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AGGREGATES OF ALTERNATE AMPHIPHILC POLYANION TO CARRY ZWITTERIONIC DRUG IN AQUEOUS MEDIA

Constain Salamanca *, Andrés Quintero, Diego Pineda and Andrés Andrade

Natural Research Group, Faculty of Natural Sciences, Universidad ICESI-Colombia

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Correspondence to Author:
Dr. Constain Salamanca

Associate Professor, Laboratory of Pharmaceutical Physicochemical, Pharmaceutical Chemistry Program, Faculty of Natural Sciences, Universidad ICESI. Calle 18 # 122-135, Cali- Colombia

E-mail: chsalamanca@icesi.edu.co

ABSTRACT: The effect of amphiphilic polyanion Poly(maleic acid-*alt*-octadecene)sodium salt on solubility of a zwitterionic drug (ampicillin trihydrate) in aqueous media was evaluated. This polymeric salt was obtained by an equimolar reaction of parent copolymer poly Poly(maleic acid-*alt*-octadecene)with an aqueous solution of NaOH. The obtained amphiphilic polyanion was purified by dialysis and lyophilization and was characterized by FTIR spectroscopy. Secondly, a method for the model drug quantification by UV spectrophotometry at 37°C was standardized. Also the grade of association drug-polymer in aqueous media using ultrafiltration methodology at three levels of temperature (20, 30 and 40°C) and two levels of pH (1.2 and 7.4) was determined. Finally, the variation of aqueous solubility of ampicillin trihydrate in ultra-pure water and two buffer media with pH of 1.2 and 7.4 with an ionic strength of 0.15 M at 37°C in presence and absence of amphiphilic polyanion was evaluated. It was found that maximum solubility is reached at a pH value of 1.2 in absence of polymeric material with a value of 36 mg/mL, which diminishes dramatically to a value of 8.3mg/mL when the polyanion is added. In the other side, when the system has a pH value of 7.4, the drug solubility slightly increases from 13 to 16mg/mL in presence of polymeric material, describing that solubility of this kind of drugs is strongly influenced by aqueous media and this type of polymeric materials.

INTRODUCTION: Nowadays, use of polymeric materials as specialized excipients for design and formulation of pharmaceutical products is more usual, looking for new and better physicochemical characteristics to active pharmaceutical ingredients (APIs), such as improvements in aqueous solubility [1, 2], permeability modulation [3, 4], modifications in dissolution profiles [5, 6], and looking for extend their chemical stability against environment factors that may generate degradation.

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In this respect, polymeric materials with biocompatibility and that can be solubilized in aqueous media forming functional structures and improve physicochemical characteristics in APIs, represent a big potential as new pharmaceutical excipients. One of this polymeric materials corresponds to poly (maleic acid-*alt*-octadecene) sodium salt, denominated as PAM-18Na [7, 8], which is an amphiphilic polyanion with the ability to pass from an ionized form to a neutral one depending on pH of the medium, as is presented in **Fig.1**.

These ionization changes in PAM-18Na in an aqueous medium leads to a variation in characteristics of the polymeric material, where it could be completely solubilized, forming hydrophobic intramolecular aggregates, in a similar process that occurs in the aggregation of surfactant molecules, or could be as insoluble material when

is in its neutral form. Both situations may significantly affect the aqueous solubility of a third component added to this same mixing system. For example, when the polymer is forming hydrophobic intramolecular aggregates, this may improve the solubility of the third component for association process directly with the polymeric aggregate, serving as a drug carrier system [9, 10].

