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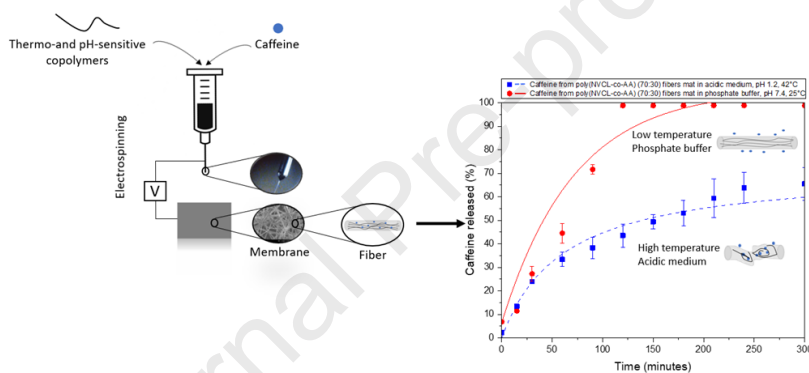
Hydrophilic drug release of electrospun membranes made out of thermo and pH-sensitive polymers

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Abstract: Electrospun fibers of the thermo-and pH sensitive poly(N-vinylcaprolactam-co-acrylic acid) (Poly(NVCL-co-AA) loaded with 10% and 30 wt% caffeine (Caf) were obtained by electrospinning and evaluated as potential drug delivery systems. Caffeine was used as a hydrophilic drug model, and poly(NVCL-co-AA) containing either 20 or 30 mol% AA was used as a carrier. The fibers morphology, as well as, their interaction with caffeine, were studied using different analytical techniques. The cytotoxicity of the different obtained fibers was evaluated by cell viability assays using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and mouse embryonic fibroblasts cell line (MEF cells). Caffeine release was studied at temperatures of 25 °C and 42°C and pH of 1.2 and 7.4. Beadless copolymer fibers with diameters ranging from 1µm to 2µm were obtained. The addition of caffeine, which was in crystalline form after being encapsulated in the fibers, resulted in an increase of fiber diameter. The obtained membranes were found to be not cytotoxic. The entrapment of caffeine was greater for the copolymer containing 30 mol% AA due to a greater affinity of AA to caffeine. At a pH of 1.2 and at both temperatures of 25°C and 42°C, as well as, at a pH of 7.4 and a temperature of 42°C, a Fickian diffusion mechanism for all copolymer fiber mats was observed. At a pH of 7.4 and 25°C the release profile showed a high rate and followed a zero-order model, due to the fast dissolution of caffeine in water. These results indicated that thermo-and pH-sensitive poly(NVCL-co-AA) are promising candidates for controlled release of hydrophilic drugs.

1. INTRODUCTION

Electrospun fiber scaffolds are widely used in biomedical applications, especially in tissue engineering [1][2][3][4], and delivery of pharmaceutical compounds [5][6][7][8]. Indeed, these scaffolds, can be obtained with a large variety of polymers, are flexible and consist of micro-to nano-size fibers [9][10], therefore providing a large surface area. All these properties make them interesting candidates for drug delivery systems. Concomitantly, in drug delivery systems, polymer-drug affinity is very critical because it determines the possible drug-loading amount, the drug dispersion within the carrier, and the drug release rate. In the particular case of electrospun fibers, their drug encapsulation can be achieved using different techniques such as mixing the drug in the to be electrospun polymer solution, attaching the drug to the fibers surface, confining the drug in the fibers core or encapsulating it in nanostructures [2][9][11].

Thermo-sensitive and pH-sensitive polymers have attracted particular attention as drug delivery systems over the last decade or so [12][13] as they can present a variable affinity to chemical substances, depending on their temperature and pH environment. Thermo-sensitive polymers

undergo distinct water soluble/non-water-soluble phase transitions at a specific temperature: lower critical solution temperature (LCST), or upper critical solution temperature (UCST). Poly(N-vinylcaprolactam) (PNVCL) is one of these thermosensitive polymers with an LCST of 32° C [14][15][5]. Being a nontoxic polymer compared to other thermo-sensitive polymers such as poly(N-isopropylacrylamide) (PNIPAm), PNVCL is a potential candidate for drug delivery systems [16][17][18]. PNVCL systems have been studied by different authors [19][20][21][24][25] in the form of membranes with thermoadjustable porosity [21], microgels [23][22][24], core-shell structures with metal ions [20]. PNVCL was modified to become pH and thermosensitive [19][26]. These modifications modulate the cloud point temperature of PNVCL, turning the polymer versatile for drug delivery applications.

In addition to temperature-sensitivity, pH-sensitivity can be an interesting stimulus to control drug release in drug delivery systems [27][28][22]. Polymers sensitive to both temperature and pH is even more advantageous. Concomitantly, copolymerization is also an interesting method to modulate the cloud point temperature of PNVCL, depending on comonomer type as previously reported by Ponce-Vargas, Cortez-Lemus and Licea-Claverie (2013) [25]. In the particular case of PNVCL, it is possible by incorporating acrylic acid (AA) segments, into the thermo-sensitive backbone (PNVCL), to obtain a thermo- and pH-sensitive copolymer such as poly(N-vinylcaprolactam-co-acrylic acid) (poly(NVCL-co-AA)) [29]. These thermo- and pH-sensitive copolymers are able to change their conformation quickly and reversibly, once in water, under the effect of temperature and pH simultaneously [29].

In this work, electrospun thermo- and pH-sensitive membranes were prepared by electrospinning two different poly(NVCL-co-AA) copolymers containing 20 and 30 mol% AA, respectively. The fibers were loaded with caffeine. Caffeine (Caf) is a model hydrophilic drug, highly soluble in aqueous solutions [30] [31] [32]. Caffeine is commonly used in many studies as a hydrophilic model drug due to its low price and availability. Furthermore, it is of common knowledge that caffeine affects the cognitive function and feelings of mental alertness, mood, and arousal [33] [34]. The objective of the work was to study the electrospinnability of the two copolymers to which 10% and 30 wt% caffeine was added, and to characterize the obtained membranes by evaluating their capacity to be used for controlled drug release. For that, the electrospinnability of the systems was optimized, the influence of caffeine addition on the LCST of the copolymer was evaluated, the cytotoxicity of the obtained membranes was verified, and the drug entrapment efficiency and release quantified. The developed biocompatible fibers were capable to modify the *in vitro* caffeine release rate, showing potential for applications in controlled release of hydrophilic drugs, including oral and topical administration route, especially in sublingual and wound healing membranes, in view the proven temperature- and pH sensitivity and the capacity of the copolymers to modulate the kinetics of *in vitro* drug release.

2. Experimental:

2.1 Material:

The copolymers used in this study, Poly(N-vinylcaprolactam-co-acrylic acid) (Poly(NVCL-co-AA) containing 17 and 28 mol% AA, were previously obtained and characterized in our research group [29]. For the sake of simplicity, they are referred to 20 and 30 mol% [poly(NVCL-co-AA) (70:30) and poly(NVCL-co-AA) (80:20)], which corresponds to the desired molar ratios of the monomers introduced in the syntheses. Caffeine, a crystalline hydrophilic substance, was obtained from Sigma-Aldrich. Ethanol and N,N-Dimethylformamide (DMF) were also supplied by Sigma-Aldrich and used as solvents for the electrospinning process.

Poly(NVCL-co-AA) (70:30) and poly(NVCL-co-AA) (80:20), named for the sake of simplicity P_(70:30) and P_(80:20) in the rest of the manuscript.

