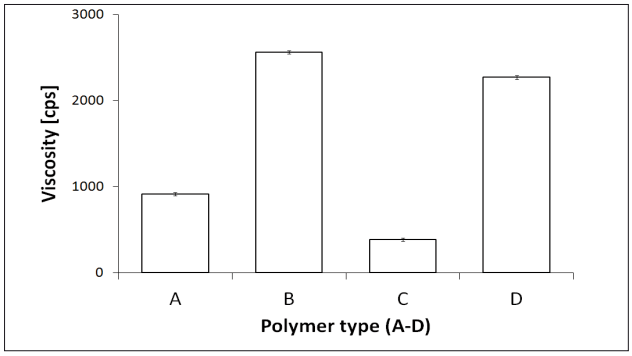


Figure 4. Viscosity of assessed polymeric hydrogels with calcium dobesilate. A – MC, B – HPMC, C – PC11, D – PA, the Y-bar represents SD, n=3

DISCUSSION

The study of the kinetics of calcium dobesilate release from differentiated polymeric hydrogels sought to determine the effect of the hydrogel on the release rate and released amount of API through the semi-synthetic membrane. The only factor that differed between the release in the standard tests (I) and non-standard tests (II) was the orientation of the extraction cell. In the course of the API release kinetics study from a potential transdermal, or diadermal drug delivery system, we used special extraction cells that were placed on the bottom of the vessel so as to investigate the release of API in a standard device. These extraction cells are closed by a semi-permeable membrane. The pharmacopoeial monograph does not specify explicitly the extraction cell arrangement, i.e. if the extraction cell must face the bottom or the top of the release vessel with regard to the positioning of its orifice. Recommendations inside the USP and Ph.Eur., however, clearly suggest that the membrane should face the stirrer [4,13]. In this case, the membrane may be affected by the high water induced by the movement of the paddle. The question is whether the qualitative study, which is the study of the API release, may be intentionally affected by the opposite orientation of the extraction cell so as to better reflect conditions at the surface of the skin. In the typical arrangement of the extraction cell, we observed a distinct differentiation of release between the series A, B, C and D.

In accordance with the course of the curve and in accordance with the designated release rates, the fastest release of API was from the formulation B, HPMC, while the lower rate of API release was observed in the case of the formulation MC (shown in Figure 3 and summarized in Table 2). Both of these are considered to be nonionic polymers. These hydrophilic polymers are often used as carriers for an API in a hydrogel. Changes in the concentration of the polymer, and, thus, the viscosity, as well as the addition of various additives to the matrix of MC and HPMC can significantly modify the release of the API from the hydrogel [6,11]. Differentiation between these bases is due to the presence within HPMC of an extended functional group – a hydroxypropyl group. Moreover, the HPMC used in the study has a lower molecular weight when compared to the molecular weight of MC; the weights of these compounds are 63000 Da and 88000 Da, respectively. Both of these factors may affect the ability to release the API – calcium dobesilate – from the hydrogel.

In the case of the anionic polymers, we observed differences between the C and D products. The C batch, produced on the basis of PC11, was characterized by demonstrating a relatively rapid release of the drug substance (Note: it is possible to distinguish a phase of very rapid release of the drug substance, followed by a plateau phase). This situation is seen as being associated with the presence of numerous neutralized acid groups in the polymer structure. This leads to a poor binding of calcium dobesilate within the polymer chain. PC11 is a polymer with a wide range of potential applications, but to date, few studies have been published using this excipient, in contrast to the widely studied carbopols.

Regarding formulation D, a polymer of acrylic acid, this demonstrated close to sustained release. This is thought to be due to the held neutralized carboxyl groups significantly affecting the binding of calcium dobesilate to the polymer PA.

A similar arrangement of the release rates was determined during the examination of the above mentioned formulations when the testing cells were in the non-standard position (II): HPMC > MC, PA < PC11. In this case, the amounts of API released were significantly higher in formulation B – based on HPMC, in comparison to formulation A – based on MC (Figure 5-1). Hence, the reversal of the position of the cell applied in the release study resulted in an increased difference between the release rates of products A and B. What is more, in the test formulations C and D, prepared on the basis of ionic polymers: PA and PC11, a greater difference between release rates was observed in the study performed with an atypical arrangement of the cell, than in the test with a typical arrangement of the cells. The increase in the value of diversity is also confirmed by the cumulative percentage of released calcium dobesilate, as shown in Figure 5-2.

