Physico-chemical Characterization, Rheological Behaviour and Evaluation of Antifungal Activity of Propiconazole Nitrate Gels

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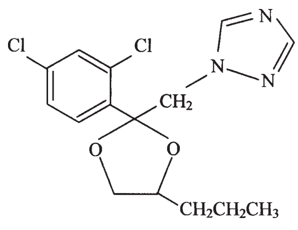
There were studied six variants of type suspension gels with 1.5% propiconazole nitrate, with various ointment bases: 4% hydroxypropyl cellulose (HPC-H), polyethylene glycol modified base and hydrated cetylic alcohol base, using as wetting agent and permeation enhancer glycerol as well as propylene glycol. The aim of this research is the physico-chemical characterization of gels: aspect, pH, homogeneity, rheological behaviour, viscosity and antifungal activity. The formulas based on HPC-H 4% have a plastic behaviour and an increased yield value; the formula sbased on modified PEG are rheopectic type, having a higher degree of thixotropy generated by propylene glycol. The formulae on hydrated cetylic alcohol base have a thixotrope behaviour (the formula including glycerol as wetting agent) and respectively thixotrope – antithixotrope behaviour (the formula including propylene glycol). The values of viscosity are higher in case of gel formulas having propylene glycol as wetting agent. Propiconazole nitrate has a good antifungal activity against clinical isolates of Candida spp., including strains resistant to other antifungal agents. All tested propiconazole nitrate gels have similar activity against Candida spp.

Keywords: propiconazole nitate, gels, rheological behaviour, antifungal activity

Increasing incidence of fungal diseases, both in immunocompromised and immunocompetent hosts, has led to an intensification of the pharmaceutical research in order to obtain new chemical structure with antifungal properties [1-3].

Patients with fungal infections usually suffer from metabolic or immunosuppressant (e.g. AIDS) diseases, leukaemia, neoplasm, patients treated with cytostatics and immunosuppressant drugs, patients exposed to radiotherapy. In the class of antifungal drugs, imidazoles have an important role in the treatment of diseases caused by different fungal infections [4–8]. Among these, propiconazole is an azole antifungal agent synthesized in 1979 by Janssen Pharmaceuticals (Belgium) and it has been used for the first time in agriculture as a systemic foliar fungicide [4]. It is now being produced by Ciba, and studied as fungistatic [6–10].

The active ingredient propiconazole is a triazole fungicide that has protective, curative and systemic activity, with the following chemical structure:



1–[[2–(2,4–dichlorphenyl)–4–propyl–1,3–dioxolan–2–yl]–methyl]–1–H–1,2,4–triazol

Propiconazole is an odourless yellowish, clear, viscous liquid [10]. After its application on rabbit skin and eye, there was noticed a slight irritation, but sensitivity studies have shown it has no allergic effect. Propiconazole nitrate is yellowish white, solid, water insoluble, but methanol (7.09 %) and ethanol (7.01 %) soluble. It was synthesized by an original method at State University of Medicine and Pharmacy "N. Testemitanu", Chisinau, Republic of Moldova and University of Medicine and Pharmacy "Gr. T. Popa" Iasi [11]. In order to develop new antifungal pharmaceutical formulation, for topical administration, we formulated and prepared ointments with 1.5wt % propiconazole nitrate, which were characterized by physico – chemical methods.

In a previous paper we published the results concerning the mechanical characterization – studies of the adhesion and extension capacity of 1.5 wt % propiconazole nitrate ointments [12].

In this study we investigated the physicol-chemical and rheological properties of propiconazole nitrate 1.5 wt % ointments, factors involved in the "in vitro" and "in vivo"drug availability. Also, we investigated"in vitro"antifungal activity of gels.

Experimental part

Material and methods

Hydroxypropyl cellulose H (HPC-H, Nisso, Japan, high dynamc viscosity 1.000–4.000 mPa·s), polyethylenglicol 300 and 4.000 (PEG 300, 4000, Labo-Chemie Wien, Fischamed, Osterreich), glycerol (Sigma Aldrich, Germania), propylene glycol (Merck, Schuchard, München, Germany), propiconazole (Chemicalland, China), triethanolamine (Alpa Aesar GmbH&CoKG, Germania), cetylic alcohol (Sigma Aldrich, Germania), lanoline (Sigma Aldrich, Germania), vaseline (Sigma Aldrich, Germania). All the chemicals used respect the

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Table 1
GEL FORMULATIONS WITH PROPICONAZOLE NITRATE 1.5%

Ingredients	Formula (g)					
	1	2	3	4	5	6
Propiconazole nitrate	1.5	1.5	1.5	1.5	1.5	1.5
HPC-H (Nisso)	4	4	–	–	–	–
Glycerole	10	–	10	–	10	–
Propylene glycol	–	10	–	10	–	10
PEG 300	–	–	23.5	23.5	–	–
PEG 4000	–	–	40	40	–	–
Cetylic alcohol	–	–	5	5	2.5	2.5
Lanoline	–	–	–	–	6	6
Vaseline	–	–	–	–	50	50
Triethanolamine	1	1	1	1	1	1
Distilled water	.... to 100					

quality degree required by 10[th] Romanian Pharmacopoeia [16].

