The Influence of the Preparation Method on the Characteristics of a New Cosmetic Gel Based on Hyaluronic Acid and Matrix-Forming Polymers

MAGDALENA BIRSAN¹, NELA BIBIRE², ALINA DIANA PANAINTE², OANA SILASI³, PAULA ANTONOAEA³, ADRIANA CIURBA³*, ANA CATERINA CRISTOFOR⁴, MAGDALENA WROBLEWSKA⁵, KATARZYNA SOSNOWSKA⁵

¹University of Medicine and Pharmacy Grigore T. Popa Iasi, Faculty of Pharmacy, Department of Pharmaceutical Technology, 16 Universitatii Str., 700115, Iasi, Romania
²University of Medicine and Pharmacy Grigore T. Popa Iasi, Faculty of Pharmacy, Department of Analytical Chemistry, 16 Universitatii Str., 700115, Iasi, Romania
³University of Medicine and Pharmacy George Emil Palade Targu Mureș, Faculty of Pharmacy, Department of Science and Technology, 38 Gheorghe Marinescu Str., 540139, Targu Mureș, Romania
⁴University of Medicine and Pharmacy Grigore T. Popa Iasi, Faculty of Medicine, Medical Department III, 16 Universitatii Str., 700115, Iasi, Romania
⁵Medical University of Bialystok, Faculty of Pharmacy, Mickiewicza St. 2D, 15-222 Bialystok, Poland

Abstract: Because hyaluronic acid (HA) cosmetic gels are the most commonly used gels in the cosmetic industry, the purpose of this study was to develop a new gel formulation of HA in carboxymethyl cellulose sodium (Carmellose, CMC Na), prepared in three different ways and to characterize the gel obtained in terms of texture, pH, thixotropy and decide which preparation method is optimal for obtaining a cosmetic gel. The gel formulations were prepared by dispersing CMC Na in water and glycerol and mixing it in three different ways with HA (at the same time, after gelling and 24 h after gel preparation). The pH, rheological properties and texture of hydrogels were evaluated. The study demonstrated that the formulation prepared with CMC Na has higher viscosity and stability at a pH = 6-9. The viscosity depends on the preparation method of hydrogels, the highest values of the mechanical parameters were recorded in the formulation in which CMC Na and HA were added at the same time. The present study showed that the difference on the texture was realised by the used preparation method. In conclusion the preparation method of a hydrogel with CMC Na 4% and 1% HA has a significant influence on the texture profile and the viscosity characteristics.

Keywords: Cosmetic Gels, Hydrogels, Carboxymethyl cellulose sodium, Hyaluronic acid

1. Introduction

Cosmetic products are available in many types of formulation, with hydrogels representing, due to their advantage, a significant class. Hydrogels are three-dimensional net-like structures where the hydrophilic bonds fix the liquid vehicle as the external phase. This topical form is characterized by thixotropy or ease of spreadability [1-4]. The physical and chemical networks binding the particles of the internal phase provide a relatively stable structure, which can originating either in the swelling of the solid polymers, or by decreasing the solubility of the polymer in a solution. Hydrogels are preferred for cosmetics use because they are easy to prepare, the properties and characteristics are suitable and the packaging mode is more comfortable.

The most important advantage of gel preparation used in a cosmetic product is the thixotropy and pseudoplastic low. The concentration of gelling agent required to form the gel is low and the viscosity of the gel does not undergo significant changes in storage at room temperature. When used, the hydrogels have a cooling effect on the skin, are clear in appearance and have an elegant aspect. The use on the skin leaves the film elastic, translucent after drying, with high adhesion on the support. The gels do not clog
the pores, the spreadability on the skin is very good, the drug release from hydrogels is good and they are easily washed away with water. This type of cosmetic products is ideal because they are safe and inert. The choice of gelling agent is an important step in obtaining a stable semi-solid formulation during storage. However, when subjected to mechanical agitation or used topically it can also be broken.

The characteristics of the gel must be adjusted to the intended use of the dosage form. There are fewer disadvantages than advantages, such as: the higher concentration of polymer used, which can determine a difficult removal, the high temperature necessary for formation of the gel. The gel can expand because the gelling agent can absorb the solution so that volume increases [5, 6]. The most unlikely reaction of a polymer is the phenomenon of sineresis, when the hydrophilic phase cannot stay in the network [7-9].

