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The influence of hydrophylic polymers on the release rate of calcium dobesilate in hydrogel formulation assessed *in vitro* using porcine ear skin

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ABSTRACT

A shortage of available experimental data exists in the available bibliography on the release rate of calcium dobesilate (CD) from hydrogel formulations. Thus, the aim of the study was to evaluate the effect of selected hydrophilic nonionic polymers and anionic polymers on the release rate of CD from formulation provided for dermal application, as compared to the reference product in the market. The work utilized excised pork skin, while, Methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), and anionic polymers (copolymers of acrylic acid) were used as CD carriers. The release study was executed by the pharmacopoeial paddle method, with extraction cells and fresh excised porcine skin as a membrane. CD in aqueous acceptor fluid was quantified by UV-VIS spectrometry at 300 nm. Subsequently, the kinetic curves were fitted to a zero-order kinetics model, a first-order kinetics model, a second-order kinetics model, as well as to the Higuchi model. The work saw that porcine ear skin influences the release pattern of the CD, compared to the artificial membrane. In the study, the evaluated formulations with MC, polyacrylic acid (PA) and polyacrylate crosspolymer 11 (PC-11) deliver over 60% of the active component (AC), within 250 min, through the excised porcine ear skin, to the acceptor compartment. Moreover, the release observed via porcine ear skin to the aqueous acceptor compartment is congenial to zero-order or first-order kinetics. In addition, the formulations prepared on the basis of MC and PA appear to control AC delivery, independently of actual concentration of AC.

INTRODUCTION

The *in-vitro* biological activity of calcium dobesilate (CD), as assessed in cell cultures, as well as in animal models, has been the subject of numerous authors' research [3,4,13,17]. Moreover, the antioxidant activity and reduction of apoptosis was successfully evaluated in human peripheral blood mononuclear cells [13]. In the Brunet study, it was noted that, in the rat, CD reduced microvascular permeabilization as induced by reactive oxygen species [3]. Furthermore, dobesilate ions influenced the endothelial cells nitric oxide synthase and increased the level of the protective nitric oxide in the cell culture [31]. In addition, they enhanced the endothelium-dependent relaxation in animal tissue, as induced by acetylcholine [27]. Some researchers have also seen that CD induces the apoptosis of some cancer cells [7], and down-regulates the apoptosis in varicose veins [17]. In yet another report, angiogenesis was recognized as being inhibited via calcium dobesilate, and the chemical significantly decreased vessel ingrowth into sponges with aFGF that were implanted subcutaneously in mice [4].

The vascular activity of CD includes a remarkable reduction of retinal albumin leakage, retinal carboxymethyllysine-advanced glycation end product level, as well as retinal vascular endothelial cell growth factor expression, resulting in reduced retinal hyperpermeability in diabetic rats [26]. The general field of CD application covers multidirectional activity that is linked to the function of blood vessels. What is more, the active component (AC) may indirectly increase lymphatic drainage, improve peripheral circulation and prevent stagnation of venous blood, and thus it is in use in ophthalmology and angiology [32]. The list of

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clinical application contains diabetic retinopathy, hemorrhoids, phlebitis, leg ulcers, edema of the lower limbs [20], chronic, recurrent gastritis, peptic ulcers of the stomach and duodenum [19], as well as Stargardt disease [6] and pterygium [5].

CD is a molecule with high applicative potential, however, there is a shortage of kinetic data dealing with the release rate of CD from compositions containing CD. Due to the ionic interactions between the AC and the functional groups of the polymeric carriers, release experiments may enhance the knowledge about the potential use of CD in topical formulations.

Hydrogels, because of their biocompatibility and biodegradability, as well as their hydrophilic nature, are now recognized as being very good carriers of therapeutic substances [20]. Yet, polymers applied as drug carriers may influence the AC release, and in-vitro evaluation of release rate is important for the selection of a proper polymeric carrier. In order to evaluate the course of release of the AC from the formulation, numerous kinetic of models may be applied. Some of these models allow a comparison of different formulations, but not in all cases is it possible to use only one model to generate data about the tested group of preparations.

