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PRACTICAL INVESTIGATION OF GELS CONTAINING ARISTOLOCHIA (*ARISTOLOCHIA CLEMATITIS*) EXTRACT

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ABSTRACT. The aim of this study is to embed the active ingredients of the hydroalcoholic extract from the aristolochia (*Aristolochia clematitis*) into a new semisolid preparation and a viscoelastic methylcellulose based hydrogel in order to broaden the topical applicability of the palette's range. The methylcellulose based hydroalcoholic gel formulation, preparation and quality evaluation was realized by continuously measuring the product's physico-chemical parameters. The evaluated physico-chemical parameters: the swelling degree, the equilibrium swelling degree, the swelling rate and the swelling fraction, the swelling rate parameter, the swelling kinetics order, the speed constant.

Keywords: *Aristolochia clematitis, hydrogel, methylcellulose, pH, swelling parameters*

INTRODUCTION

Over the last two decades, gels, in general, and hydrogels in particular, have been extensively studied as semi-solid pharmaceutical products, capable of ensuring an efficient delivery of the drug, after its oral, rectal, vaginal, ocular, cutaneous or subcutaneous administration.

Thus, hydrogels have become widely used in the biomedical and pharmaceutical fields, as transportation systems and biomedical devices, due to their biocompatibility, their network structure and due to the molecular stability of the incorporated bioactive compound [1]. Hydrogels can be prepared from a

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wide range of materials, such as semi-synthetic polymers (eg. Cellulose derivatives), whose practical applications are increasing day by day [2, 3]. Due to the high water content, hydrogels can dissolve hydro soluble drugs, resulting in aqueous, transparent gels.

Hydrogels are characterized by the following aspects: they chemically stable in general, but they may dissolve or disintegrate in water. The classic hydrogels are highly valued in dermatology due to their transparency and their high water content which confers them a refreshing sensation when applied, which is sought after in the treatment of some types of acute dermatitis. Hydrogels are physiological, they dry up on the application site and form a protective coating, conferring softness to the skin and they are preferred by patients with seborrheic skin. They are easily removed by washing the area. The pH of the hydrogels can be easily adjusted by adding buffer solutions. A great advantage is that temperature does not influence the consistency of hydrogels, with the exception of the thermo sensitive ones. They are well tolerated by the skin and mucous membranes and also have a cooling effect [4].

The birthwort can be used only externally for various conditions, as a tincture, cream, poultice or sitting baths. The birthwort tincture is prepared from thoroughly crushed or ground leaves, which are mixed with alcohol over 70°; it is then left to soak for two weeks. The resulting tincture may be used for up to two years since the date the plants were harvested on.

The hydro alcoholic extract has antiseptic, healing and detoxifying properties due to chemical composition in bioactive substances [5]. It is recommended in allergies, thrush, angina, bronchitis, an irritable bowel syndrome, hemorrhoids, varicose veins, varicose ulcers, psoriasis, vaginal candidiasis, gonorrhea, uterine fibroids, breast cysts, abscesses, breast infections, tonsillitis, rhinitis, lymphangitis, adnexitis (pelvic inflammatory disease), eczemas, dermatitis, ulcerative infections, wounds, cuts, burns, scalding, skin regeneration, hair loss and cancer diseases [6, 7].

It is used as a compress on the affected areas, thus healing severe, festering wounds, which have withstood other treatments.

RESULTS AND DISCUSSIONS

The Organoleptic Analysis

The organoleptic analysis tells us that the hydrogel is washable, that it has a homogenous, translucent aspect with a smell characteristic to the hydroalcoholic medicinal plant extract. The organoleptic characteristics: aspect, viscosity, aroma and color of the freshly prepared hydrogel were preserved up to 6 months.

The pH variation

The pH variation of the freshly prepared hydrogel, after 3 months and 6 months since its preparation is depicted in Figure 1.

