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Porous PVA microspheres for Oxytetracycline delivery

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Abstract:*Porous PVA microspheres were prepared to investigate their adsorption and release capacity of oxytetracycline (OTC) in simulated biological fluid (SBF). The highest adsorption capacity of OTC during 2hours was obtained at pH=4, being 0.9 mg* g^{-1}*. The release profile of OTC in SBF from the microspheres showed a burst effect with a cumulative release of 29±4 % within the first thirty minutes due to the immediate release of the OTC adsorbed on the surface of the microspheres, followed by a slower release reaching 41±5% within two hours,which in terms of concentration is equivalent to 4.2 µg/mL, a value close to the minimum inhibitory concentration of pathogens susceptible to OTC in injectable administration methods of therapeutic doses from 10 mg/Kg for farm animals.*

Keywords:*Polyvinyl alcohol, Microspheres, Oxytetracycline, Drug delivery, Biopolymers.*

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I. INTRODUCTION

Controlled drug release systems are widely used in the treatment of different diseases. This technique allows local, controlled and prolonged release of the drug at the site of interest, reducing systemic toxicity (Adepu S. et al, 2021; Debjit Bhowmik et al., 2012).

Among the materials used in drug delivery, PVA microspheres stand out since they have characteristics such as atoxicity and biocompatibility. Furthermore, the versatility of the chemical structure of PVA allows the incorporation of functional groups that give it the ability to capture/release molecules and chemotherapeutic drugs used mainly in oncological treatments (Verón et al., 2021; Verón et al., 2022; Biondi et al., 2012; Heaysman et al., 2016; Chen et al., 1992).

Although this characteristic is widely used for the capture and release of oncological drugs it can be extrapolated to the release of other types of drugs (Sullad et al., 2010; Sulled et al., 2011). In this sense, in the current work, we will focus on Oxytetracycline (OTC), a bacteriostatic antibiotic from the tetracycline group used worldwide in veterinary medicine (Lin et al., 2015; Zhenxing Chi et al., 2014)). This antibiotic presents a wide spectrum, including gram-positive and gram-negative, aerobic and anaerobic species, spirochetes, mycoplasmas, chlamydiae, rickettsiae, and some large viruses (Aktas et al., 2017).

In the case of treatment of infectious diseases in farm animals and poultry, the administration of OTC is commonly carried out either orally, through feed and drinking water, or parenterally by intramuscular injection (Aguiar et al., 1985; Aktas et al., 2017). These forms of administration bring with them certain drawbacks such as the loss of effectiveness of the OTC due to front gut flora after oral administration (Aktas et al., 2017), irritability caused by OTC solvents for parenteral administration of OTC, and in the context of the farm animal, a treatment regimen that requires repeated injections, is time consuming, labor-intensive, expensive, and can be traumatic to the animal (Aguiar et al., 1985; Moretto et al., 2004). For these reasons, slow release systems are of interest in veterinary medicine.

In this work, the incipient results of the capacity of porous PVA microspheres for the capture and release of Oxytetracycline in SBF are presented.

II. EXPERIMENTAL PROCEDURE

1.1 Reagents used in the microspheres synthesis and SBF preparation

The following reagents were used to prepare the porous PVA microspheres: 2 acrylamido-2methylpropane sulfonic acid sodium salt solution 50% (AMPS solution), benzoyl peroxide (BPO, 97%), poly /vinyl alcohol) (PVA, Mw 578 kg/mol, 85% hydrolyzed) and vinyl acetate (VAc, 99%, 3-20 ppm of hydroquinone as inhibitor) were purchased from Sigma-aldrich, methanol (MeOH) was supplied by Dorwill, sodium hydroxide (NaOH, 99%) and hydrochloric acid (HCl, 36.5-38%) were obtained from Cicarelli Laboratories. The SBF was prepared by the Kokubo protocol (Kokubo et al., 2006) using the following

reagents: sodium chloride (NaCl, 99.9%), sodium sulfate (Na_2SO_4, 99.5%) and calcium chloride ($CaCl_2$, 99.9%) were supplied by Mallinckrodt. Sodium hydrogen carbonate ($NaHCO_3$, 99.9%) and potassium chloride KCl (KCl, 99.9%) by Sigma-Aldrich, dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$, 99.5%) supplied by Berna reagents and Tris hydroxymethyl aminomethane (TRIS 99.8-100.1%) by Merck. Oxytetracycline chlorhydrate (OTC) was supplied by the Instituto Nacional de Tecnología Agropecuaria (INTA). Reagents were used without prior purification.

