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Short communication

Influence of plasma on the physical properties of ointments with quercetin

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Effects of two independent variables – the content of quercetin (0 or 1 or 1.5 or 5 %) and the content of plasma (0 or 2 or 4 or 6 %) – on the organoleptic properties and rheological parameters of model formulations prepared on an amphiphilic base were estimated. The consistency of all ointments was uniform, and the content of quercetin and plasma lay within the predefined range. Tested ointments are non-Newtonian systems. The content of quercetin and plasma was found to have a significant effect on the rheological properties of the ointments. An increase in the content of plasma in ointments was accompanied by a significant increase in their hardness, viscosity and shear stress and a reduction of their spreadability. The best rheological properties were shown by formulation F-3, containing 1.5 % of quercetin and 2 % of plasma.

Keywords: quercetin, plasma, ointment, rheological property

Plant raw materials are not used only as cosmetic preparations, but also in different prescription forms, including ointments. Flavonoids used in ointments are a valuable complement to antibiotic therapy, especially in the chronic and persistent course of certain infections (1).

One of such promising substances is quercetin. It is one of major bioflavonoids in human diet. Quercetin can be found in many vegetables, fruits as well as tea and grains (2). Many studies have demonstrated its biochemical and pharmacological properties (3). Depending on the concentration and location in the cell, it can exhibit both pro- and antioxidant properties. Antioxidant properties of quercetin are employed to scavenge ROS (reactive oxygen species) and find therapeutic application in the treatment of various diseases such as cancers as well as cardiovascular, ocular, autoimmune and infectious diseases (2, 3).

Studies in transgenic mice have demonstrated that quercetin and its derivatives reduce atopic dermatitis (4, 5). Phenolic extracts of *Sapium sebiferum* leaves exhibited inhibitory effects on edema induced by dinitrofluorobenzene. Application of extracts also decreased ROS and malondialdehyde levels and increased the GSH/T-GSH ratio of ear tissue

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in mice (6). Quercetin derivatives can be used in prevention and treatment of diseases mediated by ultraviolet radiation such as photoaging and skin cancer.

Many studies have demonstrated antibacterial and antifungal activity of quercetin (7, 8). Cushnie and Lamb (7) reported that flavonoids are capable of causing major structural and molecular changes in bacteria, including damage to the membranes, and inhibition of nucleic acid synthesis and cellular energy metabolism. Quercetin applied at a concentration of 16 μg/mL inhibited biofilm formation and virulence factors of *Pseudomonas aeruginosa*. Mostafa and Ibrahem (8) administered quercetin in the form of a cream to patients with aphthous ulceration in the mouth and they showed that the time required to complete their cure was significantly shorter compared to patients treated with a solution of benzydamine hydrochloride mouth wash.

Quercetin is a strongly hydrophobic substance and is most rapidly released from a hydrophilic base (9). Because of its low solubility in water, quercetin in blood plasma is bound to proteins, mainly albumin. The presence of hydroxy groups at C3 and C7 quercetin molecules results in the formation of a complex of albumin, a protein which is the main component of plasma (10). The compound of quercetin with albumin provides its transport system and determines their greater biological activity and efficacy (10, 11). The study conducted by Papadopoulou *et al.* (11) addressing the use of tryptophan fluorescence quenching revealed that of the four flavonoids examined (catechin, epicatechin, rutin, and quercetin), quercetin exhibited the strongest binding affinity at pH 7.4. Besides, the evidence suggested that binding of flavonoids to BSA did not change the molecular conformation of BSA.

Plasma is used in surgery, sports medicine and traumatology for the treatment of chronic wounds and in aesthetic medicine (12, 13). Autologous platelet-rich plasma (PRP) is a source of many growth factors that play a key role in the process of normal wound healing, such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) (13). In addition, the leukocytes present in the preparation increase its antibacterial properties (12). Having been administered plasma, patients may take fewer standard drugs, which is particularly important in the case of concurrent diseases.

In the literature, there are no data on the use of plasma in ointment formulations with quercetin. The efficacy of an ointment formulation is affected by many factors, including the composition and nature of the substrate, as well as their rheological properties.