Gels preparation

There were prepared six types of propylene glycol 1.5% gel, using three ointment bases in two variants: the first one including 10% glycerole as wetting and permeation enhancer agent and the second one including propylene glycol 10%, instead of glycerole:

-two types of gels based on HPC-H 4 % (formula 1 and 2);

-two types of gels with modified base of polyethylene glycol (formula 3 and 4);

-two types of gels with hydrated alcohol cetylic base (formula 5 and 6) (table 1);

The formulas 1 and 2 were prepared using 4 grams HPC – H, wetted with 5 g of glycerole (formula 1) and 5 g of propylene glycole (formula 2); after mixing it we added, drop by drop, gently stirring, distilled water up to 90 g. After 24 h in the prepared gel, the mixture of 1.5 g propiconazole nitrate dispersed in 5 g of wetting agent and 1 gram of triethanolamine was included, stirring continuously in order to homogenize.

The formulas 3 and 4 were prepared with cetylic alcohol, PEG 4000 and PEG 300 which were fluidized on water bath. In these fluidized compositions it was added 5 g of wetting agent and 70°C heated distilled water. Finally, the propiconazole nitrate was dispersed in the ointment base as previously. The formulas 5 and 6 were prepared also on water bath, fluidizing cetylic alcohol, lanoline and vaseline. The resulted mixture was hydrated and homogenized with heated distilled water containing triethanolamine. Propiconazole nitrate was dispersed as we specified above. The gels were packed in brown bottles and stored in a cool place.

Physico – chemical characterization

The following characteristics were considered:

Aspect: colour, homogeneity, type of gel;

pH: it was potentiometrically determined, according to 10[th] Romanian Pharmacopoeia [13].

Particle size: it was evaluated using an optical microscope Axiolab Rej 902553 type, Carl Zeiss;

Rheological investigation and dynamic viscosity

It was determined using rotational rheoviscometer Rheotest type RV2 (Germany) at 25°C ±0.1°C. We traced the flow curves rheograms and the viscosity curves of the gels, we determined the yield values extrapolating the linear part of the descendent curve of rheograms on the abscisa. Plastic viscosity was also calculated, this parameter characterizing the threshold flow of gels. It was calculated the dymanic viscosity (mPa·s), according to (1), at different moments as it follows: at the first step of shearing, named initial value; at the last step of shearing, named values after shearing and the values after stopping of shearing.

$$\eta = \zeta_r / D_r * 100 \quad (1)$$

Antifungal activity

The antifungal activity of propiconazole nitrate monosubstance and gels was tested on Candida albicans ATCC 10231 and 8 yeast strains isolated from human infections of the genital or urinary tract: : C. albicans 124, C. albicans 14, C. albicans 88, C. krusei 106, C. krusei 5, C. kefyr 16, C. glabrata 15, C. tropicalis 155. Overnight culture of each strain, on Sabouraud agar at 30°C has been used. The detection of the propiconazole nitrate minimum inhibitory concentration (MIC) was performed by dillution method [12]. The testing of antifungal activity of gels containing 1.5% propiconazole nitrate was achieved using Sabouraud agar plates incubated 24 h at 30°C. After incubation, we measured and compared the diameters of the inhibition areas [13].

