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Modeling of processes of solvent diffusion from ointment bases using *in vitro* experiments

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The results of the study of the release of propylene glycol (PG) and macrogol 400 (M400) from a water-soluble ointment base and the absorption of water by this base *in vitro* experiments through a semipermeable membrane using vertical diffusion chambers are presented. The content of hydrophilic solvents in the dialysate from 0.5 to 6.0 h of the experiment was determined by the gas chromatography method, calculated per cm^2 of the membrane area. Parameters such as release rate, cumulative content, dialysate concentration, and percentage of selvent realeased (after 6 h) were calculated. It is shown that the diffusion of PG through a semipermeable membrane is much more intense than the diffusion of M400, which is primarily due to its lower molecular weight. Ointment base absorbs water due to high-molecular macrogols and poloxamer, which do not penetrate through the membrane. Based on the results of the research, it can be predicted that the introduction of a low-molecular-weight solvent into the ointment base will eliminate the non-specificity of the dehydrating effect of the ointment base *in vivo*. The ointment base will ensure the absorption of purulent exudate in the absence of a dehydrating effect on viable tissues.

Keywords: propylene glycol, macrogol 400, ointment base, release.

Моделювання процесів дифузії розчинників з мазевої основи в дослідах *in vitro*. *О.П.Безугла, М.О.Ляпунов, І.О.Зінченко, О.А.Лисокобилка, А.М.Ляпунова*

Наведено результати дослідження вивільнення пропіленгліколю (PG) і макроголу 400 (M400) з водорозчинної мазевої основи та абсорбції води цією основою в дослідах *in vitro* крізь напівпроникну мембрану з використанням вертикальних дифузійних камер. Методом газової хроматографії встановлено вміст гідрофільних розчинників в діалізаті з 0.5 до 6.0 год експерименту в розрахунку на $см^2$ площі мембрани. Розраховані такі параметри, як швидкість вивільнення, кумулятивний вміст, концентрація в діалізаті та ступінь вивільнення (через 6 год). Показано, що дифузія PG крізь напівпроникну мембрану значно інтенсивніша, ніж дифузія M400, що повязано, в першу чергу, з меншою молекулярною масою. За рахунок високомолекулярних макроголів та полоксамеру, що не проникають крізь мембрану, мазева основа абсорбує воду. За результатами досліджень можна прогнозувати, що введення до мазевої основи низькомолекулярного розчинника дозволить усунути в умовах *in vivo* неспецифічність дегідратуючої дії мазевої основи. Мазева основа забезпечить абсорбцію гнійного ексудату при відсутності дегідратуючої дії на життєздатні тканини.

1. Introduction

Bases-vehicles for ointments are an important functional material for the development of preparations for the local treatment of purulent wounds. When applying an ointment to a wound, 2 oppositely directed processes should occur: absorption of puru-

lent exudate by the ointment and diffusion of active substances and penetration enhancers into the wound [1]. Studying such a process *in vivo* can be challenging. In recent years, regulatory authorities of the USA and the EU have been implementing *in vitro* release test (IVRT) [2, 3] for topical semisolid medicinal products. These tests, as a part of evidence of pharmaceutical equivalence, may be performed to omit the clinical trials in certain cases. It was important to study in the model conditions the diffusion of hydrophilic solvents from the ointment base into the receptor chamber with water and water into the chamber with a water-soluble ointment base. In this case water simulated the exudate.

2. Experiment

Materials

A hydrophilic ointment base, containing 47.0 % m/m propylene glycol (PG) and 18.0 % m/m macrogol 400 (M400) (solvents and penetration enhancers), 20 % m/m macrogol 1450, 10.0 % m/m macrogol 4000 (these substances are thickeners for the ointment base) and 5.0 % m/m poloxamer 338 (surface-active component and thickener), was under study. The above mentioned substances ("BASF", Germany) met the requirements of the relevant monographs of the European Pharmacopoeia [4]. The components were heated to 70–75°C, melted and homogenized with degassing at a vacuum (of –0.5 to –0.6 MPa), then cooled with stirring to 23–25°C.