Therefore, the aim of this study was to determine the influence of the content of quercetin (0 or 1 or 1.5 or 5 %) and plasma (0 or 2 or 4 or 6 %) on the organoleptic and rheological parameters (hardness, viscosity, shear stress and spreadability) of model ointments prepared on the base of Lekobaza®. The latter was selected as the base because of its rheological and amphiphilic properties. This medium makes it possible to obtain an ointment with the consistency of a soft foam and good spreadability. Furthermore, this base is often used in practice due to its lack of interaction with the components introduced.

EXPERIMENTAL

Chemicals

Quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one, was purchased from Cayman Chemicals, Michigan, USA. Factor VIII free plasma, as spray dried

powder (protein content 89–94, water 2.5–7 and salt ≤ 2 %) was a gift from Biochefa Pharmaceutical Research and Production Plant (Poland). Lekobaza® was purchased from Fagron (Pharma Cosmetic, Poland). Lekobaza® is an amphiphilic ointment comprising liquid paraffin 3.0 %, vaseline petrolatum jelly 32 %, glyceryl monostearate 3.0 %, cetostearyl alcohol 9 %, propylene glycol 5 %, Tween-40 7 %, miglyol 912 2.0 %, aerosil 0.1 %, sorbic acid 0.2 % and water 38.7 %. All materials used in the study were *p.a.* and satisfy the requirements of standards and certificates.

Preparation of ointment suspension

Based on the preliminary results, a suspension of 7 ointment formulation types was prepared, which differed in composition-concentration of quercetin (0, 1, 1.5, or 5 %) and plasma (0, 2, 4, or 6 %). Particles of quercetin were micronized using a pestle and mortar. Formulation F-6 contained only quercetin, 1 %, while F-7 only plasma, 4 %. All ointment formulations contained Lekobaza® as the base. Compositions of the different variants are shown in Table I. An appropriate amount of micronized quercetin (0 or 1 or 1.5 or 5 %) was mixed with the base in the 1:1 ratio (phase 1). Next, an adequate amount of plasma (0 or 2 or 4 or 6 %) was mixed with the base in the 1:1 ratio (phase 2). Phase 1 concentrate was mixed with phase 2 concentrate in a Unguator® (UNGUATOR® 2100, Gako, Poland) and added to the rest of the base to obtain a final mass of 100.0 g of ointment. All preparations were additionally homogenized for 6 minutes in the Unguator® in order to obtain uniform consistency. Each of the variants was done in 5 replications.

Analysis of selected physicochemical properties of ointments

Particle size analysis. – An optical microscope (MT 4200 Series Meiji Techno Co. LTP, Japan) equipped with a camera, was used to study the particle size of quercetin. 100 particles of quercetin were measured applying total magnification of 400×. Microscopic features of ointments were characterized by applying a small amount of the sample to a microscope slide, covering it with a cover slip, and observing under an E-600-Pol polarizing microscope (Nikon Corporation, Japan) at 1000× magnification. Images were captured with a digital camera (Olympus PD 26).

Spectrophotometric determination of quercetin and plasma. – To determine the content of quercetin, 10.0 mg of a relevant variant of ointment was weighed and dissolved in 10 mL of ethanol 96 %. For plasma, 10 mg of ointment was suspended in 10 mL of H_2O, and subsequently the supernatant was separated by centrifuging and the absorbance of the sam-

Table I. Composition (g) of preparations with quercetin

Ingredient	Formulation code						
	F-1	F-2	F-3	F-4	F-5	F-6	F-7
Quercetin	1.5	5.0	1.5	5.0	1.0	1.0	–
Plasma	6.0	2.0	2.0	6.0	4.0	–	4.0
Lekobaza® ad	100	100	100	100	100	100	100

ples was measured. Absorbance (x) was measured in 1 cm quartz cuvettes using a CE 3021 UV-Vis spectrometer (Cecil, UK). Concentration (y) was calculated on the basis of regression equations (quercetin $y = 0.0744x + 0.0045$ $R^2 > 0.999$; plasma $y = 0.0105x + 0.0221$ $R^2 > 0.999$). Absorbance was measured at wavelengths (λ_{max}) at which maximum absorbance was observed (λ_{max}(quercetin) = 373.0 nm, λ_{max}(plasma) = 280.0 nm). Photometric accuracy of the spectrophotometer was ± 0.005.