1.2 Synthesis of porous PVA microspheres

The microsperes were synthetized by a two-step procedure that we have previously reported (Verón et al., 2021; Verón et al., 2022).

The first stage consists of the free radical copolymerization of VAc and AMPS, resulting in porous poly(VAc-co-AMPS) microspheres. The second stage aims to replace the acetate groups in the poly(VAc-co-AMPS) microspheres with hydroxyl groups, generating an external PVA layer wich interacts with the drug and the surrounding medium. Table 1 summarizes the synthesis conditions.

Table1Synthesis conditions

Synthesis stage	Reagent	Reagent amount	Synthesis parameters
Suspension Polymerization	PVA	0.04 g	Temperature: 70 °C
	Deionized water	50 mL	Stirring rate: 30 rpm
	VAc	24 mL	Atmosphere: Nitrogen
	Mixture: AMPS/Methanol	8 mL/12 mL	Total reaction time: 120 minutes
	BPO	0.5 g	
	0.1 M NaOH	amount needed to adjust the pH of solution	
	0.1 N HCl	amount needed to adjust the pH of solution	
Saponification	2M NaOH	25 mL	Temperature: 30 °C Stirring rate: 30 rpm Reaction time: 120 minutes

1.3 Microspheres characterization

The morphology and surface of the microspheres were examined by Scanning Electron Microscopy (SEM), using a Zeiss Crossbeam 340 SEM-FIB equipment. In order to improve the quality of the images, the samples were coated with a thin layer of gold using a Blazers Sutter Coater SCD 050. To determine the chemical composition of the micropsheres, the samples were coated with carbon.

As a complementary technique for determining the chemical composition of the mirospheres, the Particle Induced X-ray Emission (PIXE) technique was used. The measurements were performed in a 1.7 MV tandem ion accelerator 5SDH Pelletron NEC. For this, tablets of 5 mm diameter and 2 mm thickness were prepared. They were fixed to an aluminum support and kept under vacuum for 30 minutes. A 3.0 MeV H^+ particle beam struck the sample surface perpendicularly and the emitted X-rays were collected by a Sirius SD detector (Peltier-cooled silicon detector). The spectral analysis was performed with the GUPIX software (Verón et al., 2022; Limandri et al., 2014).

The chemical structure was analyzed by Fourier Transform Infrared Spectroscopy (FTIR) using a Perkin Elmer Spectrum 400 FTIR spectrometer. The spectra were obtained in the wave number range from 3500 to 450 cm-1 during 30 scans and 4 cm-1 resolution.

X-ray diffraction was performed using an XRD Bruker D8 Advance diffractometer, using CuKα radiation (λ= 1.54 Å), 40 kV/40 mA beam voltaje/current, 2θ from 10° to 90°, with a step width of 0.0205°.

The glass transition temperature (Tg) of the microspheres was determined by differential scanning calorimetry (DSC) using a TA Instruments Q2000 DSC. About 10 mg of microspheres were placed in aluminum capsules and sealed. These capsules were placed in the equipment, cooled and stabilized for 5 minutes at -20 °C, and then scanned from -20 to 140 °C at 10 °C min^{-1}. This value was used to calculate the degree of hydrolysis of the microspheres obtained after the saponification process, using the equation1.

