

## Research Article

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## Papain Hydrogel with Polysorbate: Critical Micelle Concentration and Thermodynamics Stability

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### ABSTRACT

Critical Micelle Concentration is a surfactant concentration above which micelles form. Micelle formation favors solubilization of poorly soluble substances in a required middle. Papain is a proteolytic enzyme used in wound care. It is partially soluble in water and slightly stable in formulations. Hydrogel has been prepared with papain and polysorbate 80 as a solubilizing agent serially, with and without L-cysteine. This study aimed to determine the critical micelle concentration (CMC) by measuring surface tension (Du Nouy) and thermodynamic stability using zeta potential technique. The use of L-cysteine caused a decrease in CMC, and polysorbate 80 almost achieved a zero zeta potential. Nevertheless, in both serials, papain hydrogels were homogeneous and thermodynamically stable.

**Key-words:** Critical Micelle Concentration; Surface Tension; Zeta potential; Papain.

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## Introduction

Phenomena related to the interaction of surfactants and solutions are commonly studied through the physical-chemical changes in this system. The use of surfactants is a great technical resource for the structuring of insoluble systems since they act as solubilizing agents of various substances in aqueous systems. A critical micelle concentration (CMC) indicates a surfactant concentration range within which saturation occurs at the air-liquid interface with surfactant molecules, and micelles start to form. In micelles, there is a polar mantle (shell) covering an apolar inside; these apolar parts are unfavorable to the solubilization by a polar solvent – dissolving one another, while the polar heads interact favorably with the external solvent. Thus, there will also be a reduction in the free energy of the system when the CMC is reached and, according to the Gibbs equation, adsorption increases.<sup>(1,2,3)</sup> Several interfacial phenomena undergo alterations within the CMC, as surfactant absorption, micellar solubility, surface tension, surface charge (zeta potential), as well as solute-solvent and solute-solute interactions.<sup>(2)</sup> The determination of CMC values can be performed by a variety of techniques such as surface tension, conductivity, solution density and viscosity, fluorescence, light scattering, ultrasound absorption, ion-selective electrode, capillary electrophoresis, and fiber optic refraction.<sup>(1, 2)</sup>

The aggregates formed above the critical concentration show various morphologies. Micelles formed from nonionic surfactants have increasing nucleus polarity towards the water-polyoxyethylene surface. The nucleus- aqueous solution interface (polar part) is a highly hydrated palisade layer and such anisotropic distribution favors the insertion of various molecules, i.e. allows solubilization.<sup>(3,4)</sup> In micelles, the hydrophobic nucleus serves as a reservoir for drugs, while the hydrophilic shells form a spherical barrier against micelle aggregation, ensuring its solubility in aqueous media.<sup>(5)</sup> Other approaches to drug solubilization, used together or individually, could be pH control, formation of water-soluble molecular complexes, and use of surfactants and/or co-solvents.<sup>(6)</sup>

Solubilization of drugs by surfactants varies according to the chemical composition of the surfactant concerned and the location of the drug in the micelle. With amphiphilic molecules in water, the apolar part occupies the inner region of the micelle; and on the outside will be polar portions of the surfactant, being in contact with water molecules of the continuous phase.<sup>(3,7,8)</sup> Thus, polysorbates can interact with proteins and colloidal polymers in different ways.<sup>(9-11)</sup> Proteins tend to adsorb and accumulate at the interfaces, and the use of non-ionic surfactants, such as polysorbates, can minimize this phenomenon.<sup>(10,12,13)</sup> Surfactants will cover and protect proteins from other undesirable interactions by increasing their solubilization through surface direct interaction and hydrophobic bonds.<sup>(13)</sup> Surfactants increase the conformational stability of the protein and the unfolding of the free energy associated with the denaturation/aggregation.<sup>(12)</sup> This system will also be energetically unfavorable to protein adsorption on the interface.<sup>(14)</sup> Protein protection by surfactants may occur to prevent adsorption and/or stabilization in solution, inhibiting approach and consequently aggregation.<sup>(10,12)</sup> Surfactant protective effect for proteins is correlated with the CMC, by either micellar formation or simple solubilization via hydrophobic interactions.<sup>(12-15)</sup> However, at high concentrations, the surfactant may destabilize these macromolecules.<sup>(16)</sup>