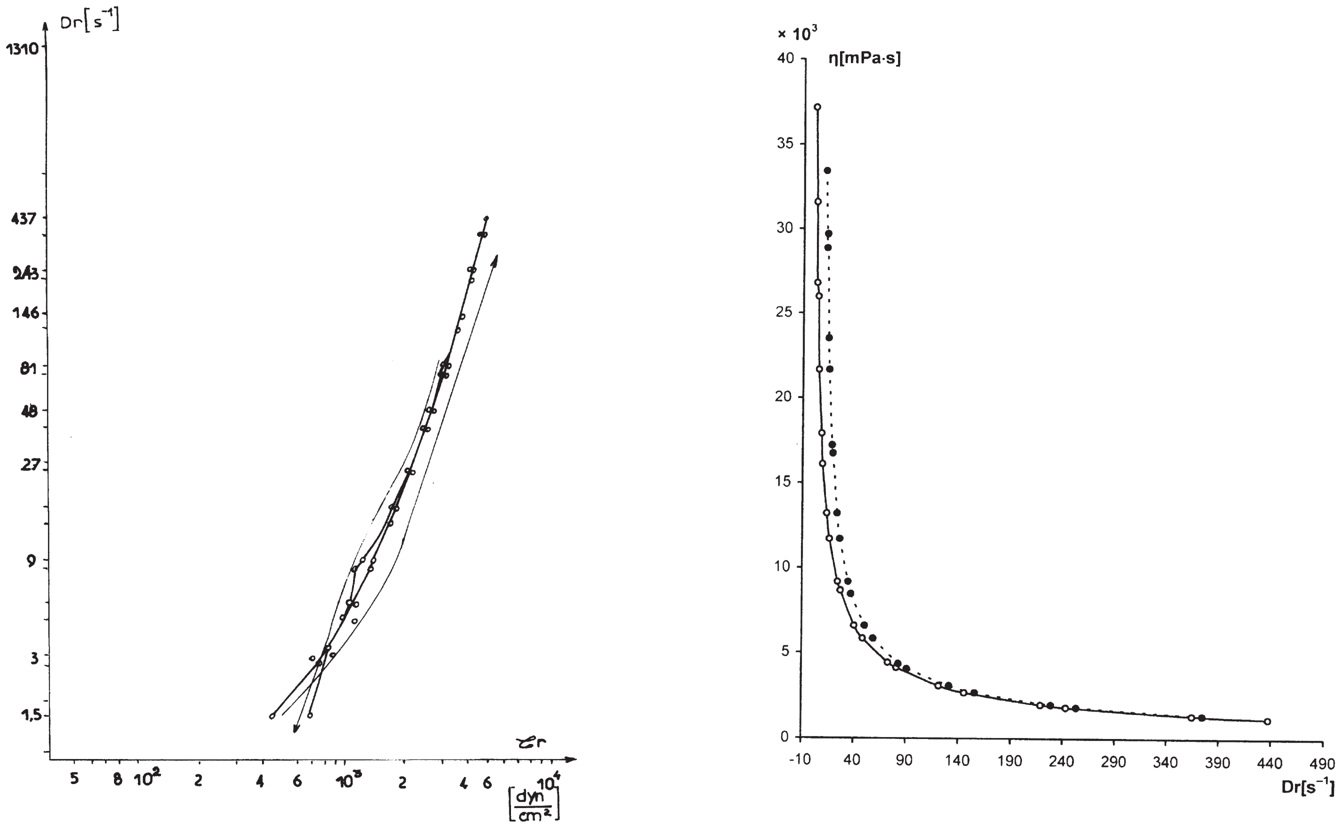


Fig. 1. Rheogram (a) and relative viscosity curve (b) of 1.5% propiconazole nitrate gel based on 4% HPC-H (with glycerol)

Results and discussions

The formulated gels have the following characteristics:

Aspect: uniform, homogenous, formulas 1 and 2 are transparent, translucent and formulas 3 and 6 are white and opaque. Due to the fact that propiconazole nitrate is insoluble in the ointment base, all the prepared gels are suspension type.

pH: the gel *p*H values (5.21 – 6.00) are within the physiological *p*H skin values (4 – 6.5)

Rheological behaviour

It has been studied the influence of the wetting agent (glycerol and propylene glycol) and propiconazole nitrate on the gel viscosity. The gel rheograms based on the shear stress applied during the increase and decrease of the shear stress rate show values which do not overlap. Subsequently, the formulas 1 and 2 have a plastic behaviour, and the flow increases related to the increase of the applied shear stress (fig. 1).

In order to start the flow, a certain shear stress τ_r, named by BINGHAM yield value is needed. The rheogram does not start from zero point. When this limit is surpassed, the behaviour of the gel becomes pseudoplastic, while its structure begins to deform.

By decreasing progressively the shear stress, the gel returns approximatively to its initial structure. Formula 1, based on HPC- H 4% with glycerol 10% as wetting agent, shows τ_r = 600 dyn/cm^2 yield value, corresponding to a plastic viscosity in value of 325.5 mPa·s (table 2).

Table 2

RHEOLOGICAL PARAMETERS OF 1.5% PROPICONAZOLE NITRATE GELS

Gel formula	Dynamic viscosity (mPa·s)	Yield value (dyn/cm^2)	Plastic viscosity (mPa·s)	Dr (s^{-1})
1	37,133.00	600	325.50	1.5
2	53,843.33	650	26.28	1.5
3	119,965.72	140	15.33	0.1667
4	621,416.66	650	65,64	0,1667
5	16,650.00	45	178.02	0.5
6	80,210.00	40	21.65	0.5

