THE EFFECT OF CHITOSAN ON THE PHYSICOCHEMICAL PROPERTIES OF DERMATOLOGICAL PREPARATIONS CONTAINING *HUMULUS LUPULUS* L. EXTRACT BASED ON CELUGEL

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Abstract

This study evaluated hydrogels containing Celugel, 3% hop cone extract obtained from Humulus lupulus L. using supercritical carbon dioxide extraction substrate, and 1% and 2% chitosan for dermatological application to treat inflammatory skin conditions. The presence of chitosan significantly affected the rheological properties of the formulations, including their dynamic viscosity, hardness, consistency, cohesiveness, and blurring time. The formulations containing 2% chitosan showed the best application possibilities. The formulations were evaluated for dissolution of cohumulone, which is an analogue of humulone contained in hop cone extract. The concentration of chitosan in the formulations had a significant effect on the dissolution testing parameters of the active ingredient: as the chitosan concentration increased, the desired effect of prolonged release time of the active ingredient was achieved while maintaining the membrane-forming properties of Celugel.

Keywords*: chitosan, Celugel, hop extract, Humulus lupulus, Supercritical CO_2 extraction, carrier*

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1. Introduction

Traditional herbal medicines have been widely researched as alternative treatments for many diseases to minimise potential adverse effects and antibiotic resistance. Evaluation of hop (*Humulus lupulus* L.) extract has revealed efficacy for acne as well as other dermatological conditions [1]. Hop cone extract has high antibacterial, antioxidant, anti-collagenase, anti-inflammatory, and even antifungal activity thanks to active ingredients such as humulones, lupulones, isohumulones, xanthohumols, and polyphenols, among others.

Dermatological application of hop extract requires a suitable hydrophilic carrier as an alternative to absorbent, lipophilic substrates so that the affected skin is not further lubricated with an oily formulation [2]. The carrier should be miscible with the hop extract and have appropriate physicochemical properties to allow the most favourable bioavailability of the hop extract from the semi-solid drug formulation. Celugel (hydroxyethylcellulose gel) was used in the study. This gel has the aforementioned properties and is recommended to treat exudative lesions, burns, acne, and oily skin disorders. It can be applied to hairy skin and mucous membranes, among other areas. When applied to the skin, Celugel has cooling and protective properties [3].

As the hop extract is quite fluid, I decided to improve its texture properties by adding chitosan to the formulations. Chitosan is distinguished by its biocompatibility, biodegradability and non-toxicity, and exhibits – depending on the degree of deacetylation (DDA) – antimicrobial, antioxidant, immunostimulant, and antifungal activity, which may also lead to synergistic effects in the treatment of dermatological conditions [4, 5]. I performed this study to determine the optimal qualitative and quantitative composition of a hydrogel to treat inflammatory skin conditions. I obtained hop extract from *H. lupulus L.* using supercritical carbon dioxide (CO_2) extraction and introduced it as an active ingredient into Celugel with and without the addition of chitosan [6]. I also evaluated the physicochemical properties and pharmaceutical availability of cohumulone, an analogue of humulone present in hop cone extract, to identify the formulation with the most favourable parameters.

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: chitosan high molecular weight (CHIT) with a DDA of 75% and a viscosity 800–2000 cP; extract of the Polish Marynka hop variety (Hopesteiner, Germany); Celugel (batch no 041120, Actifarm, Poland); glacial acetic acid (FCC, J.T. Baker, USA); and aqua purificata as required by the Polish Pharmacopoeia XII.

2.2. Methods

2.2.1. Preparation of Dermatological Formulations

Dermatological formulations containing 3% hop extract were prepared by mixing the extract with Celugel using a Gako Unguator e/s (Germany). For this purpose, 97 g of Celugel was weighed into the unguator container, and 3 g of hop extract was added to the top of the substrate. The components were stirred for 2 min at 900 rpm. The remaining 3 g of substrate was then added and stirred by gradually increasing the speed every 30 s to 1630 rpm. The time needed to reach the highest speed was 13 min. The formulation was stirred for a total of 15 min. After mixing, the formulations were deaerated by sonication for 2 h and stored at 6–8°C. In addition, Celugel formulations were prepared with 1% and 2% chitosan. Table 1 shows the Celugel composition and Table 2 shows the components of each prepared formulation.