2.2 Method:

2.2.1 Synthesis and characterizations of poly(N-vinylcaprolactam-co-acrylic acid) (poly(NVCL-co-AA))

This work reports the use temperature and pH-sensitive poly(NVCL-co-AA) with different AA proportions for the development of nanofibers via electrospinning and encapsulation of a hydrophilic drug, caffeine. The synthesis and characterization of random copolymers P_(70:30) and P_(80:20) were reported by Medeiros et al. (2017). In summary, an initial acrylic acid mol% of 20 and 30 with 80 and 70 mol% of NVCL, was used to synthesize the copolymers P_(70:30) and P_(80:20), respectively. The final AA mol% was assessed via titration and the chemical composition of the copolymers was further confirmed by Hydrogen Nuclear Magnetic Resonance (¹H NMR)[29].

2.2.2 Caffeine Entrapment and Electrospinning

Caffeine suspensions were prepared by adding 10 wt% and 30 wt% of caffeine powder to a mixture of ethanol and DMF in a ratio of 50:50 (v:v). Then, P_(70:30) or P_(80:20) in a 35 wt% and 45 wt% concentration, respectively, was dissolved in homogeneous caffeine suspensions for 3 hours using a magnetic stirrer at room temperature (21°C).

The obtained suspensions were electrospun using a commercial electrospinning machine (L100), produced by Bioinicia, Spain. The processing parameters were optimized and found to be 100 kV/m electric field, 2 mL/h flow rate, and 10 cm working distance. The humidity and the temperature during the electrospinning were set at values ranging from 35% to 40% and 21°C, respectively.

2.2.3 Characterization of Electrospun Suspension

The kinematic viscosity and surface tension of the electrospun suspensions were measured using an Ubbelohde viscometer (Cannon Instrument Company, Zeitfuchs cross-ARM Viscometer) with 1.100 cSt/s constant and a pendant drop apparatus equipped with DROPFIT software (UMR 5257), respectively.

2.3 Membrane Characterization

After being coated with gold, the obtained membranes were characterized by scanning electron microscopy (SEM, Hitachi S3600N). Poly(NVCL-co-AA) membranes loaded with 0wt%, 10wt% and 30wt% caffeine, were also characterized with Ultra-high Resolution SEM (HITACHI SU8230) to verify the presence of caffeine particles at the surface of the fibers. The diameter of the fibers was then evaluated using the Image J software.

Fourier transform Infrared spectrometer (FTIR, Perkin Elmer spectrometer) was used to verify the presence of trapped caffeine in the fibers, and the indications of caffeine-copolymer interaction. The spectra were collected using 1 cm² of the fiber's membrane and scans were taken over 3500–1500 cm⁻¹ with a resolution of 2 cm⁻¹ and an average of 32 scans.

Differential Scanning Calorimetry (DSC, Perkin Elmer differential calorimeter (Pyris 1)) was used to evaluate the crystallinity and LCST of the poly(NVCL-co-AA), and the caffeine loaded poly(NVCL-co-AA) membranes. The samples were heated from 10°C to 250°C at a heating rate of 15°C/min under nitrogen atmosphere. Distilled water was used for the LCST measurement.

Wide-angle X-ray diffraction (XRD) measurements were performed on a Panalytical X'pert Pro X-ray diffractometer equipped with copper, CuK α , tube at 45 kV and 35 mA. Scans were performed in the 2 θ range of 10–70°.

2.4 Cell viability assay

The membrane cytotoxicity was evaluated carrying out viability assays of mouse embryonic fibroblasts (MEF) cells incubated in the presence of the membranes. After cells incubation with

copolymers membranes loaded with 0, 10 or 30wt% of caffeine, cell viability was measured using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide referred to as (MTT) .

150 μ L Dulbecco's Modified Eagle Medium (DMEM) containing 20% fetal bovine serum (FBS), followed by 100 μ L of DMEM containing a cell suspension at 10^5 cells/mL were added to each well of a 96-well plate to achieve a final concentration of 10^4 cells per well. Then, the 96-well plate was incubated at 37 °C in a 5% CO₂ humid atmosphere during 24h. After 24h, the DMEM was removed using a micro-pipette, leaving the cells in the wells. 100 μ L of phosphate buffer saline solution (PBS) was added to each well to rinse the cells. Immediately PBS was removed using a micro-pipette and replaced by 250 μ L of fresh culture medium (DMEM containing 20% FBS).

After that, membranes to be tested cut into 6 mm diameter round-shaped samples needed to meet the ISO 10993-12 (1.4 cm²/mL) standard, were placed in contact with the cells inside each well. For comparison, a group of cells was incubated with an aqueous solution of caffeine at 1.5 mg/mL corresponding to the maximum caffeine concentration (30wt%) release. The plate containing the samples was then incubated again at 37°C in a 5% CO₂ humid atmosphere during 24h. Afterwards, the culture medium was removed from the wells and the cells were washed with 100 μ L of PBS, which was immediately replaced by 100 μ L of MTT solution with a concentration of 0.5 mg/mL prepared in PBS. The plate containing the samples was then incubated again for 3h at 37 °C in a 5% CO₂ humid atmosphere. The MTT solutions were carefully withdrawn, so that the formazan salt could be preserved inside the well. 100 μ L of dimethyl sulfoxide (DMSO) was then added to each well to solubilize the formazan salt and kept for 30 minutes.

Finally, the plate was stirred at 410 cycles per minute for 3 minutes and absorbance was measured at a wavelength of 540 nm in a Microplates Reader – (Hybrid Multidetector Synergy H1). Cells incubated in the absence of membrane samples were used as a negative control and cells incubated with 20% of DMSO were used as a positive control of cytotoxicity. Cell viability was calculated by using the average value of the absorbance at 540 nm of the negative control as 100 % viability. All the samples were used in four replicates and the cell viability values are presented as average values along with the standard deviation.

2.5 Caffeine Entrapment and Release

Two milligrams of caffeine-loaded membranes were dissolved in 40 ml of a basic solution pH 10 (aqueous solution of monosodium phosphate and sodium hydroxide) under magnetic stirring during 48h. The solutions were analysed as a function of time by UV-visible spectroscopy (Varian Cary 300 Bio), using the characteristic absorption peak of caffeine at 273 nm.

The Encapsulation Efficiency (EE) was calculated as the ratio of the real amount measured by UV-visible spectrum, W_d , over the weight of caffeine used in the preparation of fibers mat, W_t , eq. (1)[1]:

$$EE (\%) = (W_d / W_t) \times 100 \quad (1)$$

2.6 In vitro Caffeine release study

The *in-vitro* release studies for 10 and 30 wt% caffeine loaded in P_(70:30) and P_(80:20) were made as follows:

First, the electrospun membrane (10 mg) was placed in the dissolution medium (40 mL) of phosphate buffer saline PBS (pH 7.4), or in an acidic medium of sodium chloride and hydrochloric acid in water (pH 1.2). The samples were then magnetically stirred at 100 rpm at two different temperatures: below the LCST at 25°C and above the LCST 42°C. Subsequently, aliquots of 3 mL were removed from the dissolution medium (at suitable intervals), and immediately replaced by a fresh ones, and analyzed with UV-visible spectroscopy (Varian Cary 300 Bio) at 273 nm. To quantify the released caffeine concentration and to calculate its release

rate from each of the formulations, a calibration curve, and the average of two determinations were used respectively.

2.7 Caffeine Release Profiles

Two types of methods are commonly used to study the kinetics of controlled drug release from formulations: model-independent and model dependent. Model independent methods use a difference factor and a similarity factor to compare dissolution profiles and calculate the dissolution efficiency (DE). Model dependent methods describe the dissolution profile based on mathematical functions which are fitted to experimental data obtained from *in vitro* drug release as a function of time (Table 1). Caffeine release profiles were analyzed using the independent [dissolution efficiency (DE)] and the model-dependent (curve-fitting) methods. KinetDS, free open-source software, was used to calculate the DE and to fit the release curves to several models. The Korsmeyer–Peppas model was found to give the best fit, eq (2) , eq (3), in Table 1 [35]. KinetDS was used to analyze the accuracy and prediction ability of this model by calculating the coefficient of determination (R^2), the root mean-square error (RMSE), and the Akaike's information criterion (AIC), eq (4), eq (5), in Table 1[36].

