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STUDY OF FACTORS AFFECTING THE *IN VITRO* RELEASE OF DEXPANTHENOL FROM SOLUTIONS AND TOPICAL SEMI-SOLID PREPARATIONS

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The aim. *To identify the factors influencing the in vitro release of dexpanthenol (DP) from solutions and semi-solid preparations.*

Materials and methods. *Dispersed systems containing 5.0 % DP were studied: solutions that were Newtonian liquids and semi-solid preparations (creams, gels and ointment) with non-Newtonian flow behaviour. Rheological studies were performed by rotational viscometry. In vitro release tests were performed using vertical diffusion cells. The content of DP in the receptor medium was determined by liquid chromatography.*

Results. *It has been shown that the greatest values of release parameters of DP were characteristic of its aqueous solution; they decreased when propylene glycol (PG), macrogol 400 (M400), and poloxamer 338 (P338) were added but remained at a high enough level. The inclusion of cationic surfactant and cetostearyl alcohol (CSA) (0.5:4.5 % m/m) into the Newtonian liquid led to the formation of disperse system with a plastic flow behaviour and to significant decrease in the DP release. In the case of a cream containing a non-ionic surfactant and CSA, the release parameters of DP were also at a low level. The release of DP from the w/o emulsion-based ointment was minimal. Compared to DP aqueous solution, the rate of DP release from a carbomer-based gel decreased by 2.8 times; when 20 % of a mixture of PG and M400 (10:10 % m/m) was added to such a gel, the rate of drug release decreased by another 1.5 times. The fastest and most complete release of DP was observed in the case of the P338-based disperse system, which transformed from a Newtonian liquid into a gel at 32 °C.*

Conclusions. *In vitro release of DP depended on the type of base; rapid and complete release of DP was characteristic of its aqueous solution, and minimal release was observed in the case of hydrophobic ointment. The use of CSA in combination with a surfactant or carbomer to create bases for semi-solid preparations with plastic flow behaviour was a considerable factor that significantly slowed down the release of DP from them. The greatest values of the release parameters of DP were observed in the case of a gel based on P338.*

Keywords: *dexpanthenol (DP), liquid, gel, cream, in vitro release test (IVRT).*

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1. Introduction

Dexpanthenol ((2R)-2,4-Dihydroxy-N-(3-hydroxypropyl)-3,3-dimethyl-butanamide) is very readily soluble in water, readily soluble in ethanol (96 %) and practically insoluble in heptane [1]. In contrast to pantothenic acid, dexpanthenol (DP) is rapidly absorbed when applied topically [2], provided that it is properly released from the medicinal product.

Dexpanthenol is a component of many topical preparations in dosage forms such as creams, gels, ointments, lotions, etc. [3].

First of all, DP is used in preparations for skin moisturizing and improving its barrier function, as DP promotes epidermal regeneration by enhancing epidermal differentiation and lipid synthesis [4]. Particularly noteworthy is the effectiveness of treating atopic dermatitis using preparations with DP, which eliminates the deficiency of skin barrier function, reduces transepidermal water loss (TEWL) and increases the hydration of the stratum corneum [3, 4]. Preparations with DP are effective in the treatment of diaper dermatitis [5, 6]. DP-containing lotions are recommended for skin care for people with diabetes [2].

In addition, DP is an important component in wound healing products [2]. The positive effect of DP on wound healing is the result of its influence on fibroblast proliferation and epithelialization; both processes are important for the treatment of both deep and superficial wounds and burns [2, 7]. Ointments with DP are widely used to treat cracked nipples when breastfeeding [2].

Thus, medicinal products with DP are widely used in medicine for various pathological processes [3]. Scientific publications regarding preparations with DP are mainly devoted to their pharmacological properties and the results of clinical trials [2]. However, there is almost no data on the methodology of pharmaceutical development and research of preparations with DP taking into account a specific pathological process, individual needs and the age of patients. There are only a few publications on the DP release from drugs, which is important for their effectiveness [1, 8, 9].

The effect of three non-ionic and ionic surfactants on the porcine skin penetration of dexpanthenol was studied by Laffleur F. et al. [8]. The results showed that

the nature of surfactant as a penetration enhancer greatly impacts cutaneous barrier impairment.

In vitro release study was conducted by Sipos E. et al. [9] to compare the release characteristics of dexpanthenol in the case of the hydrogels based on two carbomers and poloxamer 407 (Lutrol® F 127) with the cream. According to their data, dexpanthenol was released in lower amounts from the cream than from the three test gels. No significant differences were observed in the amount of active substance released from the carmomer-based and poloxamer-based gels. The highest amount of dexpanthenol was released from the gel with Lutrol® F 127 base.

Studies on the *in vitro* release of active substances are primarily aimed at proving the extended pharmaceutical equivalence of hybrid products and reference drugs [10–12]. Based on the results of comparative *in vitro* release tests (IVRT), a medicinal product may be registered without comparative clinical trials. In addition, such tests are used in the case of post-approval changes to the topical products [12, 13].

In vitro release studies can be an important tool at the stage of pharmaceutical development of semi-solid preparations. To develop the composition and establish the target quality profile of the product, it is important to identify the factors that affect the *in vitro* release of active substances. Among these factors are the dispersity of the active substance, the type of base and the composition of excipients. At the next stage, it is rational to study the effect of release parameters on the pharmacological activity of the medicinal product, penetration of active substances through the skin or their pharmacokinetics [14, 15].

The aim was to identify the factors influencing the *in vitro* release of dexpanthenol from solutions and semi-solid preparations.

2. Planning (methodology) of the research

The study of various disperse systems (DS) containing 5.0 % DP was planned, namely:

1) DP aqueous solutions and DP solutions in mixed solvents containing PG and/or M400, P338 [1, 16];

2) creams that additionally contained both cationic surfactant and cetostearyl alcohol (CSA);

3) carbomer-based and P338-based gels;

4) commercially available an o/w emulsion-based cream and a w/o emulsion-based ointment.

To characterize these DS, it was necessary to study their rheological properties by rotational viscometry [1].

In vitro release of DP should be studied using vertical diffusion cells [17]. For this purpose, the analytical procedure for the determination of DP concentration in the receptor medium by liquid chromatography (HPLC) should be developed and validated in appropriate ranges as well as the validation of the IVRT method should be conducted [10, 11].

It is necessary to identify factors affecting the *in vitro* release of DP. Such factors could include the composition of excipients, the type and rheological properties of DS and bases for semi-solid preparations.