Polysorbates interact with colloidal molecules such as carbomers. Such association is influenced by the non-polar moiety, ionic character, water solubility, structural conformation, and gelation of surfactants, besides the influence of other substances and salts in the medium.<sup>(11)</sup>

Therefore, in this study, surface tension (Du Nouy's method) was used to evaluate the CMC, and zeta potential for the thermodynamic stability of the colloidal system of papain, in two test sets, with and without cysteine, using polysorbate 80 as a solubilizing agent.

Papain is a proteolytic enzyme of plant origin, being extracted from latex of *Carica papaya* green fruit. It has been used in treating of wounds of various etiologies.<sup>(17-22)</sup> Despite its great potential in cosmetics and medicine, this enzyme has limited solubility and stability.<sup>(23)</sup>

## Methods

### Materials

Carbomer 940, L-cysteine monohydrate, propylene glycol, and polyoxyethylene 20 sorbitan monooleate (polysorbate 80) were purchased from Farnos (Farnos Comércio e Indústria Ltda, Rio de Janeiro, RJ, Brazil). Sodium hydroxide microbeads were acquired from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Disodium ethylenediaminetetraacetate (EDTA) and papain (6000 U mg<sup>-1</sup>) were obtained from Fagron (Fagron do Brasil Farmacêutica Ltda, São Paulo, SP, Brazil).

## Hydrogel preparation

Hydrogel base was prepared with 1.5% (w/w) carbomer 940, 0.1% (w/w) methylparaben, with pH being adjusted to 6.0 with 10% sodium hydroxide solution (w/w). The gel was stored for 24 hours for further use in all studied preparations.

The study was performed by analyzing two sets of 4.0% papain gel formulations (w/w) with polysorbate 80, one with L-cysteine, and one without (Table 1). The solid ingredients were weighed and transferred to a porcelain grail, being suspended with a propylene glycol solution plus polysorbate 80 to the desired concentration for each sample. Then, an incorporation in 1.5% carbomer 940 gel (w/w) proceeded through homogenization by geometric dilution under mechanical stirring. The samples were kept under refrigeration at 5°C ( $\pm 2$ ), with the purpose of preserve papain properties. Surface tension and zeta potential measurements were made at room temperature (25°C  $\pm 2$ ), 24 hours after incorporation of papain and coadjuvants into the gel. The samples were prepared in triplicate.

Table 1: Sets 1 and 2 of papain hydrogel formulations with polysorbate

|       | Formulations | Components (% p/p) |       |        |       |       |
|-------|--------------|--------------------|-------|--------|-------|-------|
|       |              | Papain             | EDTA  | PPG    | CYS   | P80   |
| Set 1 | H1           | 4.000              | 0.160 | 10.000 | -     | -     |
|       | H2           | 4.000              | 0.160 | 10.000 | -     | 0.050 |
|       | H3           | 4.000              | 0.160 | 10.000 | -     | 0.100 |
|       | H4           | 4.000              | 0.160 | 10.000 | -     | 0.200 |
|       | H5           | 4.000              | 0.160 | 10.000 | -     | 0.300 |
|       | H6           | 4.000              | 0.160 | 10.000 | -     | 0.500 |
|       | H7           | 4.000              | 0.160 | 10.000 | -     | 1.000 |
| Set 2 | HC1          | 4.000              | 0.160 | 10.000 | 0.050 | -     |
|       | HC2          | 4.000              | 0.160 | 10.000 | 0.050 | 0.050 |
|       | HC3          | 4.000              | 0.160 | 10.000 | 0.050 | 0.075 |
|       | HC4          | 4.000              | 0.160 | 10.000 | 0.050 | 0.100 |
|       | HC5          | 4.000              | 0.160 | 10.000 | 0.050 | 0.300 |
|       | HC6          | 4.000              | 0.160 | 10.000 | 0.050 | 0.500 |
|       | HC7          | 4.000              | 0.160 | 10.000 | 0.050 | 1.000 |