Determination of hardness. – Hardness tests were performed with the aid of a texture analyzer Shimadzu AUTOGRAPH, series EZ-LX/EZ-SX (Shimadzu Corporation, Analytical & Measuring Instruments Division, Japan) at 25 °C. A conical probe was immersed in the container containing the sample to a depth of 10 mm and moved at a speed of 2 mm s^{-1}.

Viscosity measurements. – Ointment samples of 0.5 cm^3 were subjected to shear stress in the space between the cone performing rotation and the fixed plate of a digital viscometer Brookfield CAP 2000+ (Brookfield Engineering Inc., USA) at 25 °C. The shear rate range from 66.66 to 1267 s^{-1} determined the size of shear stress and viscosity of the formulations.

Spreadability. – Spreadability of ointments was assayed using an extensometer. Increases of the surface areas (Πr^2) of ointment formulations stretched between the extensometer plates were measured at one-minute intervals with increasing loads (14). The correlation equation of the type $y = ax + b$ describing the course of the above dependencies allows for the application of an integration method to calculate the areas under extensibility curves. For comparative analysis, the spreadability index (S) was calculated by equation (*1*):

$$i(S) = \frac{S_{test}}{S_{con}} \quad (1)$$

where S_{test} is the surface area of the formulations tested, and S_{con} is the surface area of the control formulation without active drug (Formulation F-7) expressed in mm^2.

Statistical analysis

The results were calculated as mean values ± SD of five replicates. Statistical analysis was carried out using the Statistica (StatSoft Inc.) software. The effect of two independent variables was determined: the concentration of quercetin and plasma concentration on density, viscosity, spreadability and shear stress of ointment formulations. Correlations between the parameters were analyses by the Pearson correlation coefficient (r). Statistical significance of the differences between the means was analyzed using the Fisher-Snedecor test. The level of $p \leq 0.05$ was adopted to indicate statistical significance.

RESULTS AND DISCUSSION

Analysis of microscopic images showed that natural quercetin had a crystalline structure with a particle size of 10.70 ± 4.84 μm. The crystals observed under the microscope were straight, elongated, with blunt ends and no local deformations. Crystals were not found to form aggregates (Fig. 1a). After micronization, the average size of quercetin was 5.62 ± 2.46 μm (Fig. 1b), and 4.42 ± 1.87 μm in prepared formulations.

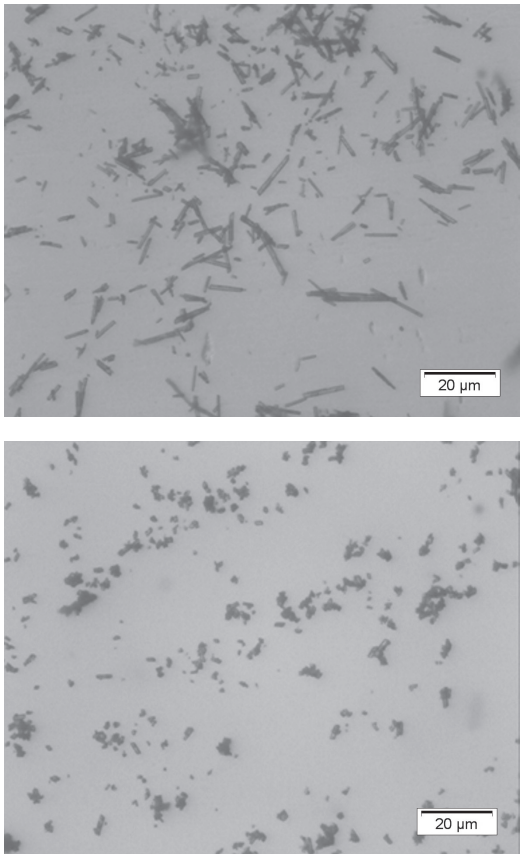

Fig. 1. Optical microscope images of quercetin (magnification 400×): a) before micronization, and b) after micronization.