3. Materials and methods

Dexpanthenol, cetostearyl alcohol, poloxamer 338, propylene glycol, macrogol 400 («BASF»), purified water (hereinafter referred to as water) were used in the experiments [15]. They met the pharmacopoeial requirements [1, 18]. The substance benzyldimethyl[3-myristoylamino)propyl] ammonium chloride monohydrate (UA/17990/01/01) (hereinafter referred to as cationic surfactant) was also used [19]. Carbopol® Ultrez 21 Polymer (hereinafter referred to as carbomer) («Lubrizol») [20] and 30 % sodium hydroxide solution were also used to prepare the gels.

The formulations of the studied systems are presented in Tables 1, 2.

Table 1

Composition of disperse systems

Component	Content (% m/m):									
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
DP	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
P338	–	6.0	–	–	3.0	6.0	9.0	6.0	17.0	17.0
PG	–	–	12.0	–	12.0	12.0	12.0	12.0	10.0	10.0
M400	–	–	–	12.0	12.0	12.0	12.0	12.0	9.0	9.0
Cationic surfactant	–	–	–	–	–	–	–	0.5	0.5	0.5
CSA	–	–	–	–	–	–	–	4.5	–	6.0
Water	95.0	89.0	83.0	83.0	68.0	65.0	62.0	60.0	58.5	52.5

Table 2

Composition of gels

Component	Content (% m/m):		
	Gel No. 1	Gel No. 2	Gel No. 3
DP	5.0	5.0	5.0
Carbopol® Ultrez 21 Polymer	0.85	0.85	–
P338	–	–	20.0
PG	–	10.0	–
M400	–	10.0	–
Sodium hydroxide solution, 30 %	1.00	1.00	–
Water	93.15	73.15	75.0

The results of some studies of Newtonian liquid No. 9 and cream No. 10 were presented in the article [21].

The medicinal products *Rjativnik® cream* («Arterium»; batch 0038631) with o/w emulsion base and *Bepanthen® ointment* («Merck», batch GP02A7V) with w/o emulsion base were also studied [19].

Rheograms (plots of the shear stress (τ_r) vs the shear rate (D_r)) were obtained at 25 °C and 32 °C by rotational viscometry (2.2.10) [1, 18] using a rotating viscometer Rheolab QC with coaxial cylinders CC-27 (for creams and gels) and DG-42 (for liquids) («Anton Paar GmbH»; software RHEOPLUS, 2.66 version). A circulating thermostat Julabo F12-ED («Julabo Labortechnik GmbH») was used to maintain a necessary temperature (with an accuracy of ±0.1 °C).

In order to study the release of DP, the IVRT method was used. The IVRT experiments were performed using vertical diffusion cells (capacity of receptor chamber was 6.3 ml; orifice area was 1 cm^2; «Copley Scientific Ltd.») and cellulose membranes (GOST 7730-89); the membranes were pre-soaked in the receptor medium (*water R*) for 24 hours. The tests were performed at 32 °C; in order to evaluate the robustness of the IVRT method to minor perturbations in temperature, two additional IVRT runs were conducted at temperatures 30 °C and 34 °C. The medium in the receptor chamber was stirred by a magnetic stirrer with a mixing rate of 600 rpm; in order to evaluate the robustness of the IVRT method to minor perturbations in mixing rate, two additional IVRT runs were conducted at 540 rpm and 660 rpm. Samples (0.3 ml) were collected from the receptor chamber at 0.5, 1, 2, 3, 4, 5, and 6 h after application of the tested product and the volume withdrawn was replaced with stock receptor medium.

The results were assessed according to the requirements of EMA draft guidelines [12] and USP General Chapter <1724> [13].

The IVRT method was validated by assessing membrane inertness, the solubility of DP in the receptor medium, and the linearity, precision, reproducibility, sensitivity, specificity, selectivity, and robustness of the method. The released amount of DP was calculated relative to its content in the test sample (recovery) [10, 11].

Quantitative determination of DP in the samples of receptor medium was performed by HPLC (2.2.29) [1, 18] according to developed analytical procedures using Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with a diode-array detector («Shimadzu»; software: LabSolutions Lite version 5.82). For the analytical studies, *Dexpanthenol RS* of State Pharmacopoeia of Ukraine (cat. No. D0123; batch 5; content 99.9 %) was used.

Analytical procedure for the quantitative determination of DP in the receptor medium.

Test solution. Filtered sample (receptor medium with released dexpanthenol).

Reference solution 1. Dissolve 60 mg of *Dexpanthenol RS* in 160 ml of *water R* and dilute to 200 ml with the same solvent (0.3 mg/ml DP).

Reference solution 2. Dissolve 40 mg of *Dexpanthenol RS* in 16 ml of *water R* and dilute to 20 ml with the same solvent (2.0 mg/ml DP).

Note. Reference solution 1 should be used in the case of DP concentrations from 0.03 mg/ml to 0.75 mg/ml, and reference solution 2 should be used in the case of DP concentrations from 0.2 mg/ml to 4.5 mg/ml.

Chromatographic conditions:

– mobile phase: 6.8 g/l *potassium dihydrogen phosphate solution – acetonitrile for chromatography R* (96:4);

– column: stainless-steel chromatographic column, 4.0×125 mm, packed with *octadecylsilyl silica gel for chromatography R* (5 µm) LiChrospher® 60 RP-select B;

– flow rate: 1.5 ml/min;

– detection: 206 nm;

– temperature: 30 °C;

– injection: 5 µl;

– chromatography time: about 5 min.

System suitability (reference solution): column performance calculated by the peak due to DP should be at least 1000 theoretical plates; symmetry factor for the DP peak should be from 0.8 to 1.5, and relative standard deviation (RSD) for areas of DP peaks should meet the requirements of State Pharmacopoeia of Ukraine (2.2.46(N)) [18].

Validation studies in regard to the procedure for the quantitative determination of DP were carried out according to the accepted methodology [18, 22]. Acceptance criteria for validation characteristics were calculated in accordance with the requirements of State Pharmacopoeia of Ukraine [22].

