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Cholesterol oxyethyleneation products as modifiers of the absorption base in anti-inflammatory ointments

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Summary:

Introduction: The assumption behind the study was to attempt modification of pharmacopoeial anhydrous absorption base, i.e. hydrophilic vaseline, by the addition of novel cholesterol oxyethyleneation products. Novel base with variant compositions was proposed as a carrier for ketoprofen – a medicinal substance with analgesic, anti-inflammatory and antipyretic activity.
Material and methods: 5 model ointments were prepared with the absorption base consisting of hydrophilic vaseline modified with cholesterol oxyethyleneation products. Extensometric method was used to test spreadness of the preparations, gravimetric method to determine the rate of volatile components loss, while viscosity parameters were determined with cone-plate digital rheometer. The test for ketoprofen pharmaceutical availability was performed with spectrophotometric method.
Results: Modification of the absorption base (hydrophilic vaseline) by introduction of novel cholesterol oxyethyleneation products increased the extensibility parameters of all prepared ointments. Viscosity tests showed that all hydrophilic vaseline-based ointments with variant compositions were tixothropic and rheologically unstable. Introduction of cholesterol derivatives into the formulae of hydrophilic vaseline-based ointments reduces their structural viscosity values. Comparison of the areas under the curves of the release of ketoprofen lysinate showed that the active substance was best released from ointments with cholesterol oxyethylenates with the lowest n umber of segments (n_{TE}), regardless of the type of the catalyst used to produce these oxyethylenates (M-Ch-10 Na and M-Ch-10 Ca).
Conclusions: As shown by the conducted studies, the use of novel oxyethylate-containing products affects optimization of the rheological parameters of the ointments and the efficacy of the release of ketoprofen lysinate into a model recipient fluid.

Key words: ointments, skin inflammation, hydrophilic vaseline, cholesterol oxyethyleneation products.

Introduction

The objective of rheological studies in drug formulation technology is to determine correlations between structure-related physicochemical properties of the drugs and the usability of these drugs [1,2,3]. Rheological parameters of topical pharmaceuticals, such as ointments, creams, gels, or pastes, are determined by components included in the base [4]. Appropriate selection of these components impacts

pharmaceutical availability of medicinal substances, and thus the efficacy of treatment.

The assumption behind the study was to attempt modification of pharmacopoeial anhydrous absorption base, i.e. hydrophilic vaseline, by the addition of novel cholesterol oxyethyleneation products manufactured by the Surface Active Agents Production Plant ICSO Blachownia in Kędzierzyn-Koźle [5,6,7]. The novel base with variant compositions was proposed as a carrier for ketoprofen — a medicinal substance with analgesic, anti-inflammatory and antipyretic activity [8].

The main indications for local application of ketoprofen include muscle and joint pains, inflammatory conditions caused by injuries such as joint dislocations, sprains or sports injuries, and tendonitis [9]. Local administration of ketoprofen determines its activity in pathological tissues and minimizes the risks of adverse events reported for oral use [10].

Objectives

The goal of the study was to assess the effects of cholesterol oxyethyleneation products on rheological parameters of hydrophilic vaseline-based ointments and the efficacy of the release of a non-steroidal anti-inflammatory drug available as lysinate salt into the external compartment.

Reagents and equipment

Reagents:
- hydrophilic vaseline (Coel);
- ketoprofen lysinate (Sigma);
- cholesterol oxyethyleneation products containing the following numbers of oxyethylenic segments (n_{TE}); 10, 20, 30, 40 (products manufacture using a sodium catalyst) and 10 for calcium catalyst (Surface Active Agents Production Plant ICSO Blachownia in Kędzierzyn-Koźle);
- distilled water.

Instrumentation:
- MR 200 formulation mixer (Alpina);
- water bath MLL 147/6 AJL (Electronic Krakow, Poland);
- Cone/plate DV-III digital rheometer, version 3.0 (Brookfield);
- bath thermostat PGW E1 (Medingen);
- extensometer, pH-meter N5170E with ERH-131 electrode (Hydromet Gliwice, Poland);
- drug release apparatus Erweka DT 600 (Erweka), apparatus accd. to Mutimer et al.;
- Visking Dialysis Tubing C/100 membrane, wall thickness 74 μm and pore diameter 75 μm (Serva Electrophoresis GmbH);
- spectrophotometer Nicolet Evolution 300, version 1.0 (Spectro-Lab);
- technical balance (Radwag, Precision Mechanics Plant in Radom, Poland), analytical balance (Radwag, Precision Mechanics Plant in Radom, Poland).