Table 3
VISCOZITY OF 1.5% PROPICONAZOLE NITRATE GELS

Formula	Dynamic viscousity (mPa·s)		
	Initial values	Values after shearing	Values after the shear stress stopped
1	37,133.30	1,171.65	33,420.00
2	53,843.33	772,66	51,986.66
3	119,965.72	1,920.44	143,958.87
4	621,416.66	1,229.08	6,222.22
5	16,650.00	746.41	8,638.00
6	80,210.00	653.11	12,366.66

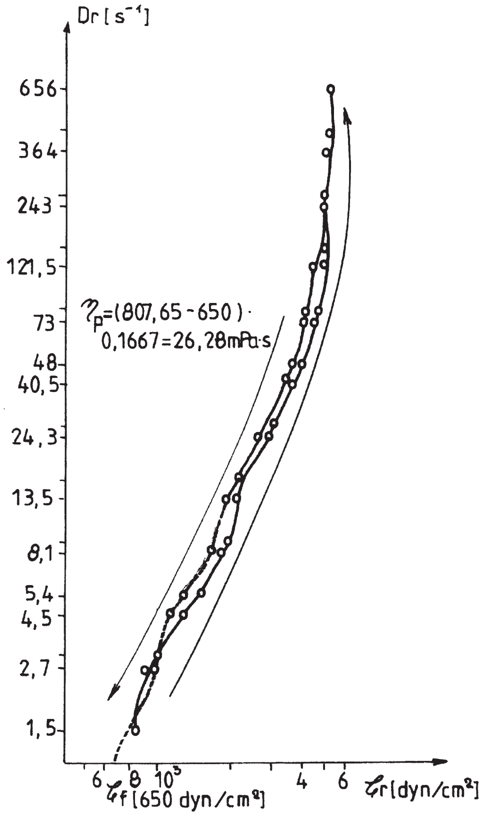
It can be observed that the two rheogram curves (the ascendent and the descendent one) do not overlap. This indicates that the gel structure does not immediately return to its initial state after the canceling of the shear stress.

Formula 1 can be included in plastic systems [16], and the hysteresis loop is extremely reduced; the gel shows a thixotropic – rheopectic character (combined flow).

Studying the dynamic viscosity of the gel, it can be observed that this parameter decreases as the shear stress increases. The value of the initial viscosity 37 133.00 mPa·s, after the application of the shear stress, decreases to 1.171,659 mPa·s, and after canceling the shear stress it reaches the value of 33 420.00 mPa·s (table 3).

In formula 2, using propylene glycol instead of glycerole as wetting agent determines the increase of viscosity, and the rheogram has the same aspect as in formula 1. The difference consists in the yield value of 650 dyn/cm^2 and a plastic viscosity of 26.28 mPa·s (fig. 2).

In time, the gel structure continues to deform, and after the progressive decrease of the shear stress, the gel returns to the initial state, aproximatively.



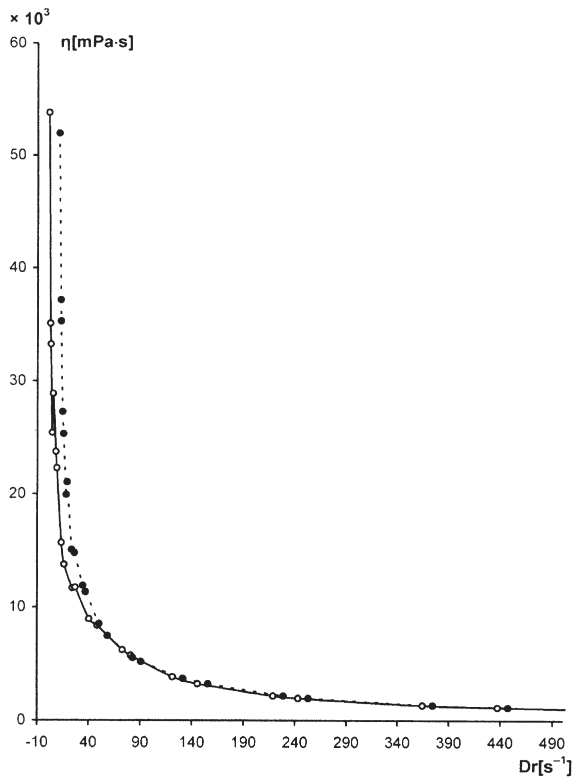


Fig. 2. Rheogram (a) and relative viscosity curve (b) of 1.5% propiconazole nitrate gel based on 4% HPC-H (with glycerol)