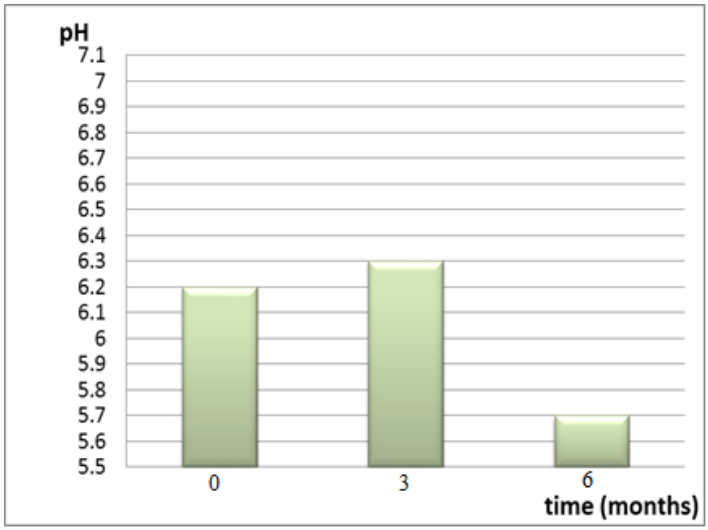


Figure 1. The pH variation depending on the time, at 0, 3 and 6 months

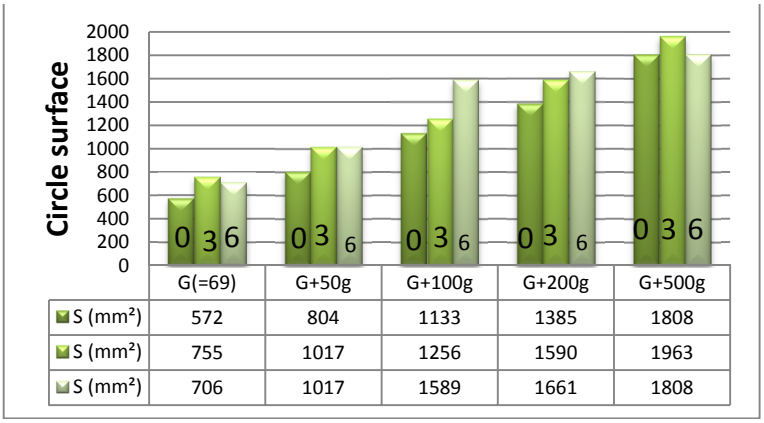
Probably the pH varies because of the partial hydrolysis processes of methyl 4-coumarate, magnoflorine iodide in the presence of aristolochic acid, ferulic and 4-coumaric acids. One should observe that, after a slight increase at 3 months, after 6 months since its preparation, it still is within the RP X (Romanian Pharmacopoeia X) provisions

The spreading capacity

The spreading capacity of an ointment is a very important property since it provides information regarding the ease with which the ointments can be applied on the skin or on the mucous membranes. It is characterized by the force that needs to be applied in order to spread the ointment and it depends on the viscosity and consistency of the semi-solid product.

As shown in Figure 2, even after 6 months, the circle surfaces fall within the accessibility limits regarding the spreading capacity of the semi-solid products, according to the RP X.

One can observe a stabilization of the consistency and of the spreading capacity of the hydrogel containing the hydroalcoholic birthwort extract, after 6 months as well.



	G(=69)	G+50g	G+100g	G+200g	G+500g
S (mm²)	572	804	1133	1385	1808
S (mm²)	755	1017	1256	1590	1963
S (mm²)	706	1017	1589	1661	1808

Figure 2. Determining the spreading capacity of the hydrogel containing the hydroalcoholic birthwort extract, via the Ojeda-Arbussa method

Determining the physico-chemical swelling parameters of the hydrogel

The degree of swelling (Q) represents the amount of liquid absorbed by the mass unit of the hydrogel:

$$Q = \frac{m_t - m_0}{m_0} \quad (1)$$

where m_0 is the mass of dry hydrogel and m_t is the mass of swollen hydrogel at the time *t*. Table 1 shows the data on the degree of swelling of the *Aristolochia clematitis* extract hydrogel.

Table 1. The degree of swelling of the *Aristolochia clematitis* extract hydrogel

Hydrogel mass (g)	Time (min.)	Degree of swelling Q (%)
m_0=1.3344	0	-
m_1=3.0894	30	131.51
m_2=3.6317	60	172.15
m_3=3.8805	90	190.80
m_4=3.8207	120	186.32
m_5=3.8947	150	191.86
m_6=3.8621	180	189.42
m_7=3.8561	210	188.97
m_8=3.5456	240	165.70 Obs.: hydrolyses

Figure 3 shows the variation of the hydrogel's swelling degree over time.