Legend: PPG = Propylene glycol; CYS = L-cysteine; P80 = Polysorbate 80

## Surface tension measurement

CMC values and surface parameters are determined from surface tension measurements ( $\text{mN m}^{-1}$ ) using a Du Nouy detachment tensiometer (KRÜSS, Model K20) with a platinum (gold joint) ring at  $25 \pm 2^\circ\text{C}$ . Three successive readings of two aliquots were made for each concentration.

## Zeta potential measurement

Zeta potentials of hydrogels were measured in a quartz cell placed between two Pd electrode chambers, using dynamic light scattering (DLS; Zetasizer nanoseries – Nano ZS90 – Malvern Instruments, CITY, COUNTRY). The values were transmitted to a computer and analyzed with a Zetasizer Software 7.01.

Measurements of zeta potential (mV) by electrophoresis were performed on the same day as sample dilution to 2.0% (w/w) in ultra-pure water (Purelab Flex - Veolia).

## Statistics

The statistical study was made through the descriptive analysis of variables in terms of mean and standard deviation using a spreadsheet software (Excel 2003 - Microsoft Office). Graphic treatments were developed by OriginPro 8.0 software.

## Results and discussion

### Determination of CMC by surface tension measurements

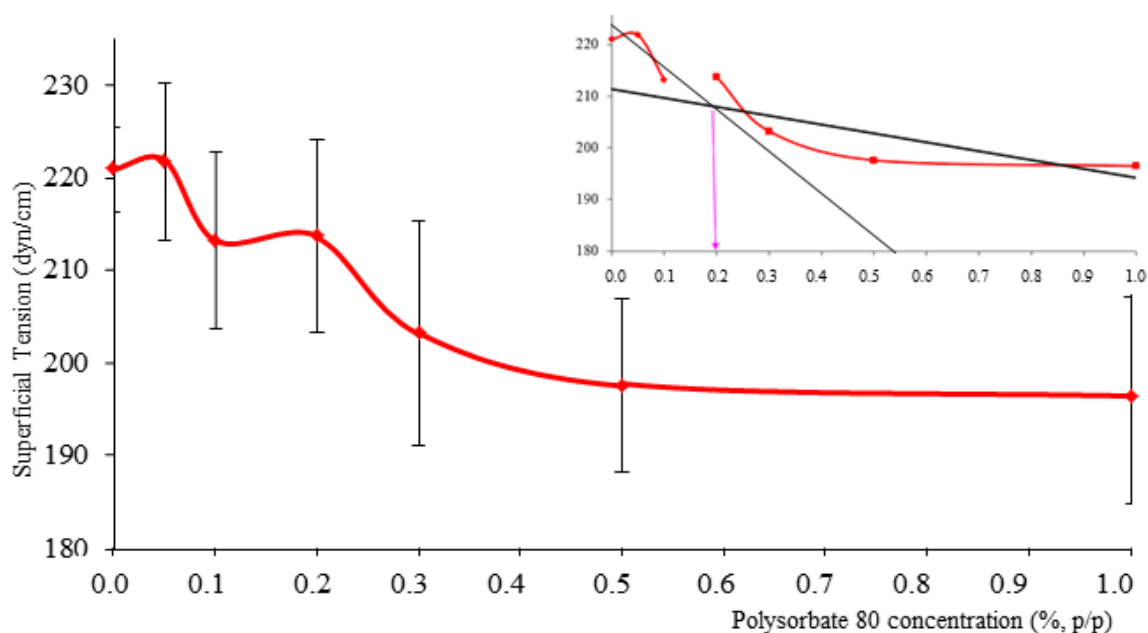
Tables 2 and 3 show the surface tension measurements of samples with and without L-cysteine respectively.

The CMC values of samples via surface tension were defined as the intersection between two straight lines. Figures 1 and 2 show the surface tension variation with polysorbate 80 concentration in preparations without L-cysteine for both set 1 and set 2, respectively.