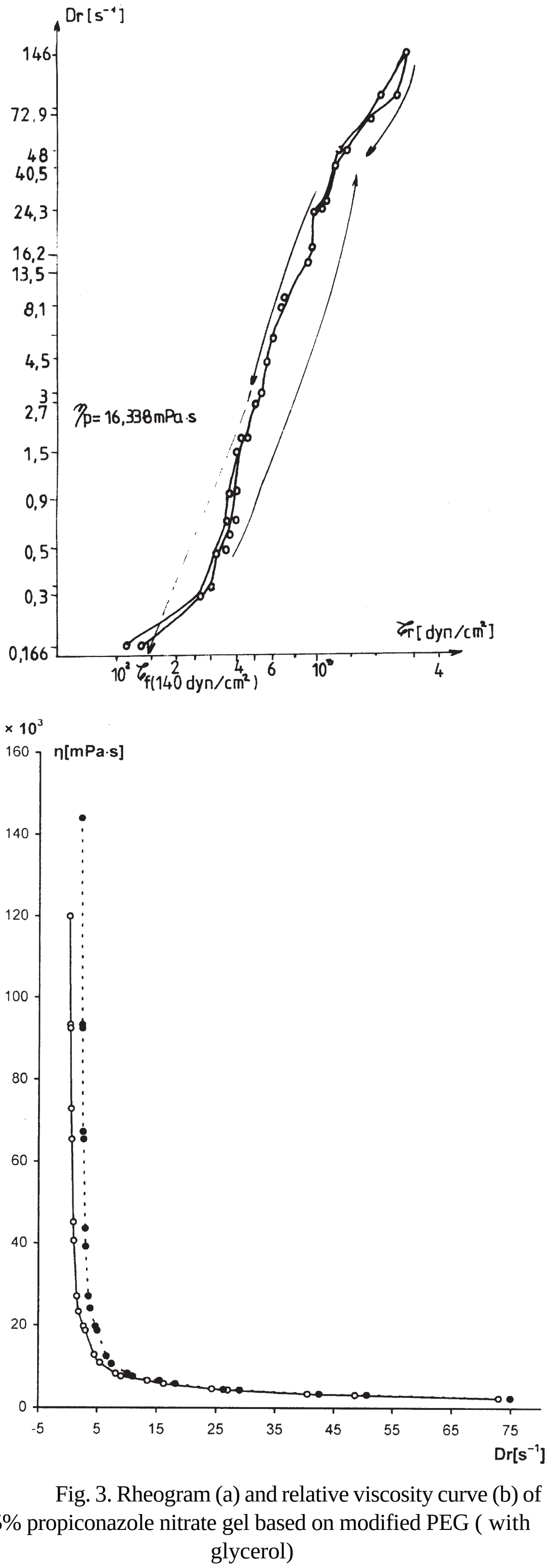


Fig. 3. Rheogram (a) and relative viscosity curve (b) of 1.5% propiconazole nitrate gel based on modified PEG (with glycerol)