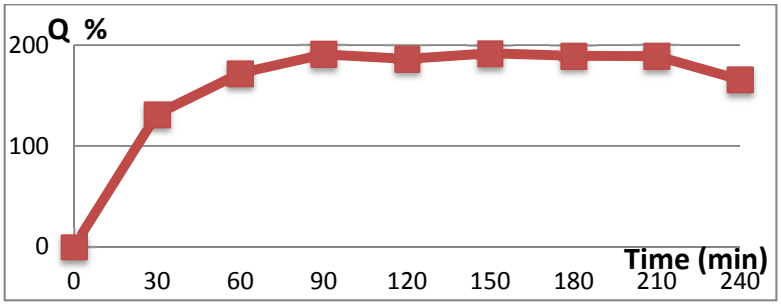


Figure 3. The variation of the hydrogel's swelling degree over time

The equilibrium water content (EWC%) [9] is calculated using the formula (2):

$$Q_e\% = \frac{m_{tf} - m_0}{m_{tf}} \cdot 100 \quad (2)$$

where m_{tf} is the mass of the swollen hydrogel in equilibrium (in the end) and m_0 is the mass of the dry hydrogel and is 189,47%.

The variation of the swelling content, per time unit, of the hydrogel is known as **swelling rate**, $\mathbf{Q_R}$ [10] and is calculated using the formula (3):

$$Q_R = \frac{Q_{t+\Delta t} - Q_t}{\Delta t} \quad (3)$$

where Q_t is the swelling degree at any given time and $Q_{t+\Delta t}$ is the swelling degree based on the dry content at $t+\Delta t$. For Δt (min) = 30 it is 0,0103 and for Δt (min) = 60 its value is 0,0015.

Based on the Davidson – Peppas model [14] the swelling of the hydrogels can be characterized by the **F swelling fraction** equation (4)

$$F = \frac{Q_t}{Q_e} = kt^n \quad (4)$$

where F is the swelling fraction, Q_e is the equilibrium swelling degree, n is the solvent's diffusion exponential and k is the constant that changes depending on the structure of the gel's network [10,13]. In order to determine the type of diffusion, one must know the value of n. The diffusion's exponential can be determined from the slope of the line obtained from the graphic representation

of the lnF, depending on the lnt; one should take into consideration the data from the graph in the region where the swelling has not reached equilibrium, where just 60% of the solvent's mass has permeated the gel's structure. The n, k values are calculated using the slope of the resulting line. If the value of n is below 0,5, we are talking about a Fickian diffusion; anything above 0,5 implies a non-Fickian diffusion of the water. **Figure 4** represents the lnF variation, depending on the lnt, only for 60% of the solvent's mass which permeated the gel's structure.

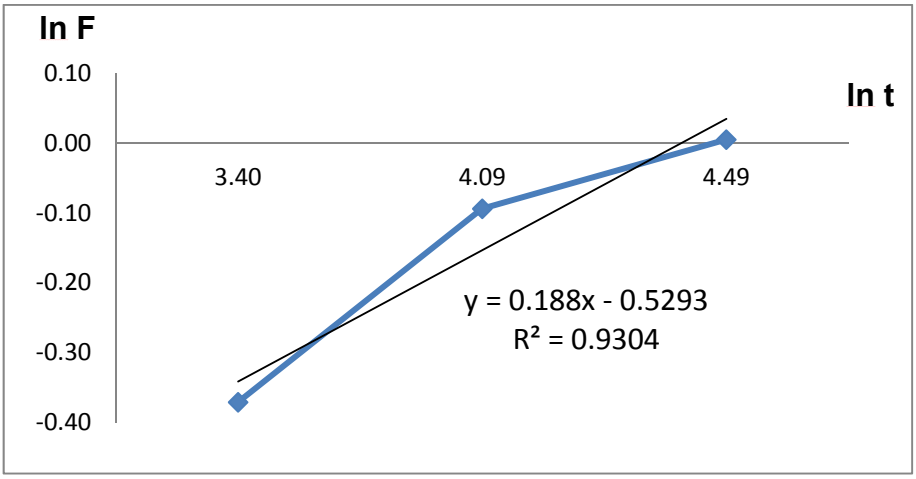


Figure 4. The lnF variation depending on the lnt for 60% of the solvent's mass which permeated the gel's structure.