Equation 1: Fox equation to determine the Tg of porous PVA microspheres.

$$\frac{1}{Tg} = \sum \frac{\alpha_i}{Tg_i}$$

where Tg is the glass transition temperature of the PVA microspheres obtained at the end of the synthesis process, and Tg_i the theoretical glass transition temperature of each of the components of the copolymer, α_i is the mass fraction of component i in the copolymer particles (Verón et al, 2021; Verón et al, 2022; Peixoto et al, 2011).

1.4 Oxytetracycline adsorption

The adsorption capacity of OTC by the microspheres was investigated using the batch equilibrium tecnique. 0.2 g of porous PVA microspheres were placed in conical tubes and contacted with 10 mL of an aqueous OTC

solution with a concentration of 40 ppm. To investigate the effect of pH on this process, the OTC solution was adjusted to pH 3, 4, 6.5 and 10 using NaOH 1M and HCl 0.1N. The samples were shaken at 350 rpm for 2 hours at room temperature and in the dark to avoid drug decomposition. After that time, adsorbent and supernatant were separated by centrifugation. The concentration of OTC in the supernatant was analyzed by UV-Vis spectroscopy monitoring absorbance changes at 360 nm, using a PG instruments T60 UV-Vis spectrophotometer.
The amount of OTC adsorbed per gram of adsorbent (Q_e) was calculated using equation 2.
Equation 2:

$$Q_e = \frac{V_m}{m}\,(C_0 - C_e)$$

whereC_0 and C_e (mg L^{-1}) are the initial and equilibrium concentrations of dye solution, respectively; V_m is the volume of dye solution and m is the weight of sulfonated PVA microspheres. All samples were prepared and measured in duplicate.

1.5In vitro release of OTC into SBF solution

The elution profile of OTC from the sulfonated PVA microspheres was evaluated in SBF solution at 37 °C. 1000 mL of SBF were prepared according to the Kokubo protocol (Kokubo et al., 2006) and 10 mL of SBF solution were contacted with 0.2 g of OTC loaded microspheres at bidestilated water pH (pH= 5.5). The samples were kept stirring for 22 hours at 37 ºC, and OTC release was measured at different time intervals between 0.5 and 3 hours by UV-Vis spectroscopy at 360 nm.
The percentage of OTC released over time was determined using the equation 3.
Equation 3:

$$OTC released(\%) = \frac{mass of OTC released in SBF solution}{mass of OTC adsorbed in the microspheres} \times 100$$

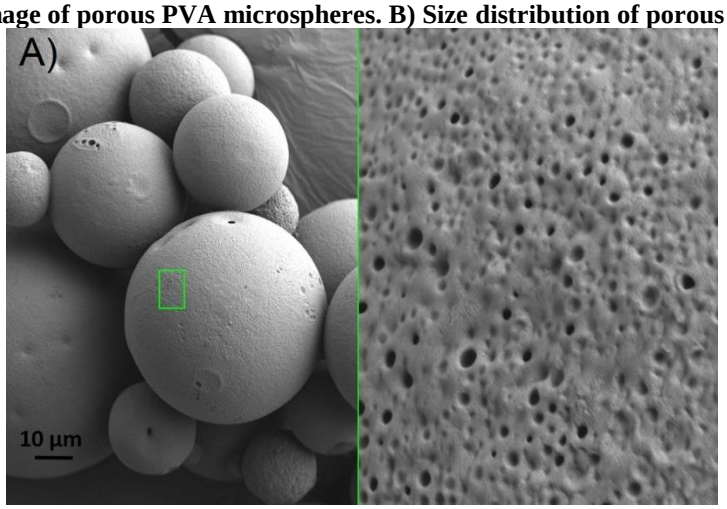
In vitro drug release tests were performed in duplicate.

III. RESULTS AND DISCUSSIONS

3.1. Synthesis and characterization of porous PVA microspheres

Porous microspheres were obtained with a size distribution in the range of 8 to 124 µm, and an averge diameterof 37 µm as seen in Figures 1A and 1B.

Figure1. A) SEM image of porous PVA microspheres. B) Size distribution of porous PVA microspheres.