The most frequently used models include the kinetics of zero, first and second order, as well as the Higuchi model. In the case of zero order kinetics, the reaction rate is largely independent of the concentration of substrate, while, first order kinetics is the process in which the rate of reaction depends linearly on the concentration of one substrate engaged in the process. The kinetics of the second order rate is, however, dependent on the product of the concentrations of the two substrates process, and the rate equation is a second degree monomial. A mathematical model to describe the targeted drug release from matrix system was first proposed by Higuchi. In this model, the concentration of the AC in the matrix is much higher than its solubility, hence, diffusion AC is performed only in one dimension, the AC molecules are much smaller than the thickness of the system, the swelling and dissolution of the matrix are negligible, the diffusion coefficient of the drug is stable, and the conditions are sink-maintained in the fluid acceptor to which the test substance is released [8,29].

Our previous experiments revealed interesting release patterns of CD from hydrogels with various functional groups (Fig. 1). These were prepared with methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), carbopol (PA), and polyacrylate crosspolymer 11 (PC11), using a synthetic membrane in an extraction cell [23].

The use of artificial membranes in release experiments comes with numerous advantages, however, animal skin may better reflect the diffusion kinetics of the AC to the application place on the skin surface. Thus, porcine ear skin has been evaluated as being an advantageous, quantitative and reproducible model for dermatopharmacokinetic assessments of topical bioavailability, and was seen as being comparable to data obtained in human subjects [15,18,28].

The aim of the study was to evaluate the effect of selected hydrophilic nonionic polymers and anionic polymers, using excised pork skin, on the release rate of CD from formulations provided for dermal application, as compared to a marketed reference product.

MATERIALS AND METHODS

Materials

The following polymers were used for the preparation of the gels: MC (Sigma Aldrich), HPMC (Ashland), and PC11 (Clariant). The marketed formulation, Galvenox Soft (Galena, Poland), comprising in its composition, CD and PA, was applied as a reference. The composition of the prepared gels was analogous as in former research [23] and is presented in Table 1. In our work, 2.0 g of CD was dissolved in 96.5 g of deionized water, and, consequently, the mixture was supplemented by 1.5 g of the respective polymer: MC, HPMC, or PC-11. All the formulations were left for 24 hours in a refrigerator at 8°C. The samples were then conditioned at a temperature of 22°C, for 2 hours before the start of the experimental procedure.

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 in the Polish market

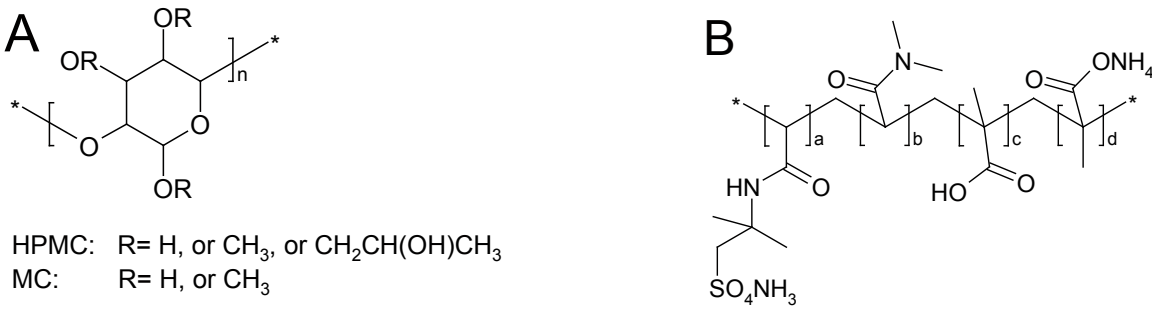

Figure 1. Hydrogels with various functional groups: methylcellulose (MC), and hydroxy propyl methyl cellulose (HPMC) – A, and polyacrylate crosspolymer 11 (PC11) – B

Methods

The release study was executed by the pharmacopoeial paddle method, at 100 rpm. Herein, the gel was placed in the extraction cells (Perspex, Pharma Test Apparatenbau, Germany) in a manner compatible with the dissolution test for transdermal patches [10]. The drug dissolution tester Erweka DT706 (Germany) was used in the experiment, with purified water as an acceptor fluid, in a volume of 900 ml, at 37°C.

In our experiment, the temperature was maintained at 37°C to reflect the conditions applied in our former research regarding artificial semipermeable membranes [23]. Moreover, a temperature of 37°C was maintained i.a. in contemporary studies of transdermal absorption of tadalafil [14], phenolic acids [33] and clopidrogel [25].

