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RHEOLOGICAL PROPERTIES OF INDOLIC ALKALOIDS OBTAINED FROM NATURAL PRODUCTS

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ABSTRACT

Indole chemistry is a fascinating and complex field. The foul-smelling indole molecule is the building block for various natural and chemical products. Moreover, some indole derivatives are vital pharmaceutical products or essential intermediates. Indole ring compounds are part of the class of aromatic heterocyclic compounds with one heteroatom, and the indole ring is the most widespread heterocycle. This paper presenting a rheological indole alkaloids from natural products. In this article, we analysed twelve formulations of the H/L (water-oil) or L/H (oil-water) emulsion type, carrying out a comparative study with alcoholic extracts of different concentrations of Vinca alkaloids obtained from the leaf and stem of the *Vinca minor L.* plant. This plant is collected to the Dobrogea area.

Keywords: rheology of alkaloids, natural components, modeling, experimental blending of natural resource

INTRODUCTION

Indole ring compounds are part of the class of aromatic heterocyclic compounds with one heteroatom, and the indole ring is the most widespread heterocycle [1].

Indole (benzopyrrole) - the representative of the class, is found in coal tars, namely in the fraction with m.p. 220°C- 26°C (3-5%) from where it is isolated industrially in the form of potassium salt [2]. It occurs freely in small amounts in some flower essences (orange blossoms) but also naturally in human feces and has an intense odor. At very low concentrations, however, it has a floral aroma and is a constituent of some floral odors (such as orange blossom) and perfumes [3].

The name indole is formed from the words *ind* (indigo) and *ole* (oleum) because indole was first isolated in the reaction of indigo dye with oleum (sulfuric acid). Indole

chemistry began to develop with the study of the dye called indigo. Indigo can be transformed into *isatin* and then into *oxindole*.

In 1866, Adolf von Bayer reduced oxindole to indole using zinc dust, and three years later, in 1869, he proposed the formula for indole [4,5].

Obtaining natural (vegetable) compounds to create excipients presents many unique benefits due to their specific properties: biocompatibility, non-irritability, innocuousness, and low price [6].

At the same time, research in the field of biochemical technology aims to increase the therapeutic agent's bioavailability by approaching strategies to increase the release of substances used in drug treatment at the site of action.

The major advantages of topical administration of some natural substances are the avoidance of side effects and the liver barrier that exists in the oral administration of chemical drugs.

A starting point in the formulation of semi-solid preparations with topical application of indolic compounds extracted from the *Vinca minor L.* plant (Figure 1) was represented by the numerous benefits that these compounds highlight externally [7,8].

Figure 1. *Vinca minor plant to Dobrogea area*

Until now, there is no data regarding including these compounds in pharmaceutical forms with topical administration. However, studies have indicated that indolic compounds contribute to the healing of open wounds by speeding up the healing process [9].

Thus, we designed and prepared 12 new pharmaceutical formulations using extractive alcoholic solutions from the leaf and stem of the *Vinca minor* plant.

The leaves and stems of the *Vinca minor* plant were harvested locally in the Dobrogea area, according to their growth periods [10,11,12].

The plant material was manually selected and subjected to a treatment that consisted of repeated washings with potable and distilled water.

The first stage in the development of these pharmaceutical forms with topical application was the preparation of extractive alcoholic solutions by shredding 50 g of plant product obtained from the leaf and stem of the *Vinca minor* plant.

Then, ethyl alcohol was added in a concentration of 40%, 70%, and 96% until 500 mL (ratio 1 to 10) [13,14,15]. The extracts were left in a cool place for 10 days in optimal conditions, away from light and moisture.

During the 10 days, the extracts were carefully monitored and stirred 2-3 times a day.

Finally, they were filtered using cotton fabric filters to separate them from the plant material.

The ethyl alcohol concentrations of 40% and 70%, respectively, necessary for the preparation of the extracts were obtained as follows: to 1000 mL of ethyl alcohol 96%, add 1443 mL of distilled water to obtain 40% ethyl alcohol and 391 mL of distilled water to obtain 70% ethyl alcohol.

The extractive alcoholic solutions of concentrations 40%, 70%, and 96% were prepared in triplicate to obtain the amount required to prepare the 12 semi-solid pharmaceutical formulations with topical application.