The specificity of the analytical procedure was confirmed by the fact that on the chromatograms obtained with the solvent (blank) and solution of placebo (receptor medium at time point 6 h in the case of IVRT test using bases without DP) there was no peak with a retention time (*Rt*), which would coincide with the *Rt* of the DP peak on the chromatograms obtained with the reference solutions and test solutions (Fig. 1). There was no difference in the Rt of the DP peaks on the chromatograms obtained with the test solutions and reference solutions (difference was less then acceptance criterion≤1.85 %) DP peaks on the chromatograms obtained with the reference and test solutions were spectrally pure.

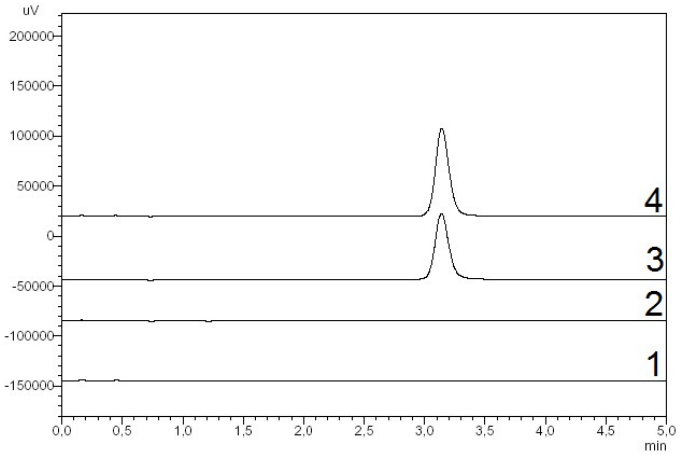

Fig. 1. Representative chromatograms obtained with solvent («blank») (1), solution of placebo (2), reference solution 1 (3) and test solution (4) (peaks with *Rt*≈3.14 min are due to DP)

According to the results of the linearity study (Table 3), the limit of quantification (LOQ) of DP in the concentration range from 0.03 mg/ml to 0.75 mg/ml in normalized coordinates was [18]:

$$LOQ=10\times S_{\alpha}:b=10\times 0.40855:1.00162=$$
$$=4.08\ \%<9.375\ \%.$$

According to the results of the linearity study (Table 4), the limit of quantification (LOQ) of DP in the concentration range from 0.2 mg/ml to 4.5 mg/ml in normalized coordinates was [18]:

$$LOQ=10\times S_{\alpha}:b=10\times 0.22297:0.99865=$$
$$=2.23\ \%<9.375\ \%.$$

LOQ, which was 4.08 % of the nominal concentration of DP in the reference solution 1, corresponded to its concentration of 0.012 mg/ml in the receptor medium; LOQ, which was 2.23 % of the nominal concentration of DP in the reference solution 2, corresponded to its concentration of 0.045 mg/ml in the receptor medium.

DP model solutions were stable during the entire period of analysis: the difference between the obtained values of Z_i for the first and last analysis was ΔZ_i=1.06 % and did not exceed the critical value:

1.06 %<√2×3 %=4.24 % (Table 5).

Table 3

Validation characteristics of the analytical procedure for the DP assay in the receptor medium in the concentration range from 0.03 mg/ml to 0.75 mg/ml and their evaluation against the acceptance criteria [22]

Parameter	Value	Criterion (n=9)	Conclusion
Linearity			
b	1.00162	–	–
S_b	0.00283	–	–
α	0.20532	1) ≤$\lvert S_\alpha \times 1.8946\rvert$=|0.77|; 2) if it does not meet the criterion (1), then ≤|1.07|	Pass
S_α	0.40855	–	–
S_0	0.70131	–	–
SD_{rest}	0.70018	≤|1.580|	Pass
r	0.99997	≥|0.99984|	Pass
Repeatability			
Standard deviation $SD_{\Delta Zi}$, %	1.23	–	–
Confidence interval: $\Delta_{\Delta Zi}=t(95\ \%, 9–1)\times SD_{\Delta Zi}$	2.29	2.29 %≤3.0 %	Pass
Accuracy			
Mean value ΔZ, %	0.15	–	–
1) statistical insignificance |ΔZ|	0.15	$\lvert\Delta Z\rvert \le \frac{t(95\ \%, 9-1)}{\sqrt{9}} \times SD_{\Delta Zi} = 0.76\ \%$	Pass
2) practical insignificance |ΔZ|	0.15	$\lvert\Delta Z\rvert \le 0.32 \times 3.0\ \% = 0.96\ \%$	

Table 4

Validation characteristics of the analytical procedure for the DP assay in the receptor medium in the concentration range from 0.2 mg/ml to 4.5 mg/ml and their evaluation against the acceptance criteria [22]

Parameter	Value	Criterion (n=9)	Conclusion
Linearity			
b	0.99865	–	–
S_b	0.00171	–	–
α	0.16956	1) ≤$\lvert S_\alpha \times 1.8946\rvert$=|0.42|; 2) if it does not meet the criterion (1), then ≤|1.07|	Pass
S_α	0.22297	–	–
S_0	0.37107	–	–
SD_{rest}	0.37157	≤|1.580|	Pass
r	0.99999	≥|0.99979|	Pass
Repeatability			
Standard deviation $SD_{\Delta Zi}$, %	0.68	–	–
Confidence interval: $\Delta_{\Delta Zi}=t\ (95\ \%, 9–1)\times SD_{\Delta Zi}$	1.26	1.26 %≤3.0 %	Pass
Accuracy			
Mean value ΔZ, %	0.30	–	–
1) statistical insignificance |ΔZ|	0.30	$\lvert\Delta Z\rvert \le \frac{t(95\ \%, 9-1)}{\sqrt{9}} \times SD_{\Delta Zi} = 0.42\ \%$	Pass
2) practical insignificance |ΔZ|	0.30	|ΔZ|≤0.32×3.0 %=0.96 %	

Table 5

Data on the stability of model solutions

Substance (concentration range)	Z_{first}, %	Z_{last}, %	$\lvert\Delta Z_i\rvert$, %	≤√2×maxΔ_{As}, %	Conclusion
Dexpanthenol (from 0.03 to 0.75 mg/ml)	97.20	100.04	2.84	4.24	Pass
Dexpanthenol (from 0.2 to 4.5 mg/ml)	101.18	100.13	1.05	4.24	Pass

According to the results of validation studies, the procedure for the quantitative determination of DP by HPLC in the receptor medium in the established ranges of application met the acceptance criteria for linearity, repeatability and accuracy (Table 3 and Table 4), and the tested solutions were stable (Table 5).

4. Research results

Research by rotating viscometer method.