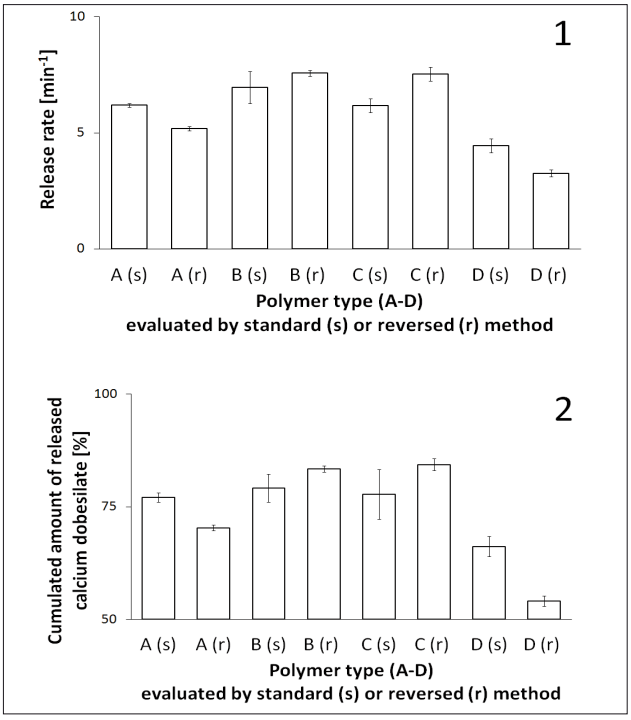


Figure 5. Variation of release rates (1) and cumulated percentage of released calcium dobesilate following the 4th hour of the process start-up; (2) for hydrogels prepared with the use of MC (A), HPMC (B), PC11 (C), and PA (D), the Y-bar represents SD, n=3

The variation in release rates of calcium dobesilate from the evaluated formulations depends on the structure of the used polymers, and on the presence of different functional groups, as well as on the molecular weight of the polymers. The amount of released API is also affected by the concentration of the polymer, which affects the viscosity. Moreover, the addition of excipients (present in formulation D) may affect the release rate.

In the case of preparations B and C based upon branched polymers, we observed an increase in the rate of release of API as a result of the reversal placement of the extraction cell used in the study. In contrast, preparations made from polymers with a moderately branched chain, A and D, showed a decrease in the rate of release of drug into the acceptor compartment, when the cell was placed reversibly to the standard position. The ground of this phenomenon may be, on the one hand, the limited circulation of the acceptor fluid under the membrane. On the other hand, the swelling capacity of the polymer during testing may differ. The movement of the acceptor fluid inside the extraction cell, and low adherence of the polymeric system to the interior surface of the extraction cell may result in an increase of the released API amount. In formulations of MC and PA, however, adhesion may be greater than in the case of formulations based on HPMC and PC11.

CONCLUSIONS

Calcium dobesilate is released from the hydrogels prepared on the basis of hydrophilic polymers, in varying quantities. In the group of nonionic polymers, the release rate is greater in the case of the HPMC preparation, and smaller in the case of the MC formulation. In the group of anionic polymers, the release rate is higher in the formulation prepared with PC11, while release from a reference formulation comprising a polymer of acrylic acid PA is more extended. Placement of the extraction cell in standard position or reversible position does not affect the alignment of the formulations investigated in terms of the release rate in the group of non-ionic formulations: B > A, and in the preparation of ionic group C > D. However, the results obtained in the standard arrangement of the extraction cell (B > A > C> D) differ from the results obtained in the course of measurements carried out with the extraction cell directed towards the bottom of the vessel used for release testing (B > C > A> D).

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REFERENCES

1. Brown W.E., Marques M.R.: USP and Dissolution – 20 Years of Progress. *Diss. Technol*., 21, 24, 2014.
2. Cuevas P. *et al*.: Topical dobesilate eye drops for ophthalmic primary pterygium. *BMJ Case Rep*., doi: 10.1136/bcr.12.2011.5449, 2012.
3. Cuevas P. *et al*.: Treatment of Stargardt disease with dobesilate. *BMJ case Rep.*, pii: bcr2012007128. doi: 10.1136/bcr-2012-007128, 2012.
4. European Pharmacopoeia 7.0, 2.9.4 Dissolution test for transdermal patches, 01/2008:20904, 2011.
5. Jafferany M. *et al*.: Geriatric dermatoses: a clinical review of skin diseases in an aging population. *Int. J. Dermatol.*, 51, 509, 2012.
6. Joshi S.C., Lam Y.C.: Modeling heat and degree of gelation for methylcellulose hydrogells with NaCl additives. *J. Appl. Polym. Sci*., 101, 1620, 2006.
7. Kamath K.R., Park K.: Biodegradable hydrogels in drug delivery. *Adv. Drug Deliv. Rev*., 11, 59, 1993.
8. Kaur C. *et al*.: An open trial of calcium dobesilate wit venous ulcers and stasis dermatitis. *Int. J. Dermatol*., 42, 147, 2003.
9. Lam Y.C., Joshi S.C., Tam B.K.: Termodynamic characteristic of gelation for Methyl – cellulose hydrogel. *J. Therm. Anal. Calorim*., 87, 475, 2007.
10. Rosiak J., Yoshi F.: Hydrogels and their medical applications. *Nucl. Instr. Meth. Phys. Res*., 151, 56, 1999.
11. Shah N. *et al*.: Prediction of Drug release from Hydroxypropyl Methylcellulose (HPMC) Matrices: Efect of Polymer Concentration. *Pharm. Res.,* 10, 1693, 1993.
12. Tejerina T., Ruiz E.: Calcium Dobesilate: Pharmacology and Future Approaches. *Gen. Pharmac*., 31, 357, 1998.

13. The United States Pharmacopeial convention {711} Dissolution, Stage 6 Harmonization, 2011.
14. Uddin R., Saffoon N., Sutradhar K.B.: Dissolution and Dissolution Apparatus: A Review. *Int. J. Curr. Biomed. Pharm. Res.,* 1, 201, 2011.