The structure of the preparations are shown in the following table.

Table 1. *Natural preparations obtained*

Preparation no./composition	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Citric acid	2,6			1			2,3					
Distilled water	10,3	20,5	26,5	21,2	26,8	27,1	18,7	20	28	26,4	27,6	26,8
Alcoholic extract 96% from the stem of the *Vinca minor* plant	16,2	16	24,8	24,8	25	21,3	14,8	15,6	24,3	24,8	17,3	21
Hyaluronic acid	1						1					1,3
Lanolin	62,1	36	37	57	37,5	27,1	56	35	36,3	31,7	44,1	26,8
Ag-Sulfadiazine	2,6		5,3	5,3		6,8	2,7	5	5,2	5,3		6,7
Cetyl alcohol	2,6	5,1	3,7	3,7	10,7	4,1	2,3	3,4	3,1	3,2	11	4
Vegetable collagen	2,6	3				6,8	2,2		3,27			6,7
Zinc oxide		3		5,3		6,8		5				6,7
Vaseline		10,3			25			10				
Shea butter		5,1						5				
Vitamin E		1						1				
Stearic acid			2,7	2,7					3,1	5,33		

MATERIALS AND WORKING METHOD

The method used in the rheological analysis of semi-solid preparations consisted of obtaining the following rheological parameters: obtaining viscosity values (η) at different shear stresses (τ) due to certain shear speeds (D).

The velocity gradients were generated by different rotational speeds (ω) of the R-pivots corresponding to the apparatus used.

Equation of rheological is [13,14,15]:

$$\eta = f(D) \quad (1)$$

$$D = f(\tau) \quad (2)$$

$$D = \omega * R \quad (3)$$

$$\tau = \eta * D \quad (4)$$

The viscosity value corresponding to a given value of the shear rate is called apparent viscosity and can be evaluated with the formula (1), where η represents the viscosity and D represents the shear rate or the velocity gradient measured in s^{-1}.

The graphical realization of equation (1) represents the flow curve.

Equation (2) allows the evaluation of the velocity gradient (shear rate) as a function of the shear stresses, and the graphical representation renders the rheogram of the semi-solid formulations.

The shear rate is determined based on the rotational speeds using equation (3), where, R represents a characteristic of the viscometer pivot used.

The shear stress (τ) is obtained with equation (4) based on the recorded shear rate and viscosity values.

The rheological method makes flow curves and rheograms from viscosity measurements at different shear rates and at different shear stresses.

The shape of the rheograms and flow curves obtained can be used to appreciate the rheological behavior of the pharmaceutical formulations.

About fitting the results into a rheological model, the Ostwald de Waele model is observed (respectively, the power law is followed) which is represented by the equation [16]:

$$\tau = k \, x \, D^n \text{ or } \eta = k \, x \, D^{n-1} \quad (5)$$

Appropriate equipment was used to perform the rheological tests.

A. The Reováscostar;

B. Laboratory utensils,

C. Glassware laboratory,

C. Balance analytical to measurements.

The operational conditions maintained constantly throughout the rheological experiments were:

- Working temperature 25 °C ± 0.1 °C corresponding to the storage temperature of the studied samples;
- Before starting readings on the device, each sample was left at rest in the thermostated vessel to ensure thermal and mechanical balance and the reproducibility of the results. The thermostat time was about 15 minutes before the start of the experiment;

Viscosity (η) is measured in cP, and its values are different depending on the values of the rotation speed ω, measured in rpm. The shear stress (τ) is measured in Pa, and for its calculation, the values of the shear rate (D), measured in s-1, and of the viscosity (equation 4) are needed. All rheological experiments were performed in triplicate.

To confirm the Ostwald de Waele model's rheological parameters, a statistical analysis (the mean values, the standard deviation, and the coefficient of variation CV%) was calculated before the start of the experiment.

The materials used in the rheological study are represented by samples from the 12 new semi-solid preparations using natural extracts from the *Vinca minor L.* plant formulations using the extractive alcoholic solutions 96%, 70%, and 40% obtained from the stem and leaf of the *Vinca minor L.* plant.

RESULTS AND DISCUSSIONS

Calculations of the other parameters were performed in Microsoft Excel 2016 and in Figure 2-13 is presenting linearization of rheological parameters and Figure 14 is presenting preparates.