Table 1: Independent and Dependent Kinetic Model Applied to Analyze the Drug Release Data Approach

Approach	Method	Equation
Model-independent	Dissolution Efficiency (DE)	$DE = \left(\frac{\int_0^t M_t dt}{M_{t_{max}} \times t} \right) \times 100$ (2)
Model-dependent	Korsmeyer-Peppas	$M_t = K \times t^n$ (3)
Best Model Criteria		$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_{i_{obs}} - y_{i_{pred}})^2}{n}}$ (4)
		$AIC = (2 \times K) + n \times \left[\ln \left(\sum_{i=1}^n (y_{i_{obs}} - y_{i_{pred}})^2 \right) \right]$ (5)

M_t is the amount of drug released in time t ; K is the release constant incorporating structural and geometric characteristics of the drug-dosage form; n is the diffusional exponent indicating the drug-release mechanism; M_{tmax} is the maximum amount of drug released (=100%)

3. RESULTS AND DISCUSSION

3.1 Synthesis of Poly(NVCL-co-AA)

The synthesis and characterization of $P_{(80:20)}$ were done according to the method described in detail by Medeiros et al (2017). The chemical structure of the random poly(NVCL-co-AA) copolymer is now shown in Figure 1a. Figure 1b presents the effect of pH and temperature on the polymer structure.

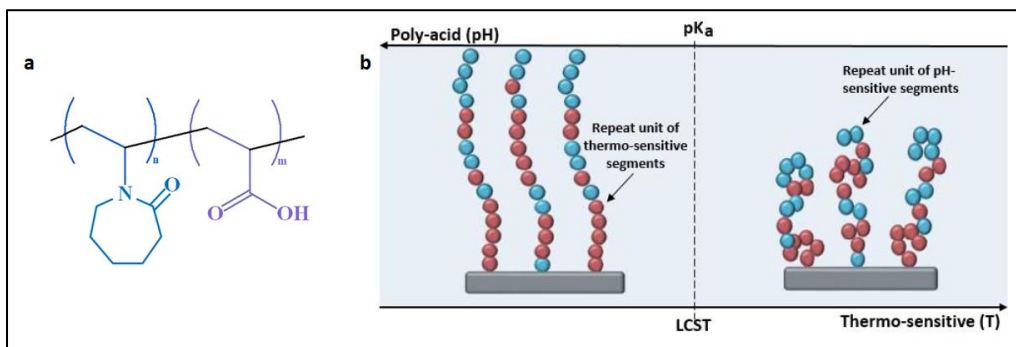


Figure 1: Poly(NVCL-co-AA) chemical structure (a), the effect of pH and temperature on the polymer structure (b).

The content of AA in the poly(NVCL-co-AA) copolymer was determined by titration and ^1H NMR. For the two polymers studied, it was found equal to 17 mol% (for $\text{P}_{(80:20)}$) and 28 mol%, (for $\text{P}_{(70:30)}$) respectively. For both copolymers, the content of AA was slightly less than the nominal one. The LCST of these both polymers were determined using UV-vis and found to be equal to 39 and 40 °C respectively. For $\text{P}_{(70:30)}$ the molar mass (M_n) and dispersity (\mathcal{D}) were 6247 g/mol and 1.59, respectively.

3.2 Preparation of caffeine containing fibers

3.2.1 Morphology and average diameters of the fibers

The viscosity, and surface tension of the polymer solutions containing caffeine in different concentrations were measured prior to electrospinning. The values are listed in Table 2. Polymer solutions without caffeine were also prepared for both poly(NVCL-co-AA) copolymers.

Table 2: Properties of $\text{P}_{(70:30)}$ and $\text{P}_{(80:20)}$ solutions with different Caffeine concentrations

Caffeine Concentration (w/v)	Poly(NVCL-co-AA) (70:30)		Poly(NVCL-co-AA) (80:20)	
	Viscosity (cSt)	Surface tension (mN/m)	Viscosity (cSt)	Surface tension (mN/m)
0%	550	12	840	9.9
10%	590	9.8	860	13.3
30%	850	8.8	990	11.4

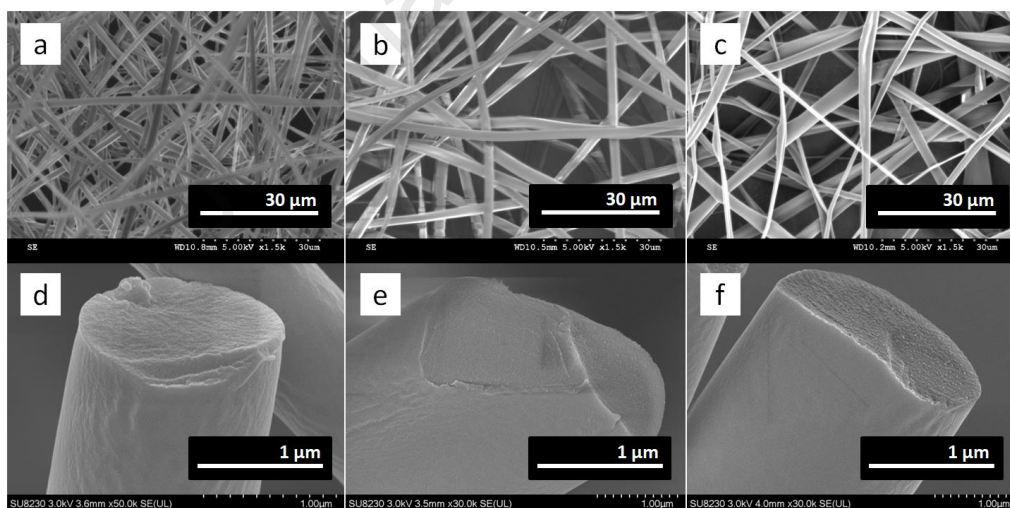


Figure 2 : SEM images of $\text{P}_{(70:30)}$ fibers with different caffeine concentrations (a, d) Poly(NVCL-co-AA) fibers, (b, e) Poly(NVCL-co-AA) fibers with 10%wt caffeine, (c, f) Poly(NVCL-co-AA) fibers with 30wt% caffeine

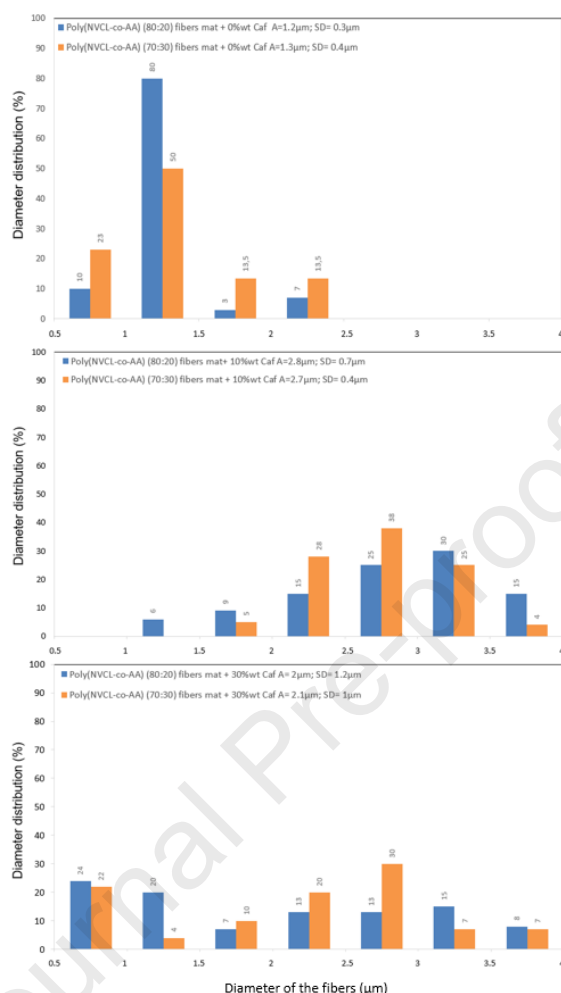


Figure 3: Fiber diameter distribution of P_(70:30) and P_(80:20) with 0 wt%, 10 wt% and 30 wt% caffeine loaded fibers concentrations

Figure 2 shows typical morphologies of P_(70:30) electrospun fibers containing 0, 10 and 30wt% caffeine obtained in this work. Figure 3 reports the fiber diameter distribution for each type of fibers obtained.