The disperse systems No. 1–7 (Table 1) were liquids with Newtonian flow behaviour and low viscosity. For example, the dynamic viscosity of the solution No. 6, whose rheograms are shown in Fig. 2, was 12.2 mPa·s at 25 °C and it was 11.5 mPa·s at 32 °C. Solution No. 6 in its composition corresponded to the dispersion medium of the DS No. 8, whose rheological parameters were much greater (Table 6). The disperse system No. 8 was a non-Newtonian fluid with a plastic flow behaviour and minor thixotropic properties (Fig. 3), which was due to the cationic surfactant and CSA in its composition [21, 23]. The disperse systems No. 9 and No. 10 were, respectively, Newtonian fluid and non-Newtonian system with a plastic flow behaviour and thixotropic properties [21].

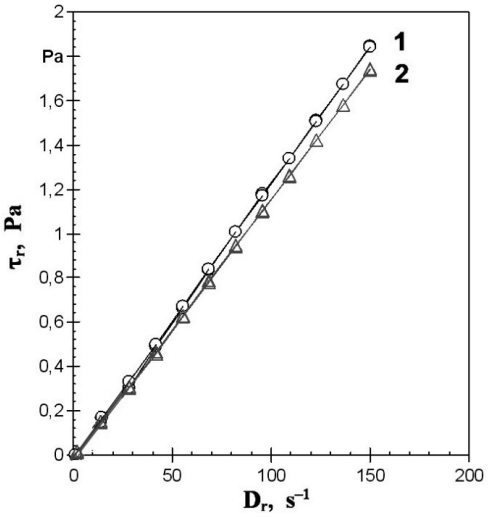

Fig. 2. Rheograms of DS No. 6 at 25 °C (1) and 32 °C (2)

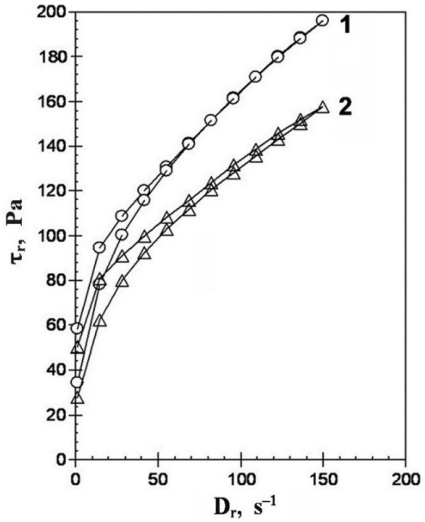

Fig. 3. Rheograms of DS No. 8 at 25 °C (1) and 32 °C (2)

Table 6

Yield stress (τ_0), thixotropic relative area (A_N) and apparent viscosity (η) for DS No. 8 at different temperatures (t)

t, °C	τ_0, Pa	A_N, Pa·s^{-1}	η (Pa·s) at D_r		
			14.6 s^{-1}	41.6 s^{-1}	82.3 s^{-1}
25	58.1	608.4	6.49	2.88	1.84
32	50.0	965.4	5.54	2.40	1.50

The base vehicle of the *Rjativnik® cream* is an o/w emulsion containing non-ionic surfactant and CSA as emulsifiers [19]. Therefore, plastic flow behaviour and thixotropic properties are characteristic of this preparation (Fig. 4). Compared to the disperse system No. 8, the *Rjativnik® cream* had more significant thixotropic properties, as evidenced by the larger areas of hysteresis loops (Tables 6, 7).

The carbomer-based gels No. 1, 2 (Table 2) had a plastic flow behaviour (Fig. 5). The rheological parameters of these gels differed little at 25 °C and 32 °C (Table 8).

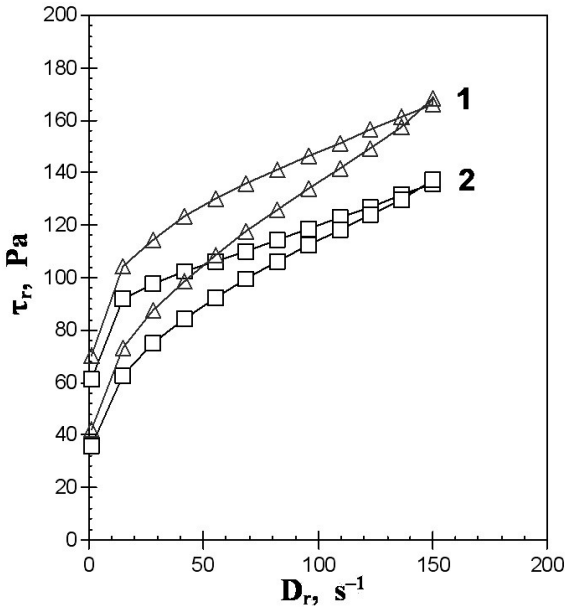

Fig. 4. Rheograms of *Rjativnik® cream* at 25 °C (1) and 32 °C (2)

Table 7

Yield stress (τ_0), thixotropic relative area (A_N) and apparent viscosity (η) for *Rjativnik® cream* at different temperatures (t)

t, °C	τ_0, Pa	A_N, Pa·s^{-1}	η (Pa·s) at D_r		
			14.6 s^{-1}	41.6 s^{-1}	82.3 s^{-1}
25	70.0	2488.1	7.18	2.96	1.72
32	61.3	1755.4	6.32	2.46	1.39

It should be noted that with an increase in temperature by 7 °C, the values of the rheological parameters of the study objects, the rheograms of which are shown in Fig. 2–4, decreased. In contrast, P338-based gel No. 3 at 25 °C was characterized by a Newtonian flow behaviour (Fig. 6) with low values of rheological parameters (Table 5), but at 32 °C,

the flow behaviour changed to plastic, and the values of rheological parameters increased sharply and became comparable to those for gels No. 1 and No. 2 (Table 8). The results of the study of this sol→gel transition by rotational viscometry and spin probes were published in [24].

Table 8

Yield stress (τ_0) and apparent viscosity (η) for gels at different temperatures (t)

Gel	t, °C	τ_0, Pa	η, Pa·s at D_r		
			14.6 s^{-1}	41.6 s^{-1}	82.3 s^{-1}
No. 1	25	210.9	19.64	8.41	4.92
No. 1	32	205.6	18.80	8.16	4.92
No. 2	25	195.1	20.18	9.01	5.45
No. 2	32	195.7	18.64	8.49	5.06
No. 3	25	0.49	0.17	0.17	0.17
No. 3	32	255.1	20.25	7.20	3.70

Validation of the IVRT method.