Table 2: Surface tension values of papain hydrogels with polysorbate, set 1

| Preparations | Concentrations<br>Polysorbate 80 (% p/p) | Surface Tension<br>(dyn/cm)* |
|--------------|--|------------------------------|
| H1           | 0.000                                    | 221.00 (±4.60)               |
| H2           | 0.050                                    | 221.83 (±8.51)               |
| H3           | 0.100                                    | 213.13 (±9.45)               |
| H4           | 0.200                                    | 213.70 (±10.23)              |
| H5           | 0.300                                    | 203.21 (±12.08)              |
| H6           | 0.500                                    | 197.60 (±9.24)               |
| H7           | 1.000                                    | 196.47 (±11.64)              |

\*Average of three readings for each concentration with a standard deviation



**Fig. 1** Graphic representation of the straight line equations used for CMC determination on surface tension graph for papain hydrogels with polysorbate of set 1

CMC will be observed through surface tension if there is a change in the angle between the lines, curve inflection, which had been decreasing and begins to form a plateau, even after a constant addition of surfactants. (3,8,12,24-26)

Each line led to an equation (Equations 1 and 2); and by calculating the straight-line equations, an accurate CMC value could be obtained for set 1 of 4.0% papain hydrogels (w/w) without L-cysteine (0.20495).

Equation 1:  $y = -78.667x + 222.59$

SD = 3.89

N = 3

Equation 2:  $y = -2.9915x + 207,08$

SD = 2.93

N = 3

Where:

y = Surface Tension;

x = Surfactant concentration (polysorbate 80) (%; w / w);