Table 1. The celugel composition.

Substance	Quantity	Properties and applications
Hydroxyethylcellulose (HEC 10 000)	Proprietary information	Gelling agent, non–ionic
Water	≥ 80%	Solvent
Glycerol	Proprietary information	Prevents immediate drying Plasticiser – eliminates differences in elasticity
Sorbic acid	Approximately 0.1%	Preservative
Potassium sorbate	Approximately 0.1%	Preservative

Table 2. Composition of the prepared formulations.

Formulation	Celugel content [%]	Hop extract content [%]	Chitosan content [%]
Celugel + chitosan 1%	99	0	1
Celugel + chitosan 2%	98	0	2
Celugel + hop extract	97	3	0
Celugel + chitosan 1% + hop extract	96	3	1
Celugel + chitosan 2% + hop extract	95	3	2

2.2.2. pH of the Prepared Formulations

The pH of the prepared formulations was measured using a CP-505 pH-meter with an EPS-1 electrode (Elmetron) adapted to determine the pH in semi-solids. The substrate and formulation were measured in triplicate at room temperature (21.0°C ± 1°C). The results are shown in Table 3.

2.2.3. Blurring Time Test

The blurring time was estimated by adding 1 ml of gel to 30 ml of distilled water in a beaker and shaking the components at 37°C and 100 shakes per minute in a thermostatic shaker (WB22, Memmert, Germany). The results are shown in Table 4.

2.2.4. Consistency Test

The consistency test was performed using a cone penetrometer at room temperature (21 ± 1°C) according to Polish Pharmacopoeia XII guidelines. The samples were placed in a container measuring 75 mm in diameter and 62 mm in height. The penetrating element was released for 5 s and the penetration depth was read. The results are shown in Table 4.

2.2.5. Dynamic Viscosity Test

Rheological tests were performed using a RVDV-III+CP cone/plate rheometer (Brookfield, USA) with a CP51 cone (angle 1.565°, radius 1.2 cm); the temperature was controlled using a Brookfield AP7LR-20 thermostat. Gel samples (0.5 ml) were applied with sterile Becton Dickinson Discardit II syringes (2 ml). The apparent viscosity (V) and shear rate dependence (SR) were measured at 37 ± 0.1°C. Based on this, hysteresis loops were determined for each formulation – the main shear started at the shear rate of 0.384 s^{-1} up to a maximum shear rate of 21.12 s^{-1}; the rotational speed (RPM) then decreased at the

same interval to the initial value. This starting point at the lowest rotational speed and the point at the highest speed were used for statistical analysis. The shear rate was calculated based on the spindle speed using Equation 1:

$$\text{Shear rate} = \text{RC} \times \text{RPM}, \tag{1}$$

where the shear rate constant (RC) for the CP51 cone is 3.84. Three trials were performed for each formulation. The results are shown in Table 4.

2.2.6. Hydrogel Carrier Texture Test

The texture of the prepared formulations were assessed with the TA.XT Plus Texture Analyser, using the Texture Exponent 32 software. The following gel texture parameters were examined: hardness, consistency, and back extrusion, which involves the process of retracting extrusion. The texture analysis software setting were speed, 1.5 mm/s; distance, 10 mm; pressure force, 5.0 g; and disc diameter, 40 mm. The measurements were performed three times at room temperature (22 ± 1°C). The results are shown in Table 5.

2.2.7. Spreading Test

The spreading test was performed using an extensometer to check the spreadability of the prepared formulations. A 0.5–ml sample was applied to the base of the apparatus with a syringe. The top plate (mass = 126 g) was lowered and the radius of the surface occupied by the substrate was measured after 1 min. The top plate was then loaded with 150 g weights every 1 min, up to a total weight 1050 g (1176 g when including the top plate). The average radius was calculated, and then the surface area of the spread preparation was calculated using Equation 2:

$$P_{wheel} = \pi r^2, \tag{2}$$

where:

P_{wheel} – area of the wheel field [cm^2];

r – radius of the wheel [cm].