From the analysis of the data obtained and presented in this table, the following can be highlighted:

- All preparations in the form of H/L or L/H type emulsions: P1-P12 show the linearization of flow curves with excellent correlation coefficients, with values higher than 0.99.
- About fitting the results into a rheological model, it is found that all the preparations P1-P12 in the form of H/L or L/H type emulsions follow the Ostwald de Waele model (concerning the power law), with flow index n<1, in the case of all formulations. Thus, it is confirmed that the preparations show the behavior of pseudoplastic fluids, and the apparent viscosity decreases with increasing shear rates.
- It is observed that the preparations made with vegetable extracts from the stem of the *Vinca minor* plant obtained in low-concentration alcohol presented the lowest values for viscosity.
- It is found that by adding ZnO to the composites with vegetable extracts in alcohol of low concentration (40 %), comparable values were recorded compared to those without ZnO in the composition so that its presence does not influence the rheological parameters.

- The highest values of viscosities were recorded in the case of preparation P3 (30800 cP – 105100 cP), which contains plant extract from the stem of the *Vinca minor* plant made in 70% alcohol, followed by preparation P8 (12800 cP – 35200 cP) which has in its composition vegetable extract obtained from the leaf of the *Vinca minor* plant made in 96% alcohol.
- The lowest values of viscosities were recorded in the case of preparation P5 (609 cP – 45212 cP), which has in its composition plant extract from the stem of the *Vinca minor* plant made in 40% alcohol. Although the shear speeds varied in an extensive range (1.7 s^{-1}- 68 s^{-1}), the smallest range of variation in viscosities is presented by preparation P1 (1493 cP – 17541 cP) obtained from the stem of the *Vinca plant* minor in high concentration alcohol respectively, 96%.
- By comparison, the preparations made with plant extracts obtained from the *Vinca minor* plant record viscosities with high value ranges for all extracts from the leaf, compared to those made from the stem of this plant, a fact that demonstrates differences in the composition of the Vinca alkaloids in the two plant materials studied.