Calculations showed *n=0,33* and the diffusion constant (the front factor) *k=1,65* (min^{-n}).

In order to study the swelling kinetics of hydrogels, one must apply the Voigt-Kelvin viscoelastic kinetic model [11], having the following equation:

$$Q_t = Q_e(1 - e^{-\frac{t}{\tau}}) \quad (5)$$

where Q_t is the swelling degree for the t period, Q_e is the equilibrium swelling degree and ***τ*** is the **swelling rate parameter (time retardation).** The τ value is the measure of the swelling rate in the sense that: the lower the τ value is, the higher the swelling rate will be. In order to calculate the parameter of the swelling rate τ, by the linearization of the equation (5) $\ln(1-\frac{Q_t}{Q_e})$ one must graphically represent it, depending on the time t. Using the slope of the line

(slope = -1/τ) we can calculate the τ swelling rate parameter. The swelling rate represents the time necessary to obtain a swelling degree equal to 0,6321 of the equilibrium swelling degree's value. Figure 5 shows the evaluation of the swelling rate.

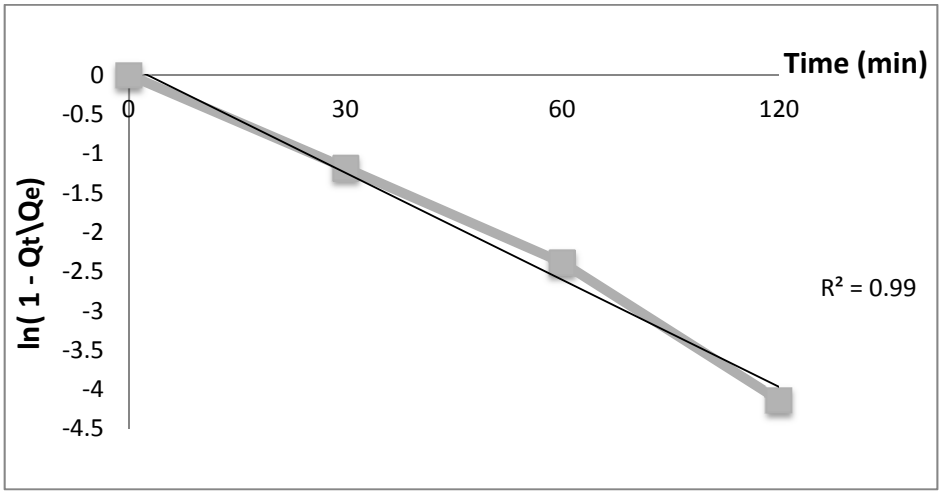


Figure 5. The evaluation of the swelling rate.

After the calculations, τ = 26,5 min.

In order to determine the order of the swelling kinetics, we must resort to the procedure of Quintana & al. [11,12], regarding the model – dependency approach. For a **level one kinetics**, the differential of the swelling speed is as equation (6):

$$\frac{dQ}{dt} = K(Q_e - Q) \tag{6}$$

where Q is the swelling degree at the time t. By integrating the (6) equation, we have the formula (7):

$$\ln\left(\frac{Q_e}{Q-Q_e}\right) = K \cdot t \tag{7}$$

If the swelling occurs via a level one kinetics, through a graphical representation of $\ln\left(\frac{Q_e}{Q-Q_e}\right)$ depending on the time, **Figure 6**, we need to analyze the value of the regression coefficient R, which should be close to 1;