Freshly collected porcine ears were purchased from a certified abattoir. The hairiness was carefully removed and the organ was washed in deionized water. The skin was then accurately excised from the adhering tissues using a Humby transplantation knife, and the obtained skin thickness was ca. 500 μm.

Three parallel measurements were performed in the extraction cells, by sampling the acceptor fluid volume of 2 ml, every 10 minutes, over 4.5 hours. The acceptor fluid was not supplemented – so as to reflect the conditions of a parallel experiment on artificial membranes [23]. A spectrophotometer UV-VIS T60 (PG Instruments, USA) at 300 nm, was used for quantification of the CD in the acceptor fluid, by way of a standard curve. The wavelength was determined in accordance with a previously outlined absorption spectrum of calcium dobesilate in an aqueous solution.

The prepared standard curve was based on three independent series of assessments, with five measurement points at concentrations between 0.025 and 5.00 μg/mL. Statistical evaluation of the results was performed using Statistica software. The kinetic curves were analyzed as a zero-order kinetic equation, a first-order kinetic equation, and a second-order kinetic, as well as the Higuchi model, as presented in a former paper [23] and detailed by other authors [8]. Subsequently, the following equations presented in Table 2 were evaluated for the presentation and discussion of the data.

Table 2. Kinetic formulae applied for evaluation of obtained data

Kinetic model	K	$t_{0.5}$
0 order	$K_{(0)} = \frac{Q_0 - Q_t}{t}$	$t_{0.5} = \frac{Q_0}{2K_{(0)}}$
1st order	$K_{(1)} = \frac{1}{t} ln \frac{Q_0}{Q_t}$	$t_{0.5} = \frac{0.693}{K_{(1)}}$
2nd order	$K_{(II)} = \frac{Q_0 - Q_t}{Q_0 Q_t} \frac{1}{t}$	$t_{0.5} = \frac{1}{K_{(1)} Q_0}$
Higuchi	$K_{(H)} = \frac{Q_t}{t^{0.5}}$	$t_{0.5} = \left(\frac{Q_t}{2K_{(H)}} \right)^2$

K – rate constant, respectively for zero order kinetics ($K_{(0)}$), 1st order kinetics ($K_{(1)}$), 2nd order kinetics ($K_{(II)}$), and for the Higuchi model ($K_{(H)}$), t – time, $t_{0.5}$ – half release time, Q_0 – initial percentage of the released drug, Q_t – percentage of the released drug after time t, Q_i – initial percentage of the drug in the formulation

RESULTS

The initial level of CD in the prepared formulations evaluated in the study, is given in Table 1. As revealed in the graphs seen in Figure 2, the largest quantity of CD was released from the polymer-based hydrogel preparation C with PC-11. Here, the released amount of CD reached almost 88% of the initial level of AC in the formulation. Intermediate amounts of released AC were observed in the test formulations A and D, corresponding respectively to, ca. 68% and ca. 70%, after 250 min. Our work demonstrated that the decidedly lowest amounts of CD, i.e. only 51%, were released from formulation B, prepared with the non-ionic polymer HPMC. As seen in Fig. 2, formulations A and D present a release pattern close to a linear process. Contrarily, formulations B and C reflect a process that is similar to a first order process.

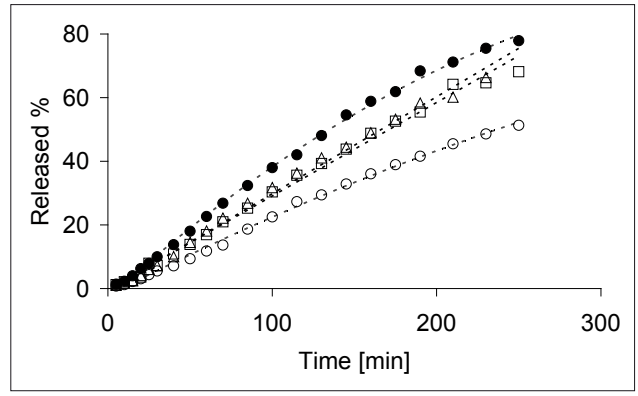

Figure 2. Course of the release of calcium dobesilate from hydrogels prepared with the use of MC (□), HPMC (○), PC11 (●), and PA (Δ), the dotted lines are the visual guide, n = 3, the standard deviation was in the range between 1.3-8.4 for the MC formulation, 0.2-9.7 for the HPMC formulation, 0.9-7.7 for the PC-11 formulation, and 0.2-5.3 for the PA formulation