As shown in Figs. 2a-c, quercetin solids in the ointments were fragmented and dispersed by mixing.

The ointment was uniform in all variants. Results revealed that the drug substance was generally evenly and uniformly dispersed. Contents of quercetin and plasma were within the predefined range in all formulations. Drug content was evaluated by calculating the mean recovery (%) from three samplings while homogeneity was evaluated by determining the relative standard deviation (% RSD). Mean recoveries (from 99.6 to 110 %) and RSD values (< 4 %) are in agreement with Polish Pharmacopeia (15).

Hardness of the ointment base was observed to increase with increasing the content of solid matter in it: the lowest hardness was achieved for formulation F-6, and the highest for F-4 (Table II).

Spreadability is the measure of increase in ointment surface under pressure. Highly spreadable ointments cover the surface of a lesion easily, which improves the diffusion of

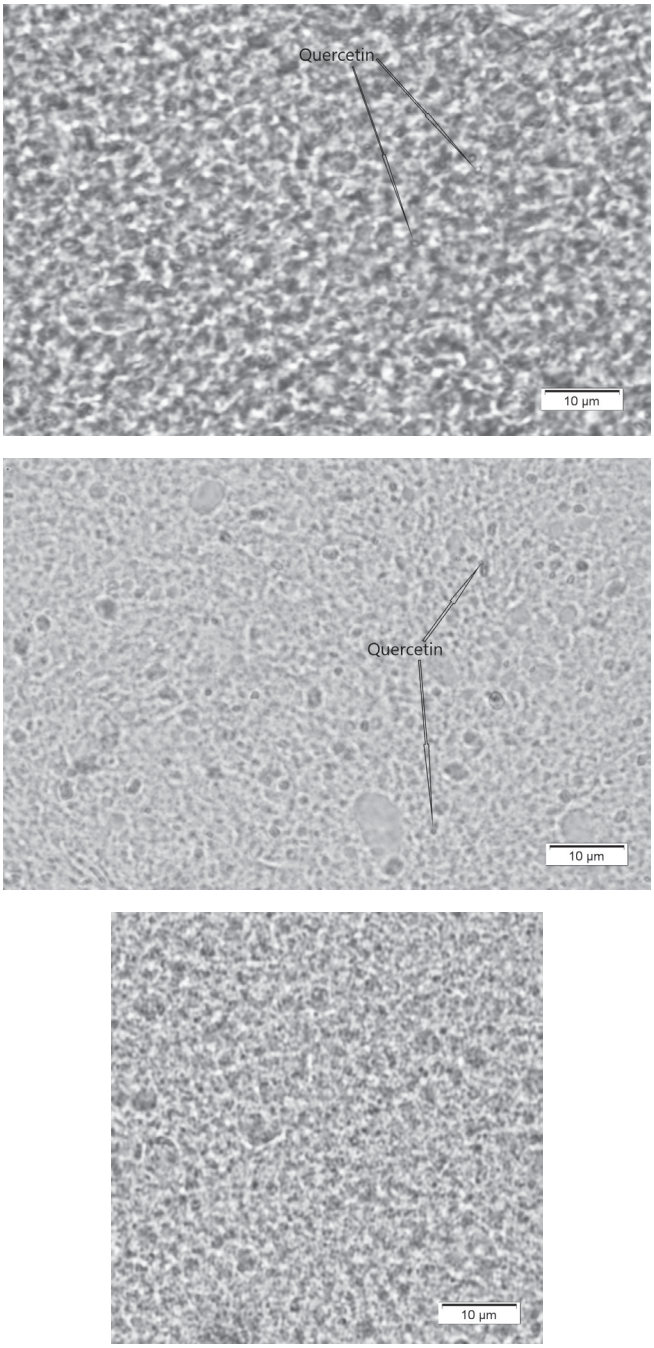

Fig. 2. Polarized microscope images of quercetin ointments (magnification 1000×): a) F-3, b) F-6 and c) F-7.