It can be seen that electrospinning poly(NVCL-co-AA) without caffeine leads to beadless cylindrical fibers with a smooth surface morphology (Figure 2-a). In contrast, electrospinning poly(NVCL-co-AA) with caffeine leads to ribbon-like fibers. Ultra-high resolution SEM images (Figure 2, d-f) of the cross-section caffeine-loaded fibers seem to indicate the absence of caffeine crystals on the fiber surface. This indicates that the caffeine was, probably, incorporated inside the fiber matrix.

Incorporating caffeine into the fibers resulted in increasing the average fiber diameter from $\sim 1\mu\text{m}$ to $\sim 2\mu\text{m}$ (Figure 3). This was observed for both copolymers. This increase can be related to the increase of electrospun solutions viscosity, from 550 up to 850 cSt for P_(70:30), and for P_(80:20), from 840 up to 990 cSt, upon caffeine addition (Table 2). It can be observed (Figure 2) that increasing the loading amount of caffeine generated more deformation of the fiber morphology and non-homogeneity of its diameter. This phenomenon can be attributed to the low solubility of caffeine

in Ethanol and DMF, and weak caffeine-copolymer interaction. A similar effect was reported in the research of Sti et al. using polylactic acid (PLA) fibers [37]. In their research, Sti et al. showed that by increasing the caffeine loading to 50% by weight, the PLA fibers lose their cylindrical shape and surface smoothness, which they attributed to the growth of crystalline regions inside the fibers [37].

Although not shown here, the same phenomenon was also observed with P_(80:20) caffeine loading fibers.

3.1.2 Fourier transform infrared spectroscopy (FTIR) analyses

Good affinity between a model drug and a polymer is important for the formation of smooth and homogenous fibers, and for a steady drug release. The interactions between the drug model and the polymer can be evaluated using IR spectroscopy.

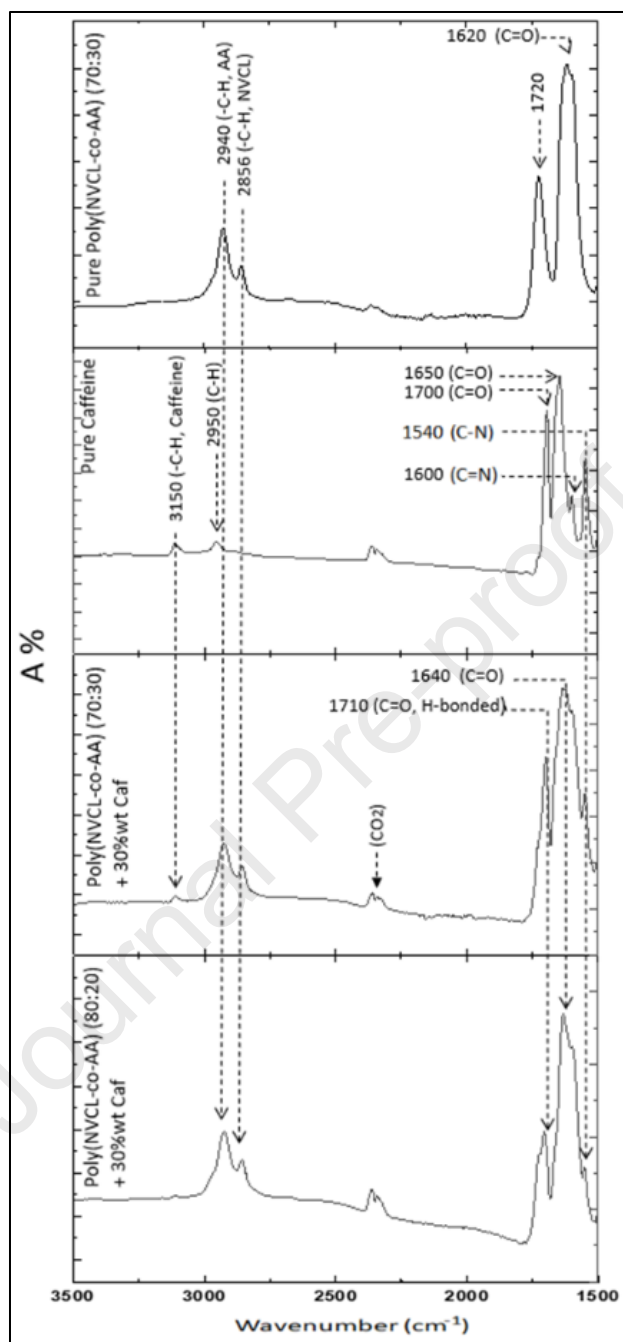


Figure 4: FTIR spectra of pure $P_{(70:30)}$, pure caffeine and $P_{(70:30)}$ and $P_{(80:20)}$ with 30 wt% caffeine

Figure 4 shows the FTIR spectra of pure $P_{(70:30)}$, pure caffeine and 30 wt% caffeine-loaded $P_{(80:20)}$ and $P_{(70:30)}$ fibers.

Pure $P_{(70:30)}$ infrared spectra present two characteristic bands at 1620 cm^{-1} and 1720 cm^{-1} corresponding to (C=O) stretching vibration, and two other bands at 2856 cm^{-1} and 2940 cm^{-1} corresponding to absorption and related to the aliphatic (C-H) band of NVCL and AA, respectively.

The caffeine spectra show characteristic absorbance at 1700 cm^{-1} and 1650 cm^{-1} corresponding to (C=O) stretching of the amide group. Other bands were also observed around 1600 cm^{-1} and 1540 cm^{-1} assigned to (C=N) and (C-N) amide stretches [38][39]. The bands at 2950 cm^{-1} and 3150 cm^{-1} may be ascribed to the asymmetric stretching of C-H bonds of methyl ($-\text{CH}_3$) groups in the caffeine molecule and the (C-H) stretching vibrations.

The spectra of $\text{P}_{(80:20)}$ and $\text{P}_{(70:30)}$ fibers to which 30 wt% caffeine was added, show the presence of the (C-N) stretches from caffeine at 1540 cm^{-1} which confirms a homogeneous encapsulation of caffeine into the fibers during electrospinning. The small shifts in band positions from 1720 cm^{-1} for the pure copolymers and 1700 cm^{-1} for the pure caffeine to 1710 cm^{-1} in the caffeine-loaded fibers may indicate the formation of a hydrogen bond between the copolymer and the caffeine molecules. The small shift of carbonyl stretching band of poly(NVCL-co-AA) from 1620 cm^{-1} to 1640 cm^{-1} also suggests that there may be a small intermolecular interaction between the caffeine and the copolymers [37][40]. A band at 2350 cm^{-1} is attributed to CO_2 molecules in the atmosphere [41]. The same results were obtained for the fibers loaded with 10wt% caffeine.