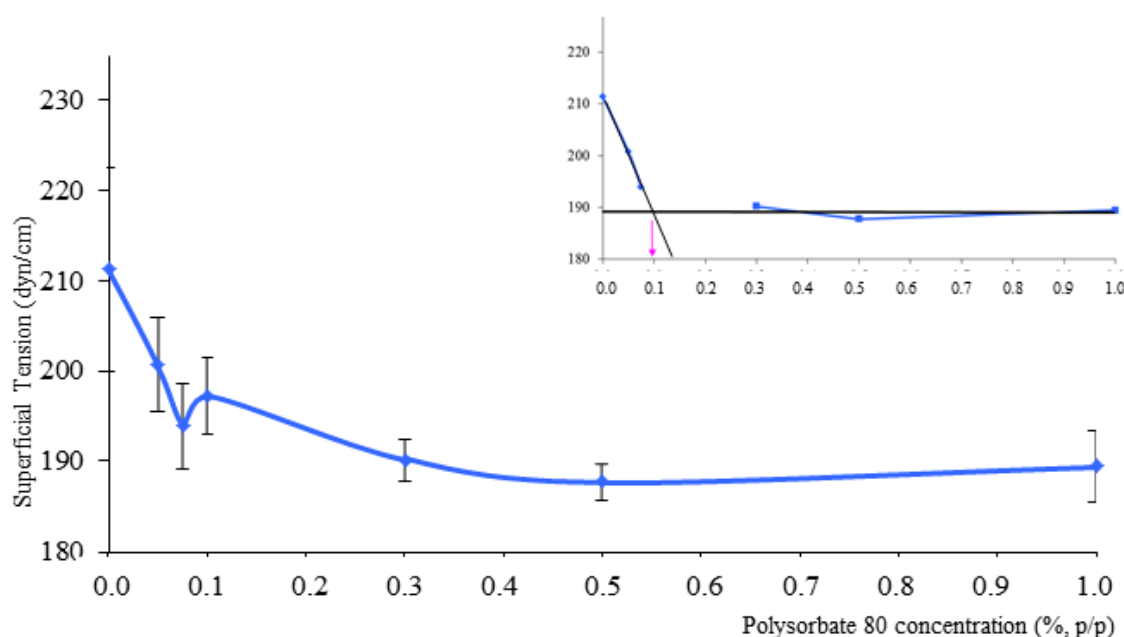
SD = Standard deviation

N = Number of points to draw the straight-lines

Table 3: Surface tension values of papain hydrogels with polysorbate, set 2

| Preparations | Concentrations<br>Polysorbate 80 (% p/p) | Surface Tension<br>(dyn/cm)* |
|--------------|--|------------------------------|
| HC1          | 0.000                                    | 211.38 ( $\pm 11.32$ )       |
| HC2          | 0.050                                    | 200.79 ( $\pm 5.26$ )        |
| HC3          | 0.075                                    | 193.97 ( $\pm 4.69$ )        |
| HC4          | 0.100                                    | 197.31 ( $\pm 4.24$ )        |
| HC5          | 0.300                                    | 190.17 ( $\pm 2.28$ )        |
| HC6          | 0.500                                    | 187.73 ( $\pm 1.96$ )        |
| HC7          | 1.000                                    | 189.43 ( $\pm 3.87$ )        |

\*Average of three readings for each concentration with a standard deviation



**Fig. 2** Graphic representation of the straight line equations used for CMC determination on surface tension graph for papain hydrogels with polysorbate of set 2

Surface tension graphs of cysteine added hydrogels were analyzed likewise, generating the straight-line equations 3 and 4. The intersection of these lines indicated an accurate value of 0.09765.

Equation 3:  $y = -229.28x + 211.60$

SD = 0.81

N=3

Equation 4:  $-0.2077x + 189.23$

SD = 1.77

N=3

Where:

y = Surface Tension;

x = Surfactant concentration (polysorbate 80) (%; w / w);

SD = Standard deviation

N = Number of points to draw the straight-lines

CMC depends on several factors such as ionic structure, environmental temperature, and salts affecting aggregation process.<sup>(27)</sup> Another study on the interaction between polysorbate 80 and carbomers<sup>(11)</sup> explains the high surface tension in these systems. With carbomer, polysorbate 80 concentration on the surface decreases as the surfactant is adsorbed onto the polymer, in order to stabilize itself inside the polymer net. The polymer-surfactant interactions lead to an increased surfactant activity on the surface, consequently, without reducing the surface tension. This increasing concentration of surfactant causes the polymer to become saturated. After saturation, surfactant activity increases again, reducing surface tension to a constant level, from which micelle formation starts.<sup>28</sup> Carbomer and polysorbate interactions occur especially through hydrogen bonds, changing polymer conformation. Such a conformational amendment reduce the area occupied

by the colloid thickening the interfacial layer of the surfactant, what justifies the higher surface tension values compared to those of solutions with surfactant only.<sup>(11)</sup>

The CMC difference between both sets of hydrogels (with and without cysteine) might have occurred due to effective charges from cysteine in solution, altering the organization of micelles. This finding is consistent with that of another study where the influence of anions on the physical properties of liquids was assessed, highlighting the solubility of ionic liquids in water, micelle formation and, consequently, surface tension.<sup>(26)</sup>

### Thermodynamic stability via zeta potential

The stability of systems containing dispersed particles can be verified through zeta potential ( $\zeta$ ), which governs the degree of repulsion between dispersed particles of similar loads. The attraction forces would overlap repulsion ones if zeta potential were below  $|30|$  mV (negative or positive), increasing aggregation among them.<sup>(3)</sup> In addition, a greater stability of the system could be expected when zeta potential is above  $|30|$  mV. Tables 4 and 5 displays the zeta potentials of samples under study for both sets.