Each formulation was tested three times. The test was performed at a room temperature of 21.0°C ± 1°C. The results are shown in Table 5.

2.2.8. Diffusion of Cohumulone from the Prepared Formulations

The diffusion rate of cohumulone from the formulations was evaluated using the membrane method in a Varian VK7025 paddle apparatus according to the Polish Pharmacopoeia XII. The gel was inserted into a fixed-volume dialysis container, which was placed in a thermostated vessel containing 400 ml of methanol as the acceptor fluid. The solution was then stirred with a paddle stirrer at 100 rpm. The rate of release was assessed by measuring the amount of diffusing extract per cohumulone into the acceptor fluid. At 1, 3, 6, and 24 h after the start of the test, a 1-ml sample of the solution was removed and replaced with 1 ml of methanol. The collected samples were filtered using filters with a 10 μm pore size. Six trials were performed for each prepared formulation.

The collected samples were analysed with high-performance liquid chromatography (HPLC). The prepared samples were applied to a 15-cm-long Phenomenex column with a diameter of 4.6 mm and a bed grain size of 5 μm. The flow through the column was at a rate of 0.8 ml/min, on a Thermo Scientific Dionex UltiMate 3000 apparatus. The assay was performed in an isocratic system, in the presence of an eluent of 85% methanol in 0.1% formic acid. The measurements were made at a wavelength of 256 nm. The resulting chromatogram was analysed using Dionex CHROMELEON 7 software. The

drug concentration in the samples and the mean percentage of dissolved cohumulone were calculated using a linear regression equation for the drug: y = 4.7244x – 5.9931. Figure 1 shows the drug dose released versus time and Figure 2 shows a chromatogram of the hop extract.

3. Results and Discussion

3.1. pH of the Prepared Formulations

The hop extract had a pH of 2.65 (Table 3). The addition of the extract to Celugel lowered the pH from 4.98 to 4.25. The highest pH among the analysed formulations was Celugel with 2% chitosan, with a pH of 7.00. The addition of hop extract to Celugel and chitosan reduced the pH to 5.67 (1% chitosan) and 5.83 (2% chitosan). The physiological pH of the skin ranges from 4.1 to 5.8. In view of the above analysis, the combination of Celugel, chitosan, and hop extract produces an acidic product that is close to the skin's pH. Hence, these formulations could have beneficial dermatological properties.

Table 3. The pH of the prepared formulations.

Formulation	Sample number			Avg. pH	Standard deviation
	First	Second	Third		
Hop extract	2.62	2.67	2.65	2.65	0.025
Celugel	5.02	4.96	4.95	4.98	0.038
Celugel + hop extract	4.32	4.23	4.19	4.25	0.067
Celugel + chitosan 1%	6.94	6.91	6.91	6.92	0.017
Celugel + chitosan 2%	7.02	6.98	7.01	7.00	0.032
Celugel + chitosan 1% + hop extract	5.66	5.67	5.68	5.67	0.010
Celugel + chitosan 2% + hop extract	5.81	5.72	5.98	5.83	0.029

3.2. The Influence of Hop Extract and Chitosan on the Viscosity, Consistency, and Blurring Time of Celugel Formulations

The average blurring time of Celugel with hop extract was 22 min (Table 4). The addition of 1% chitosan increased the blurring time to 110 min; 2% chitosan produced an even longer blurring time of 160 min (Table 4). The Celugel formulation containing 2% chitosan and hop extract had the highest viscosity, while the Celugel formulation with only hop extract showed the lowest shear stress and viscosity among the tested samples (Table 4).

Table 4. The shear stress, viscosity, blurring time and consistency of the prepared formulations at 37°C.