3.1.3 Differential scanning calorimeter (DSC) analyses

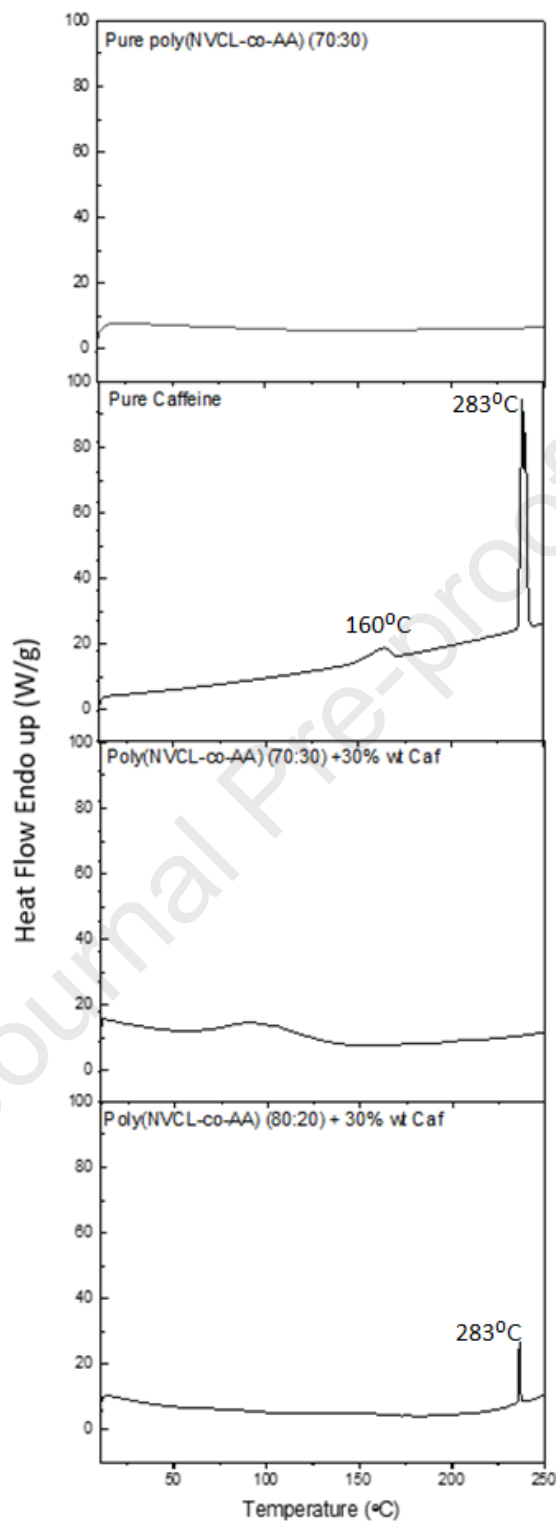


Figure 5: DSC curves of pure P_(70:30), pure caffeine and P_(70:30) and P_(80:20) fibers with 30 wt% caffeine

Figure 5 shows the DSC thermograms of pure poly(NVCL-co-AA) powder, pure caffeine and the fibers of both copolymers loaded with 30wt% caffeine (Caf). The thermogram of the pure copolymer is typical of an amorphous polymer. The thermogram for pure caffeine presented the

expected small endothermic peak at around 160°C (phase transformation from Form II to Form I) and a large one at 238°C corresponding to a fusion peak [42]. The DSC thermograms of the 30 wt% caffeine-loaded $P_{(70:30)}$ fibers did not show any caffeine fusion peak, suggesting that caffeine was not in crystalline form in the fibers [40]. Similar curves were also obtained with 10 wt% caffeine-loaded $P_{(70:30)}$ fibers.

Conversely, the DSC thermogram of the 30 wt% caffeine-loaded $P_{(80:20)}$ fibers shows a caffeine melting peak (Form I) at 238°C, which indicates the presence of caffeine in the crystalline state in the fibers. Similar curves were also obtained with 10 wt% caffeine-loaded $P_{(80:20)}$ fibers. These results may suggest that a better caffeine-copolymer interaction was obtained in the case of $P_{(70:30)}$ most likely due to the higher proportion of AA present in this copolymer compared to $P_{(80:20)}$.

3.1.4 X-ray diffraction (XRD) analysis

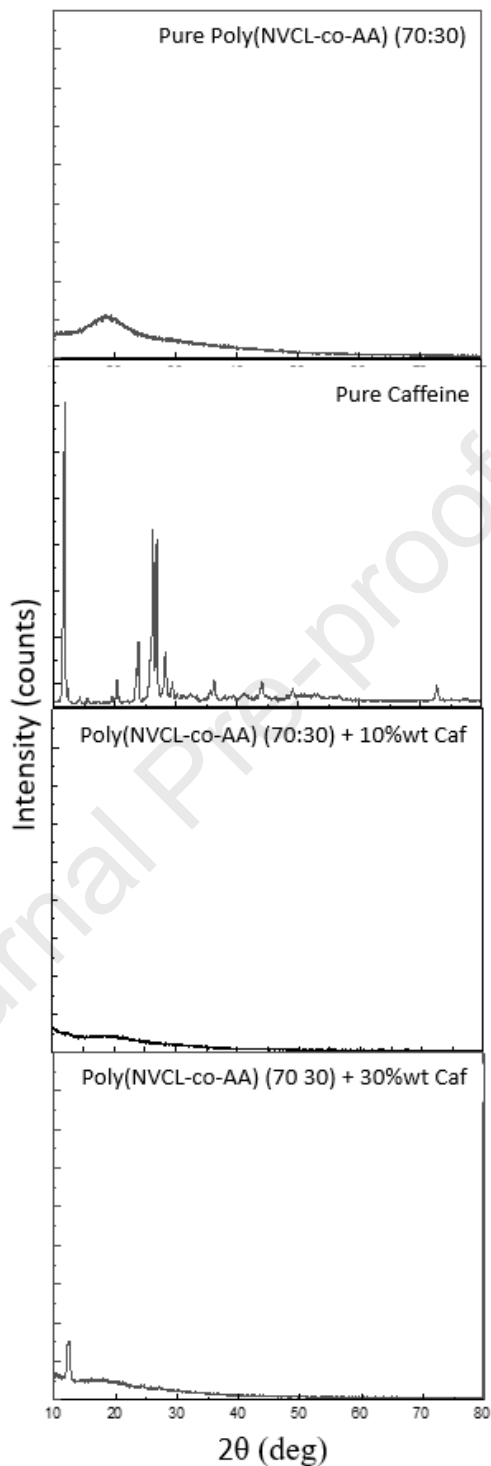


Figure 6: XRD patterns of pure $P_{(70:30)}$, pure caffeine and $P_{(70:30)}$ fibers with 10 wt% and 30 wt% caffeine

Figure 6 shows the XRD patterns of pure $P_{(70:30)}$ powder, pure caffeine and the caffeine-loaded $P_{(70:30)}$ fibers. The patterns of the caffeine-loaded $P_{(80:20)}$ for both concentrations have not been shown because they confirm the DSC results.

The pattern of fibers containing only pure $P_{(70:30)}$ showed absence of any diffraction peaks, only a broad halo was observed. This confirms that $P_{(70:30)}$ is an amorphous copolymer. The two characteristic diffraction peaks of caffeine appeared at 2θ angles of 11.25° , and 26.25° which confirms the crystallinity of the caffeine. In the pattern of 10 wt% caffeine-loaded fibers, the characteristic diffraction peaks of caffeine could not be observed. A characteristic hump of an amorphous materials was observed. This suggests that caffeine was present in amorphous form inside the fibers confirming the result obtained with the DSC [40]. However, the 30 wt% caffeine-loaded $P_{(70:30)}$ fibers pattern shows a diffraction peak at a 2θ angle of 11.25° , which indicates that caffeine maintains its crystallinity during the electrospinning process. These results are different from what was obtained with the DSC, most likely due to the difference of sensitivity of both techniques, and this may be explained by the low dispersion of caffeine in the fibers and the lack of interaction between the copolymer and the caffeine at high concentrations. In a similar fashion Sti et al. reported the XRD pattern of PLA fibers containing 10 wt% caffeine did not show any crystallinity, unlike the case of PLA containing 50wt% caffeine [37].