Experimental method

Development of model formulae

5 model ointments were prepared with the absorption base consisting of hydrophilic vaseline modified with cholesterol oxyethyleneation products. The formula of the ointments is presented in Table 1.

Table 1: Formula of the ointments with the absorption base consisting of hydrophilic vaseline modified with cholesterol oxyethyleneation products.

Ingredient	Quantity (g)
Ketoprofen lysinate	2.5
Hydrophilic vaseline	76.5
Cholesterol oxyethyleneation product*	1.0
Distilled water	20.0

* Oxyethyleneation products used included products prepared using a sodium catalyst had the following numbers of oxyethylenic segments: n_{TE}=10 (M-Ch-10 Na ointment), n_{TE}=20 (M-Ch-20 ointment), n_{TE}=30 (M-Ch-30 Na ointment), n_{TE}=40 (M-Ch-40 Na ointment) and the product of n_{TE}=10, prepared using a calcium catalyst (M-Ch-10 Ca ointment).

For comparison, an ointment containing no cholesterol oxyethyleneation products was also prepared (M-0 ointment). The formula of the ointments is presented in Table 2.

Table 2: The formula of the ointments with hydrophilic vaseline base.

Ingredient	Quantity (g)
Ketoprofen lysinate	2.5
Hydrophilic vaseline	77.5
Distilled water	20.0

Ointment extensibility test
The test was conducted at 25 °C using the extensometric method.

Determination of ointment viscosity parameters
Ointment viscosity tests were conducted at 32 °C±0.1 °C using a cone/plate digital rheometer coupled with a bath thermostat [12].

Determination of the kinetics of the loss of volatile ointment components
Plates with the diameter of d=9cm and total surface area of P=63.59 cm2 were covered with uniform ointment layers. Thus prepared samples were placed in a dryer-balance at 32±0.1 °C for 2.5 and the percentage weight loss readings were taken every 15 minutes.

Determination of the kinetics of the release of lysinate from the ointment
The test was conducted using the technique used for transdermal therapeutic systems according to Ph.Eur. 6 requirements [13].

A 5 g ointment sample was placed in a dialysis container with the mass exchange surface P=19.625 cm^2 (apparatus accd. to Mutimer *et al.*). Next, the ointment surface was covered by an appropriately prepared Visking dialysis membrane. Before the test, the dialysis membrane was exposed to distilled water over 24 h. The entire system was closed with the lid and tightened with nuts. Thus prepared dialysis container was placed in a thermostated vessel (32°±0.1 °C), containing 0.25 dm^3 of distilled water as a recipient liquid. The solution above the container was put in continuous rotational motion using a stirrer rotating at 100 rpm. The mass exchange rate was measured by spectrophotometric analysis of ketoprofen lysinate released from the ointment. Measurements were made at nine time points over 6 hours (samples collected at 40-minute intervals). The amount of the released ketoprofen lysinate was determined at λ=260 nm using the following equation: A= 0.4249·c+ 0.0677 (r=0.9991), where: A is absorbance and c is the concentration of the pharmaceutical substance.

Results and Discussion

Results of extensometric tests

Extensometric tests (measurements of extensibility) allowed to assess products' capacity to increase its surface area under the pressure force. Figure 1 presents an example extensibility curve (relationship between the extended ointment surface area and the force load applied).

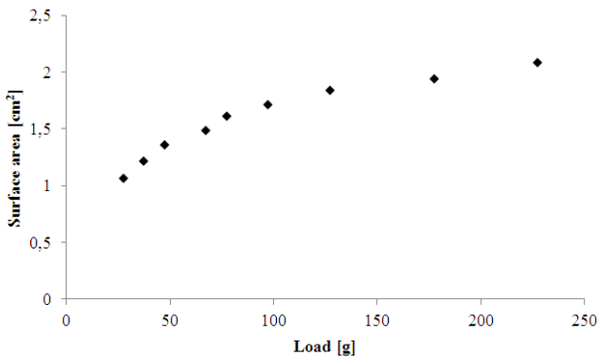

Figure 1: The relationship between the applied load and the observed increase in M-Ch-30 Na ointment surface area.