IVRT

The *in vitro* experiments in regard to PG and M400 release from ointment base were performed using vertical diffusion cells and cellulose membranes (GOST 7730-89); the membranes were pre-soaked in the purified water (hereafter — water) for 24 hours. The tests were performed at 32°C. The medium (water) in the receptor chamber was stirred by magnetic stirrer with mixing rate 600 rpm. Samples (1.0 ml) were collected from receptor chamber at 0.5, 1, 2, 3, 4, 5, and 6 h after application of the ointment base and the volume withdrawn was replaced with stock receptor medium (water). The concentrations of PG or M400 in receptor medium at different sampling time were measured and solvent amount (mg) released at given time per unit area (cm^2) was calculated for each sample. The results were assessed according to the generally accepted approaches [5, 6].

Quantitative determination of PG and M400

Quantitative determination of PG and M400 in the samples of the dialysate was performed by Gas chromatography (GC) [4] using gas chromatograph Shimadzu GC-2014 with FID detector and AOC-5000 autoinjector ("Shimadzu"; software: GC solution version 2.30.00). The analytical procedure for M400 is described below; this procedure has been validated previously [7].

Analytical procedure for quantitative determination of M400

Test solution. The filtered sample (receptor medium containing released M400) to be tested.

Reference solution. The filtered solution of M400 ("Sigma-Aldrich", cat.No. 202398) in water 1 mg/ml.

Chromatographic conditions:

— column: material — fused silica; size l = 30 m, d = 0.32 mm; stationary phase — poly(dimethyl)(diphenyl) siloxane; film thickness — 0.25 μm;

— *carrier gas*: nitrogen for chromatography;

— *linear velocity*: 50 cm/min;

— *split ratio*: 1:30;

— *detection*: flame ionisation;

— *injection*: 1 μl of the test solution and the reference solution;

— *temperature*: column — 150°C hold for 1 min, then increase 5°C/min up to 270°C and hold for 40 min; injection port — 270°C; detector — 270°C;

System suitability (reference solution): resolution between peaks due to any two M400 oligomers should be minimum 5; symmetry factor of the peak of any M400 oligomer should be in the range from 0.8 to 1.5 and relative standard deviation for sum of the peak areas of the all M400 oligomers should not exceed 4.0 %.

Analytical procedure for quantitative determination of PG

Test solution. The filtered sample (receptor medium containing released PG) to be tested.

Reference solution. The solution of PG CRS (CRS of State Pharmacopoeia of Ukraine, cat. No. P0347) in water 40 mg/ml.

Chromatographic conditions:

— column: material — glass; size l = 110 cm, d = 3.2 mm; stationary phase — ethylvinylbenzene-divinylbenzene copolymer (8-100 mesh);

— carrier gas: nitrogen for chromatography;

— flow rate: 25 ml/min;

— detection: flame ionisation;

Table 1. Validation characteristics of the analytical procedure for the PG quantification in the dialysate by GC and their evaluation against the acceptance criteria [8]

Parameter	Value	Criteria (n = 10)	Conclusion
Linearity			
b	0,98925		
S_b	0,00505		
α	−0,08167	1) ≤ $\lvert S_\alpha \times 1.8595\rvert = \lvert 0.69\rvert$ 2) if does not pass (1), then ≤$\lvert 1.03\rvert$	Pass according to both criteria
S_α	0,36878		
S_0	0.80444		
S_0/b	0.81318	≤$\lvert 1.72\rvert$	Pass
r	0.9999	≥$\lvert 0.9998\rvert$	Pass
Repeatability			
standard deviation $SD_{\Delta Zi}$, %	1.68		
confidence interval: $\Delta_{\Delta Zi}$ t (95 %10 −1)SDΔZi	3.08	≤3.2 %	Pass
Accuracy			
mean value ΔZ, %	−0.83		
1) statistical insignificance $\lvert\Delta Z\rvert$:	0.83	$\lvert\Delta Z\rvert$ ≤3.08 : $\sqrt{10}$ = 0.97 %	Pass
2) practical insignificance $\lvert\Delta Z\rvert$:	0.83	$\lvert\Delta Z\rvert$ ≤ 0.32×3.2%=1.02%	