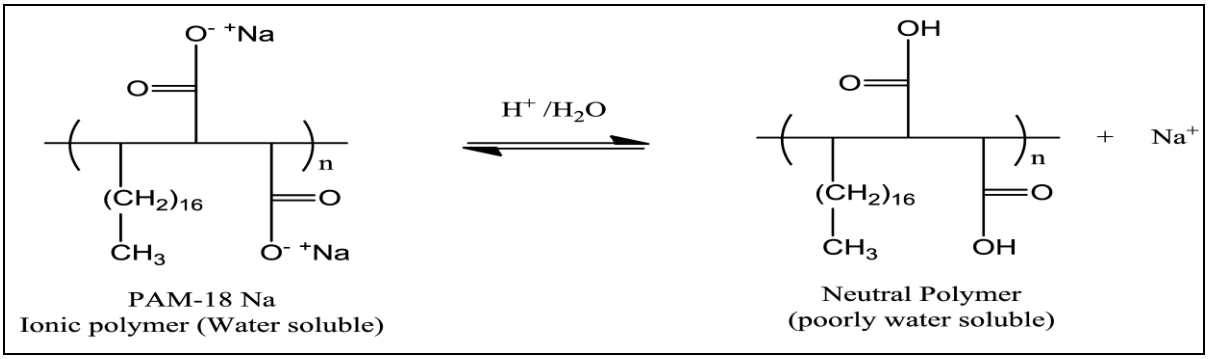

FIG. 1: STRUCTURE OF PAM-18Na AND THE PROCESS OF IONIZATION EN AQUEOUS MEDIA

Furthermore, when the material is in a neutral form, this can compete with the third component by solvating effect leading to a decrease in solubility of the component. This process is very important to know in the design stages of pharmaceutical formulations, where polymeric materials, ionic or hydrophobically modified, are used with other ingredients (IFAs or other excipients), which can affect the physicochemical and biopharmaceutical properties of the latter ones. Thus, this study focused on evaluating the incidence of the amphiphilic material PAM-18Na on the solubility of a zwitterionic molecule model (ampicillin trihydrate) in ultra-pure water and two buffer media at pH 1.2 and 7.4 with a constant ionic strength of 0.15 M at 37°C.

MATERIALS Y METHODS:

Materials:

Poly (maleic anhydride-*alt*-octadecene), called as PAM-18, with an average Mn of 30000-50000 was acquired on Sigma-Aldrich®. Ampicillin trihydrate Fersinsa Gb® was provided by laboratories Tecnoquímicas S.A. from Colombia. NaOH, HCl, KCl, KH_2PO_4 and K_2HPO_4 were purchased from Merck®, each of these was used as received. Ultra-pure water was used obtained by a purification system Elix Essential Millipore®, with mean values of pH of 5.5 and conductivity of 1 μS/cm.

Obtention of the amphiphilic polyanion:

The amphiphilic polyanion PAM-18Na was obtained from a commercial copolymer of Poly(maleic anhydride-*alt*-octadecene), referred to as PAM-18, which was reacted with an aqueous solution of NaOH at a slightly higher amount than the molar equivalent amount given between the carboxylic acid groups present in the polymer structure, after the process of the maleic anhydridering opening. This modification was carried out at room temperature for a time of 24 hours with moderate agitation. Subsequently, the polymer solution was dialyzed using cellulose membranes by Sigma Chemical Co® and lyophilized on an Eyela®Frezeer Dryer model FDU 1110.

Description of the crystal habit of study materials:

Due to the solubility of a compound depends significantly on the crystal habit [11], we proceeded to describe the external morphology that presented the study materials (ampicillin trihydrate, PAM-18 and PAM-18Na). For this, photographs 600x were taken by a SEM Phenom G2, where it was found that the ampicillin trihydrate presents morphology needles, while the PAM-18 and PAM-18Nahave rhombic and plate forms, respectively **Fig. 2**

Preparation of buffer systems:

The buffer solutions with values of pH 1.2 and pH 7.4 with ionic strength of 0.15 M were prepared using mixtures of HCl/KCl, and KH_2PO_4/K_2HPO_4, respectively. The adjusting on the ionic strength was performed with KCl following the methods described in the United States Pharmacopeia (USP 35-NF 30, 2012)[12].