Formulation	Shear stress [N/m^2] at 21.12 [s^{-1}]	Average Viscosity [mPa·s]	Blurring time [min] ± SD	Penetration depth [mm^{-1}]
Celugel + hop extract	940.49	4453.08	22 ± 3.04	314.0
Celugel + chitosan 1% + hop extract	1762.34	8344.42	110 ± 2.12	303.0
Celugel + chitosan 2% + hop extract	2627.12	12350.46	160 ± 4.11	291.0

The blurring time and viscosity of the gels are closely related parameters. As viscosity increases, the blurring time of a hydrogel increases. If the viscosity decreases under shear stress, the ointment can spread on the skin while the return to baseline prevents the ointment from running off the skin. Based on the results, Celugel formulations with chitosan show the best application possibilities. This view is also confirmed by the results of the consistency test. In the case of Celugel with hop extract, which has a semi-fluid consistency, and the cone penetration depth was 314.0 mm. The addition of 1% and 2% chitosan resulted in a noticeable decrease in the cone penetration depth, specifically 303.0 and 291.0 mm, respectively. These results demonstrate an improvement in application consistency.

3.3. The Effects of Chitosan on the Texture Parameters of the Prepared Formulations

The texture parameters of a gel allow assessing its applicability. The high cohesiveness of the carrier indicates its resistance to damage, while hardness and consistency reflect the ease of application of the drug formulation. Based on the results of tests using a texturometer (Table 5), the tested parameters increased as the chitosan content in the gel increased. The formulation with the best texture parameters – hardness, cohesiveness, and consistency – contained Celugel, 2% chitosan, and hop extract. The texture parameters were reduced in the formulation with 1% chitosan. The Celugel formulation with hop extract but without chitosan showed the best spreadability (9.26 mm). This parameter is related to the consistency of the gel and is indicative of its application possibilities. This formulation covered a large area, spread easily, and formed a film on the surface. The presence of chitosan reduced the spreading of the hydrogel as the concentration increased.

The choice of substrate should depend on how it will be applied to the affected skin, namely spot application or application to an extensive area. Celugel did not bind easily to hop extract, perhaps due to the hydrophilicity of Celugel and the hydrophobicity of the hop extract. The addition of chitosan significantly improved the consistency of the formulation, improved the application properties and rheological properties, and prolonged the release while maintaining the film-forming characteristics of Celugel.

Table 5. The texture parameters of the prepared formulations.

Formulation	Hardness [g]	Consistency [g·s]	Cohesiveness [g]	Viscosity index [g·s]	Wheel field [cm^2]
Celugel + hop extract	32.497	134.743	–16.924	–43.046	9.26
Celugel + chitosan 1% + hop extract	38.185	159.308	–20.452	–56.770	8.99
Celugel + chitosan 2% + hop extract	43.872	183.873	–23.862	–69.125	8.64

3.4. Diffusion of Cohumulone from the Prepared Formulations

The release curves of cohumulone, as an active ingredient, from the prepared formulations are shown in Figure 1, while the pharmacokinetic parameters of the release profiles are shown in Table 6. The release of cohumulone depended on the chitosan concentration and decreased as the polymer content in the carrier increased.