— injection: 1 μl of the test solution and the reference solution;

— temperature: column — 220°C; injection port — 250°C; detector — 250°C;

— run time: ~ 4 min; Rt of PG peak ~ 2.4 min.

System suitability (reference solution): column performance calculated by the peak due to PG should be at least 300 theoretical plates; symmetry factor of PG peak should be in the range from 0.8 to 2.0 and relative standard deviation should be ≤3 %.

Validation of procedure for quantitative determination of PG and calculation of the acceptance criteria were carried out according to the methodology outlined in the State Pharmacopoeia of Ukraine [8].

The specificity of the analytical procedure for PG quantification was confirmed by the fact that on the chromatogram of the solvent (water) there was no peak with a retention time, which would coincide with the retention time of the PG peak (Rt≈2.4 min) on the chromatograms of the reference solution and model solution (dialysate sample at time point 6 h). In addition, there was no difference in the retention times of the PG peaks on the chromatograms of the model solution and reference solution.

The range of the analytical procedure was chosen taking into account the results of determining the minimum and maximum concentrations of PG in the samples of the dialysate. The validation of the procedure for quantitative determination of PG was performed in the range of PG concentrations in model solutions from 0.31 mg/ml to 60.82 mg/ml (from 0.8 % to 152.0 % of the nominal concentration of PG in the reference solution — 40.0 mg/ml). The procedure for quantitative determination of PG in the studied range met the acceptance criteria in regard to linearity, repeatability and accuracy [8] (Table 1). The solutions were stable for more than 24 hours. The minimum limit of PG quantification (MLQ), calculated from the signal/noise ratio on the chromatogram of the model solution (0.31 mg/ml PG), was 15.31 μg/ml [30].

Water absorption by the ointment base

The water content in the chamber with the ointment base can be determined by K.Fisher titration [4, 8], but, generally, in the case of model process, it could be done by evaluating of change in mass (Δm) of the contents in the chamber with an ointment base. The chamber, in which 3.0 g of the

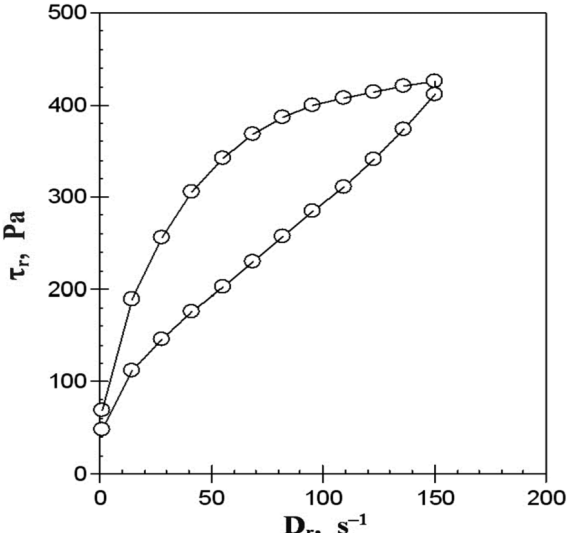

Fig. 1. Rheogram of ointment base (at 32°C).