3.1.5 LCST measurements

Table 3: LCST Measurements of $P_{(70:30)}$ and $P_{(80:20)}$ fibers mat with 0, 10 and 30 wt% of caffeine

Copolymer	Poly(NVCL-co-AA) (70:30)			Poly(NVCL-co-AA) (80:20)		
Caffeine concentration (wt %)	0	10	30	0	10	30
LCST ($^\circ\text{C}$)	40	41	41	39	40	40

Table 3 presents the LCST of $P_{(70:30)}$ and $P_{(80:20)}$ with 0 wt%, 10 wt% and 30 wt% caffeine concentrations. The increase of the caffeine concentration from 10% to 30%wt did not result in a noticeable difference in LCST values within the limits of the experimental error, and this may be explained by the low caffeine-copolymer interaction.

3.3 Caffeine entrapment

Table 4: Caffeine Entrapment into Poly(NVCL-co-AA) Electrospun Fiber Mats

Caffeine fibers mat	EE (%)
Poly(NVCL-co-AA) (70:30)	10 wt% Caffeine 98.8
	30 wt% Caffeine 100
Poly(NVCL-co-AA) (80:20)	10 wt% Caffeine 75
	30 wt% Caffeine 75

Table 4 presents the caffeine entrapment into $P_{(70:30)}$ and $P_{(80:20)}$ fiber mats. It can be seen that, the EE values for $P_{(70:30)}$ were above 98% for both 10 wt% and 30 wt% caffeine loading. This confirms the successful encapsulation of the caffeine into the fibers. However, this was not the case for $P_{(80:20)}$, for which only 75% caffeine was encapsulated with 10 wt% and 30 wt%. This may be related to lower of interaction between the copolymer $P_{(80:20)}$ and the caffeine, confirming the results of DSC, XRD and LCST. They, also, support the use of poly(NVCL-co-AA) electrospun fibers as a potential hydrophilic model carrier, since the amount of caffeine used during the formulations is almost totally encapsulated in the $P_{(70:30)}$ fibers during electrospinning. This emphasizes the importance of the drug-model affinity to the copolymer in the encapsulation process.

3.4 Cell viability and cytotoxicity assays

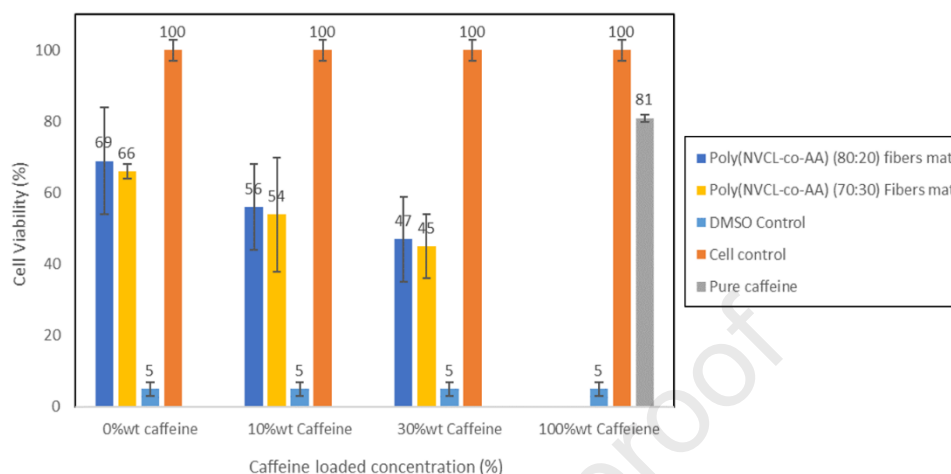


Figure 7: Cell viability of MEF cells incubated with P_(70:30) and P_(80:20) at different caffeine concentrations

Figure 7 shows the values of viability of MEF cells incubated in pure caffeine and poly(NVCL-co-AA) fiber mats containing 0%, 10%, and 30 wt% caffeine. For all the studied materials, the cell viability values were found to be lower than 70% indicating that the fibers are cytotoxic [ISO:10-953-5]. As expected, the cytotoxicity of P_(70:30) and P_(80:20) fiber mats without caffeine was quite similar, 69% and 66%, respectively. Although the average cell viability values found in the cells, incubated with the fiber mats were lower than 70%, the copolymer poly(NVCL-co-AA) was reported by several authors to be a non-toxic material [29]. In most of these works, cytotoxicity was measured by the indirect method and different types of probes were used to measure cell viability. Herein, the cytotoxicity was assessed by the direct method, where the material was directly in contact with the cells that had grown on the bottom of the well plate. Therefore, it is reasonable to assume that the fiber mats disintegrated under incubation and, most probably, the small fragments had compromised the cell viability. The lowest values of cell viability were observed in cells incubated with caffeine loaded fiber mats. These findings support our claim that the cytotoxicity observed in this assay was in fact a result of the fiber mats fragmentation. Therefore, considering that *in vivo* tissues would not be as fragile as *in vitro* cell culture, regarding small fragments of fiber mats, it would be possible to conclude that these results suggest that the P_(70:30) and P_(80:20) fibers mat loaded with caffeine have considerable potential as a caffeine drug delivery system.

3.5 *In vitro* release study

The fibers prepared introducing 10 wt% caffeine (with respect to the polymer) during electrospinning were used to evaluate the kinetic behavior of the active ingredient.

Figure 8 (a,b) shows the caffeine *in vitro* release profiles as a function of time for the two poly(NVCL-co-AA) ratios in fiber mats obtained in acidic medium (pH 1.2) and PBS (pH 7.4). The lines are meant to be just a guide for the eye and not a result of a mathematical function. For each pH, two different temperatures were evaluated, 25°C (figure 8-a) and 42°C (figure 8-b). The temperatures, at which drug release was studied, were chosen to evaluate the drug release behavior from the fibers below and above the LCST of the copolymers. 42°C may seem elevated but corresponds to temperatures that are reached in some wounds and the results could be

interesting for wound healing applications [43]. The choice of pH for each dissolution test was based on the copolymer LCST, i.e., below and above this parameter. For comparison, dissolution tests were also performed for pure caffeine at the same conditions as can be observed in Figure 9. It can be seen from Figure 9 that fast pure caffeine dissolution was observed, over than 65 % within 20 seconds of the test, for all conditions, as a result of the high drug water-solubility. The caffeine dissolution was slightly favored by temperature, as expected.

By comparing Figure 8-a and b, the effect of temperature on caffeine release from $P_{(70:30)}$ and $P_{(80:20)}$ copolymers, mainly at pH 7.4, due to the thermo-sensitive behavior of PNVCL segments is clearly demonstrated. At this pH, the effect of PNVCL and PAA are similar, i.e., both segments are in a swollen state. For example, 100 % of caffeine was released from $P_{(70:30)}$ after 120 minutes, while at 42°C, 72 %. For $P_{(80:20)}$, the release at 25 °C was 70 % and at 42 °C, (39%), which is related to the higher amount of PNVCL. Above the LCST, PNVCL chains tend to collapse into aggregates entrapping the caffeine molecules. In acidic medium (pH 1.2), the same trend was observed for both copolymer ratios, i.e., the caffeine release rate decreased as the temperature of the medium increased from 25 to 42 °C. However, the difference in the release rate at the two evaluated temperatures was smaller at pH 7.4, compared with the same temperatures at pH 1.2. At pH 1.2, we have different competitive behaviors governing caffeine release. While the PNVCL segments change from a swollen to a collapsed state with increasing temperature, the PAA segments remain in a collapsed state due to the low pH. This difference in release rate at pH 1.2 at different temperatures is even smaller for the copolymer $P_{(70:30)}$ with higher amount of PAA. The results obtained in the present work are in good agreement with those obtained by Liu et al. [8], who studied the effect of temperature on captopril (hydrophilic drug) release from poly(NVCL-co-MAA) fibers. The authors evaluated the drug release at different temperatures above and below the lower critical solution temperature (LCST), and concluded that the drug release profiles were found to be greatly influenced by temperature.