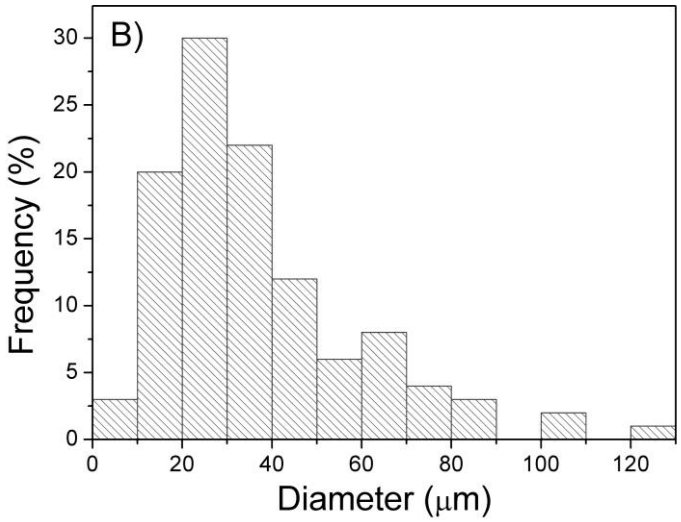
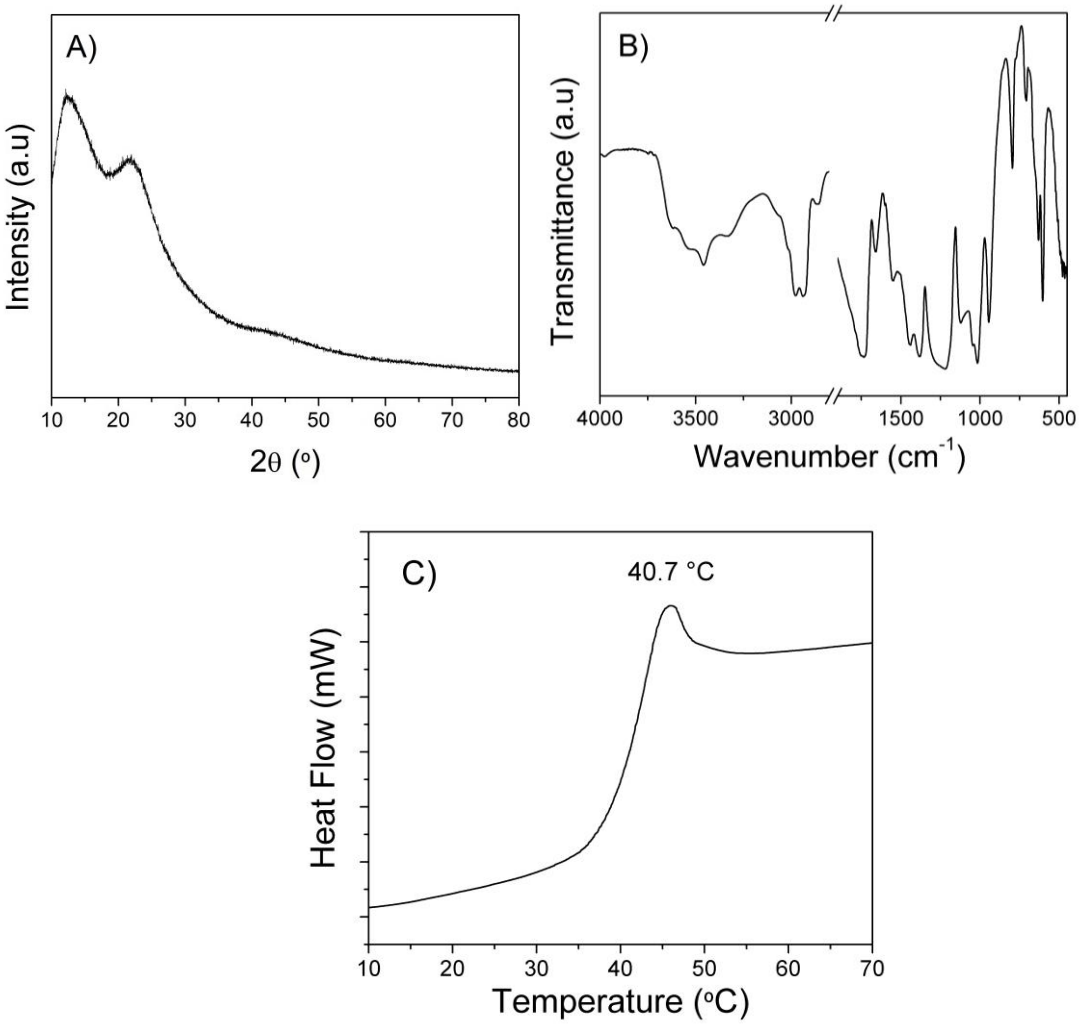