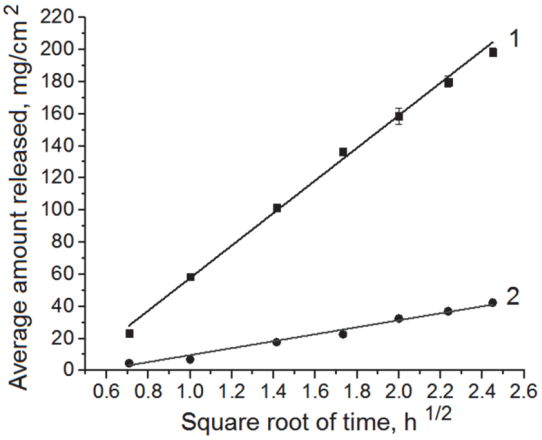

Fig. 2. PG release rate plot (*1*) and M400 release rate plot (*2*) at 32°C (solvent amount (mg) released per unit area (cm^2 vs square root of time ($h^{1/2}$)).

ointment base was initially placed, was weighed at certain time points and the change in mass (Δm) of the contents of the chamber was calculated. If the chamber contains solutions of hydrophilic high molecular weight substances that are not able to diffuse through the membrane, this approach is quite correct. But this approach does not take into account the diffusion of low molecular weight hydrophilic substances (PG and M400) into the receptor chamber with water. In this case, the researcher does not receive exact quantitative results, but can estimate the kinetics of the model process.

Rheological properties of the ointment base

Rheogram (plot of the shear stress (τ_r) versus the shear rate (D_r) were obtained at 32°C by rotating viscometry [4] using a rotating viscometer "Rheolab QC" with coaxial cylinders CC-27 ("Anton Paar GmbH"; software RHEOPLUS, version 2.66). Rheogram (Fig. 1) was used to characterize the flow behaviour as well as to determine the hysteresis (thixotropic) area (A_H), yield stress (τ_0) and the apparent viscosity (η) at various shear rates (Table 2).

The experiments were performed at 32°C (skin temperature) according to the requirements regarding IVRT [2, 3]. A circulating thermostat Julabo F12-ED ("Julabo Labortechnik GmbH", Germany) was used to maintain a necessary temperature.

3. Results and discussion

The object under study at 32°C had the consistency of an ointment base. It was a non-Newtonian liquid characterized by a plastic flow (τ_0 = 68.9 Pa), thixotropic properties (A_H = 14541.6 Pa·s^{-1}) and different values of apparent viscosity at different shear rates (Table 2).

Water and the hydrophilic ointment base, separated by permeable membrane, cause two oppositely directed diffusion processes: 1) PG and M400 penetrate into the chamber with water; 2) water diffuses into the chamber with the ointment base [9].

According to the presented plots (Fig. 2) and values of correlation coefficients (Table 3) the relationship between PG or M400 amount released per unit area of the membrane versus the square root of time was linear and allowed us to adequately determine the release rates. Coefficients of determination were greater than 0.97 (acceptance criterion $R^2 > 0.90$) [5, 6].

Table 2. Rheological parameters of ointment base at 32°C

Rheological parameters:					
τ_0, Pa	A_H, Pa·s^{-1}	η, Pa·s, at D_r:			
		14.55 s^{-1}	28.09 s^{-1}	41.64 s^{-1}	82.27 s^{-1}
68.9	14541.6	13.01	9.10	7.34	4.70

Table 3. Parameters of PG and M400 release from ointment base

Parameter	Results in the case of:	
	PG	M400
Release rate (R), mg/cm^2/h$^{-1/2}$	100.05±2.02 SD: 1.00	22.59±0.39 SD: 0.19
Cumulative amount (A) (at time the point 6 h), mg/cm^2	198.43±4.92 SD: 2.44	42.42±0.89 SD: 0.44
Content (C) in the dialysate (at the time point 6 h), mg/ml	23.37±0.58 SD: 0.29	4.00±0.10 SD: 0.05
Correlation coefficient r	0.9977 SD: 0.001	0.9919 SD: 0.002
Coefficient of determination R^2	0.995	0.984
Percentage of solvent released (at the time point 6 h), %	99.43±2.47 SD: 1.22	55.51±1.17 SD: 0.58

The release rate of PG from the ointment base was greater than the release rate of M400 by approximately 4.4 times, which was due to the difference in their molecular weights (by 5 times) [4]. The minor difference between the ratio of release rates and the ratio of molecular weights was probably due to the interaction of hydroxyl groups of PG with high-molecular macrogols and poloxamer [10], which slightly delayed the release of PG.