Regarding the effect of pH on the release rate of caffeine from $P_{(70:30)}$ and $P_{(80:20)}$, the results clearly show that it, also, strongly influences the *in vitro* release kinetics of the active ingredient. Caffeine itself is a drug that has basic groups in its structure, and therefore, has a pH-dependent dissolution behavior, as shown in Figure 9. After 60 seconds of testing, the entire amount of the caffeine was dissolved at pH 1.2, while this percentage decreased to 92 % in pH 7.4. Concerning the caffeine release from $P_{(70:30)}$, at the same temperature (25°C), it can be observed from Figure 7-a, that the caffeine release decreased from 100 to 46%, after 120 minutes, by changing the pH from 7.4 to 1.2. From $P_{(80:20)}$, the release percentage decreased from 70 to 53 %, by changing the pH from 7.4 to 1.2, at 25 °C and after 120 minutes of testing. These results evidence the effect of PAA segments on drug release. Similar results were reported by SÓti et al. [37], who performed dissolution studies on releasing caffeine from PLA fibers. The results described by Eranka et al. [40] concerning the release of caffeine from polyvinylpyrrolidone (PVP) fibers, also, corroborates our results.

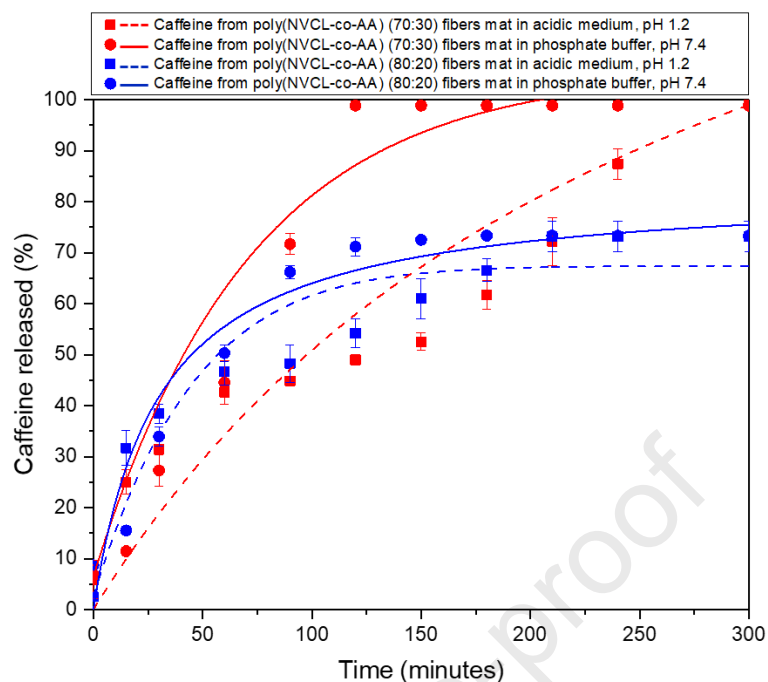


Figure 8-a: *In vitro* release profile of 10 wt% caffeine from P_(70:30) and P_(80:20) fiber mats at 25°C in acidic medium, pH 1.2 and in phosphate buffer, pH 7.4

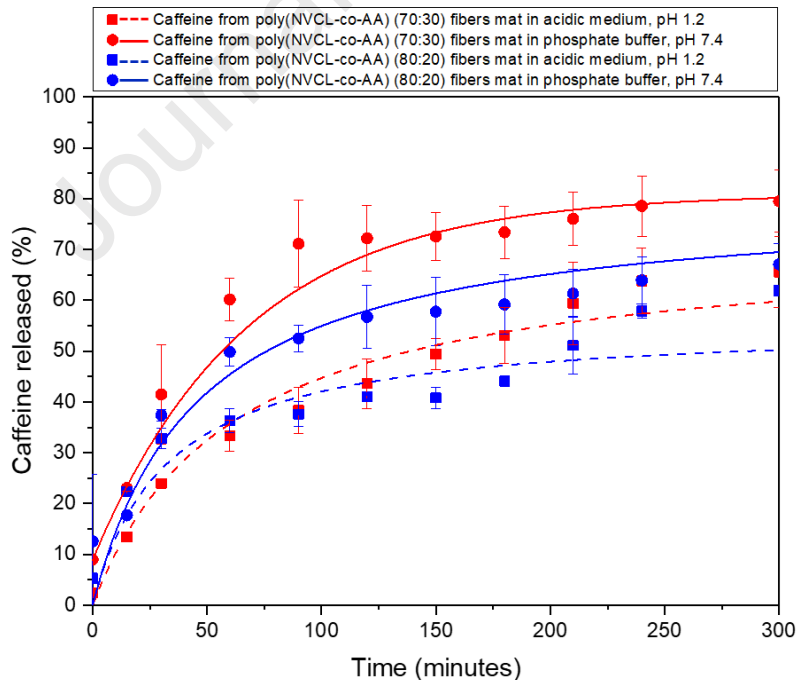


Figure 8-b: *In vitro* release profile of 10 wt% caffeine from P_(70:30) and P_(80:20) fiber mats at 42°C in acidic medium, pH 1.2 and in phosphate buffer, pH 7.4

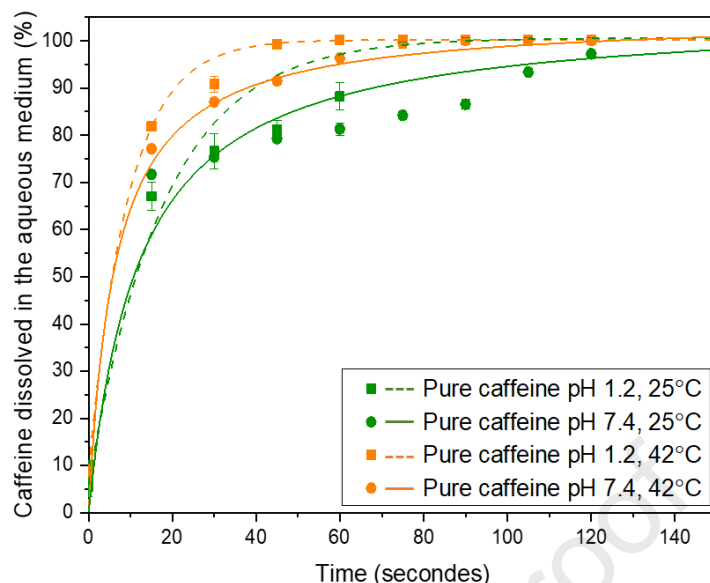


Figure 9: Dissolution rate of pure caffeine in acidic medium (pH 1.2) and phosphate buffer (pH 7.4) at 25°C and 42°C

3.6 Release mechanism

Due to the ability of poly(NVCL-co-AA) chains to swell or collapse as a function of temperature and pH variation, it was concluded that diffusion is the main mechanism governing the caffeine release from the fiber mats. To determine the mechanism of drug release, the cumulative drug (until 150 min) release from the poly(NVCL-co-AA) fibers was fitted to the Korsmeyer–Peppas model using KinetDS software. Table 5 presents the coefficient of correlation (R^2), root-mean-square error (RMSE), Akaike's information criterion (AIC), kinetic constant (K), and the release exponent (n), obtained by a model-dependent method. In this model, the kinetic constant (K) incorporates structural and geometric characteristics of the polymer system, and the release exponent (n) characterizes the mechanism of drug release [eq. (1) and Table 2].

The dissolution efficiency (DE) values are also presented [eq. (2) and Table 1]; however, this parameter was calculated by a model-independent method.