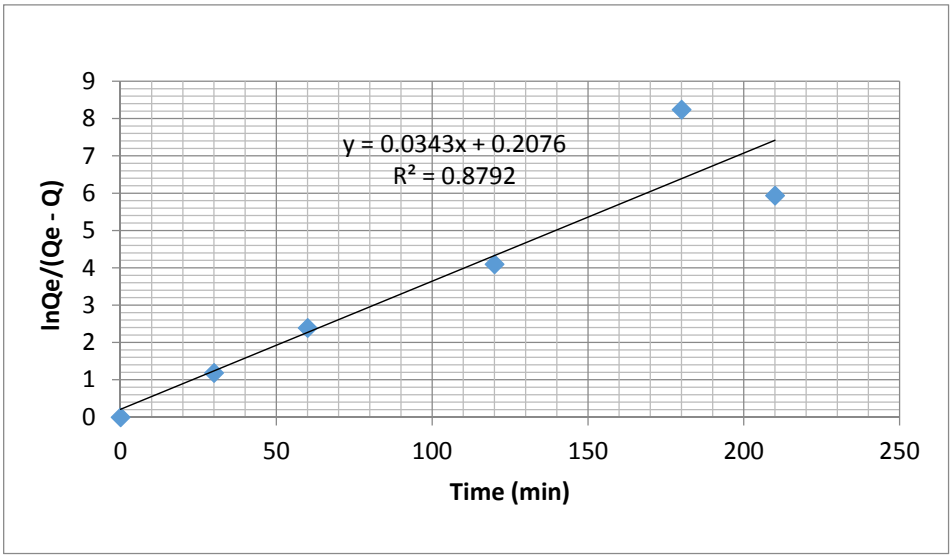


Figure 6. Graphical representation of the $\ln\left(\frac{Q_e}{Q-Q_e}\right)$ variation, depending on the time

otherwise, the kinetics of the swelling is not a level one. A level two swelling kinetics has the differential equation (8)

$$\frac{dQ}{dt} = K(Q_e - Q)^2 \tag{8}$$

After the integration and linearization of the equation, we obtain the following:

$$\frac{1}{Q} = \frac{1}{K \cdot Q_e^2} + \frac{1}{Q_e} \cdot t \tag{9}$$

If, for the graphic representation of 1/Q, depending on the time t, the linear regression coefficient is close to 1 more than in the case of a level one kinetics, the swelling kinetics is a level two type. Figure 7 shows the variation of the reciprocal value of the swelling degree, depending on the time. The graph also allows us to determine the speed constant.

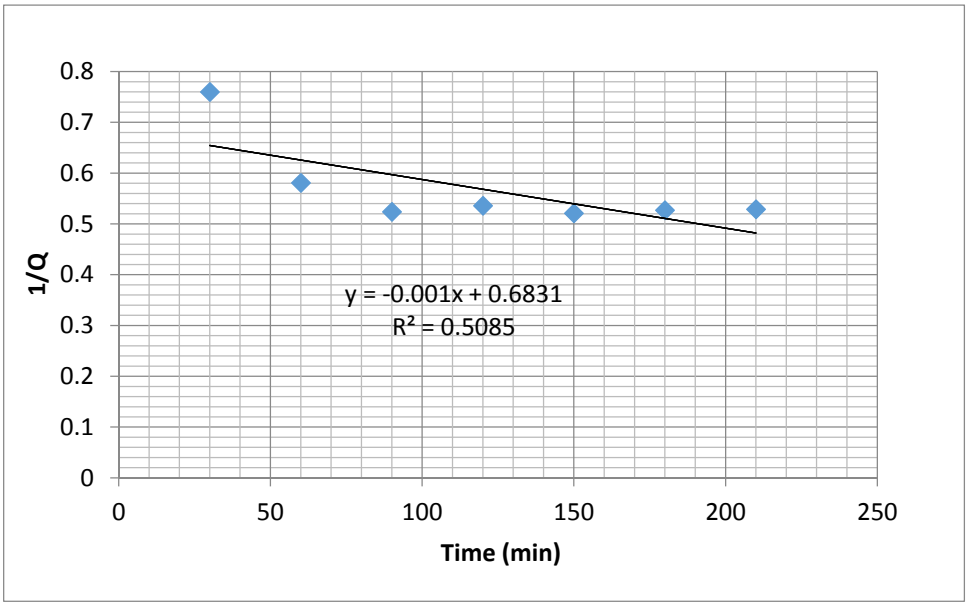


Figure 7. The variation of the reciprocal value of the swelling degree, depending on the time.