In the cosmetic and pharmaceutical industry, the following polymers are often used, when producing gels: carboxymethyl cellulose sodium (carmellose sodium, CMC Na), methyl cellulose and hydroxypropyl cellulose [10, 11]. Cellulose ether gels are prepared by dispersing in cold water. In order to increase the swelling degree of the polymer matrix, it is recommended to pre-soak it with a solvent facilitating water retention, such as glycerin.

Cellulose derivatives are widely used in the development of ophthalmic (artificial tears), dermatological preparations. They are also frequently used for covering burned tissues (minimize water loss and are easily removed). It is known that CMC Na as a carrier helps with the healing of diabetic ulcers.

The cosmetic and pharmaceutical industry is interested in gel products prepared with different types of Carbopol because they are easy to prepare and inexpensive. Carbomers are synthetic high-molecular-weight polymers of acrylic acid that are cross-linked with either allyl sucrose or allyl ethers of pentaerythritol [12-15]. Carbomer can be irritating to the eyes, mucous membranes, and the respiratory tract. As a syntetic product, in contact with the eye, carbopol is not able to create a similar natural fluid. It is a polymer which is difficult to remove with water because of the gelatinous film that forms. It is necessary to work with gloves, eye protection, and a dust mask during handling. An important disadvantage after application of a carbomer gel is the intense film-forming effect. After evaporation of the hydrophilic phase, the network-like gel structure produces a film, slightly occlusive, which creates an unpleasant sensation. For this reason, this study decided to formulate other types of gel, which included CMC Na as the sodium salt of a polycarboxymethyl ether of cellulose (Figure 1). It is included in the FDA Inactive Ingredients Guide as less irritant [16]. Typical molecular weight is 90 000-700 000.

![Figure 1. Structure and SEM image of the CMC Na](image)

CMC Na with 3-6% average viscosity grade is commonly utilized to produce gels that can be used as a topical base.

Hyaluronic acid (HA) is a natural biopolymer, an ingredient heavily used in cosmetology due to its exceptional ability to retain and attract water. It is humectant and a moisturizer, helping the skin tissue redense and restructure. It stretch the skin, reducing wrinkles and fine lines. HA acts as a protection
against dehydration, being well tolerated by the skin [8]. HA is a polymer of disaccharides [19, 20], themselves composed of D-glucuronic acid and N-acetyl-D-glucosamine, linked via alternating β-(1→4) and β-(1→3) glycosidic bonds (Figure 2).

![Figure 2. Chemical structure of HA [18]](https://doi.org/10.37358/MP.20.2.5358)

The aim of this paper was to evaluate a formulation with HA in CMC Na, which were prepared in three different ways. After the preparation, the texture profile, the pH and the thixotropy were assessed, in order to decide which preparation method was better for obtaining a cosmetic gel.

2. Materials and methods
   2.1. Materials
   CMC Na and glycerol were purchased from Sigma Aldrich (Steinheim, Germany), HA LMW and Cosgard were purchased from Elemental (Oradea, Romania). Water is obtained using a reverse osmosis Mili-Q Reagent Water System (Billerica, MA, USA).

2.2. Preparation of hydrogels
   The composition of hydrogels is shown in Table 1.

   Table 1. Formulation of gel and preparation method

   | Formulation code | FHA-1 | FHA-2 | FHA-3 |
   |------------------|-------|-------|-------|
   | HA               | 1     | 1     | 1     |
   | CMC Na           | 4     | 4     | 4     |
   | Glycerol         | 15    | 15    | 15    |
   | Cosgard          | 1     | 1     | 1     |
   | Water up to*    | 100   | 100   | 100   |
   | Preparation method | HA and CMC Na have been added in the same time | HA has been added after gellation of CMC Na | HA has been added after 24 h after CMC Na gel preparation |

   *The amount of components is expressed in g.