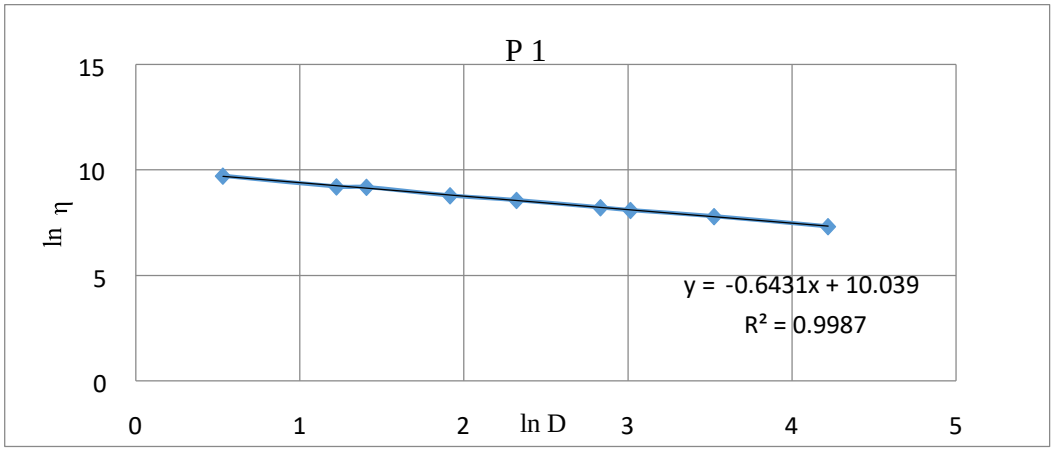

Figure 2. *Linearization of rheological parameters, preparation 1*

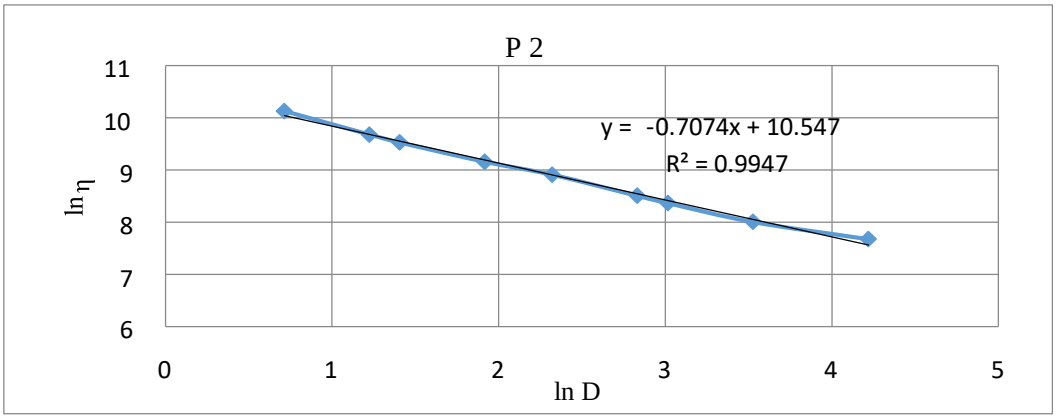

Figure 3. *Linearization of rheological parameters, preparation 2*

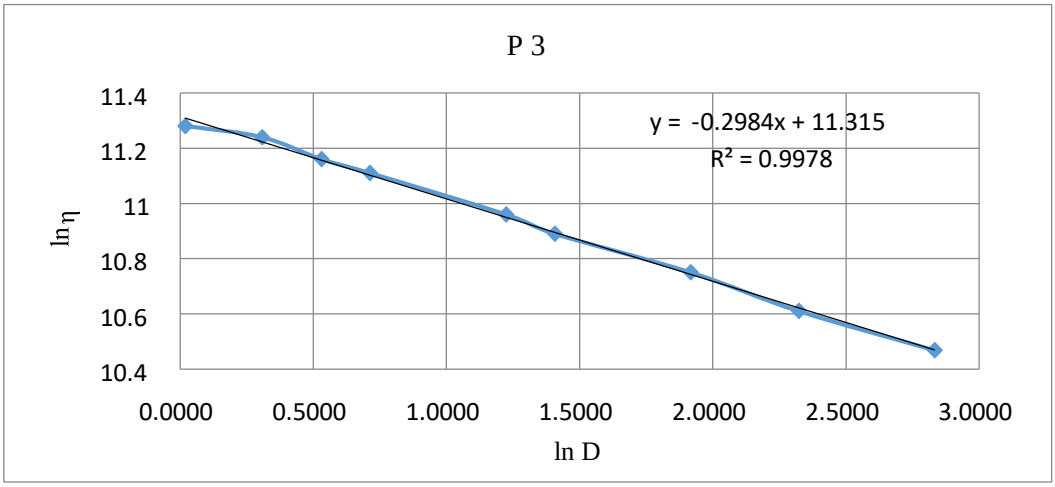

Figure 4. *Linearization of rheological parameters, preparation 3*

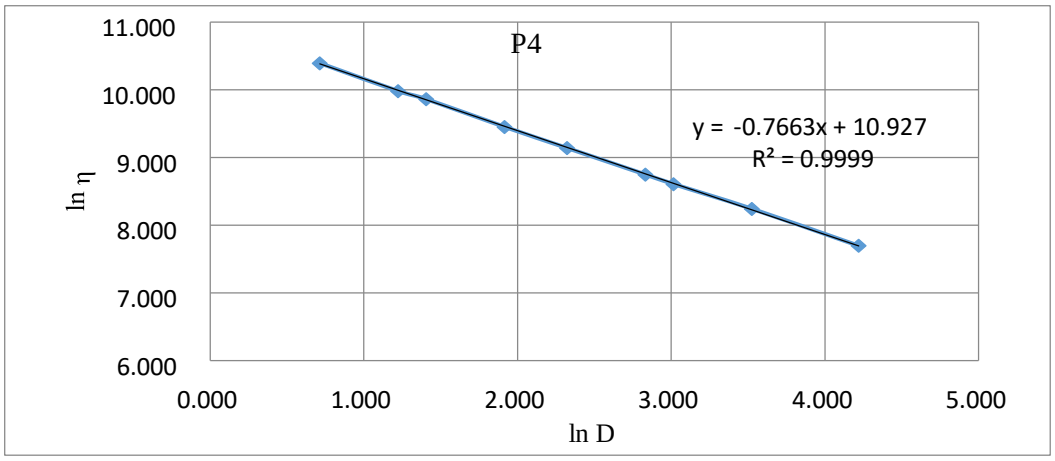

Figure 5. *Linearization of rheological parameters, preparation 4*

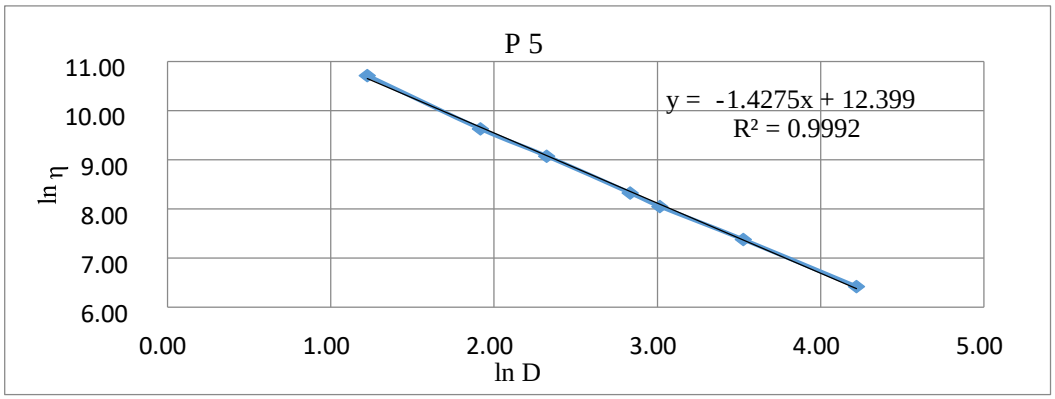

Figure 6. *Linearization of rheological parameters, preparation 5*

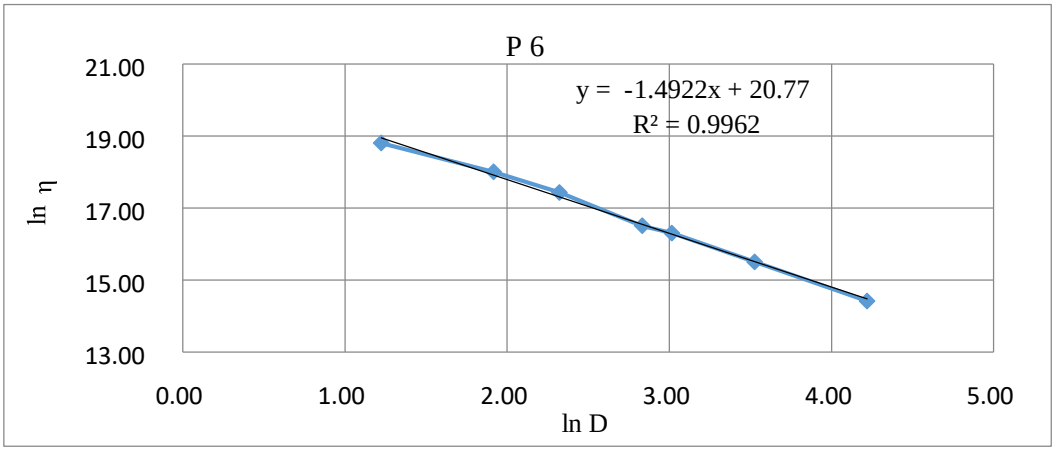