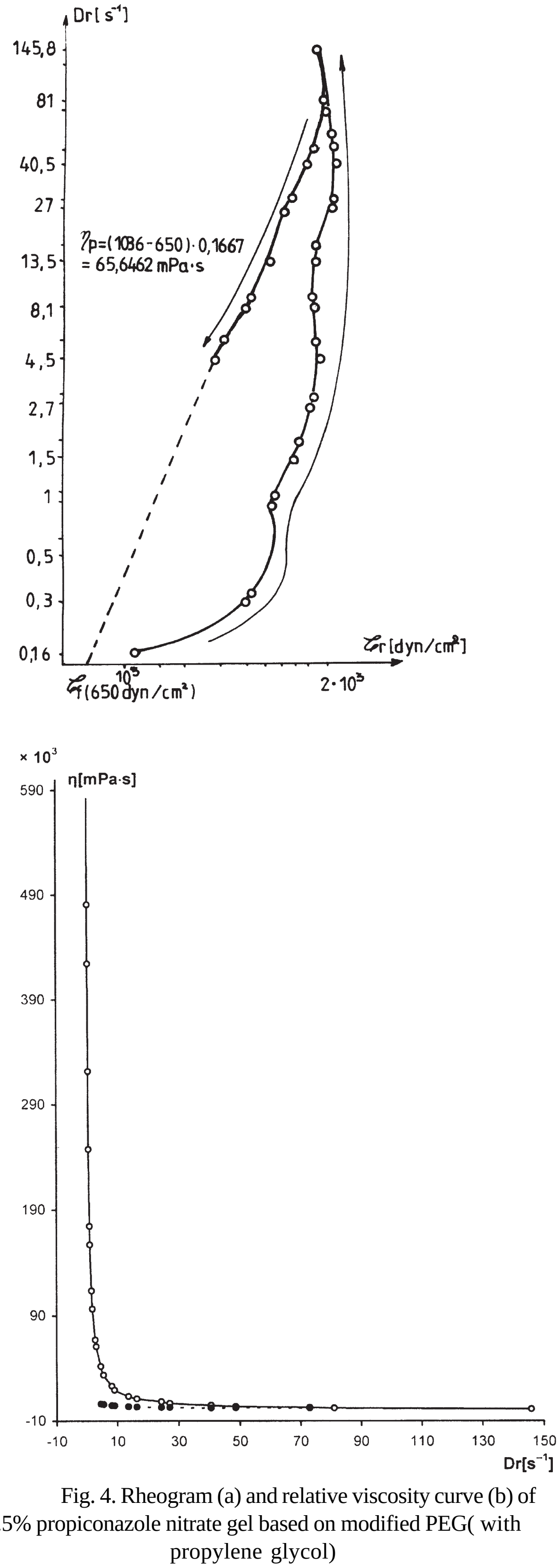


Fig. 4. Rheogram (a) and relative viscosity curve (b) of 1.5% propiconazole nitrate gel based on modified PEG(with propylene glycol)

Analyzing the dynamic viscosity curves of the gels based on HPC- H 4 %, but with different wetting agents, it can be noticed that propylene glycol provides a higher value of relative viscosity 53.843,33 mPa·s (formula 2) compared to 37.133,33 mPa·s (formula 1). After the shear stress has stopped for both formulas, the viscosity returns to values related to the initial ones: 33.420,00 mPa·s (formula 1) respectively 51.986,66 mPa·s (formula 2) (table 4).

These values are according to the rheograms, where the yield value of formula 1 is lower than the one of formula 2.

Formulas 3 and 4 are based on polyethylene glycol PEG 300 and PEG 4000 (table 1). The aqueous dispersions of PEG with high molecular weight show a pseudoplastic behaviour [3]. When applying high shear stress on these gels, it can be observed the rheopexy phenomenon, followed by a slight thixotropy, associated with the polymeric molecular structure, as a result of temporary cross-linked bonds (fig. 3).

The addition of some fat alcohols, such as cetilyc alcohol 5%, as in case of formulas 3 and 4, as well as the presence of propylene glycol 10% (formula 4) provides a high degree of thixotropy and a yield value of 650 dyn/cm^2 (formula 4) compared to formula 3 containig glycerole 10 %, which has a lower yield value, 140 dyn/cm^2.

There can also be observed some different values for plastic viscosity: 65.46 mPa·s (formula 4) respectively 15.33 mPa·s (formula 3).

By applying high shear stress on these two formula gels, the passing from laminar flow to turbulent flow is associated with an increase of aparent viscosity, PEG characteristic phenomenon.

In this area of turbulent flow, it can be observed a decrease of friction, flow curves showing a loop, more evident in formula 4, compared to newtonian flow curves (fig. 4).

Usually, the addition of electrolyts, for instance propiconazole nitrate, determines the decrease of absolute viscosity of PEG dispersion, but the presence of polyols, such as glycerole and propylen glycol, provides a continuous increase of viscosity up to a point, where the disociated molecules of propiconazole nitrate have a similar configuration with dispersed PEG molecules. Afterwards, the disociated propiconazole nitrate molecules show a similar behaviour with aqueous solutions under shear stress.

Thereby, formula 3 shows an initial viscosity of 119.965,72 mPa·s being maintained at high values up to an applied shear stress with the value of 406 dyn/cm^2, followed by a decrease of viscosity. The decreasing curve of formula 3 underlines the increase of viscosity above the initial value up to 143.958,87 mPa·s.

In the case of formula 4, the initial viscosity is higher (due to propylene glycol) having a value of 621.416,66 mPa·s. After that, it decreases in relation with the increase of the applied shear stress, continuing to have high value (113.866,66 mPa·s per shear stress of τ_r 1708 dyn/cm^2).

The decresed viscosity curve of formula 4, generates an evident hysteresis loop, having its final point at 6.222,22 mPa·s, corresponding to a shear stress of τ_r = 1890 dyn/cm^2.

The formulas 5 and 6, based on hydrated cetilyc ointment, also characterized by different wetting agent (glycerole and propylene glycol) are H/L emulsion type (H – hydrophilic, L- lipophilic). Lipophilic excipients are thixotropic gels with plastic behaviour, cetilyc alcohol and lanoline confer a display and adhesion capacity higher than the first four gel formulas. The data results were published in a previous paper [15]. $F_6 > F_5 > F_4 > F_2 > F_3 > F_1$.

For the H/L gel types, formulas 5 and 6, having a nonionic emulgator (cetilyc alcohol), the flow curve displays a high degree of thixotropy as well as large hysteresis areas (fig. 5).

Compared to formula 5, formula 6 has a combined thixotrope – antithixotrope character, provided by the propylene glycol (fig. 6).

Generally, the viscosity of H/L or L/H emulsion gels depend on their hydratation degree. The formulas 5 and 6 have 30 % hydratation (according to table 1), which confers the maintaning of the structure, as well as a corresponding display degree on the skin.