The validation was carried out according to the common methodology [10, 11]. For validation of the IVRT method, DS No. 8 was used since among the studied disperse systems (Table 1), it was characterized by the lowest release of DP, which had been established in previous experiments.

In preliminary experiments, *water R*, *phosphate buffer solution* pH 5.8, and *phosphate buffer solution* pH 7.2 were used as receptor media. Since the results of DP release into these media did not differ considerably, *water R* was chosen as the receptor medium.

According to the monograph «Dexpanthenol» [1], the solubility of DP in *water R* at temperatures from 15 °C to 25 °C is more than 1 g per 1 ml (very soluble), which is significantly greater than the highest concentration measured during the validation of the IVRT method – it was 0.48 mg/ml in the case of the gel with DP content of 7.5 %. These data indicated that the solubility of DP in *water R* was sufficient to ensure a «sink condition».

The release of DP into *water R* was studied using membranes:

– cellulose membrane (GOST 7730-89);

– PVDF membrane (cat. No. 7270; «Copley Scientific Ltd.»);

– Supor Polyethersulfone membrane (cat. No. 7274A; «Copley Scientific Ltd.»).

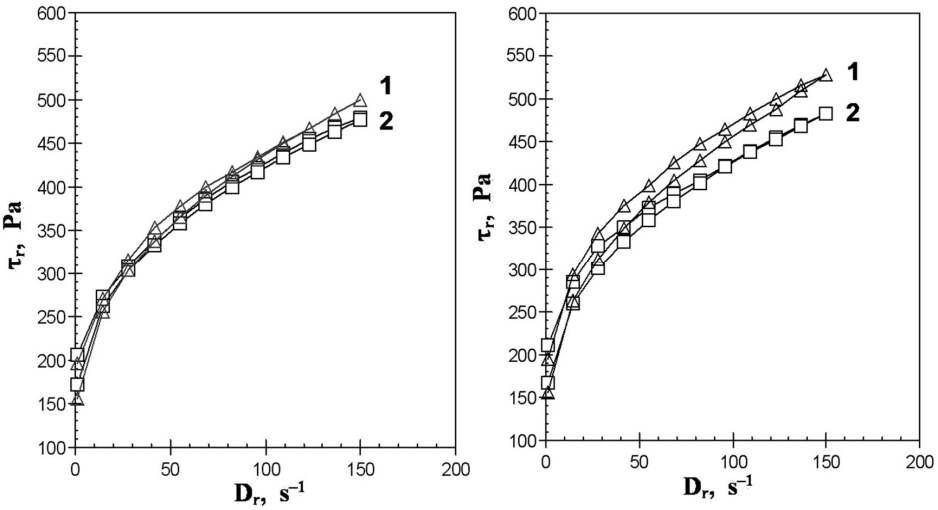

Fig. 5. Rheograms of gel No. 1 and gel No. 2 at 25 °C (1) and 32 °C (2)

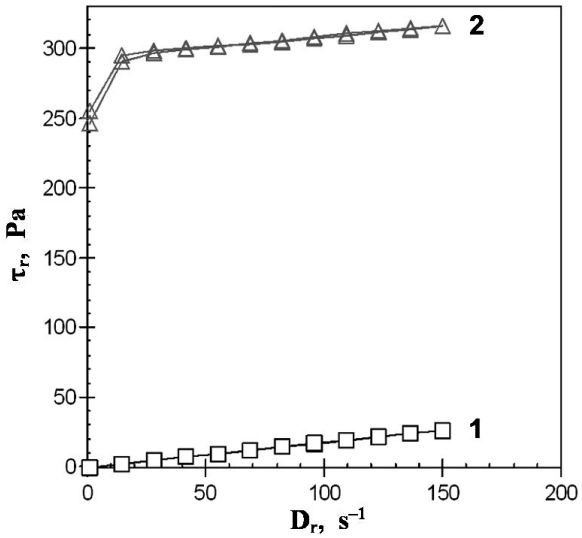

Fig. 6. Rheograms of gel No. 3 at 25 °C (1) and 32 °C (2)

In the experiment, positive results regarding the linear dependence of the released amount of DP per unit area of the membrane on the square root of time were obtained only with the cellulose membrane, which was chosen for further experiments.

The results of the validation studies are shown in Fig. 7–11, and their evaluation against the acceptance criteria is summarized in Table 9. Fig. 7 shows plots characterizing the linearity, precision and reproducibility of the IVRT method; plots characterizing the sensitivity, specificity and selectivity of the IVRT method are presented in Fig. 8, 9; Fig. 10, 11 show plots characterizing the robustness of this method.

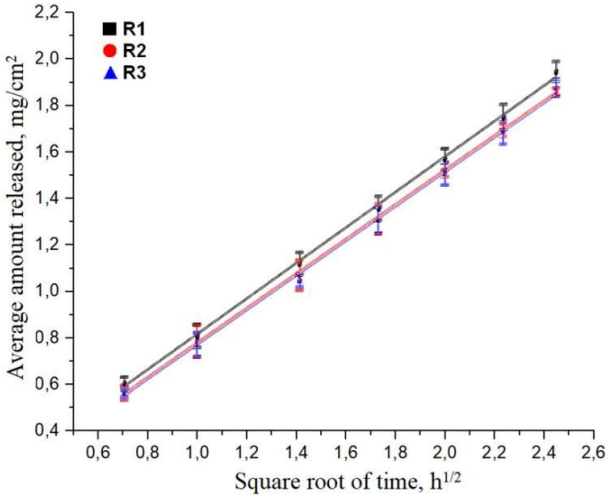

Fig. 7. Release rate plots obtained from the three IVRT runs using 5 % DP DS No. 8

The IVRT technique met the established acceptance criteria for all validation characteristics (Table 9).