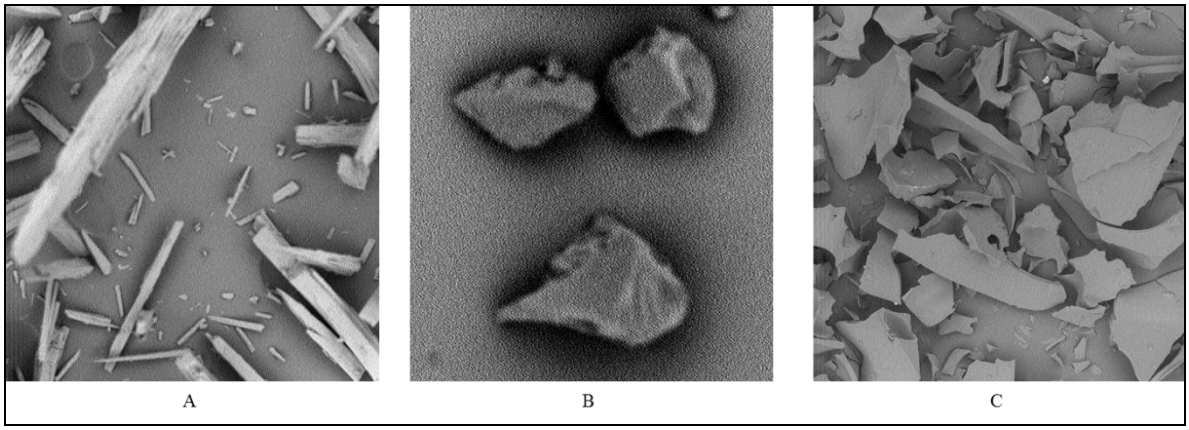

FIG. 2: SEM PHOTOGRAPHS OF: A. AMPICILLIN TRIHYDRATE. B. PAM-18. C. PAM-18Na.

Methods:

Ampicillin trihydrate quantification by UV spectrophotometry:

Ampicillin trihydrate in different media (ultra-pure water, buffer pH 7.4 and 1.2) were quantified by UV spectrophotometry at 234 nm, using a computer Shimadzu® coupled to a temperature control system, where each assay was performed at 37°C. Standardization parameters obtained were: selectivity, repeatability of equipment, repeatability of the method and linearity. For this last parameter, were prepared an ampicillin trihydrate serial solutions with concentrations between 0,1-0,7mg/mL in triplicate for each mean, where linear relationships between absorbance and concentration were found according to the Lambert Beer model (ultra-pure water: Y = 0,004 + 0,842X with R^2 = 0,999, pH 7,4 buffer solution: Y = 0,015 + 0,747X with R^2 = 0,999 and pH 1,2 buffer solution: Y = 0,017 + 0,831X with R^2 = 1,000), where Y = molar absorbance and X = the molar concentration of ampicillin trihydrate.

Association polymer-drug in aqueous media:

The percentage of association of the PAM-18Na and ampicillin trihydrate in aqueous medium was carried out with an Amicon® ultrafiltration cell 202 modified for testing. The cell had a membrane PES NMWL of 10 kDa and was immersed in a water bath. Each test was performed with dilute concentrations of ampicillin trihydrate (5-45 mg/L) in two buffer solutions pH 7.4 and 1.2 at various temperatures (20, 30 and 40 °C) and containing the amphiphilic polyanion completely dissolved at fixed concentration of 45mg/L. Quantification of the drug was performed using the same UV spectroscopy equipment, but because of the low drug concentrations, wavelength of 204 nm was used for quantitation of the same, after verification of the selectivity and sensitivity of the method. Finally the percentage of polymer-drug association (% A) was obtained by the equation 1:

$$\%A = \left(\frac{C_t - C_w}{C_t} \right) \times 100 \qquad \text{Eq. 1.}$$

where C_t corresponds to the total molar concentration of drug added to the ultrafiltration system and C_w is the molar concentration of the ultra-filtrated drug .

Solubility assays:

An orbital shaking methodology for solubility assays was standardized, where a shaker Heidolph®-Unimax 1010 coupled to a thermostat system for incubation was used. For each test, mixtures of 10.0mL of ampicillin trihydrate and ultra-pure water were prepared in various proportions ranging from 2 to 20mg/mL at 37 °C, with a constant agitation rate of 350 rpm and a maximum test length of 45 minutes, in order to avoid the effect of chemical hydrolysis of ampicillin trihydrate [13].