Table 4: Zeta potential values of papain hydrogels with polysorbate, set 1

| Preparations | Concentrations<br>Polysorbate 80 (% p/p) | Zeta Potential (mV)*  |
|--------------|--|-----------------------|
| H1           | 0.000                                    | -48.05 ( $\pm 0.85$ ) |
| H2           | 0.050                                    | -46.49 ( $\pm 4.19$ ) |
| H3           | 0.100                                    | -45.01 ( $\pm 0.65$ ) |
| H4           | 0.200                                    | -46.51 ( $\pm 0.19$ ) |
| H5           | 0.300                                    | -42.11 ( $\pm 1.50$ ) |
| H6           | 0.500                                    | -41.23 ( $\pm 0.04$ ) |
| H7           | 1.000                                    | -44.55 ( $\pm 2.76$ ) |

\*Average of three readings for each concentration with a standard deviation

Firstly, when comparing the zeta potentials of preparations with and without L-cysteine, we observed that samples with the same concentration of surfactant of both sets had different values, being those containing cysteine always lower. As an anionic molecule, cysteine adds negative charges to the system, modifying the distribution of loads and making the particles less negative and, hence, reducing zeta potential. The closer to zero the value of this quantity, the smaller the repulsive forces and, therefore, an increasing aggregation trend of particles will occur, with further precipitation. When evaluating aqueous systems, the presence of bromide anion promotes adsorption on particle surfaces, reducing electrostatic repulsion and facilitating aggregation.<sup>(26)</sup>

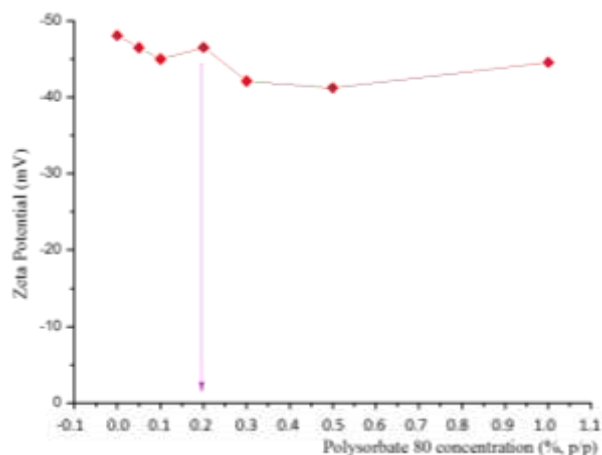
Table 5: Zeta potential values of papain hydrogels with polysorbate, set 2

| Preparations | Concentrations<br>Polysorbate 80 (% p/p) | Zeta Potential (mV)*  |
|--------------|--|-----------------------|
| HC1          | 0.000                                    | -38.20 ( $\pm 0.95$ ) |
| HC2          | 0.050                                    | -38.01 ( $\pm 4.72$ ) |
| HC3          | 0.075                                    | -35.00 ( $\pm 1.84$ ) |
| HC4          | 0.100                                    | -39.16 ( $\pm 0.80$ ) |
| HC5          | 0.300                                    | -39.11 ( $\pm 5.32$ ) |
| HC6          | 0.500                                    | -36.84 ( $\pm 2.49$ ) |
| HC7          | 1.000                                    | -38.45 ( $\pm 1.63$ ) |

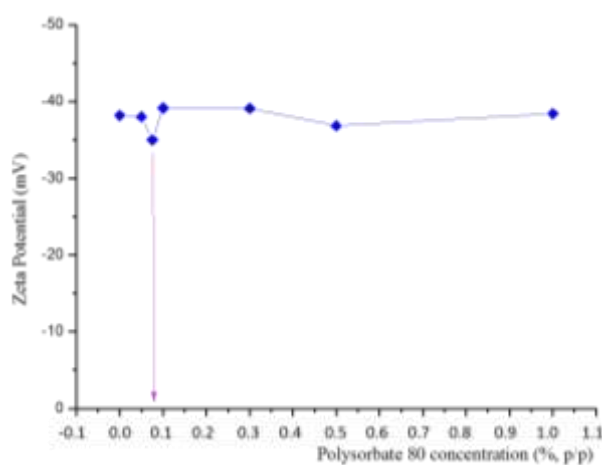
\*Average of three determinations for each concentration with a standard deviation

It is noteworthy that all 4.0% papain hydrogels (w/w) in this study reached zeta potentials (in modulus) above 30, indicating that these dispersed systems have a stable load distribution.

In both sets, a significant curve behavior change is highlighted by the graphs of zeta potential graphs versus polysorbate 80 concentration (Figures 3 and 4). Interestingly, this phenomenon occurs in the vicinity of the concentrations pointed on the surface tension graphs (Figures 1 and 2) as being the CMC.