   The CMC Na was dispersed in water and glycerol and mixed using a magnetic stirrer (MR Hei-Standard, Heidolph, Schwabach, Germany). Mixing was conducted until a transparent gel was obtained. Cosgard, used for its preservative function, was added to the gel. In FHA-1 the HA was added with CMC Na sodium at the same time and mixed using a magnetic stirrer until a transparent gel was obtained. In FHA-2 the HA was added after gellation, after which it was mixed together three min. at 1000 rpm. In the FHA-3 the HA was added 24 h after the CMC Na gel preparation and the product was mixed three min at 1000 rpm.

   In previously published papers several tests were conducted determining certain parameters of the topical products in relation to the mixing time. Other studies have shown that, by increasing the mixing time an optimal pharmaceutical form was obtained [22]. A study such as the present one, intent on analyzing the rheological properties in relation to the gel transformation moment of the semi-solid form, has not yet been undertaken. The formulations of the cosmetic products are usually not focused on the
preparation method, but rather on the fixed formula. If we analyse the results we will notice how different the properties of a gel can be, depending on its preparation method. The same quantities of excipients and different methods of preparation result in different products with different sensorial properties.

2.3. Methods of gel formulation characterization

2.3.1. pH Measurement

The pH of hydrogels was determined using Orion 3 Star pH-meter with glass electrode (Thermo Scientific, Waltham, MA, USA). The data shown is the average of six experiments.

2.3.2. Rheological properties

The viscosity and thixotropy of formulated hydrogels were analyzed using a programmable viscometer RV DV-III Utra Brookfield (Brookfield, Middleboro, MA, USA) with a cone-plate device (CP-52Z, 24 mm diameter). Viscosity was measured at different shear rates, whereas thixotropy was studied at an increasing shear rate ranging from 0.5/s to 12/s and then a decreasing shear rate from 16/s to 0.5/s. All the measurements were conducted at 25°C±0.5°C [22].

2.3.3. Texture Analysis

Textural properties of prepared gels were examined by a TA.XT Plus Texture Analyser (Stable Micro System, Godalming, UK) using Texture Exponent 32 software.

3. Results and discussions

3.1. pH of gels

The gels with CMC Na have higher viscosity and stability at a pH between 6-9, although they are stable over a broader pH range between 2-10. A pH less than 2 could cause the precipitation of CMC and with a pH higher than 10 the viscosity decreases rapidly [23-26]. For topical application gels need a pH between 4.5-8.5, but if it is a cosmetic product the gels need a pH similar to that of the skin, between 4.5-5.5.

All formulas have an optimal pH. If hyaluronic acid is added after the gelling process, but on the same day the obtained product has the lowest pH value, respectively 4.85. Th pH value increases if the hyaluronic acid is added 24 h after gel preparation. The pH of all the formulations was found to range between 4.85 and 5.26 (Table 2).

| Table 2. pH and viscosity of the prepared formulation |
|------------------------------------------------------|
| **Formulation** | **pH** |  | **Viscosity** (mPa·s) |  |  |
|                | Mean  | STD | %RSD | Mean  | STD | %RSD |
| FHA-1          | 5.12  | 0.02 | 0.56 | 33244.5 | 37.15 | 0.11 |
| FHA-2          | 4.85  | 0.05 | 1.17 | 35865.0 | 41.56 | 0.12 |
| FHA-3          | 5.26  | 0.04 | 0.82 | 90099.3 | 121.72 | 0.13 |

*Viscosity was tested at 12.00 s⁻¹

3.2. Rheological properties

The viscosity and the rheological properties of the gels are correlated with the spreadability of the hydrogel, the application behavior and contact time with the skin surface. The viscosity depends on the preparation method of hydrogels and the concentration and molecular weight of the polymer, the gel structure and the interchain interaction. CMC Na is a polyelectrolyte and is, as a result, sensible to pH and ionic strength variations. Cellulose derivates hydrogels are semi-stiff gels, viscoelastic, exhibiting thixotropic behavior compared to methyl cellulose and hydroxymethyl cellulose which exhibit a pseudoplastic flow [7].