Then an aliquot was taken and filtered through cellulose acetate membranes of 0.45 micron Millipore® and it absorbance was determined at 37°C, to subsequently be compared relative to a calibration curve. Once the methodology was standardized, the solubility of the zwitterionic drug in aqueous and buffer media with and without the PAM-18Na was determined, as previously

described. For assays in the presence of amphiphilic polyanion, a weight ratio of polymer:drug 1:1 was used, wherein the amount of ampicillin trihydrate corresponded to the value obtained in the solubility test in absence of polymer. Finally, each test was performed in triplicate.

Data processing and analysis:

The data was statistically analyzed using analysis of variance (ANOVA) of two factors, corresponding to the media (ultra-pure water, buffer pH 7.4 and buffer pH 1.2) and the absence and presence of the polymeric material, with a percentage of 95% confidence using the computer program Microsoft Excel for each experimental condition evaluated.

RESSULTS AND DISCUSSION:

Obtention of amphiphilic polyanion

The amphiphilic polyanion PAM-18Na was obtained from the PAM-18 according to the reaction process that is presented in **Fig.3**

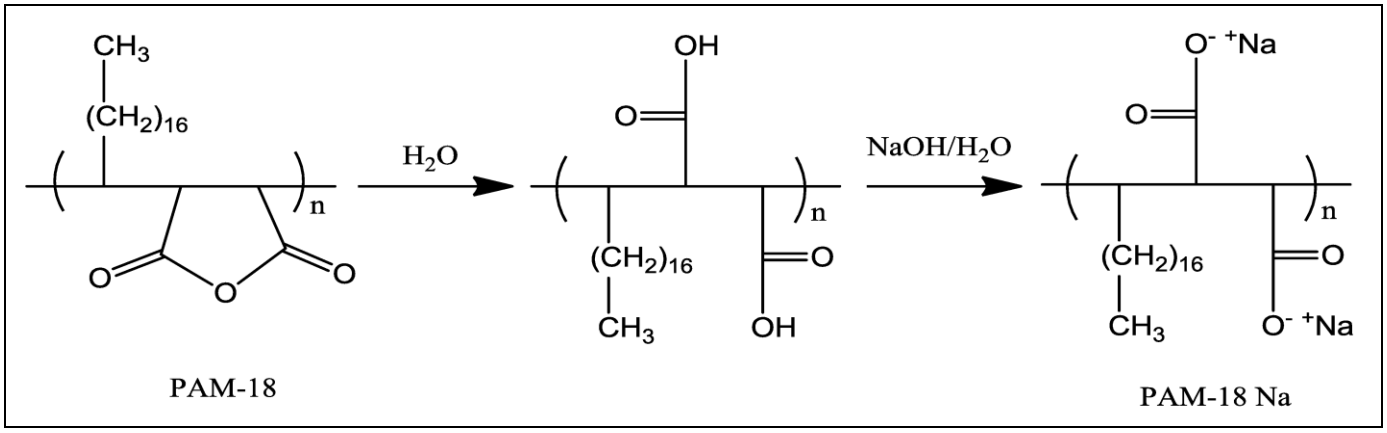

FIG. 3: OBTENTION OF PAM-18Na.

Amphiphilicpolyanion formation was evidenced by a qualitative variation of the solubility of the polymer, which changed from heterogeneous mixture state to a completely homogeneous aqueous light yellow solution. This change is given by opening of the maleic anhydride ring in the PAM-18 and leads to the formation of two carboxylic acid groups, which then are converted to carboxylates. This process was observed by comparing the FTIR spectra of PAM-18 and PAM-18Na, as shown in **Fig.4**.

