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# Formulation of Casein Hydrogels †

**Vanina A. Guntero [1,*], María C. Acuña [1], Yamile S. Aon [1], Leandro G. Gutierrez [2] and Cristián A. Ferretti [2]**

1 Group of Natural Products and Materials (ProNaM), Universidad Tecnológica Nacional (UTN), Facultad Regional San Francisco, San Francisco 2400, Argentina; candelaacu@facultad.sanfrancisco.utn.edu.ar (M.C.A.); yamiaon@gmail.com (Y.S.A.)
2 Group of Organic Synthesis and Materials (GSOM), Laboratorio Fester—Química Orgánica (FIQ), Instituto de Química Aplicada del Litoral (IQAL) (UNL-CONICET), Universidad Nacional del Litoral, Santa Fe 3000, Argentina; guti.lg@live.com (L.G.G.); cferretti@fiq.unl.edu.ar (C.A.F.)
* Correspondence: vguntero@facultad.sanfrancisco.utn.edu.ar; Tel.: +54-9-3564-623116

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**Abstract:** Protein-based hydrogels have gained considerable interest due to their properties such us biocompatibility, nontoxic, biodegradability, renewable, inexpensive and easy to obtain. Hydrogel properties depend on temperature, polymer concentration, pH, crosslinking levels, salt concentrations and aging. Casein is a natural protein present in the bovine milk (about 80%) which exists in the form of various micelles, it is composed of $\alpha$-s1, $\alpha$-s2, β- and κ-casein and tends to self-assembly. Casein based hydrogels are suitable for use in biomedical applications. Considering their potential applications in the field of medicine, in this work, we have the objective to find the best conditions to development a casein hydrogel with tetracaine hydrochloride such active compound. The tetracaine hydrochloride has anesthetic properties so would allow a painless and comfortable treatment for the patient. So, different hydrogel formulations were proposed. The selected components were casein, glycerol, tetracaine hydrochloride, potassium carbonate and sodium alginate. Stability test, apparent density, pH, moisture content and swelling test were investigated. The formulation that allowed us to obtain a hydrogel with the desired properties was composed of tetracaine hydrochloride 1%, casein 2%, glycerol 50%, sodium alginate 4% and potassium carbonate solution 18% (The percentages are expressed on a casein basis). Casein hydrogel showed potential for use as anesthetic in medicine.

**Keywords:** hydrogel; tetracaine hydrochloride; properties; optimization

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## 1. Introduction

Biopolymer hydrogels are versatile soft materials typically assembled from entangled or crosslinked proteins and/or polysaccharides. Biopolymer hydrogels have found a wide range of practical applications, including in the food, cosmetics, personal care, agrochemical, pharmaceutical, and biomedical industries [1].

Hydrogels are characterized by containing excessive amount of water in their structure. The swelling properties of hydrogels are mainly related to the elasticity of the network, the presence of hydrophilic functional groups in the polymer chains, the extent of cross-linking, and porosity of the polymer [2]. Hydrogel porosity or microchannel opens a fascinating area in delivering bioactive molecules, including drugs, peptides, and proteins to cells. Hydrogels may be chemically stable or unstable, depending on the interactions or crosslinking of the polymer chains [3]. Hydrogels are crosslinked 3D networks comprising of either natural or synthetic polymers. It has been observed that synthetic hydrogels have more controllable physical properties and are more reproducible, but show poor biocompatibility, lack of bioactivity and little resemblance to the natural environment [4].

Protein-based hydrogels are especially welcomed from different sectors due to their outstanding properties, such as high nutritional value, excellent functional properties, amphiphilic nature, biocompatibility, biodegradability, and lower toxicity in comparison with synthetic polymers [5].

Hydrogels can be formulated by using a wide range of polymers, including those of food origin. The benefits of hydrogels made from food-grade biopolymers include safety, low cost, and commercial availability. One possibility for creating food-based hydrogels is the use of caseins, alone or in combination with others food-grade polymers [6].

Casein, as the main component of milk protein, is a major animal protein resource. Casein is a calcium-phosphate-binding protein composed of $\alpha$s1-casein, $\alpha$s2-casein, β-casein and κ-casein. The low solubility of casein is a key determinant of the suitability of proteins for use in food systems. At a pH of 4.6 (the isoelectric point) the total net charge of casein is zero and there is no electrostatic repulsion between the molecules, at which point solubility is at its lowest. Therefore, the closer the pH of casein is to the isoelectric point, the more likely it is to precipitate and coagulate [7].

On the other hand, Tetracaine hydrochloride, TH, is an amphiphilic compound that also possesses colloidal properties and is one of the most used local anesthetic drugs. Since tetracaine is a poorly water-soluble compound, it is usually formulated as tetracaine hydrochloride [8]. Considering the pharmacological properties of tetracaine to reduce pain caused by skin irritations from minor burns, we selected this compound for the development of a hydrogel for topical use.

In this study, different casein hydrogel formulations were prepared by changing the concentrations of the reagents. The hydrogels were characterized at a macroscopic level and their stability at different temperatures was evaluated.

## 2. Materials and Methods

### *2.1. Materials*

Commercial micellar casein (CA) from bovine milk; glycerol ($C_3H_8O_3$) (Gly), potassium alginate [$(C_{12}H_{14}CaO_{12})_n$] (PAlg), tetracaine hydrochloride (TH) were obtained commercially. Buffer of pH 10.6 [anhydrous potassium carbonate, $K_2CO_3$] (AnP) was prepared using deionized water.