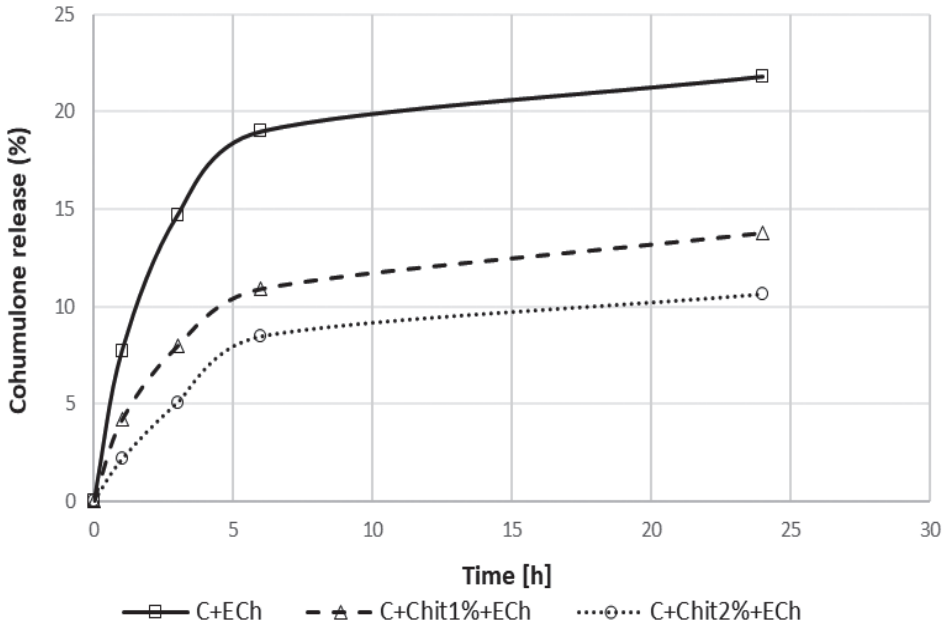

Figure 1. Cohumulone release from the prepared formulations over time. C, Celugel; Chit, chitosan; ECh, hop extract.

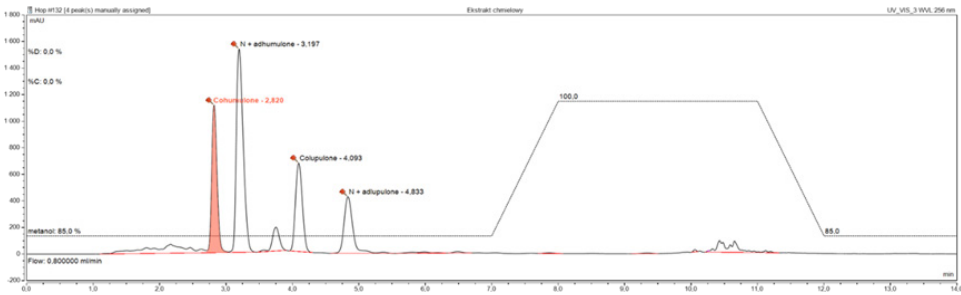

Figure 2. Chromatogram of the hop extract [7, 8].

The cohumulone release curves revealed that after 24 h, the mean percentage of cohumulone release was highest from the Celugel formulation with hop extract (21.84%), with a release rate constant of 0.007247 h^{-1}. The mean percentage of cohumulone release from the Celugel formulation with 1% chitosan and hop extract was significantly decreased at 13.81%, with a release rate constant of 0.005927 h^{-1}. The Celugel formulation with 2% chitosan and hop extract had the lowest mean percentage of medicinal substance released (10.65%), with a release rate constant of 0.004610 h^{-1}. The addition of chitosan notably decreased the percentage of cohumulone release and the rate constant. The release process is slower, which may help to prolong release when it is necessary to use Celugel to apply the hop extract.

The cohumulone release results correlate with the blurring test results (Table 4): the faster the blurring time, the better the cohumulone release. It will be so important to conduct tests with other acceptor fluids and to confirm the results *in vivo*.

Table 6. Pharmacokinetic parameters of hop extract release in a semi-logarithmic system.

Formulation	Equation describing the kinetics of the release profile	R^2	Release rate constant K [h^{-1}]
Celugel + hop extract	y = 0.007247x + 3.2128	0.9716	0.007247
Celugel + chitosan 1% + hop extract	y = 0.005927x + 4.0008	0.9971	0.005927
Celugel + chitosan 2% + hop extract	y = 0.004610x + 3.7405	0.9596	0.004610

4. Conclusions

1. As the chitosan concentration in the prepared Celugel formulations increased, the values of the texture parameters relevant to dermatological application (hardness, consistency, cohesiveness, viscosity, and blurring time) increased.
2. The spreadability of the prepared formulations depended on the presence of chitosan and decreased as the polymer concentration increased.
3. The diffusion of cohumulone, an analogue of humulone contained in hop cone extract, decreased as the chitosan concentration increased.

5. Acknowledgements

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6. References

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