Cumulative amount and concentration of PG in the dialysate were 4.7 times and 5.8 times greater compared to these parameters for M400, respectively. The percentage of PG released from the ointment base was greater compared to the percentage of M400 released by approximately 1.8 times; after 6 hours of the experiment, almost all amount of PG was released (Table 3). It should be noted the higher percentage of PG released compared to M400 release.

As Fig. 3 shows, simultaneously with the diffusion of PG and M400 into the chamber with water, water was absorbed by the ointment base containing impermeable high-molecular macrogols and poloxamer. At time point 6 h, the weight of the chamber with ointment base increased by approximately 260 %, despite almost complete release of PG and 55.5 % release of M400 from this chamber (Table 3). This indicates the ability of the ointment base to absorb exudate and cause nonspecific dehydration of tissues.

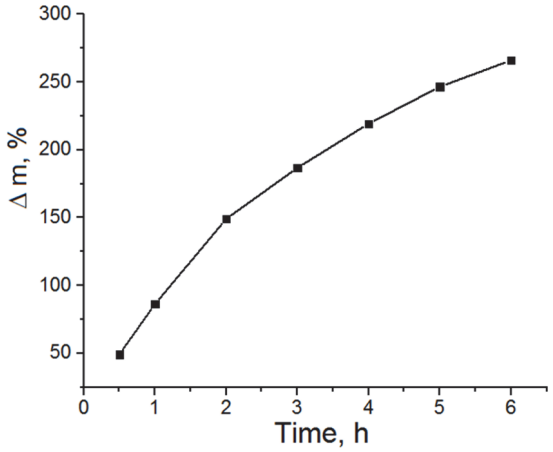

Fig. 3. Kinetics of changes in the mass of the contents in the chamber with the ointment base at 32°C.

4. Conclusions

An experiment with an ointment base containing a combination of two hydrophilic solvents differing in molecular weight was conducted for the first time. According to the results of research PG with a low molecular weight of 76.1 [5] quickly released during *in vitro* experiments, and M400 (Mr 383.5) [7] penetrated through the membrane into the chamber with water much more slowly due to its higher molecular weight. Poloxamer 338, macrogol 1450 and macrogol 6000 due to their high molecular weight did not penetrate into the chamber with water, but provided water absorption by ointment base. To a certain extent, the absorption of water by the ointment base was also provided by that part of M400 that did not penetrate into the chamber with water.

The conventional hydrophilic ointment base contains only M400 in combination with high-molecular macrogols [1]. Obviously, when applying ointment with this

base, the processes of water/exudate diffusion to the ointment base prevail due to the low release rate of M400. *In vivo* this leads to nonspecific dehydration of the biological object, when along with the absorption of exudate, the dehydration of all tissues, for example, granulation tissues, occurs.

If an ointment base contains two solvents with different molecular weights, the processes of diffusion *in vivo* will be different. PG is able to quickly penetrate into the tissues, as a result of which an osmotic balance can occur between the biological object and the ointment. This balance could then be maintained by M400, which slowly penetrates into the tissues. According to the research results, it can be assumed that a low-molecular solvent included into the composition of the ointment base should eliminate the nonspecific dehydrating effect of the ointment base *in vivo*. In this case, the ointment base can provide the absorption of purulent exudate by the medicinal product without dehydrating effect on viable tissues, in particular, granulation tissues.

Varying the ratio between the components of the ointment base makes it possible to develop medicinal products that are optimal for the treatment of various pathological processes, when the different ratio between the processes of penetration and dehydration is required.

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