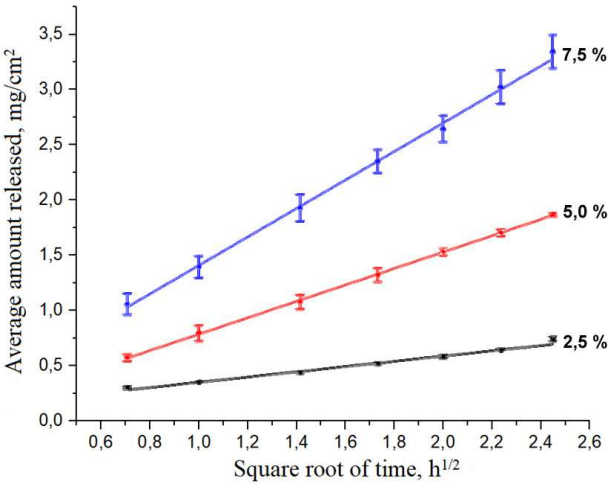

Fig. 8. Release rate plots obtained from the IVRT runs using DS with different content of DP: 2.5 %, 5.0 % and 7.5 %

The results of validation experiments confirmed the potential suitability of the IVRT method to measure the release parameters of DP from studied dispersion systems and its ability to distinguish between different releases of DP from these objects.

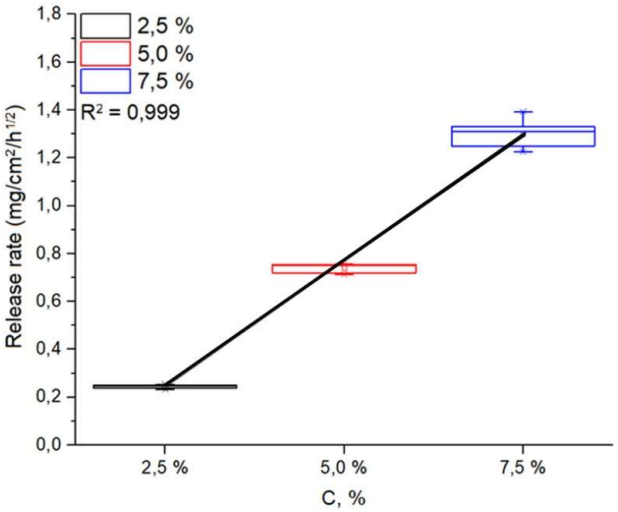

Fig. 9. Box and whiskers plot of the DP released rates for three DS with different content (C) of the dexpanthenol: 2.5 %, 5.0 % i 7.5 %

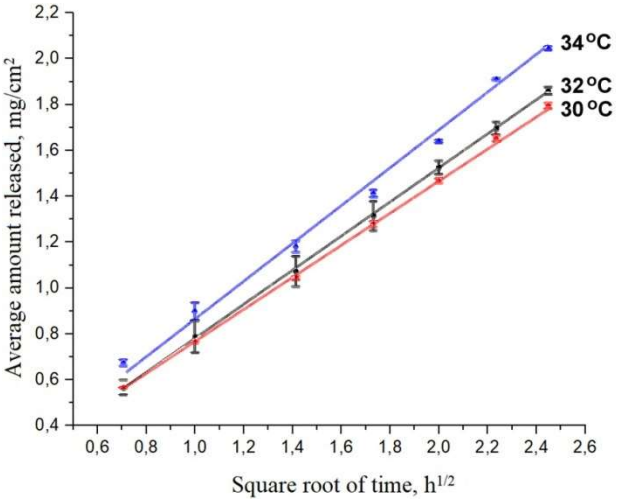

Fig. 10. DP release rate plots obtained from the three IVRT runs performed at different temperatures (30 °C, 32 °C, 34 °C)

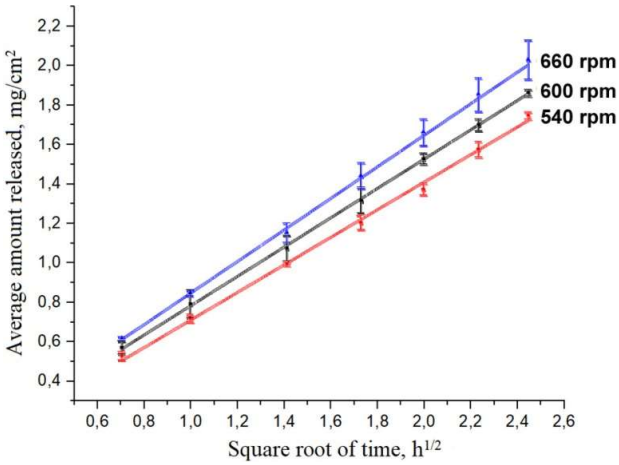

Fig. 11. DP release rate plots obtained from the three IVRT runs performed at different mixing rates (540 rpm, 600 rpm i 660 rpm)

Table 9

Main validation characteristics of IVRT method

Characteristic	Acceptance criteria	Results	Conclusion (+/–)*
Membrane inertness	DP concentration in the model solution after contact with the membrane ≥ 95 %	100.47 % (SD 0.18)	+
DP solubility in the receptor medium (*water R*)	DP solubility in *water R* should be at least 10 times higher than its highest measured concentration in the samples obtained during the validation of IVRT method	More than 1000 fold the maximum concentration of DP	+
Linearity (coefficient of determination)	R^2>0.90	R^2>0.99	+
Precision and reproducibility	$RSD_{intra\text{-}run}$<15 % $RSD_{inter\text{-}run}$<15 %	$RSD_{intra\text{-}run}$: 2.64 % $RSD_{inter\text{-}run}$: 1.75 %	+ +
Sensitivity	Mean release rate (DS 2.5 %)<mean release rate (DS 5.0 %)<mean release rate (DS 7.5 %)	0.25 mg/cm^2/h$^{-1/2}$<0.74 mg/cm^2/h$^{-1/2}$ <1.30 mg/cm^2/h$^{-1/2}$	+
Specificity	R^2>0.90	R^2=0.999	+
Selectivity	The confidence interval for two products with different concentrations of DP should fall outside the limits of 75.00–133.33 % (USP) or 90.00–111.00 % (EMA)	DS (2.5 %) *vs* DS (5.0 %): Lower limit: 31.80 %; Upper limit: 34.14 % DS (7.5 %) *vs* DS (5.0 %): Lower limit: 166.50 %; Upper limit: 184.46 %	+(USP)/+(EMA) +(USP)/+(EMA)
	The confidence interval for pairwise comparison of a product itself, in different IVRT runs, should fall within the limits of 75.00–133.33 % (USP) or 90.00–111.00 % (EMA)	DS (5.0 %) (run 1) *vs* DS (5.0 %) (run 2): Lower limit: 100.41 %; Upper limit: 105.82 % DS (5.0 %) (run 1) *vs* DS (5.0 %) (run 3): Lower limit: 100.29 %; Upper limit: 104.97 % DS (5.0 %) (run 2) *vs* DS (5.0 %) (run 3): Lower limit: 96.28 %; Upper limit: 101.67 %	+(USP)/+(EMA) +(USP)/+(EMA) +(USP)/+(EMA)
Robustness in regard to minor changes in the temperature	Mean release rates should not deviate by more than 15 % from the mean release rates at nominal parameters	Mean release rate at 30 °C: 0.70 mg/cm^2/h$^{-1/2}$; deviation – 5.41 % Mean release rate at 32 °C: 0.74 mg/cm^2/h$^{-1/2}$ Mean release rate at 34 °C: 0.82 mg/cm^2/h$^{-1/2}$; deviation – 10.81 %	+ +
Robustness in regard to minor changes in the mixing rate	Mean release rates should not deviate by more than 15 % from the mean release rates at nominal parameters	Mean release rate at mixing rate 540 rpm: 0.70 mg/cm^2/h$^{-1/2}$; deviation – 5.41 % Mean release rate at mixing rate 600 rpm: 0.74 mg/cm^2/h$^{-1/2}$ Mean release rate at mixing rate 660 rpm: 0.80 mg/cm^2/h$^{-1/2}$; deviation – 8.11 %	+ +
Recovery (amount of released after 6 h)	Ideally, not more than 30.00 % of the dose of DP in the donor chamber	~2.53 % (SD 0.20)	+