Figure 7. *Linearization of rheological parameters, preparation 6*

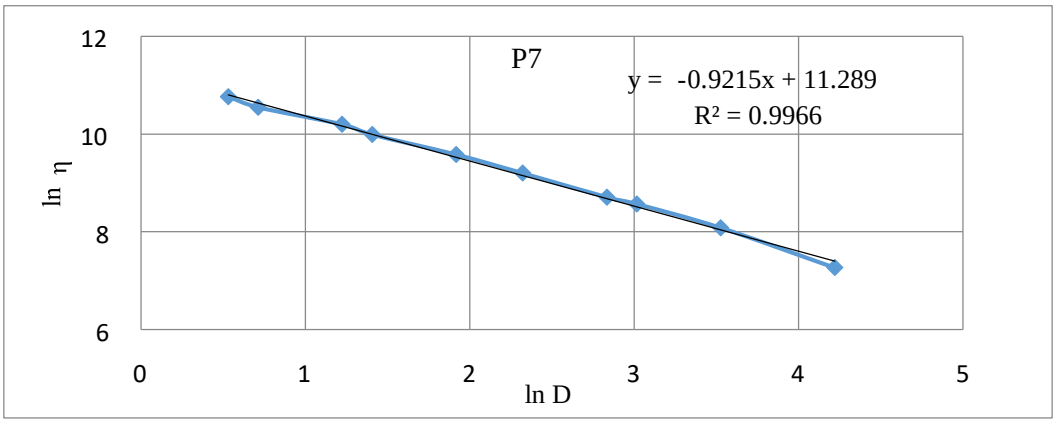

Figure 8. *Linearization of rheological parameters, preparation 7*

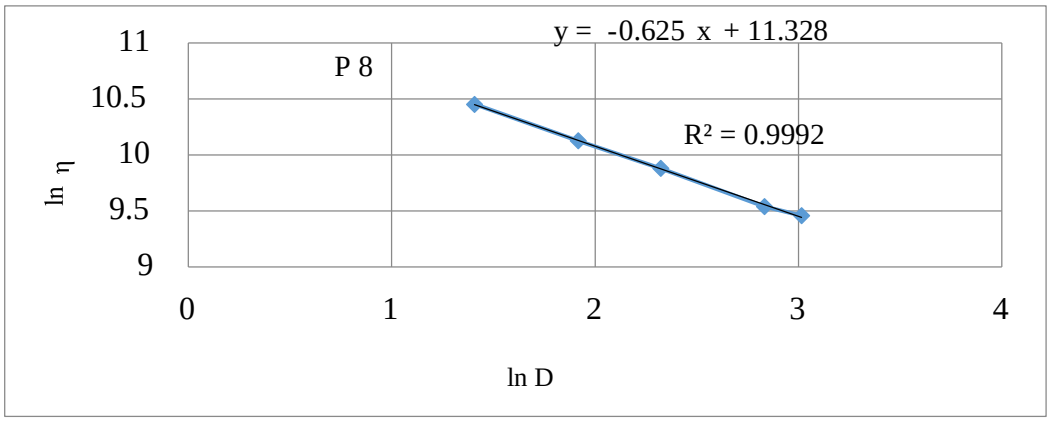

Figure 9. *Linearization of rheological parameters, preparation 8*

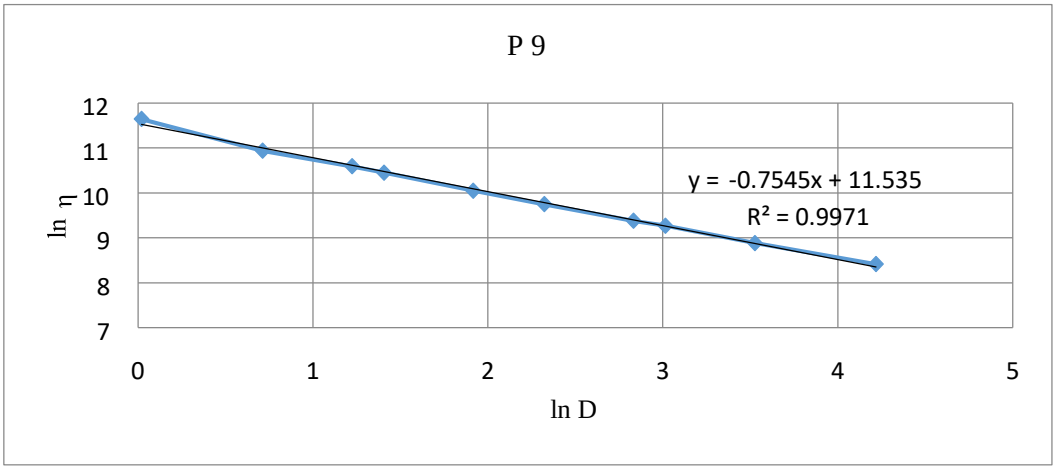

Figure 10. *Linearization of rheological parameters, preparation 9*

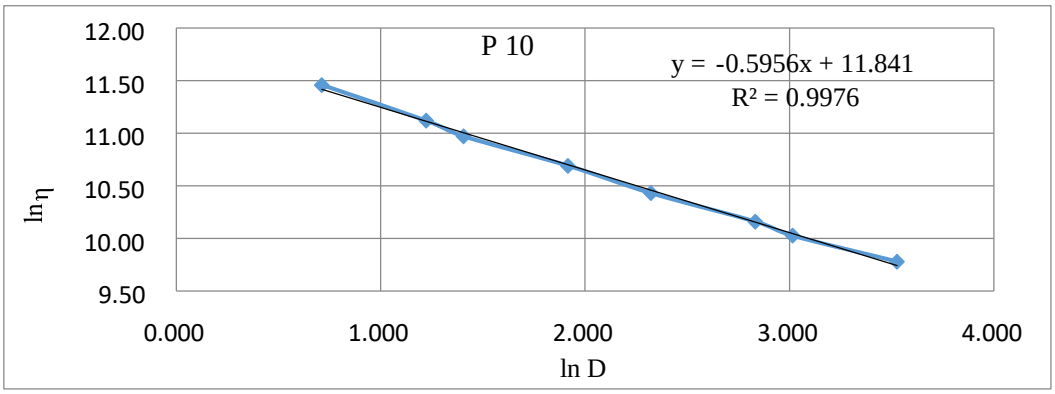

Figure 11. *Linearization of rheological parameters, preparation 10*

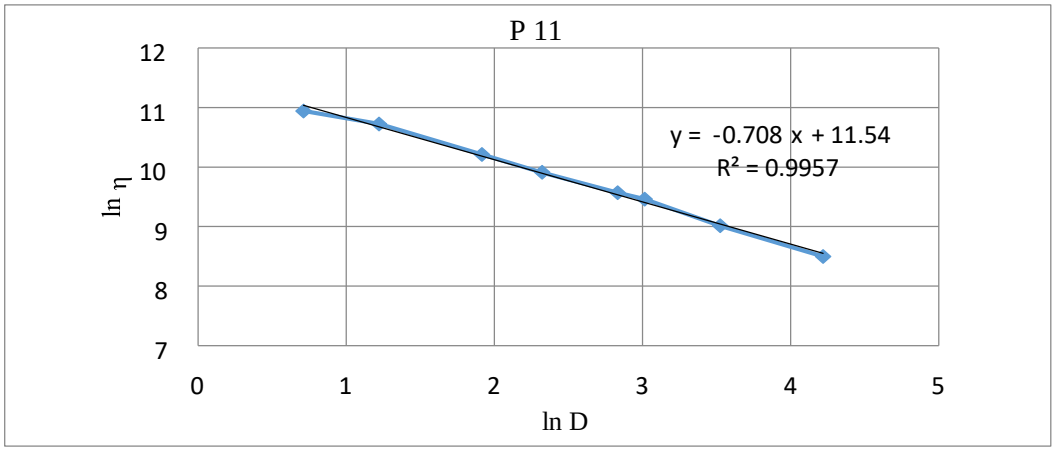

Figure 12. *Linearization of rheological parameters, preparation 11*

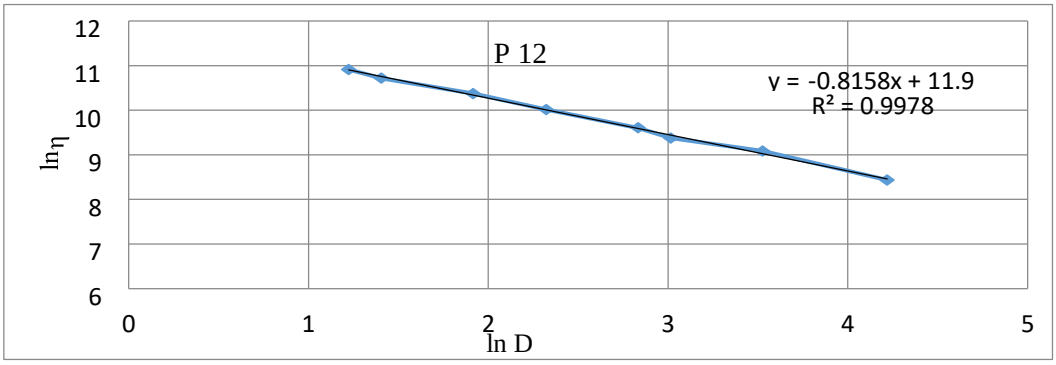