The release rate constants, calculated due to the zero-order kinetics equation were in the range 2.20×10^{-1} %×min^{-1} to 3.38×10^{-1} %×min^{-1}, respectively, for extreme formulations B and C. Calculations performed by way of the first-order kinetics equation, revealed release rate constants that ranged from 3.05×10^{-3} min^{-1} to 6.25×10^{-3} min^{-1}. In the case of the second-order process, the release rate constants were between 4.39×10^{-5} min^{-1}×%$^{-1}$ and 1.33×10^{-4} min^{-1} ×%$^{-1}$. Finally, the release rate constants determined by the method of Higuchi were arranged in a similar manner, and were between the values 4.04 min$^{-0.5}$ to 6.25, min$^{-0.5}$ for the same formulations. The calculated values for the regression coefficients are summarized in Table 3. Herein, the regression coefficients ranged from 0.9573 to 0.9979. High rates of regression were observed when employing a zero-order process model – formulations A and D, or the first-order process – formulations B and C. Low regression coefficients were observed when applying the second-order process equation for formulations A, C and D, as well as in the case of the Higuchi equation as applied for formulation B.

Table 3. The parameters determined in the course of release kinetics of calcium dobesilate gel formulations A, B, C and D, K – release rates for respective models: zero order kinetics ($K_{(0)}$), 1st order kinetics ($K_{(I)}$), 2nd order kinetics ($K_{(II)}$), and for the Higuchi model ($K_{(H)}$), r^2 – correlation coefficient for the regression fit describing the course of the release as a first order process

Parameter	Kinetics model								BF
	0 order		1st order		2nd order		Higuchi		
	$K_{(0)}$ [%×min^{-1}]	SD	$K_{(I)}$ [min^{-1}]	SD	$K_{(II)}$ [min^{-1}×%$^{-1}$]	SD	$K_{(H)}$ [min$^{-0.5}$]	SD	
Formulation type	A (MC formulation)								
Rate constant	2.94×10^{-1}	2.64×10^{-2}	4.81×10^{-3}	9.34×10^{-4}	8.74×10^{-5}	2.94×10^{-5}	5.39	4.91×10^{-1}	0
r^2	0.9953	0.0004	0.9910	0.0090	0.9629	0.0301	0.9847	0.0030	
Formulation type	B (HPMC formulation)								
Rate constant	2.20×10^{-1}	3.86×10^{-2}	3.05×10^{-3}	7.65×10^{-4}	4.39×10^{-5}	1.51×10^{-5}	4.04	7.16×10^{-1}	I
r^2	0.9969	0.0002	0.9979	0.0001	0.9898	0.0052	0.9846	0.0021	
Formulation type	C (PC-11 formulation)								
Rate constant	3.38×10^{-1}	1.48×10^{-2}	6.25×10^{-3}	4.15×10^{-4}	1.33×10^{-4}	1.29×10^{-5}	6.25	3.09×10^{-1}	I
r^2	0.9917	0.0073	0.9917	0.0029	0.9573	0.0167	0.9889	0.0031	
Formulation type	D (reference formulation)								
Rate constant	2.98×10^{-1}	2.62×10^{-2}	4.88×10^{-3}	7.76×10^{-4}	8.78×10^{-5}	2.22×10^{-5}	5.48	4.91×10^{-1}	0
r^2	0.9958	0.0019	0.9939	0.0032	0.9653	0.0135	0.9880	0.0009	