When the HA was added at the same time with the polymer, the hydrogel had the smaller value of viscosity, 33244.5 mPa S. This phenomenon could be explained by the HA’s influence on the bonding...
of the cellulose polymer with the water molecules, due to higher affinity to water molecules than carmellosse sodium. CMC Na require a minimum of 10 min for gelification. The presence of glycerol is important for fast hydration but in his absence the gelification process lasts longer. If the HA had been added after the gelification of the CMC Na, the value of viscosity would have increased with almost 220 mPa S. Because the process of gelification had not yet ended, HA extracted the water from the network of polymer. The last formulation (FHA-3), in which the HA was added after 24 h after the structuration of the CMC Na, has the highest value of viscosity. The cellulose polymer has enough time for gel formation and the HA does not extract the water from the network of polymer. The value of the viscosity in FHA-3 formulation is 2.7 times higher than in the first formulation. Formulated hydrogels have pseudoplastic characteristics with a shear-thinning property and they are characterized by thixotropy, visible as hysteresis loops (Figures 3 and 4). Thixotropy is very important for a cosmetic product, because the product should be easy to apply and after applying pressure, the gel must retain its bonds in order to be able to return to its original shape. A semi-solid product is applied on the skin by the force of touch and transforms into a liquid. The cream needs to create the bonds again quickly and restore a semisolid form, i.e. to have thixotropy. Otherwise it cannot be deemed a satisfactory cream.

All the formulations are exposed to stress. The thixotropic properties allow the restoration of the gels’ structure after the removal of shear stress (mechanical force) and increases their retention at the site of application. All the cosmetic hydrogels with hyaluronic acid were characterized by thixotropic features at 25°C: viscosity decreased with increasing shear rate and then increased with a diminished shear rate. This behavior was demonstrated by the hysteresis loops formed in rheograms (Figure 3 and Figure 4). In the rheogram of the formulation FHA-3 a slower shear rate was used, since the viscosity of product was too high and after shear rate 2 the rheometer couldn’t analyse the shear stress.

3.3. Textural properties

The texture characteristics of a semi-solid dosage form determine important parameters for a product which is easier to apply on the skin. Hardness is expressed as a maximal force attained during the downwards motion of the disc (compression of hydrogel), cohesiveness- as the process needed to deform the product during the compression. The consistency is correlated with the force with which the product resists compression during the upwards motion of the disc [21]. As shown in Table 3 and Figures 5-7, hydrogels FHA-2 and FHA-3 possessed lower mechanical properties than FHA-1, which may suggest easier application and better spreadability on the skin. The textural parameters provide information about the response to an external force and are very important in checking the removal facility of preparation from the container or their spreadability on the skin surface. Hardness is the maximal force needed to accomplish a deformation and the cohesiveness represents the work needed to deform the formulation.
during the compression of the sample. These results of the texture analysis confirmed findings from rheological measurements.

| Formula | Hardness (g) | Consistency (g) | Cohesiveness (g·s) |
|---------|--------------|-----------------|-------------------|
|         | Mean | STD  | %RSD | Mean | STD  | %RSD | Mean | STD  | %RSD |
| FHA-1   | 3891.94 | 152.30 | 3.82 | 1585.16 | 169.30 | 10.67 | 3468.85 | 64.28 | 1.85 |
| FHA-2   | 1852.84 | 88.81  | 4.79 | 920.31  | 61.46  | 6.78  | 920.31  | 61.46  | 6.67  |
| FHA-3   | 324.32  | 38.27  | 6.91 | 176.41  | 7.68   | 4.33  | 176.41  | 7.68   | 4.33  |

Figure 5. Texture profile for FHA-1

Figure 6. Texture profile for FHA-2

Figure 7. Texture profile for FHA-3

The highest values of the mechanical parameters were recorded in the formulation FHA-1 and could be due to the quality of networks between the CMC Na-water-HA. In comparison to the second or third formula, with the first formulation HA has time to create some bonds with water. When adding the polymer and HA at the same time the polymer first needs a hydration to relax the structure and after which it includes the water in the network. While the CMC Na performs the hydration, the HA quickly develops the bonds with the water. Formulation FHA-1 has the biggest values of all the texture parameters. FHA-3 has the highest values of viscosity but the lowest values of the texture characteristics. When the arm of the texture analyser is introduced in the container, the probe sticks to the entire arm, without the possibility of retraction. Practically, the highest value of viscosity makes the probe stick and decreases the values of texture analyses.
4. Conclusions

This study presents the design and characterization of a gel formulation of hyaluronic acid in carboxymethyl cellulose sodium, prepared in three different ways.