Figure 2A shows the X-ray diffraction pattern of the microspheres. The two broad peaks in the region of 2θ= 10-30º demonstrate the amorphous nature of the polymer matrix (Verón et al, 2021; Verón et al., 2022).

Figure2. A) X-ray diffraction. B) FTIR spectrum and C) DSC curve of porous PVA microspheres.



The infrared spectrum shows the characteristic bands in this type of samples (Figure 2B). The band in the zone between 3150 cm^{-1} and 3700 cm^{-1} comprises the superposition of the N-H and O-H stretching vibrations of AMPS and PVA, respectively (Verón et al., 2021; Verón et al., 2022). The bands at 1550 cm^{-1} and 1650 cm^{-1} correspond to N-H bending (Amide II) and C=O stretching (Amide I) from AMPS (Verón et al., 2021; Verón et al., 2022). In this work, as in previous works, the characteristics bands of the sulfonate groups in the region 1000-1250 cm^{-1}, corresponding to symmetric and asymmetric S-O stretching and C-S stretching around 621 cm^{-1} could not be observed due to the overlap of the PVA-PVAc bands (Verón et al., 2021; Verón et al., 2022).The bands observed between 500 and 1500 cm^{-1}, together with the intense peak at 1740 cm^{-1} (C=O stretching), correspond to the vibration frequencies associated with the acetate groups present in the polymer structure, and which constitute the core of the microspheres (Verón et al., 2021; Verón et al., 2022; Ambrosio et al., 2022).

The outer layer of the microspheres is made up of PVA. To estimate the thickness of said layer, the degree of hydrolysis was determined using equation 1 and the Tg measured by DSC (Figure 2C).

The mass fraction of PVA, α, was 0.09. This means that the microspheres are coated with 9% PVA, and are made up of a large PVAc-AMPS core.

The chemical composition of the microspheres determined by EDS is observed in Figure 3. We put main emphasis on this element, since it is what allows us to infer the concentration of AMPS in the microspheres, and what is involved in drug capture. The concentration of S detected was 0.8 ± 0.1 wt%. This result is consistent with that detected by PIXE (Figure 4).

Based on the results obtained by both methods (PIXE and EDS), and using a value of 229.23 atomic mass units for the molar mass of AMPS, we were able to estimate an AMPS content in the microspheres of 5.8 ± 0.1 wt%. This higher concentration of S in the sample, compared to previous results (Verón et al., 2021; Verón et al., 2022) can be attributed to the lower amount of NaOH used in the saponification stage. Which translates into a thinner external PVA layer, as reflected by the DSC and FTIR results.

Figure 3. EDS analysis of porous PVA microspheres.