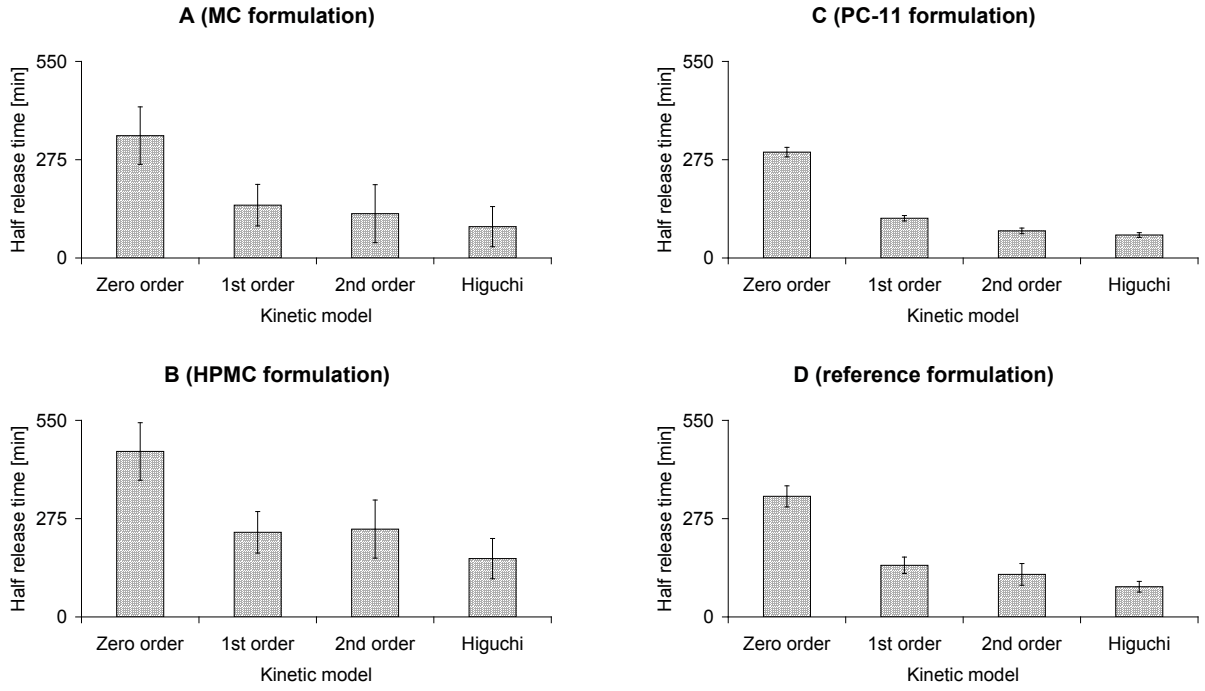

Figure 3. Variation of half release times of released calcium dobesilate for hydrogels prepared with the use of MC (A), HPMC (B), PC11 (C), and PA (D), as evaluated according to the zero order kinetic equation, the first order kinetic equation, and the second order kinetic, as well as the Higuchi model. The Y-bar represents SD, n=3

Figure 3 presents the half-release times as determined for each formulation using the different kinetic equations. The maximum half-release time was observed when the zero-order model was employed, while, congenial half- release times were obtained as a result of the calculations performed according to first-order and second-order kinetics. Significantly lower half-release times were determined on the basis of the Higuchi equation.

DISCUSSION

In the available bibliography, there is a shortage of available experimental data on the release rate of CD from hydrogel formulations, yet an exception exists, in the presence of our own previously published report [23]. In agreement with pharmacopoeial data, CD in the form of monohydrate is very soluble in water, as well as in anhydrous ethanol [23]. However, the stability of the AC may be problematic for the evaluation of release rates. As revealed in Fig. 4, CD easily undergoes reduction and oxidation reactions [16].

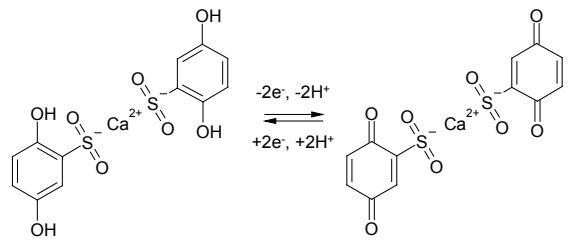

Figure 4. The scheme of red-ox reaction of calcium dobesilate

Thus, in the presented research, we proposed that experimenters utilize purified water as the acceptor compartment. In such work, the increasing level of AC in the acceptor compartment reflects the increasing amounts of AC penetrating through the excised porcine skin. At the actual state of current research, it is hard to clearly state the genuine level of AC within the living human skin tissue. However, the increase of AC level in the acceptor compartment confirms the possibility of AC penetration through the skin barrier.

In the case of both A formulation (MC), and D formulation (PA, reference), we observed releases congenial to zero-order kinetics. Contrarily, in formulations containing HPMC (B), and PC-11 (C), the release was not controlled by the membrane, and followed a first order kinetics pattern. In previous assessments [23], we employed an artificial cellulose membrane, and the observed kinetics was in all the cases congenial to the first order pattern, with characteristic curving and plateau stage. The porcine skin, consisting of specific layers, and visualized in Fig. 5A, is thought to be a good model for evaluating drug transport through the human skin [9,22,30]. However, the subcutaneous tissue (presented in Fig. 5B) is usually not used in such experimental work, and is generally cut away during the preparative stage [24]. Thus, the results may only approximate the actual fate of the CD when applied on the skin.