Table II. Hardness and viscosity parameters of formulations determined at 2 °C at two selected shear rates

Formulation code	Hardness (mN)	Shear rate 333 (s^{-1})		Shear rate 733 (s^{-1})	
		Shear stress ($N\ m^{-2}$)	Viscosity (mPa s)	Shear stress ($N\ m^{-2}$)	Viscosity (mPa s)
F-1	2592 ± 187	1178 ± 124	3533 ± 244	460 ± 22	627 ± 17
F-2	1521 ± 174	415 ± 11	1245 ± 45	318 ± 7	433 ± 21
F-3	993 ± 80	431 ± 12	1294 ± 24	139 ± 8	189 ± 11
F-4	2978 ± 138	1050 ± 94	1350 ± 60	404 ± 11	551 ± 34
F-5	1622 ± 117	643 ± 35	3150 ± 149	386 ± 9	527 ± 13
F-6	981 ± 91	370 ± 22	1110 ± 98	75 ± 6	102 ± 5
F-7	1316 ± 170	585 ± 12	1755 ± 73	240 ± 16	327 ± 16

drug substance from a dosage form to the external compartment. Ointment spreadability decreases with increasing the content of plasma (Table III). Ointments containing 2 % plasma are more spreadable compared to formulations with 6 % plasma. The results indicate that increasing the plasma content in ointments worsens their rheological properties, suggesting the need to reduce its content. The largest area under the extensibility curve is characterized by formulation F-6 (containing only quercetin) (1734.07 mm^2) and it is about 22.87 % higher compared to formulation F-7 (containing only plasma). With regard to spreadability, the resultant ointments could be grouped in ascending order: F-6 < F-3 < F-2 < F-7 < F-5 < F-4 < F-1 (where F-1 has the lowest and F-6 the highest value).

The course of flow curves of all prepared ointments indicates a non-Newtonian character. The relationship between shear stress and shear rate is not a straight line running through the origin of the coordinate system within the range of shear rates from 66.66 to 1267 s^{-1}. The highest shear stress values were obtained for F-1 and the lowest for F-6 (Table II). In the shear rate range from 66.66 to 733 s^{-1}, a sharp decline in shear stress is observed

Table III. Correlation equations (y = a + bx) that describe ointment spreadability

Formulation code	a ± da	b ± db	R^2	Surface area (mm^2)	*i*(*P*)
F-1	198.39 ± 9.78	1.9665 ± 0.097	0.9691	803.84	0.57
F-2	270.76 ± 15.24	2.9071 ± 0.164	0.9621	1589.63	1.13
F-3	254.64 ± 11.20	2.6032 ± 0.114	0.977	1607.34	1.14
F-4	157.81 ± 8.32	1.4279 ± 0.075	0.9716	1123.62	0.80
F-5	214.03 ± 15.27	2.3204 ± 0.166	0.9788	1319.59	0.94
F-6	324.48 ± 22.15	3.1664 ± 0.216	0.943	1734.07	1.23
F-7	246.14 ± 14.52	2.3029 ± 0.136	0.9566	1411.24	1.00

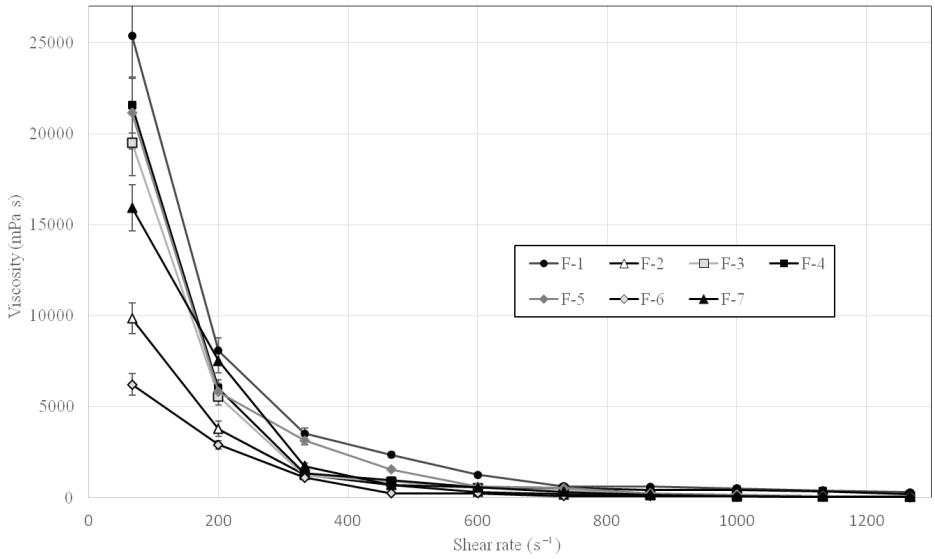

Fig. 3. Effect of formulation composition on its viscosity in dependence on the shear rate.