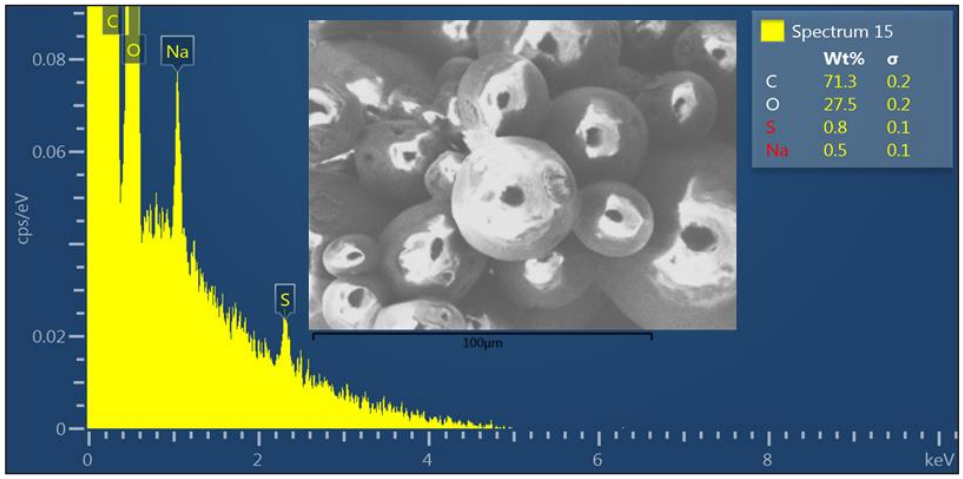
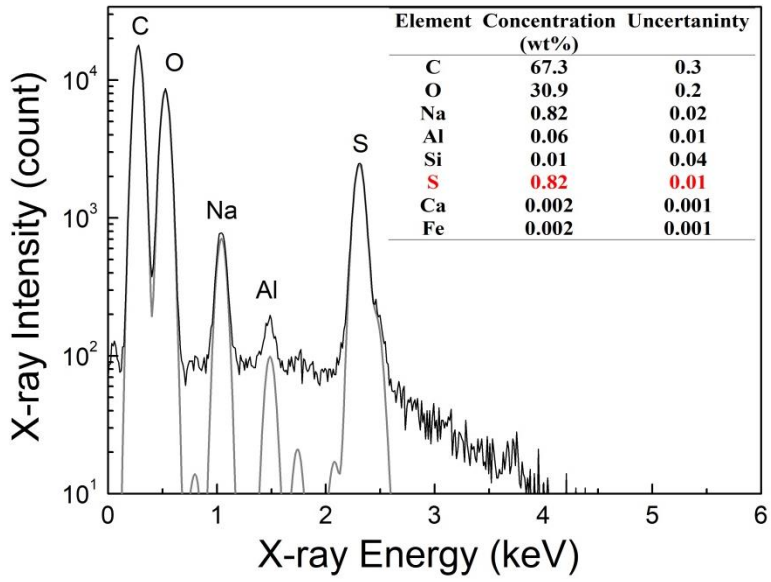


Figure 4. PIXE spectrum and composition of porous PVA microspheres.



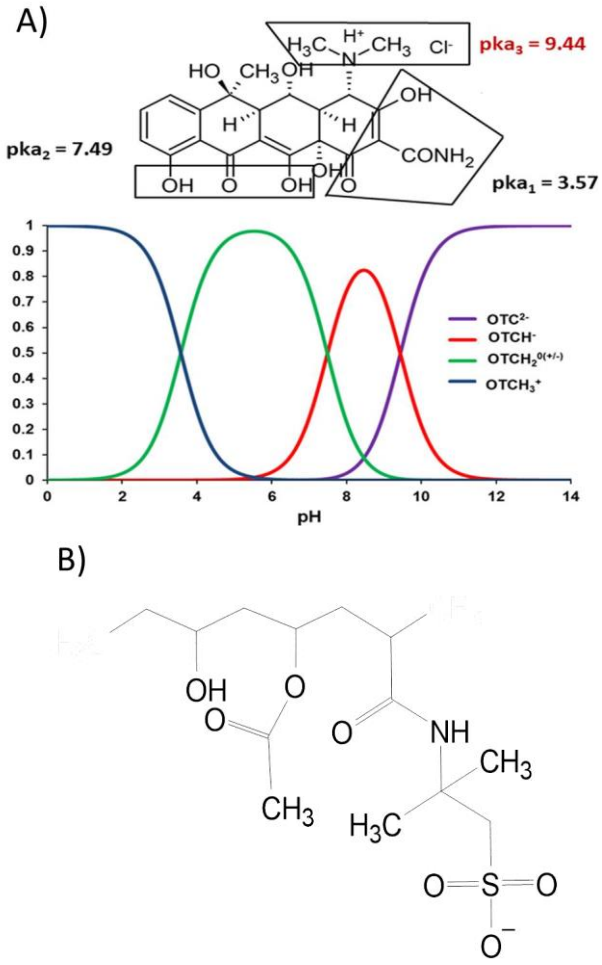
3.2 Oxytetracyclin adsorption and release