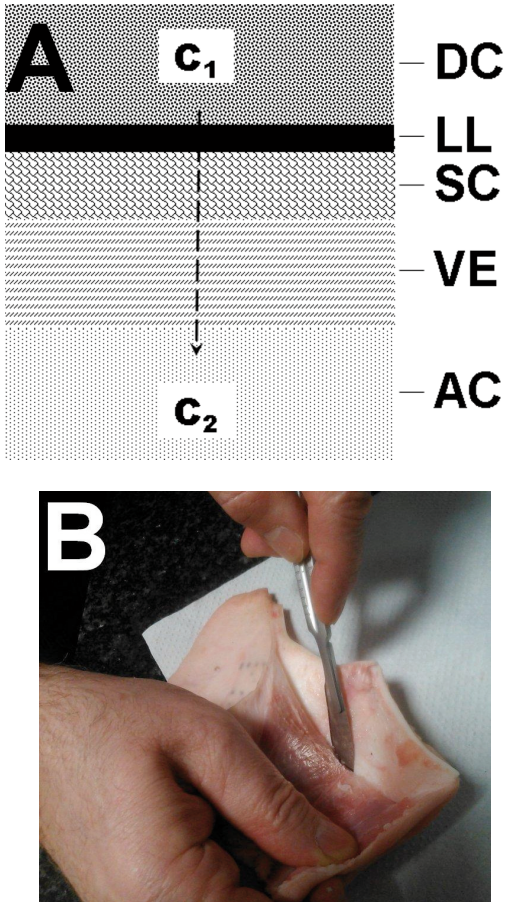

Figure 5. Visualization of the skin layers, and transport of CD through it, in the "in-vitro" conditions (A), C_1 – concentration of CD in the donor compartment at the initial phase of the process, C_2 – the concentration of CD in the acceptor compartment, DC – donor compartment, LL – lipid layer, SC – stratum corneum, VE – viable epidermis, and presentation of the porcine ear skin as used in the experiments (B)

The surprising release patterns of CD from the MC and PA formulations (A and D), may indicate some interactions between the components of the formulations and the skin. The superficial layers of the skin have outstanding barrier properties against physical and chemical factors, due to the presence of the lipophilic film, and the stratum corneum. Within the stratum corneum, the corneocytes composed of keratin filaments are closely connected with each other by way of desmosome junctions. Moreover, the intercellular space between the corneocytes is filled by lipids and adhesion proteins [11]. The application of a hydrogel formulation, as it is the case of the A-D preparations, leads to the hydration of the superficial layers of the skin [21]. Consequently, the permeability of some ACs through the skin increases significantly. However, the hydration of the SC leads to the formation of an aqueous environment. This may enable interaction between the components of the superficial non-living layers of the skin and the components of the hydrogel. In the case of the A and D formulations, the MC and PA, respectively, may interact with the components of the upper skin layers. This assumption is confirmed by way of the experiments undertaken by Anlar et al., who investigated the bio-adhesion of modified polyacrylic acid to the bovine mucosa, and who confirmed that the least substituted polymers, with low molecular weight, presented the same degree of bio-adhesion, while highly branched polyacrylic acid polymers had reduced bio-adhesion [1]. What is more, the MC, HPMC and modified polyacrylates have potential bio-adhesivity, which depends on the assessed tissue and polymer structure [12]. Thus, the varied release rate of the CD from the assessed formulations may be ascribed to the structure of the polymeric carriers and to specific interactions between the hydrophilic functional groups of the polymers and components of the skin [2].

CONCLUSIONS

The porcine ear skin influences the release pattern of the CD, when compared to the artificial membrane. In our work we saw that the evaluated formulations with MC, PA and PC-11 deliver over 60% of AC, within 250 min, through the excised porcine ear skin, to the acceptor compartment. What is more, the release observed via porcine ear skin to the aqueous acceptor compartment is congenial to zero-order or first-order kinetics. In addition, the formulations prepared on the basis of MC and PA appear to control AC delivery independently of actual concentration of AC, whereas in the case of PC-11 and HPMC, the dominant release pattern is in agreement with first-order kinetics.

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