Comparatively, formulas 5 and 6 have a lower viscosity than other emulsion gels having active anionic emulsifier (e. g. sodium lauryl sulfate, sodium cetyl sulfate) [16].

Thus, formula 5 shows an initial viscosity of 16.500,00 mPa·s, value which decreases simultaneously with the increase of applied shear stress. After the shear stress is stopped, viscosity reaches a value of 8 638.00 mPa·s.

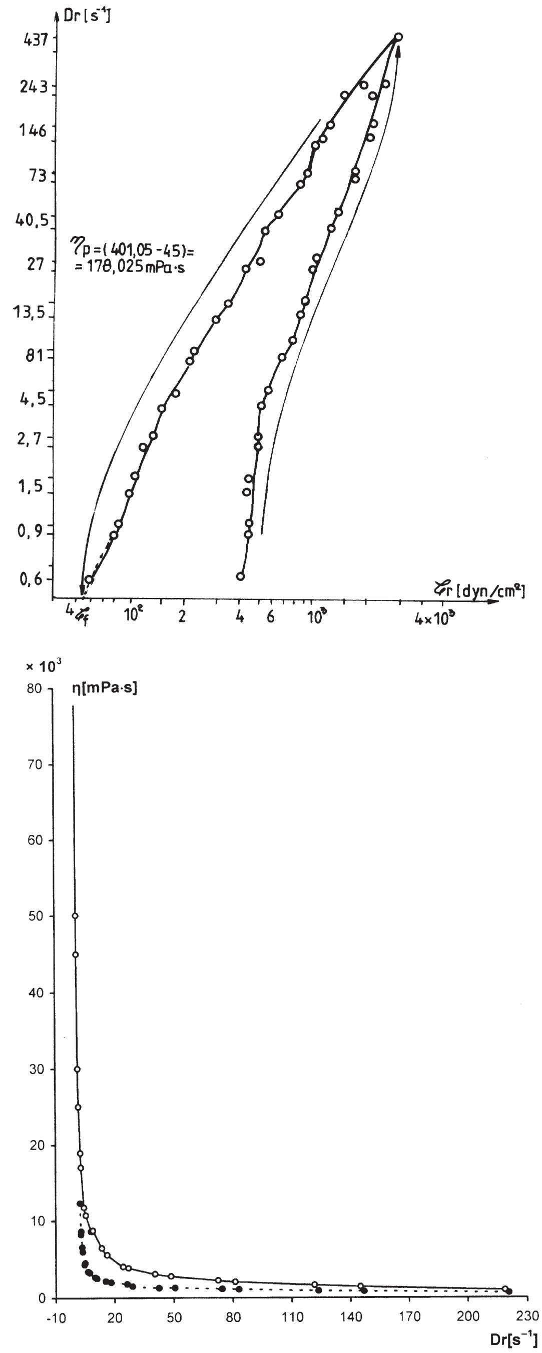


Fig. 5. Rheogram (a) and relative viscosity curve (b) of 1.5% propiconazole nitrate gel based on hydrated cetylic alcohol (with glycerol)

Formula 6 has an initial viscosity of 80 210.00 mPa·s that decreases to 12.366,66 mPa·s after the application of the shear stress.

This behaviour is in accordance with the determined yield values, which are very close: 45 dyn/cm^2 formula 5 and 40 dyn/cm^2 formula 6, corresponding to different plastic viscosities 178.02 mPa·s formula 5 and 21.65 mPa·s formula 6.

Observing the yield values presented in table 3, the following can be noticed:

-the formulas 1 and 2 based on HPC – H 4% have higher yield values, closely related, corresponding to a plastic viscosity with different values, phenomenon produced by the two different wetting agents (glycerole and propylene glycol);