Figure 13. *Linearization of rheological parameters, preparation 12*

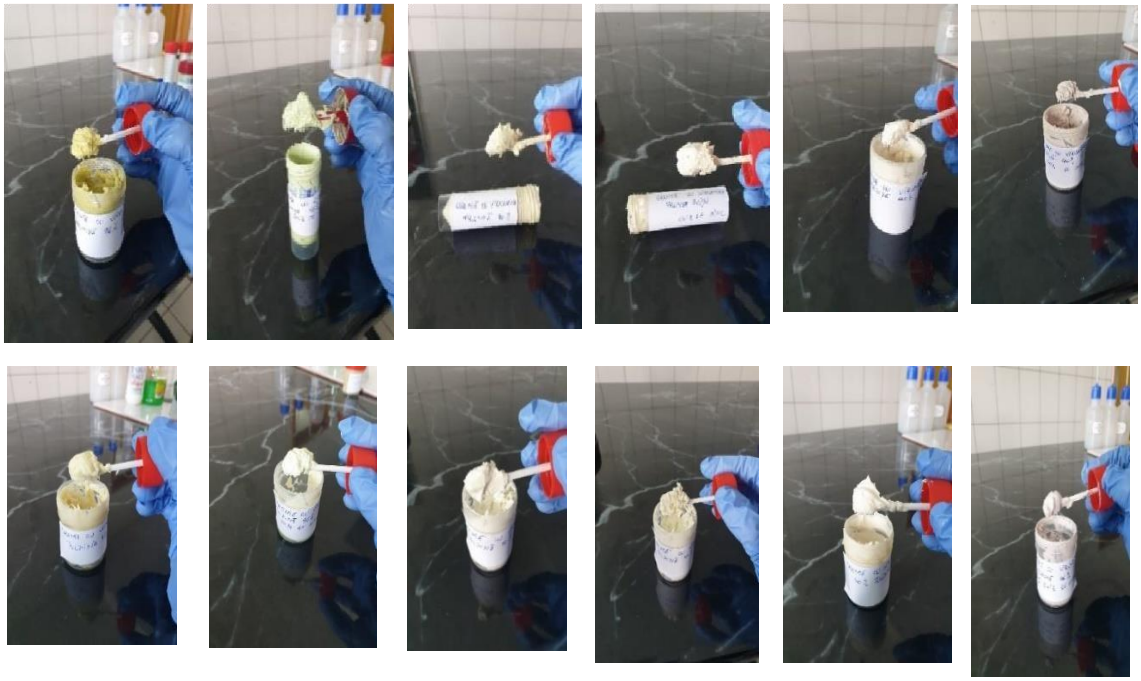

Figure 14. *Preparations prepared for rheological analysis*

CONCLUSIONS

From the analysis of the study carried out on the 12 new pharmaceutical formulations embodied in preparations P1-P12, the following important conclusions can be drawn:

- All the 12 semisolid pharmaceutical formulations studied presented a non-Newtonian, pseudoplastic, thixotropic fluid behavior;
- All preparations P1-P12 in the form of H/L or L/H type emulsions taken in the study, having rheological, pseudoplastic character and flow index n<1, show a decrease in apparent viscosities, with the increase in shear speeds,
- The rheological behavior of the preparations respects the power law given by the Ostwald de Waele model;

- From the analysis of the programs, it was observed that all these preparations made with alcoholic plant extracts taken in the study the shear stress decreased;
- Composite samples P1-P6, obtained from the stem, and P7-P12, obtained from the leaf of the *Vinca minor* plant, were comparatively analyzed.

The following conclusions can be drawn:

- The different compositions in different Vinca alkaloids obtained in alcoholic extracts, leaf versus stem, for all comparative alcohol concentrations.
- Both preparations made with plant extracts from the leaf and those made from the stem of the *Vinca minor* plant obtained low concentrations of alcohol (40%).
- ZnO added to the preparation does not influence the values obtained for the apparent viscosity.
- In the case of preparations with plant extracts made in 70% and 96% alcoholic solutions, it was found that adding ZnO increased the thixotropy of the preparations compared to those that did not have ZnO in their composition.

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