whereas at higher shear rates from 733 to 1267 s^{-1} shear stress is maintained at a similar level for each ointment formulation.

Spreadability is a function of structural viscosity: the lower the viscosity, the higher is the spreadability. Fig. 3 shows the impact of ointment composition on the viscosity (h) in dependence on the share rate.

As shown in Fig. 3, an increase in plasma and quercetin content in ointments significantly ($p < 0.05$) increases the viscosity at low shear rates. However, when the shear rate increased, the difference in viscosity of the investigated formulations was not significant. In all cases, viscosity decreased gradually with increasing the shear rate.

Results of the analysis of the correlation between the composition of ointments and their physical parameters (hardness, spreadability, viscosity, shear stress) are summarized

Table IV. Pearson coefficients between the composition of quercetin ointments and the examined parameters

Ingredient	Hardness		Spreadability		Viscosity		Shear stress	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Quercetin concentration	0.321	≤ 0.05	–0.283	≤ 0.05	0.357	≤ 0.05	0.249	≤ 0.05
Plasma concentration	0.941	≤ 0.001	–0.983	≤ 0.001	0.775	≤ 0.001	0.899	≤ 0.001

r – Pearson correlation coefficient; *p* – significance

in Table IV. It can be seen that plasma concentration in the ointment base was a factor that had a significant positive correlation with hardness ($r = 0.941$, $p \leq 0.001$), viscosity ($r = 0.775$, $p \leq 0.001$) and flow ($r = 0.899$, $p \leq 0.001$).

It was found that there was a weak positive correlation (for viscosity: $r = 0.357$, $p \leq 0.05$; for flow: $r = 0.249$, $p \leq 0.05$; for hardness: $r = 0.321$, $p \leq 0.05$) between the content of quercetin in the base ointment and rheological parameters such as viscosity and shear stress.

An inverse correlation was found between spreadability and quercetin content ($r = -0.283$, $p \leq 0.05$) as well as the content of plasma ($r = -0.983$, $p \leq = 0.001$).

Results of the comparison of the hardness, spreadability and viscosity of the ointments prepared in this study indicated that individual preparations had different physicochemical properties. Differences in spreadability and viscosity of the ointments resulted in different amounts applied to patients in clinical settings and different feel on the skin; these variations may affect the therapeutic effectiveness of the preparation (16). In the evaluation of rheological parameters, the best properties were those of formulation F-6, which does not contain plasma, and formulations F-2 and F-3 containing 2 % plasma and 5 and 1.5 % quercetin, respectively.

These data are difficult to discuss because there is no scientific literature addressing this issue. There are no ready-made formulations available on the pharmaceutical market that would contain both quercetin and plasma. Few works involve *in vitro* and *in vivo* studies of semi-solid formulations with quercetin (4, 9). Polyphenolic extracts constitute an attractive ingredient for cosmetics and pharmacy (16).

Among the prepared ointments, formulation F-3 (containing 1.5 % quercetin and 2 % plasma) showed the best rheological properties. The following stage should involve evaluation of interactions between the medicinal substance and other ointment ingredients. An effective form of therapy still requires more precise specification of the optimum concentration and determination of the stability of active substances.

CONCLUSIONS

The experiment indicates that quercetin has a significant effect on the rheological parameters of ointment formulations within the tested content range. Their hardness, viscosity and shear stress increase and their spreadability decreases with increasing the content of plasma in ointments. There is a weak positive correlation between the content of quercetin in ointment base and the rheological parameters such as hardness, viscosity and shear stress.

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