Note: «+» – pass, «–» – not comply with acceptance criteria

DP release studies.

The release parameters of DP from disperse systems No. 1–8 are presented in Table 10, and DP release rate plots for DS No. 1, DS No. 6 and DS No. 8 are shown in Fig. 12.

According to the data presented, the release rate plots were linear for all runs of IVRT. The highest values of DP release parameters were characteristic of its aqueous solution (DS No. 1). P338 and/or hydrophilic solvents slowed down the release of DP (Table 10). Thus, the release rate of DP from DS No. 6 containing 6.0 % P338, 12.0 % PG and 12.0 % M400 was 1.3 times lower than in the case of the aqueous solution of DP (DS No. 1).

However, the lowest values of DP release parameters were observed in the case of DS No. 8, which additionally contained cationic surfactant and CSA. Compared to DS No. 6, which was the dispersion medium for DS No. 8, the release rate was 15.2 times lower, the cumulative content and released amount were 12.2 times

lower. That is, the formation of a three-dimensional network from cationic surfactant and CSA molecules in the dispersion medium (DS No. 6) led to a significant decrease in the *in vitro* release parameters of DP from DS No. 8. This decrease correlated with a change in the flow behaviour and rheological parameters (Fig. 2, 3, Table 6), as well as with a decrease in PG release [21].

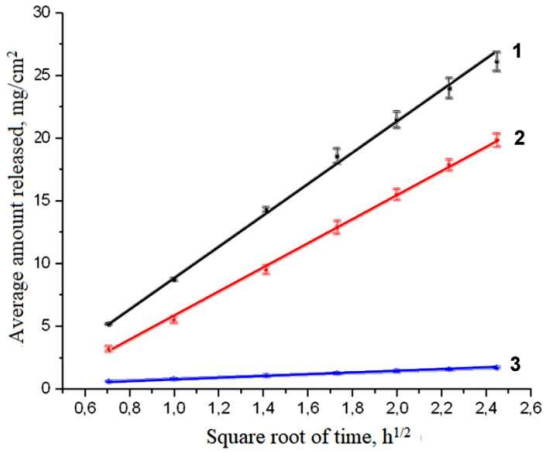

Fig. 12. DP release rate plots for DS No. 1 (1), No. 6 (2) and No. 8 (3)

The data on DP release from DS No. 9 and DS No. 10, which contained almost three times more P338 (17 %) than DS No. 6 and DS No. 8, are presented in Fig. 13 and Table 11.

Table 10

Parameters of DP release from DS No. 1–8

Object of research	Parameters				
	Release rate, mg/cm²/h$^{-1/2}$	Cumulative amount (A) (at time point 6 h), mg/cm²	r	R^2	Amount of released substance (after 6 h), %
DS No. 1	12.50	26.07	0.99946	0.99892	34.76 %
DS No. 2	12.10	25.33	0.99930	0.99860	33.78 %
DS No. 3	11.01	23.41	0.99974	0.99948	31.21 %
DS No. 4	10.91	23.23	0.99982	0.99964	30.97 %
DS No. 5	10.51	21.52	0.99977	0.99954	28.69 %
DS No. 6	9.60	19.80	0.99927	0.99854	26.39 %
DS No. 7	9.88	20.21	0.99992	0.99984	26.94 %
DS No. 8	0.63	1.62	0.99998	0.99996	2.16 %

Table 11

Parameters of DP release from DS No. 9 and DS No. 10

Object of research	Parameters				
	Release rate, mg/cm²/h$^{-1/2}$	Cumulative amount (A) (at time point 6 h), mg/cm²	r	R^2	Amount of released substance (after 6 h), %
DS No. 9	8.67	18.19	0.99400	0.98804	24.25 %
DS No. 10	0.64	1.64	0.98564	0.97149	2.18 %

The values of the DP release parameters from DS No. 6 and DS No. 9, which are Newtonian liquids (Fig. 1) [21], were similar, despite the difference in P338 concentration (6 % and 17 %, respectively). That is, a change in the concentration of P338 had a slight effect on the kinetics of DP release.

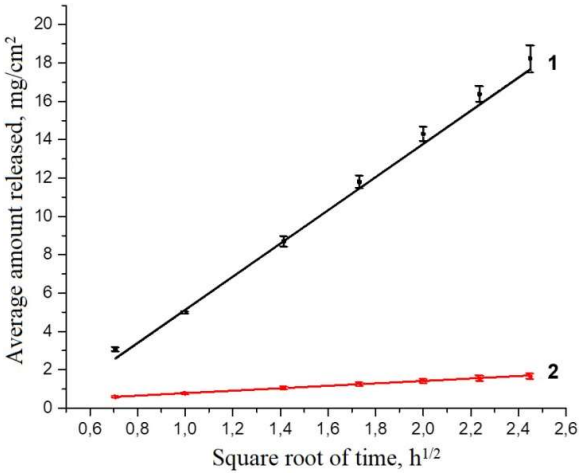

Fig. 13. DP release rate plots for DS No. 9 (1) and DS No. 10 (2)

When comparing the DP release parameters from DS No. 8 and DS No. 10 containing cationic surfactant and CSA, it can be concluded that they were almost identical (Tables 10, 11), despite the large difference in the concentration of P338. That is, the presence of surfactant in combination with CSA, which had formed a coagulation structure (Fig. 3), was a crucial factor that reduced the release of both DP and PG [21].