Table 2. Oxytetracyclin adsorption onto porous PVA microspheres at 2 hours adsorption.

pH	Q_e (mg g^{-1})
3	0.6
4	0.9
6.5	0.1
10	0.1

Table 2 summarizes the values obtained for Q_e at different pH after two hours of adsorption.
It can be seen that the highest adsorption capacity for two hours was achieved at pH 4 (Q_e = 0.9 mg g^{-1}). At pH 3, 6.5 and 10 Q_e was 0.6, 0.1 and 0.1 mg g^{-1}, respectively. These results are related to the interaction of the functional groups present in the structure of the microspheres with the functional groups in the structure of the OTC (Figure 5A), and their ionization at different pH.
The OTC is an antibiotic from the tetracycline family. Is characterized by a molecular structure comprising a tetra-benzene skeleton, three ionizable groups, and multiple acid dissociation constant. In acidic aqueous solutions (pH< 3.3) OTC assume a cationic form (TCH_3^+). Within the pH range of 3.3 to 7.3 OTC exhibits zwitterionic behavior ($H_2OTC^{\pm}$), transitions to an anionic state at pH range between 7.3 and 9.1 ($HOTC^-$), and adopts a dianionic form at pH > 9.1 (OTC^{2-}) (Figure 5A) (Wang et al., 2013; Ouahiba et al., 2023; Orellana et al., 2014; Ferchichi et al., 2023).

Figure 5. A) Structure of OTC and its distribution at different pH values extracted from Ferchichi et al., 2023. B) Structure of PVA microspheres extracted from Verón et al, 2021.



On the other hand, sulfonated PVA microspheres in the range studied contain negatively charged $-SO_3^-$ groups that allow ionic interaction with positively charged groups of OTC (Verón et al., 2021; Verón et al., 2022). In addition, the microspheres contain pendant groups -OH, $COOCH_3$, and CONH in their structure (Figure 5B), which also intervene in the adsorption process through other types of interactions (weak H-like bonding interactions).

Table 2 shows that the adsorption capacity Q_e for two hours in acidic conditions is greater than in alkaline conditions and at conditions close to neutrality, Q_e (pH 4) >Q_e (pH 3) >Q_e (pH10) >Q_e (pH 6.5) .

At pH 4, the TCH_3^+ and $H_2OTC^{\pm}$ forms of Oxytetracycline coexist, and PVA microspheres contain $-SO_3^-$ groups, this allows an electrostatic attraction between the $-SO_3^-$ and the positively charged groups of the OTC. At pH 3, H^+ ions compete with the positive forms of OTC by interaction with the $-SO_3^-$ groups forming $-SO_3H$, consequently decreasing the electrostatic attraction of OTC. Added to this, the H^+ could hydrate the pendant OH groups in the structure of the microspheres forming OH_2^+. This generates a Coulomb repulsion with the cationic forms of OTC at the pH under study (Verón et al., 2021; Verón et al., 2022). This explains the greater adsorption capacity of the microspheres at pH 4 with respect to the adsorption capacity at pH 3.