### *2.2. Preparation of a Casein Hydrogel*

The preparation of hydrogels was based on previous literature [9]. A casein solution (10 wt.%) with tetracaine was prepared by dissolving the protein in a tetracaine solution in potassium carbonate buffer (pH 10.6) at 60 °C under magnetic stirring. Then, glycerol and potassium alginate at different concentrations were added (Table 1). The pH of mixture was ajusted to 7 with acetic acid. After mixing the mixture for 30 min and cooling at 4 °C for 1 h, the hydrogels were obtained.

**Table 1.** Hydrogels formulation (wt. %).

| Form. N° | TH (%) | CA (%) | AnP (%) | Gly (%) | PAlg (%) |
|---|---|---|---|---|---|
| 1 | - | 9.49 | 85.39 | 4.74 | 0.38 |
| 2 | 1.86 | 9.31 | 83.8 | 4.66 | 0.37 |
| 3 | 1.87 | 9.33 | 83.96 | 4.66 | 0.19 |
| 4 | 0.1 | 9.5 | 85.3 | 4.70 | 0.40 |
| 5 | 0.07 | 7.4 | 88.56 | 3.69 | 0.30 |
| 6 | 0.07 | 7.1 | 85.41 | 7.12 | 0.28 |

### *2.3. Caracterization of Hydrogels*

The general appearance of the hydrogels was evaluated analizing the physical aspect, apparent density, pH, moisture content, swelling and stability considering the bibliography. The composition of tetracaine in the hydrogels was analizing by UV-Vis spectroscopy.

## 3. Results

To obtain hydrogels of adecuate consistency, different formulations were testead, changing the concentration of TH (0.07 to 1.87%), CA (7.1 to 9.5%), AnP (83.8 to 88.56%), Gly (3.69 to 7.12%), PAlg (0.19 to 0.40%).

In Figure 1, the tetracaine hydrogels obtained are shown. The consistency of these formulations was evaluated at room temperature (20 °C) and at low temperature (4 °C). All formulations were initially viscous and firm; however, after 24 h, only formulations 1–4 maintained these properties. Considering that these were the best formulations, we continued with the characterization of these hydrogels.

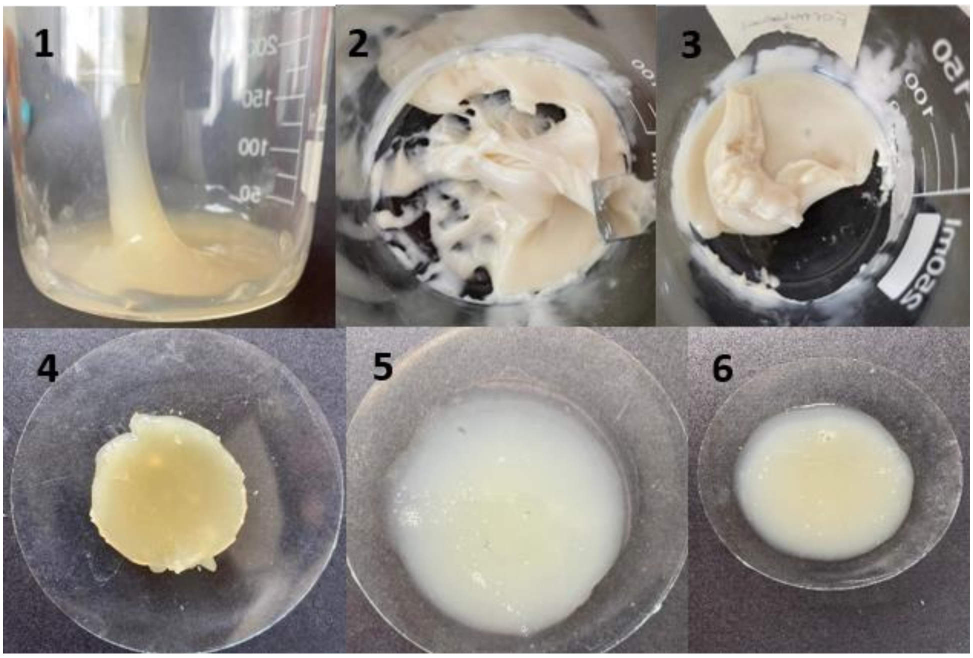


**Figure 1.** Formulations of tetracain hydrogels obtained (1 to 6).

Table 2 shows the results of the quality parameters of the casein hydrogels. Considering formulation 1 as a control, the other formulations did not show significant changes in their properties with the addition of tetracaine. In all cases, the incorporated tetracaine content was in line with the theoretical amount.

The swelling ratio of all hydrogels varied from 4.2 to 5.0 after 24 h, indicating that they are suitable for topical treatment. This result is consistent with the expressed by [10].

In addition, release assays were performed in phosphate buffer pH = 7.5, in which it was possible to evaluate that tetracaine is completely released from the hydrogels in less than one hour of testing.

**Table 2.** Hydrogels formulation (wt. %).

| Form. N° | Density (%) | pH (%) | Moinsture (%) | Swelling (%) | Tetracaine (%) |
|---|---|---|---|---|---|
| 1 | 0.51 | 7.1 | 94 | 5.0 | 0 |
| 2 | 0.57 | 7.5 | 92 | 4.5 | 1.9 |
| 4 | 0.55 | 7.4 | 93 | 4.5 | 1.8 |
| 5 | 0.56 | 7.1 | 91 | 4.2 | 0.2 |

## 4. Conclusions

In this work, casein, potassium alginate, glycerol, and tetracaine hydrochloride were used to formulate hydrogels. In these formulations, the apparent density was approximately 0.5, the pH was approximately 7, the moisture content was approximately 90%, and the swelling was approximately 4.5%. Being the optimal formulation composed of tetracaine hydrochloride at 1%, casein at 2%, glycerol at 50%, and sodium alginate at 4%, it can be concluded that this combination provides the desired properties for the hydrogel. These results suggest that tetracaine hydrogel shows potential for use as an anesthetic in medicine.

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