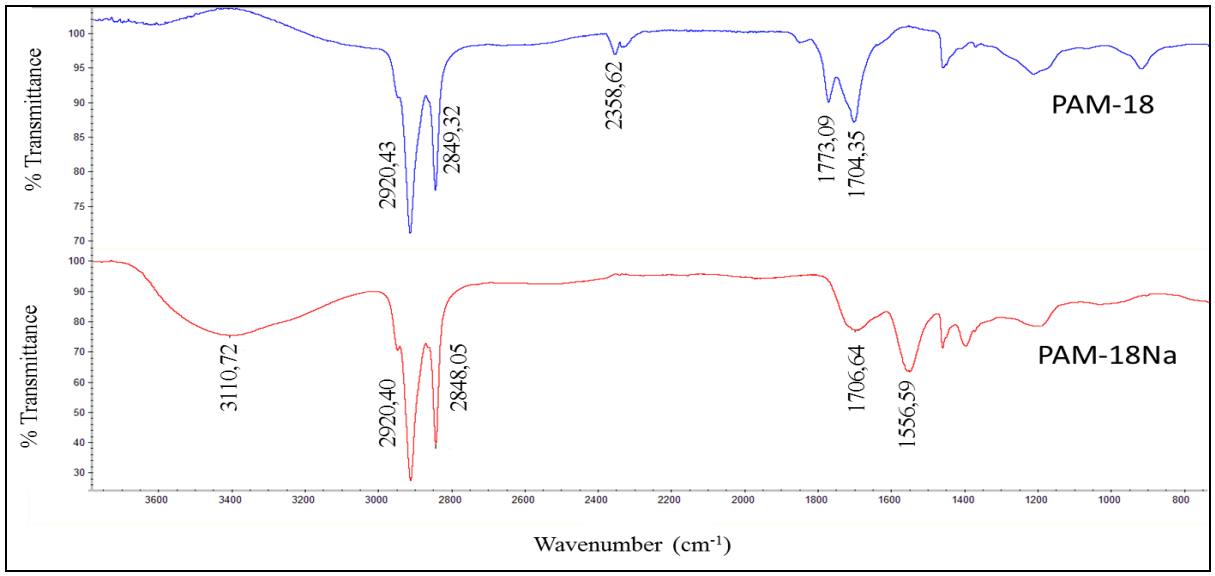

FIG.4: IR SPECTRUM OF POLYMERIC MATERIALS PAM-18 AND PAM-18Na.

The IR spectra showed characteristic signals at both the starting material (PAM-18) and the polyanion formed (PAM-18Na), corresponding to the symmetric and asymmetric stretching of the CH bonds at 2920 and 2848 cm^{-1}. Also the disappearance of the signal at 2358 cm^{-1} was observed in the PAM-18, corresponding to the opening of the maleic anhydride ring. A variation on the two signals of the carbonyl groups of maleic anhydride, 1773 and 1704cm^{-1} at 1706 and 1556 cm^{-1} was also observed when the carboxylate species are formed. Finally, the appearance of a band at 3401 $cm^{-1}$was observed, indicating the presence of a hydroxyl group, from the formation of carboxylic acid groups indicating that the ionization process from PAM-18 toPAM-18Na is not full, as expected.

Association polymer-drug in aqueous media:

The association of ampicillin trihydrate and amphiphilic polyanion PAM-18Na in buffer solution of pH 1.2 and 7.4, at 20, 30 and 40 °C of temperature showed association profiles similar to those described in Langmuir adsorption isotherms type I, where high rates of association were reached in all cases tested **Fig. 5**.

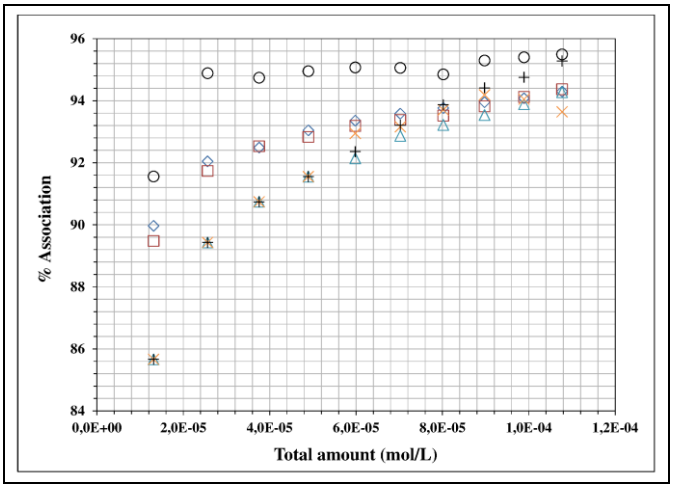

FIG.5: ASSOCIATION PERCENTAGE BETWEEN AMPICILLIN TRIHYDRATE AND PAM-18Na IN AQUEOUS MEDIA TO DIFFERENT MEDIUMS: (◇) Buffer pH 1.2 at 20°C, (□) Buffer pH 1.2 at 30°C, (○) Buffer pH 1.2 at 40°C, (△) Buffer pH 7.4 at 20°C (×) Buffer pH 7.4 at 30°C, (+) Buffer pH 7.4 at 40°C.