The adsorption capacity for two hours at pH 6.5 was only 0.1 mg g^{-1}. In this case, almost all of the OTC is in its zwitterionic form $H_2OTC^{\pm}$ with a neutral net charge and a very small fraction coexists as $OTCH^-$ (Figure 5A). As we mentioned previously, PVA microspheres contain $-SO_3^-$ groups, which can produce an electrostatic attraction for that small cationic fraction of OTC, but at the same time an electrostatic repulsion with the coexisting anionic fractions. Furthermore, some authors (Leal et al., 2018; Tongare et al., 1999) have reported that when OTC is in its neutral or zwitterionic form, it is capable of establishing intermolecular interactions forming aggregates in aqueous solution. This could hinder the diffusion of OTC through the pores of the microspheres, consequently decreasing the adsorption capacity.

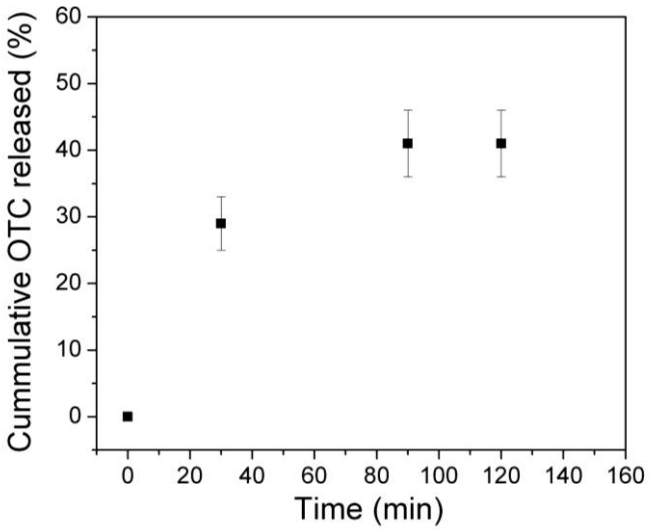
Low adsorption capacity of OTC was also observed under alkaline conditions. At pH 10 the OTC is in the anionic form OTC^{2-}, this generates electrostatic repulsion with the SO_3^- groups present in the microspheres. In addition, as we have mentioned on other occasions (Verón et al., 2021; Verón et al., 2022), the excess of Na^+

in alkaline conditions generates a "charge screening effect" that protects the anionic functional groups and avoids effective positive-negative interactions between adsorbent and adsorbate.

3.3 In vitro OTC release

The release profile of OTC in SBF at 37 °C from the porous PVA microspheres is observed in Figure 6.

Figure 6. Cumulative Oxytetracycline released in SBF at 37 º C.



Within the first 30 minutes, an initial burst occurs in which around 29±4% of the OTC loaded in the PVA microspheres is rapidly released. The release continues gradually reaching a maximum of 41±5% (about 4.2 µg OTC/mL) at 2 hours of release.This behavior could be due in the first instance to the release of the OTC molecules adsorbed on the surface of the microspheres, followed by the slower diffusion of the molecules contained inside the pores after the penetration of the SBF into the structure of the microspheres. The released OTC concentration of 4.2 µg/mL in this work, is comparable to the minimum inhibitory concentration in plasma (MIC) declared by the National Committee for Clinical Laboratory Standards (NCCLS) to make the target bacteria non- resistant to oxytetracycline (Chi et al., 2014; Sun Y. et al., 2002). This MIC is expected after injectable administration of therapeutic doses from 10 mg OTC per kg of body weight. This implies the use of a much higher amount of drug than that used in this work, to obtain an effective dose in plasma.

IV. CONCLUSION

Porous PVA microspheres were able to capture and release oxytetracycline, a broad-spectrum bacteriostatic drug of great interest in veterinary medicine.

We found that the optimal pH for the adsorption of OTC at room temperature was pH=4. These conditions favor drug capture by interaction between the sulfonate groups present in the microspheres with the ionized forms of OTC.

The in vitro release of the drug adsorbed from the microspheres was characterized with an overall release of 41±5% in 2 hours, which is equivalent to 4.2µg OTC/mL, the minimum dose of OTC required in plasma for an antibacterial effect.

These results allow us to evaluate the convenience of using these microparticles as a novel method of OTC administration.

Conflict of interest

There is no conflict to disclose.

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