The results showed that the preparation method of a hydrogel with CMC Na 4 and 1%. HA modifies the texture profile and the viscosity characteristics. If a hydrogel with higher viscosity is desired, then the FHA-2 is satisfactory. A cosmetic product easy to apply and with good spreadability is obtained by adding the HA immediately after the gelification process of the CMC Na. The FHA-2 is the optimum formula for the skin, since a higher consistency and viscosity preserves the HA as an active ingredient in the network of the polymer and facilitates the absorption.

References
1. LIN, S., CAO C, WANG, Q., GONZALE, M., DOLBOW, J.E., ZHAO, X., Design of stiff, tough and stretchy hydrogel composites via nanoscale hybrid crosslinking and macroscale fiber reinforcement, Soft Mater., 14, 2014, 7519-7527.
2. COBURN, J., GIBSON, M., BANDALINI, P.A., LAIRD, C, MAO, H.Q., MORONI, L., SELIKTAR, D., ELISSEEFF, J., Biomimetics of the extracellular matrix: An integrated three-dimensional fibre-hydrogel composite for cartilage tissue engineering, Smart Struct. Syst., 7, 2011, 213-222.
3. BAS, O., DE-JUAN-PARDO, E.M., CHHAYA, M.P., WUNNER, F.M., JEO, J.E., KLEIN, T.J., HUTMACHER, D.W., Enhancing structural integrity of hydrogels by using highly organised melt electrospin fibre constructs, Eur. Polym., 72, 2015, 451-463.
4. BIRSAN, M., VIERIU, M., BIBIRE, N., COJOCARU., I., Influence of hydroxypropyl methylcellulose with various molecular on flowing and swelling parameters in buccoskeletal tablets with miconazole nitrate, Rev. Chim., 68(10), 2017, 2346-2349.
5. BIRSAN, M., COJOCARU, I., STAMATE, M., TEODOR, V., TUCHILUȘ, C., Antifungal action of imidazole derivates from new pharmaceutical forms on various strains of Candida, Rev. Chim., 67(7), 2016, 1385-1388.
6. BIRSAN, M., APOSTU, M., TODORAN, N., ANTONOAEA, P., RUSU, A., CIURBA, A., Development of dermal films containing miconazole nitrate, Molecules, 23(1640), 2018, 1-12.
7. BENMOUFFOK-BENBELKACEM, G., Non-linear viscoelasticity and temporal behavior of typical yield stress fluids: Carbopol xanthan and ketchup, Rheol. Acta., 3, 2010, 305-314.
8. CARRETTI, E., DEI, L., WEISS R.G., Soft matter and art conservation. Rheoreversible gels and beyond, Soft Matter, 1, 2005, 17-22.
9. PICH, A.Z., ADLER, J., Composite aqueous micro gels: An overview of recent advances in synthesis, characterization and application, Polym. Int., 59, 2007, 291-297.
10. PATEL, N.A., PATEL, N.J., PATEL, R.P., Formulation and evaluation of curcumin gel for topical application, Pharm. Dev. Technol., 1, 2009, 80-89.
11. MARTINO, G., Personal care applications of starch. The Chemistry and Manufacture of Cosmetics, New York, Toronto: McGraw-Hill, 2002, 703-729.
12. PENN, L.E., Gel Dosage Form: Theory, Formulations and Processing, New York: Marcel Dekker, 1999, 338-381.
13. DIEBOLD, Y., HERRERAS, J.M., CALLEJO, S., ARGÜESO, P., CALONGE, M., Carbomer-versus cellulose-based artificial-tear formulations: Morphologic and toxicologic effects on a corneal cell line, Cornea, 17(9), 1998, 433-440.
14. CIURBA, A., LAZAR, L., ANTONOAEA, P., GEORGESCU, A.M., VARI, C.E., TODORAN, N., In vitro/in vivo performance study of new metronidazole periodontal gel formulations, Farmacia, 63(1), 2015, 11-19.
15. RAYMOND, C., ROWE, P., SHESKEY, J., SIAN, C., Handbook of Pharmaceuticals Excipients, Fifth Edition, London-Chicago: Pharmaceutical Press and American Pharmacists Association, 2006, 120-129.
16.***, https://en.wikipedia.org/wiki/Hyaluronic_acid
17. AVERBECK, M., GEBHARDT, C.A., VOIGT, S., BEILHARZ, S., ANDEREGG, U., TERMEER, C.C., SLEEMAN, J.P., SIMON, J.C., Differential regulation of hyaluronan metabolism in the epidermal and dermal compartments of human skin by UVB irradiation, J. Invest. Dermatol., 127(3), 2007, 687-697.