Table 1 summarizes the values of association between polymer PAM-18Na and ampicillin trihydrate at a fixed polymer concentration of 25 mg/L, two pH values (1.2 and 7.4) and different temperatures (20, 30 and 40 ° C).

TABLE 1: AVERAGE POLYMER-DRUG ASSOCIATION PERCENTAGE (%A)

Media	T(°C)	Average(%A)	SD
Buffer pH: 7,4	20	91,7	1,0
	30	90,6	0,7
	40	92,2	0,8
Buffer pH: 1,2	20	93,1	0,8
	30	92,9	0,7
	40	94,7	0,6

T: temperature, SD: standard deviation.

The **Table 1** shows that there is a high association between the polymer PAM-18Na and ampicillin trihydrate in the two buffer solutions evaluated, being slightly higher when the system is at a pH value of 1.2. Furthermore, it was found that the temperature does not significantly affect the rate of association in the evaluated conditions.

These results can be explained due to the formation of hydrophobic aggregates generated by alkyl side chains of PAM-18Na in the aqueous medium, which form a nonpolar pseudo-phase, which causes the drug to spontaneously migrate from the aqueous phase to this hydrophobic aggregate, thus, this polymeric material can act as an amphiphilic drug carrier system, indifferent to pH of medium, as previously described for the same polymer system with other organic substrates [8, 14].

However, it should be noted that this only take place in a dilute concentration regime, where the polymer is fully dissolved at any pH value, and in the case where the concentration begins to increase, thermodynamic equilibrium phenomena will begin to occur between the polymer in solid state and in dissolved state, as previously described in **Fig.1**. Thus, in a dilute concentration for both the drug and the polymer, high values of association rate will exist, indifferent to temperature and pH of the medium.

Solubility assays:

The concentration of ampicillin trihydrate dissolved in relation to the total amount added in the three media (ultra-pure water, buffer solutions of pH 7.4 and 1.2) with and without amphiphilic polyanion at 37°c using an orbital shaker at 350rpm is shown in **Fig.6.**

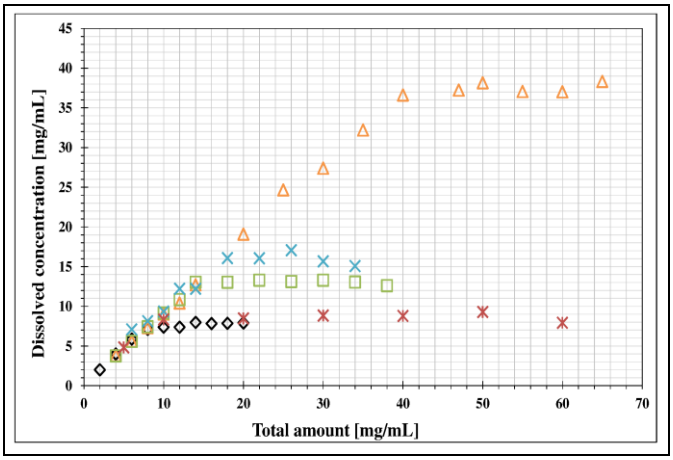

FIG.6: DISSOLVED CONCENTRATION VS.TOTAL AMOUNT OF AMPICILLINE TRIHYDRATE IN DIFFERENT AQUEOUS MEDIA. (◇) Ultra-pure water, (△) buffer solution of pH 1.2 without PAM-18Na, (✳) buffer solution of pH 1.2 with PAM-18Na, (□) buffer solution of pH 7,4 with PAM-18Na, (×) buffer solution of pH 7,4 with PAM-18Na.

Fig. 6 shows typical saturation profiles in different aqueous media, where the ampicillin trihydrate maximum is reached at a pH value of 1.2. It is also observed that the amphiphilic polyanion dramatically decreases this value. Furthermore, when the system is at pH 7.4, the solubility is slightly increased in the presence of the polymeric salt. **Table 2** summarizes the mean values of the amount of ampicillin trihydrate dissolved in the buffer solutions in the presence and absence of the PAM-18Na at 37°C, using an orbital shaker at 350rmp, with their respective standard deviation.