Figure 2 compares the extensibilities of all tested ointments under the highest load applied during the test (227 g).

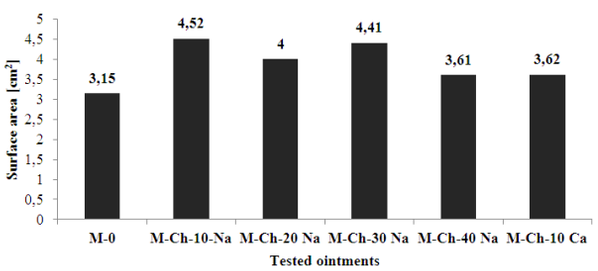

Figure 2: Comparison of extensibilities of all tested ointments under the highest load applied during the extensometric test.

Extensibility is the measure of product's capacity to increase its surface area under increasing pressure force. Products of high extensibility are easily distributed over the administration site. This affects the quality of application. Administration of ointments on inflamed tissues should not increase the pain sensation. Good extensibility of the ointment increases the rate of release of the active substance at the site of administration. Diffusion of the active substance into the external compartment may occur over a large ointment surface area that has been spread using a low pressure force.

Modification of the absorption base (hydrophilic vaseline) by introduction of novel cholesterol oxyethyleneation products increased the extensibility parameters of all prepared ointments (Fig. 2). In case of ointments prepared using novel ointments, and in case of the highest loads applied during the extensometric tests, the surface areas of extended ointments ranged from: 3.61 to 4.52 c.u. In case of the ointment with unmodified formula (M-0), the value was 3.15 c.u.

Viscosity test results

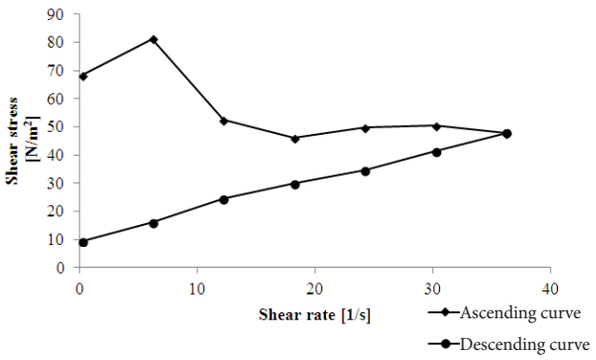

Figure 3: Hysteresis loop for the M-0 ointment.

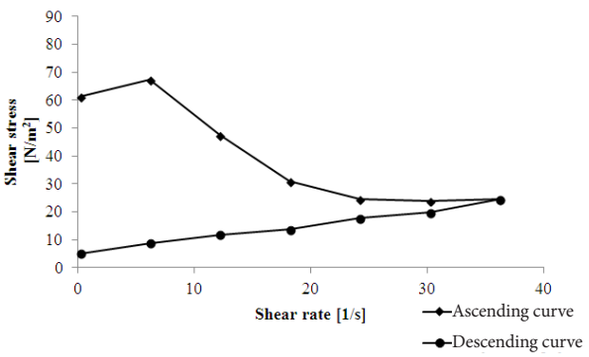

Figure 4: Hysteresis loop for the M-Ch-10 Na ointment.

Viscosity tests showed that all hydrophilic vaseline-based ointments with variant compositions were tixothropic and rheologically unstable, as confirmed by the hysteresis loop test [14]. Measurements of shear stress depending on shear rate were performed by increasing the shear rate from zero to a pre-defined maximum value and than back to zero immediately after reaching the maximum point. Figures 3 and 4 present example hysteresis loops obtained for the M-0 ointment and for an ointment containing a cholesterol oxyethyleneation product (M-Ch-10 Na).

Positive tixotrophy was observed for all prepared products. Upon isothermal flow of the fluid that has previously been in stasis for a prolonged time, the shear stress was reversibly reduced over time in these systems.

Structural viscosity of the tested ointments was compared at the ascending hysteresis loop curve fragments for three arbitrarily selected shear rates of 12.2, 24.2 and 30.2 1/s. The results are listed in Table 3.