18. BÎRSAN, M., CRISTOFOR A.C., ANTONOAEA, P., TODORAN, N., BIBIRE, N., PANAINE A.D., VLAD, A.V., GRIGORE, M., CIURBA, A., Evaluation of miconazole nitrate permeability through biological membrane from dermal systems, Farmacia, 68(1), 2020, 11-115.

19. TOMCZYK, M., SOSNOWSKA, K., PLESZCZYNSKA, M., STRAWA, J., WIATER, A., GROCHOWSKI, D.M., TOMCZYKOWA, M., WINNICKA, K., Hydrogel containing an extract of tormentillae rhizome for treatment of biofilm-related oral diseases, Nat. Prod. Commun., 12(3), 2017, 417-421.

20. CHEABURU-YILMAZ, C.N., PAMFIL, D., VASILE, C., BIBIRE, N., LUPUSORU, R.V., ZAMFIR, C.L., LUPUSORU, C.E., Toxicity, biocompatibility, pH-Responsiveness and methotrexate release from PVA/HA cryogels for psoriasis therapy, Polymers, 9(4), 2017, Article Number: 123, DOI: 10.3390/polym9040123.

21. KOWALSKA, M., WOZNIAK, M., PAZDZIOR M., Assessment of the sensory and moisturizing properties of emulsions with Hemp Oil, Acta Polytect. Hung., 14(8), 2017, 183-195.

22. TOMCZYKOWA, M., WROBLEWSKA, M., WINNICKA, K., WIECZOREK, P., MAJESWSKI, P., CELINSKA-JANOWICZ, K., SAWCZUK, R., MILTYK, W., TRYNISZEWSKA, K., TOMCZYK, M., Novel gel formulation as topical carriers for the essential oil of bidens tripartita for the treatment of candidias, Molecules, 23(2517), 2018, 2-10.

23. BÎRSAN, M., BIBIRE, N., VIERIU, M., PANAINE A.D., COJOCARU, I., Influence of hydroxypropyl methylcellulose on flowing and swelling parameters in biomucoadhesive tablets with miconazole nitrate, Rev. Chim., 68(10), 2017, 2346-2349.

24. CHEABURU YILMAZ, C.N., YILMAZ, O., AYDIN KOSE, F., BIBIRE N., Chitosan-graft-poly(n-isopropylacrylamide)/PVA cryogels as carriers for mucosal delivery of voriconazole, Polymers, 11(9), 2019, Article Number: 1432, DOI: 10.3390/polym11091432.

25. *** European Pharmacopoeia, 9th Edition. Council of Europe, Strasbourg, 2017.

26. *** Farmacopeea Româna, Ed. a X-a, Editura Medicală, Bucharest, 2008.

27. *** United States Pharmacopeia and National Formulary (USP 41-NF 36). Rockville, MD: United States Pharmacopeial Convention; 2016. https://online.uspnf.com/uspnf/document/GUID-AC788D41-90A2-4F36-A6E7-769954A9ED09_1_en-US. Accessed January 18, 2019.

28. *** Farmacopeea Româna, Ed. a X-a, Editura Medicală, Bucharest, 2008.

29. *** United States Pharmacopeia and National Formulary (USP 41-NF 36). Rockville, MD: United States Pharmacopeial Convention; 2016. https://online.uspnf.com/uspnf/document/GUID-AC788D41-90A2-4F36-A6E7-769954A9ED09_1_en-US. Accessed January 18, 2019.

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