Due to the fact that, in the case of this model, R has a value less close to 1 when compared to the level I kinetics model, the swelling of the birthwort extract hydrogel follows a level I kinetics, with a speed constant of $K = 3{,}33 * 10^{-2}\ min^{-1}$. This model can be found among other swelling kinetics models [17], therefore suggesting the complexity of this process's kinetics.

CONCLUSIONS

The hydroalcoholic birthwort extract, turned into gel with methylcellulose, forms a stable product, with an optimal release of the active ingredient.

After a slight acidification process, the pH changes remain within the scope of the safety requirement's applicability.

The Ojeda – Arbussa diagram indicates a softening of the product, over time, which becomes stable after 3 and 6 months.

The evaluated physico-chemical parameters: the swelling degree, the equilibrium swelling degree, the swelling rate and the swelling fraction, the swelling rate parameter, the swelling kinetics order, the speed constant; all these demonstrate the presence of a physical hydrogel structure.

EXPERIMENTAL SECTION

The preparation of the hydroalcoholic Aristolochia clematitis extract hydrogel, for one hundred grams of product, was done as follows: for 50 g of distilled water – vehicle, heated to 70^0 C, 5 g of methylcellulose with DS = 1,5 – 1,9 were added (Parma Produkt Srl. 1145 Budapest, Hu) as a viscosity-increasing agent under continuous stirring. In order to cool down the solution, we added 10 g of glycerol (SC Stera Chemicals Srl, Jilava, Ro) as a humectation agent [16] and 10 g of 96^0 ethyl alcohol (SC Medchim TM Srl, Bucureşti, Ro) as a dispersing agent [8]. The remaining 15 g of distilled water are to be added and one should wait until the system takes a gel-like form, which is when the 10 g of birthwort tincture need to be added (Aroma Plant – Ion Bonchiş, Bunteşti nr. 83, Bihor, Ro) 27% (V/V) as a therapeutic agent.

The organoleptic examination ensures that the most important characteristics of the hydrogel are determined, such as its aspect, consistency, its smell and its color [8].

The determination of the pH was done with a potentiometrically, according to the RP X [18]. provisions, by using a portable, digital pH-meter of the *pHep*®+ *by Hanna pH* type. The measurements were performed on samples taken from the freshly prepared hydrogel, after 3 and 6 months since its preparation.

In order to determine the spreading capacity, the Ojeda – Arbussa method was used. The method is based on the principle of measuring the spreading surface of an ointment, under the action of a determined force. The measurement is made with a common device known as an extensometer, suggested by A. Pozo-Ojeda and J.M. Sune-Arbussa. The device consists of two glass plates, with sides of 11 cm. The external part of the lower plate is covered in scale paper, with 5 concentric circles drawn on it. Starting with the first circumference, the perpendicular diameters are graduated in mm, which intersect in the center, where the first circle has been drawn, with a 1 cm diameter. On the lower plate, in the center of the first circle, 1 g of ointment is placed, over which the second plate is placed. The diameter of the circle created by the ointment is noted, a circle which resulted after the ointment was applied pressure with a glass plate weighing 69 g. After 1 minute, on the top plate of the extensometer, weights are added, in an ascending order – 50, 100, 200 and 500 g. The radiuses of the circles created by the investigated sample are read and they are noted down with r_1, r_2, r_3 etc. Afterwards, the surfaces are calculated, which are noted with S_1, S_2, S_3 etc.

In order to determine the main physico-chemical swelling parameters of this hydrogel, after its preparation it is dried to a constant weight, at a temperature of 50^0 C, after which a cylindrical shaped piece is cut out [15],

which has m_0 = 1.3344 g. This sample is immersed by means of a plastic bag in a glass containing 500 ml of distilled water and it is removed from said glass at intervals of 30 minutes; the excess water is removed from the surface of the hydrogel in the swollen phase, after which the mass is weighed with the analytical scale. This procedure is repeated until the hydrogel starts disintegrating, which is signaled by the loss in mass, after a long interval in which the mass was constant. Table 2 represents the starting values for the evaluation of the other physico-chemical swelling parameters. All the measurements of the physico-chemical parameters derived from the swelling degree were made on the hydrogel, after six months since its preparation.

Average values were reported for at least three measurements made for each sample, separately.

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