Introduction of cholesterol derivatives into the formulae of hydrophilic vaseline-based ointments reduces their structural viscosity values, as observed at all three shear rates tested in the study. Based on the Einstein-Smoluchowski equation: $D=kT/6\pi r\eta$, (where: D — diffusivity of the medicinal substance, k — Boltzmann constant, T — temperature in Kelvins, r — observed radius of the molecule of the medicinal substance, η — viscosity) [15], one may expect that the reduction in viscosity parameters would enhance diffusibility of the medicinal substance (ketoprofen lysinate) from the ointment into the external compartment, which is associated with increased anti-inflammatory efficacy of the product.

Table 3: Structural viscosity parameters for the model ointments.

Ointment	Shear rate 12.2 1/s		Shear rate 24.2 1/s		Shear rate 30.2 1/s	
	Shear stress [N/m2]	Viscosity [mPa·s]	Shear stress [N/m²]	Viscosity [mPa·s]	Shear stress [N/m²]	Viscosity [mPa·s]
M-0	52.5	4286	49.7	2054	50.5	1672
M-Ch-10 Na	47.5	3895	24.5	1010	23.9	789.9
M-Ch-20 Na	42.3	3471	30.4	1257	30.2	994
M-Ch-30 Na	44.1	3618	28.2	1167	29.6	980.8
M-Ch-40 Na	52.1	4269	39.2	1618	42.9	1422
M-Ch-10 Ca	51.1	4188	40.0	1651	38.8	1284

Water loss kinetics test results

The measurements of water loss kinetics constitute a supplement to the rheological tests (extensibility, structural viscosity). The ointment's tendency to lose water affects its structural viscosity following application on the skin, and thus, the kinetics of the release of the active substance. From this standpoint, slight viscosity changes are preferred over time. The measurements of the water loss kinetics may also be used to assess the rheological stability of the product as part of stability tests. Slight changes in the ointment mass occurring over time

affect the stability of its physicochemical parameters upon storage.

Figure 5 presents an example curve of ointment water loss.

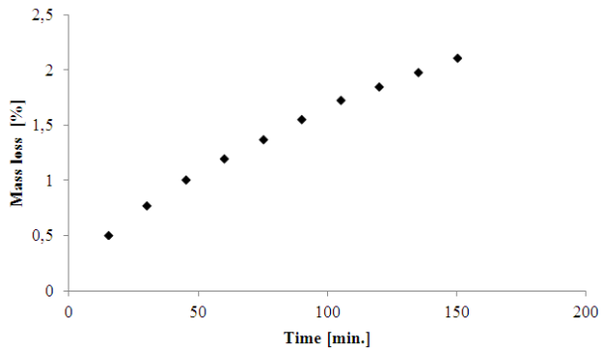

Figure 5: Kinetics of water loss for the M-Ch-10 Ca ointment.

Table 4: Parameters of the regression equation of the type y=ax+b describing the kinetics of the loss of water from the ointment

Ointment	Regression equation coefficients		Correlation coefficient r	Surface area [c.u.]
	a	b		
M-0	0.1653	0.4036	0.9916	1895.5
M-Ch-10 Na	0.1721	0.4951	0.9912	1983.6
M-Ch-20 Na	0.1560	0.4693	0.9871	1800.8
M-Ch-30 Na	0.1875	0.4776	0.9945	2152.8
M-Ch-40 Na	0.1760	0.4549	0.9921	2021.6
M-Ch-10 Ca	0.1744	0.4520	0.9927	2003.4

The relationship between the loss in the mass of the tested ointments [%] and time [min.] was described at the significance level of p=0.05 by a regression equation of the type y=ax+b. Parameters a and be were used to calculate the surface areas P under the water loss curves, expressed in conventional units [c.u.], using an integration method. The obtained values are listed in Table 4.

It was shown that introduction of cholesterol oxyethyleneation products into the ointment formula did not significantly affect the loss of water from the products. Surface areas under the curves of the loss of water from the ointment containing different cholesterol derivatives ranged from 1800.8 to 2152.8 c.u. In case of the ointment with unmodified formula (M-0), the value was 1895.5 c.u. (Table 4). As shown by the calculations, the tested ointments would be characterized by comparable changes in viscosity parameters during application on the tissue affected by inflammation. The diffusibility of ketoprofen lysinate would be at similar levels throughout the contact with the application site, as follows from th e Einstein-Smoluchowski equation (D=kT/6πrη).

Results of determination of the kinetics of the release of lysinate from the ointment

Figure 6 presents an example of the relationship between the quantity of the released ketoprofen lysinate (in mg per cm2 of the dialysis membrane) as a function of the square root of time.