**Fig. 3** Graph of zeta potential of papain hydrogels versus polysorbate concentration, set 1



**Fig. 4** Graph of zeta potential of papain hydrogels versus polysorbate concentration, set 2

The dependence of zeta potential on the surfactant concentration is divided into two parts. One is related to a progressive linear increase on zeta potential prior to CMC. The other shows that the zeta potential becomes invariant, or almost invariant, as the concentration increases after the CMC. The point of intersection of these two straight lines corresponds to the CMC.<sup>2</sup> The findings of this study corroborate with those of Cifuentes et al (1997).<sup>(1)</sup> It seems to be possible to determine easily the values of a surfactant CMC from concentration versus zeta potential curves.

Furthermore, there is a relationship between zeta potential and surface tension. However, this linear relationship could not be quantitatively confirmed. Thus, we must emphasize what has been said previously [26] that cysteine affects micellar formation.

## Conclusions

This study investigated the effect of polysorbate 80 and L-cysteine on surface tension and zeta potential of papain hydrogels. The surface tension graphs for preparations with L-cysteine showed lower CMC values than did those without this compounds. The same phenomenon is observed in the zeta potential graphs. The L-cysteine addition in these amino acid preparations modifies the values of critical micelle concentration and the zeta potential of these systems.

All the studied preparations showed to be homogeneous, i.e. without precipitates, even for preparations with L-cysteine add above the CMC. The zeta potential studies indicated thermodynamic stability of the preparations. However, systems with L-cysteine have a higher tendency to precipitate particles over time than do those without it. It is clearly noticed when zeta potential values are near  $|30|$  mV.

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## References

- 1 Cifuentes A, Bernal JL, Diez-Masa JC. Determination of Critical Micelle Concentration Values Using Capillary Electrophoresis Instrumentation. *Anal. Chem.* 1997; 69:4271-4.
- 2 Song Y, Sun R, Zhao K, Pan X, Zhou H, Li D. An induction current method for determining the critical micelle concentration and the polarity of surfactants. *Colloid Polym. Sci.* 2015;293:1525-34.
- 3 Sinko PJ. *Físico-Farmácia e Ciências Farmacêuticas. Artmed.* 5 ed.; 2008.
- 4 Prazeres TJV, Beija M, Fernandes FV, Marcelino PGA, Farinha JP, Matinho JMG. Determination of the critical micelle concentration of surfactants and amphiphilic block copolymers using coumarin. *Inorg. Chim. Acta.* 2012; 381:181-7.
- 5 Rapoport N. Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery. *Prog. Polym. Sci.* 2007; 32:962-90.
- 6 Mahato RI, Narang AS. *Pharmaceutical Dosage Forms and Drug Delivery.* 2 ed; 2012.
- 7 Ferreira AO. *Guia Prático de Farmácia Magistral.* v.1, 3ed., São Paulo: Phamabooks; 2008.
- 8 Shaw DJ. *Introdução à química dos colóides e de superfícies.* São Paulo: Editora da Universidade de São Paulo. 1975.
- 9 Silva RL da, Volpato NM. Meios para dissolução de comprimidos de nimesulida: ação dos tensoativos. *Rev. Bras. Cienc. Farm.* 2002; 38,2:163-72.
- 10 Ohtake S, Kita Y, Arakawa T. Interactions of formulation excipients with proteins in solution and in the dried state. *Adv. Drug Delivery Rev.* 2011; 63:1053-73.
- 11 Barreiro-Iglesias R, Alvarez-Lorenzo C, Concheiro A. Poly(acrylic acid) microgels (carbopol® 934)/surfactant interactions in aqueous media Part I: Nonionic surfactants. *Int. J. of Pharma.* 2003; 258:165-77.
- 12 Lee HJ, McAuley A, Schulke KF, McGuire J. Molecular origins of surfactant-mediated stabilization of protein drugs. *Adv. Drug Delivery Rev.* 2011; 63:1160-71.
- 13 Kamerzell TJ, Esfandiary R, Joshi SB, Middaugh CR, Volkin DB. Protein-excipient interactions: Mechanisms and biophysical characterization applied to protein formulation development. *Adv. Drug Delivery Rev.* 2011; 63:1118-59.
- 14 Gray WD. Surfactant Effects on Adsorption of Recombinant Factor VIII (rFVIII) at the Air-Water Interface. (Project for the degree). *Science in Chemical Engineering.* Oregon State University, University Honors College. 2011.
- 15 Wang P-L, Johnston TP. Enhanced stability of two model proteins in an agitated solution environment using poloxamer 407. *J. Parenter. Sci. Technol.* 1993; 47:183-9.
- 16 Katakam M, Bel LN, Banga AK. Effect of surfactants on the physical stability of recombinant human growth hormone. *J. Pharm. Sci.* 1995. 84,6:713-6.
- 17 Velasco, MVR. Desenvolvimento e padronização de gel contendo papaína para uso tópico. 144 p. Dissertação de Mestrado. Faculdade de Ciências Farmacêuticas de São Paulo. Universidade de São Paulo. São Paulo. 1993.