TABLE 2: DISSOLVED CONCENTRATION OF AMPICILLIN ON DIFFERENT AQUEOUS MEDIUM WITH AND WITHOUT PAM-18Na.

Medium	Average dissolved concentration of ampicillin (mg/mL)	Standard deviation
H_2O	7.1	0.1
Buffer pH:1,2	37.0	0.5
Buffer pH:1.2 + PAM-18Na	8.3	0.1
Buffer pH: 7,4	13.0	0.1
Buffer pH: 7.4 + PAM-18Na	16.0	0.5

The analysis of variance of two factors showed significant differences between the solubility of ampicillin trihydrate, respect to the type of medium and the absence and presence of the amphiphilic polyanion. For the initial case, when the ampicillin mg/mL was observed. At this pH, both ampicillin and the PAM-18Na are ionized by the acid trihydrate was mixed only with ultra-pure water, it was found that the value of the solubility (intrinsic solubility) obtained is very similar to that previously reported for this drug under similar conditions with an average value of 7.1mg/mL[15,16].

It is also noted that the drug solubility is increased significantly in the media with pH 1.2 and slightly in the medium of pH 7.4, which can be explained according to the zwitterionic nature of the ampicillin trihydrate, the theory of molecular interactions solute-solvent[17]and the degree of dissociation of the drug α. Because ampicillin trihydrate has two pK_a values, 2.65 (carboxylic acid group) and 7.24 (amino group), when it is in ultra-pure water, a proximity will exist between the isoelectric point of the drug (pK'= 5.0) and the pH of the aqueous medium (pH = 5.5), which according to the Henderson Hasselbalch theory[18], the presence of ionic species for neutral species will be very low and thus the solvation effect of ampicillin will depend mainly on the solute-solvent interactions of hydrogen bonds.

Furthermore, when the zwitterionic drug is in the buffer solutions of pH 7.4 and 1.2, there will be a considerable increase in the degree of ionization, leading to form attractive interactions (ion-dipole) with the aqueous medium and with this a major effect of solvation, increasing the solubility of the ampicillin in the medium. For the case where the amphiphilic polyanion is added to the mixing system in an amount 1:1 to the value of the intrinsic solubility of the ampicillin trihydrate at 37 ° C, two opposite effects are observed with respect to pH. In the case of buffer solution pH 1.2, there is a significant decrease in drug solubility pass from 37.0 mg/mL to 8.4 mg/mL.

At this pH, ampicillin will be strongly ionized by the amino group as previously explained, while the polymeric material will be in a neutral form at a concentrated system, where it will be in thermodynamic equilibrium between the solid phase and solution phase, generating competition for solvation effect with ampicillin. Whereas at pH 7.4, a slight increase in the solubility of zwitterionic drug passing from 13.0 mg/mL to 17.0

carboxylic groups, leading to both are completely solubilized in the medium. Thus, the dissolved

polymer may generate polymer hydrophobic aggregates, which can be carried or associated with ampicillin trihydrate and thus enhance their solubility.

CONCLUSION: The results show that the pH of the medium greatly affects the solubility of the zwitterionic molecule, increasing the solubility when it is in media with pH 7.4 and 1.2. For the case of amphiphilic polymeric material and depending its amount, this may be in a dissolved or a precipitated form, which strongly condition ampicillin solubility in the mixing system. Thus, when the polymeric material is added to a pH value of 1.2 in a high concentration, the amphiphilic polyanion adversely affect the aqueous solubility of ampicillin trihydrate. While a pH value of 7.4, the polymer will be fully dissolved to form hydrophobic aggregates, which can be carried with the drug and thereby increase its solubility. Finally it is necessary to conduct more studies focused on evaluating changes in the solubility of these compounds with amphiphilic ionic polymeric materials from a dilute regime until a concentrated regime, since the proportion of polymer shows that may significantly affect the solubility of this zwitterionic type molecules in aqueous media.

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