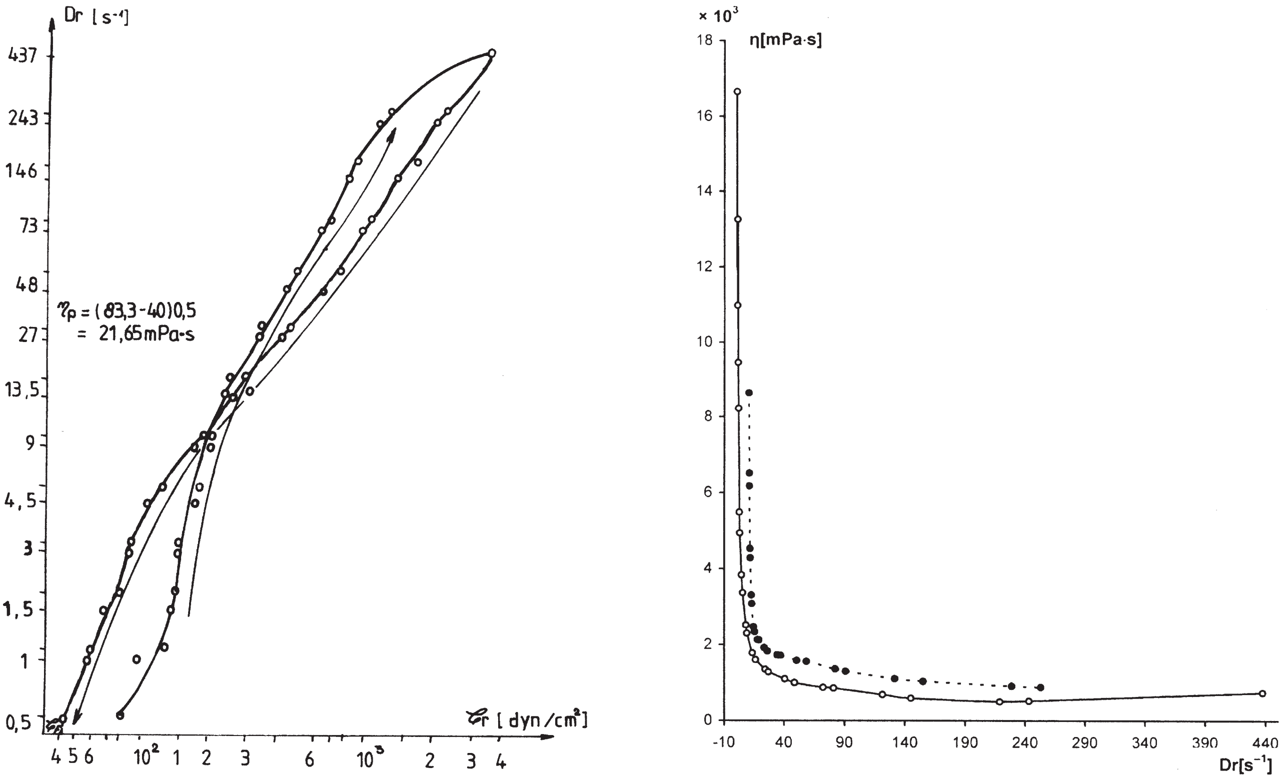


Fig. 6. Rheogram (a) and relative viscosity curve (b) of 1.5% propiconazole nitrate gel based on hydrated cetylic alcohol (with propylene glycol)

Table 4
DIAMETERS OF INHIBITION AREAS PRODUCED BY PROPICONAZOLE NITRATE GELS (3.5 mg) AGAINST CANDIDA SPP.

Strain	**Diameters of inhibition areas (mm)**					
	Gel 1	**Gel 2**	**Gel 3**	**Gel 4**	**Gel 5**	**Gel 6**
C. albicans ATCC 10231	31	31	32	32	33	33
C. albicans 124	34	33	31	32	33	33
C. albicans 14	30	29	30	31	31	30
C. albicans 88	24	24	23	25	25	24
C. krusei 106	27	26	26	27	28	27
C. krusei 5	21	22	21	22	23	23
C. kefyr 16	20	20	20	21	21	20
C. glabrata 15	21	20	21	21	21	21
C. tropicalis 155	32	32	31	32	33	32

-the formulas 3 and 4 based on PEG in addition with cetilyc alcohol 5%, show different yield values, while the plastic viscosity shows concordant values;

-the formulas 5 and 6, based on hydrated cetilyc alcohol, H/L emulsion type, show low and close yield values, while the plastic viscosity has a higher value for formula 5 compared to formula 6.

Antinfungal activity

We noticed good antifungal activity of propiconazole nitrate on reference strain and on vaginal or urinary clinical isolates, both C. albicans and non-albicans strains, both strains susceptible or resistant to other antifungal agents. The values of MIC are in range of 0.125 - 32 μg/mL. Diameters of the inhibition areas of propiconazole nitrate gels have values between 20-34 mm. There were no statistically significant differences for the tested six formulations (table 4).

Conclusions

The obtained data present the properties of the six formula gels with propiconazole nitrate 1.5 %. The rheological behaviour underlines the following characteristics: the gels based on HPC-H 4 % can be included in the class of plastic fluids and increased yield value; the gels based on modified PEG can be included in rheopex fluid, followed by the marked thixotropy due to propylene glycol;the formula 5, based on hydrated cetilyc alcohol, is a thixotrope fluid, while the formula 6 has a combined thixotropic – rheopectic behaviour.

All the gel formulas containing propylene glycol (formula 2, 4 and 6) show higher values of viscosity compared to the gels including glycerol (formula 1, 3 and 5).

Propiconazole nitrate has a good antifungal activity against clinical isolates of *Candida* spp., including strains resistant to other antifungal agents. All tested propiconazole nitrate gels have similar activity against *Candida* spp.

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