The existence of a coagulation structure in the *Rjativnik® cream* also led to a significant slowdown in the release of DP compared to its aqueous solution, but the values of the release parameters were approximately 2 times higher than in the case of DS No. 8 (Table 12).

The lowest values of DP release parameters were observed in the case of *Bepanthen® ointment* (Table 12, Fig. 14). Since the DP solution was the dispersed phase of the w/o emulsion, its contact with the membrane was minimal.

The values of DP release parameters in the case of the carbomer-based gel No. 1 were lower than those of its release from the aqueous solution (DS No. 1) (Table 12, Fig. 14). The release rate was 2.8 times lower, and the cumulative content and amount of released substance were 2.5 times lower. When the PG and M400 were added to the composition of carbomer-based gel No. 2 at concentrations of 10 % each, the values of DP release parameters decreased by 1.5 times (Table 12).

Compared to the aqueous solution (DS No. 1), the values of DP release parameters were greatest in the case of P338-based gel No. 3 (Table 12, Fig. 14). At 32 °C, this system had the rheological properties of a gel and, despite the absence of penetration enhancers, provided a rapid and complete release of DP. This distinguished it from DS No. 8 and DS No. 10, which had high values of rheological parameters and a low level of DP release (Tables 10–12).

Table 12

Parameters of DP release from the objects of the research

Object of research	Parameters				
	Release rate, $mg/cm^2/h^{-1/2}$	Cumulative amount (A) (at time point 6 h), mg/cm^2	r	R^2	Amount of released substance (after 6 h), %
DS No. 1	12.50	26.07	0.99946	0.99892	34.76 %
Rjativnik® cream	1.46	3.46	0.99997	0.99994	4.62 %
DS No. 8	0.63	1.62	0.99998	0.99996	2.16 %
Bepanthen® ointment	0.0045	0.013	0.99138	0.98283	0.02 %
Gel No. 1	4.53	10.26	0.99990	0.99980	13.67 %
Gel No. 2	2.97	6.92	0.99976	0.99952	9.22 %
Gel No. 3	11.06	23.26	0.99330	0.98664	31.01 %

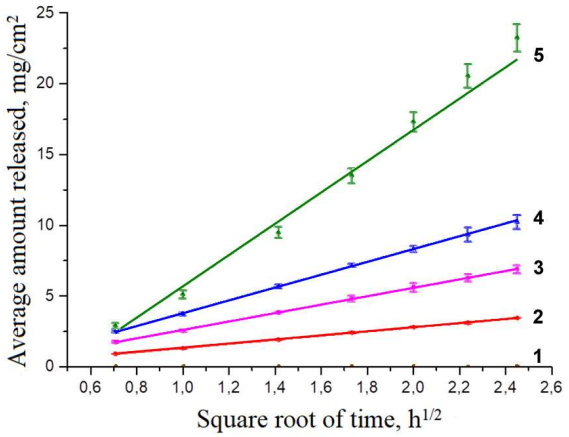

Fig. 14. DP release rate plots for *Bepanthen® ointment* (1), *Rjativnik® cream* (2), carbomer-based gel No. 2 (3), carbomer-based gel No. 1 (4) and P338-based gel No. 3 (5)

5. Discussion of research results

Two factors can be considered important for the effective use of dosage forms with DP: first, the release of DP and its subsequent penetration into the skin, which would provide moisturizing and therapeutic effects, and second, the proper consistency of the drug for applying it to the skin. Clarifying the relationship between rheological properties, *in vitro* release, and *in vivo* efficacy of the product with DP is of interest. DP was easily released from its aqueous solution during IVRT experiments. However, an aqueous solution is not the optimal form for skin applications [25]. When hydrophilic components (PG and M400) were added to the aqueous solution of DP, which could potentially promote DP penetration, and the block copolymer P338 [16], which could promote the adhesion of the product to the treated surface, a tendency to slow down the release of DP was identified.

The formation of creams with a plastic flow behaviour and thixotropic properties due to the addition of cationic surfactants and CSA into Newtonian liquids was an important factor that contributed to a significant decrease in the DP release parameters by 16–20 times. In such creams, the content of block copolymer P338 did not affect the DP release parameters. If the base vehicle was the visco-plastic o/w emulsion, the consistency of which was due to non-ionic surfactant and CSA, the DP release parameters were slightly higher but remained at a low level. From the w/o emulsion-based ointment, the release of DP was the lowest; compared to the aqueous solution, the release rate was approximately 2300 times lower, and the amount of released substance was approximately 1700 times lower.

Compared to other semi-solid dosage forms (DS No. 8 and DS No. 10, *Rjativnyk® cream* and *Bepanthen® ointment*), the DP release parameters were greater in the case of the carbomer-based gel. When 10 % PG and 10 % M400 were added to such a gel, the release parameters decreased by 1.5 times. However, the greatest values of DP release parameters were in the case of P338-based gel (Table 12, Fig. 14). At 32 °C, this system had rheological properties of a gel and, despite the absence of penetration enhancers, provided the rapid release of DP. This distinguished it from DS with high values of rheological parameters due to the presence of surfactants and CSA (DS No. 8 and No. 10). The release of DP from these dispersed systems was minimal.

Study limitations. The limitation of this work is that only one P338-based gel was used.

Prospects for further research. In the future, the release of DP from gels based on various poloxamers that differ in the composition of the dispersion medium should be studied. It is also of interest to compare, using *in vivo* experiments, the moisturizing effect on the skin of dispersed systems with different parameters of DP release. The results of this work can be used to develop topical preparations with dexpanthenol with a pronounced moisturizing effect on the skin or with a potent wound healing effect.

6. Conclusions

In vitro release of DP depended on the type of base; rapid and complete release of DP was characteristic of its aqueous solution, and minimal release was observed in the case of hydrophobic ointment. The use of CSA in combination with a surfactant or carbomer to create bases for semi-solid preparations with plastic flow behaviour was a considerable factor that significantly slowed down the release of DP from them. The greatest values of the release parameters of DP were observed in the case of a gel based on P338.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

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