- 18 Sanchez Neto, R. Aspectos morfológicos e morfométricos da reparação tecidual de feridas cutâneas de ratos com e sem tratamento com solução de papaína a 2%. Dissertação de Mestrado em Técnica Operatória e Cirúrgica Experimental da Escola Paulista de Medicina. 1991.
- 19 Ferreira, A.M. et al. Atividade antibacteriana in vitro de géis com diferentes concentrações de papaína. Rev. Elet. Enf. [Internet]. 2008b.; 10(4):1035-40, Disponível em: <<http://www.fen.ufg.br/revista/v10/n4/v10n4a15.htm>>. Acesso em: 21 mai. 2013.
- 20 Silva LM. Efeitos benéficos da papaína no processo terapêutico de lesões de pele. In: Jorge SA, Dantas SRPE Abordagem multiprofissional no tratamento de feridas. São Paulo: Atheneu, p.123-31; 2003.
- 21 Hax, G. Comparando os efeitos da utilização da papaína e AGE em lesões cutâneas: estudo experimental. Dissertação de Mestrado em Ciências da Saúde, PUC-RS. 2009.
- 22 Miura D. Desenvolvimento farmacotécnico e estudo de estabilidade de géis de papaína destinados ao tratamento de feridas. Dissertação (Mestrado). Programa de Pós-Graduação em Ciências Aplicadas a Produtos para Saúde. Faculdade de Farmácia. Universidade Federal Fluminense. 2012.
- 23 Young-Chu, S. et al. Stabilization of papain and lysozyme for application to cosmetic products. Biotechnology Letters. 2000; 22:137-40.
- 24 Aulton ME. Delineamento de Formas Farmacêuticas. 2ed. Porto Alegre: Artmed. 2005.
- 25 Florence AT, Atwood D. Princípios Físico-químicos em Farmácia. 2ª.ed. São Paulo: Pharmabooks. 2011.
- 26 Tiwari AK, Sowmiya SM, Saha SK. Study on premicellar and micellar aggregates of gemini surfactants with hydroxyl substituted spacers in aqueous solution using a probe showing TICT fluorescence properties. J. of Photochemistry and Photobiology A: Chem. 2011 ; 223:6-13.
- 27 Guzmán NM, Fernández JF, Parada M, Orbegozo C, Rodríguez A, Padrón A. Efecto del catión, del anión y del co-ión sobre la agregación de líquidos iónicos en solución acuosa. Quim. Nova. 2010 ; 33,8:1703-8.
- 28 Guerra JPVTA. Coacervação em sistemas aquosos contendo xantana, poli(etilenoimina) e dodecil sulfato de sódio. Trabalho de conclusão de curso (Graduação em Química), Centro de Ciências Físicas e Matemáticas - Departamento de Química. Universidade Federal de Santa Catarina, Florianópolis. 2008.