The obtained relationships were described by correlation equations of the types y=ax+b and lg(y)=a·lgx+b (a logarithmic form of the exponential

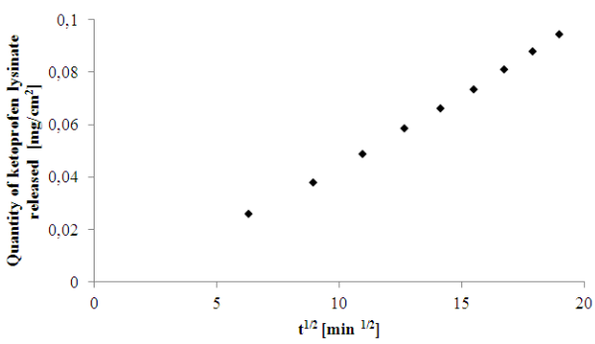

Figure 6: Kinetics of the release of ketoprofen lysinate from the M-Ch-30 Na ointment.

Table 5: Regression equations describing the kinetics of the release of ketoprofen lysinate from model ointments

Ointment	Regression equation type	Regression equation coefficients		Correlation coefficient r	Surface area [c.u.]
		a	b		
M-0	y=ax+b lg(y)=a·lgx+b	4.1365·10-3 0.6435	-2,0335·10-2 -1.8417	0.9731 0.9651	0.4044
M-Ch-10 Na	y=ax+b lg(y)=a·lgx+b	5.160·10-3 1.0252	-0.0451·10-2 -2.3003	0.9746 0.9668	0.9088
M-Ch-20 Na	y=ax+b lg(y)=a·lgx+b	5.1299·10-3 1.6352	-2.2820·10-2 -3.2015	0.9940 0.9971	0.5319
M-Ch-30 Na	y=ax+b lg(y)=a·lgx+b	5.4611·10-3 1.1811	-1.0226·10-2 -2.5368	0.9991 0.9998	0.7442
M-Ch-40 Na	y=ax+b lg(y)=a·lgx+b	6.0644·10-3 1.5758	-2.7472·10-2 -3.0646	0.9881 0.9975	0.6225
M-Ch-10 Ca	y=ax+b lg(y)=a·lgx+b	5.5206·10-3 1.4582	-2.3623·10-2 -2.9553	0.9846 0.9909	0.9746

equation y=axb). Regression equations and areas under the curves of ketoprofen lysinate release expressed in conventional units are listed in Table 5.

As shown by the high correlation coefficient for the linear equation y=ax+b at p=0.05, the process of release of ketoprofen lysinate from the ointment follows a zero-order kinetics. The exact kinetic equations based on the analysis of the diffusion process are usually complex and have the form of a sum of exponential functions [16].

The areas under the curves of release of the active substance from cholesterol derivatives-containing ointments are larger than the area obtained for the ointment obtained from hydrophilic vaseline only. For the cholesterol derivatives-containing ointments, the values ranged from 0.5319 to 0.9476, while for the M-0 ointment, the value was 0.4044 c.u.

Comparison of the areas under the curves of the release of ketoprofen lysinate showed that the active substance was best released from ointments with cholesterol oxyethylenates with the lowest n umber of segments (n_{TE}), regardless of the type of the catalyst used to produce these oxyethylenates (M-Ch-10 Na and M-Ch-10 Ca). More than a twofold increase in the efficacy of release of ketoprofen lysinate was achieved from ointments containing cholesterol derivatives including n_{TE}=10 oxyethylenic fragments.

Conclusions

Novel formulae for the ointment base, obtained after introducing oxyethyleneated cholesterol derivatives into hydrophilic vaseline may comprise a potential vehiculum for anti-inflammatory ointments. Regardless of their chemical structure (the number of oxyethylenic fragments) and the type of catalyst used in the synthesis of the oxyethyleneate, its presence in the ointment contributes to the favourable change in rheological parameters (increased surface area of extended ointment under pressure force, reduced structural viscosity with a negligible effect on the effect of the water loss). Introduction of cholesterol derivatives into the hydrophilic vaseline-based ointments enhances the efficacy of the release of ketoprofen lysinate from the ointment. The highest areas under the release curved were obtained for products containing cholesterol derivatives featuring n=10 oxyethylenic fragments.

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