From the dissolution experiments, the Korsmeyer–Peppas release model was found to best describe the release behavior of caffeine from poly(NVCL-co-AA) fiber mats at different temperature and pH. This is evident from the relatively high correlation values listed in Table 5. The kinetic constants (K) obtained for all release tests by a model dependent method can, also, be observed individually in Figure 10 for caffeine released from poly(NVCL-co-AA) fiber mats at 25 and 42 °C.

Table 5: Results of Curve Fitting and Kinetic Analysis of 10 wt% caffeine Release Data from P_(70:30) and P_(80:20) Electrospun Fiber Mats Using the Korsmeyer–Peppas Model and Dissolution Efficiency (DE) Obtained via Independent Model Analysis

Curve fitting parameters	Poly(NVCL-co-AA) (70:30)				Poly(NVCL-co-AA) (80:20)			
	25°C		42°C		25°C		42°C	
	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4	pH 1.2	pH 7.4
R^2	0.98	0.98	0.97	0.95	0.98	0.94	0.85	0.90
RMSE	1.30	6.60	1.73	5.60	1.61	6.24	2.90	2.32
AIC	17.93	37.41	21.39	35.42	20.52	36.73	27.54	24.86
K (min ⁻¹)	10.60	0.94	3.45	6.17	15.45	3.02	13.35	24.38
n	0.32	0.95	0.53	0.53	0.26	0.66	0.244	0.18
DE	39.76	58.13	32.93	56.13	44.87	51.42	36.00	50.51

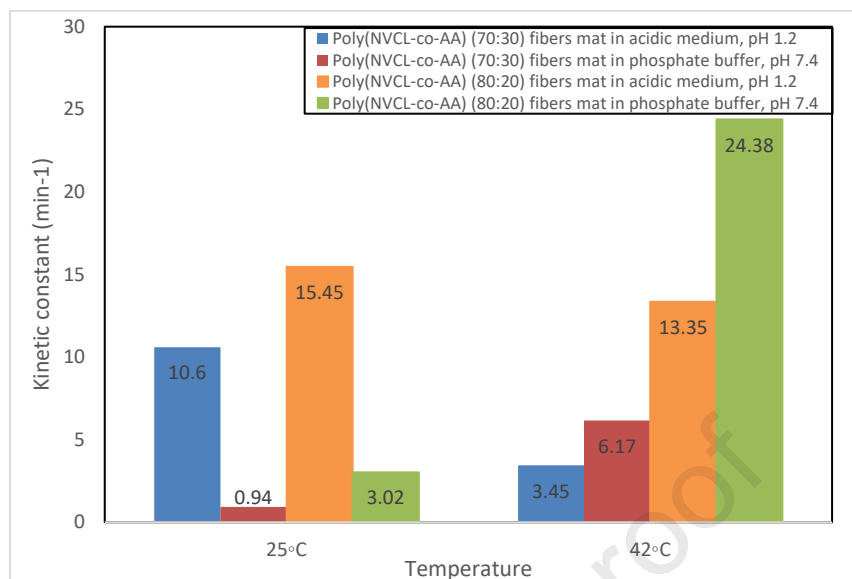


Figure 10: Kinetic constant of P_(70:30) and P_(80:20) Electrospun Fiber Mats at 25 °C and 42 °C in acidic medium, pH 1.2 and in phosphate buffer, pH 7.4

It can be seen from the data presented in Table 5 and Figure 10 that increasing the temperature from 25 °C (below the LCST) to 42 °C (above the LCST) leads to a decrease of the caffeine release rate (K) at pH of 1.2. Below the LCST, a fast release is expected due to the higher hydrophilicity of the poly(NVCL-co-AA) fiber mats even in acidic medium. At this temperature hydrogen bonds are formed between lactam seven-membered-ring molecular chains and water molecules, resulting in copolymer chain relaxation and free movement of the caffeine molecules. Above the LCST (42 °C), the copolymer chains collapse due to the polymer-polymer interaction and the loss of the hydrogen bonds caused by the water molecules vibration. This limits the caffeine molecules movement, and as a consequence sustained release rates were obtained. Unlike the acidic medium, the caffeine release rate (K) increased when the pH is 7.4, which can be related to the swelling of the PAA chains. Changing the releasing condition from acidic medium to PBS medium (basic), the PAA segments change from an agglomerated state to a swollen state. In PBS (pH 7.4) the ionization of carboxyl groups from PAA segments causes the dissociation of the polymeric complex, and the fibers swell under the effect of electrostatic repulsion.

The R^2 values higher than 0.9 obtained for both copolymers P_(70:30) and P_(80:20) release kinetics indicate that the Korsmeyer–Peppas model predicts the data with good accuracy. However, the fact that these values were not in all cases very close to unity also indicates that diffusion is not the only release mechanism governing the kinetics of caffeine release from the fiber mats. Moreover, it is known that release exponent (n) values higher than 0.5, mean that the diffusion release mechanism follows a non-Fickian pattern (anomalous diffusion). When n is smaller than 0.5 and close to 0 (flat system), this means that the release behavior resembles a Fickian model [35][44]. For all dissolution conditions except 25 °C combined with pH 7.4, the release exponents were equal to or smaller than 0.5, indicating a Fickian diffusion mechanism for all formulations. However, at 25 °C and at a pH of 7.4, release mechanism can be described by a zero order model and this is due to the fast dissolution of caffeine in water, as well as, the strong hydrophilicity of the poly(NVCL-co-AA) fiber mats, at this condition. The discussed effect of temperature and pH on caffeine release from fiber mats can be, once more, evidenced by observing DE values obtained by a model-independent method. In summary, the increase in temperature results in

reduced *DE* for the same pH conditions. On the other hand, the increase in pH for the same temperature results in increased *DE* values (Table 5).

Although the Korsmeyer-Peppas model, in general, can be applied to describe the release of caffeine in this study, the calculation of RMSE and AIC parameters indicated that for the tests performed mainly at 25°C and pH 1.2 for P_(70:30) fiber mats, as well as, for the tests performed at 25°C and pH 7.4 for P_(80:20), the diffusion was not the only mechanism governing the release of caffeine. For the release test made at 25 °C and pH 1.2 for P_(70:30) fiber mats, this result can be directly associated by the collapsed state of polymer chains in view of the low pH and the higher concentration of pH-sensitive PAA in the fibers. RMSE is a good indicator of the standard deviation and the closer to zero this parameter, the more suitable is the method chosen to evaluate the release mechanism. At 25 °C, when pH increased from 1.2 to 7.4, the high values of RMSE and low value of AIC parameters, indicate a competition between collapsed state of PAA segments and swollen state of PNVCL segments, below the LCST. Although the release mechanism needs to be further elucidated, this study demonstrated that the release of caffeine and probably other hydrophilic similar drugs can be impeded by their entrapment in poly(NVCL-co-AA) fiber mats.

4. CONCLUSION

In this study, poly(NVCL-co-AA)-based electrospun fiber mats loaded with caffeine have been successfully prepared via electrospinning. Copolymers containing different AA mol% (20 and 30) were evaluated and the caffeine concentration was kept at 10% and 30 wt% (with respect to the copolymer). Scanning electron microscopy showed that the fibers had smooth surfaces and that the average fiber diameter and morphology change as a function of caffeine concentration. IR spectroscopy results demonstrated that there were some intermolecular interactions between caffeine and poly(NVCL-co-AA). X-ray diffraction and differential scanning calorimetry studies indicated that highly-loaded caffeine maintains its crystallinity inside the fibers. The caffeine entrapment was dependent on the caffeine – copolymer proportion. P_(70:30) membranes showed the highest Encapsulation Efficiency (EE) value (100%). The caffeine kinetics release was studied at two temperatures: below and above the LCST of PNVCL, and at two pH values: acidic medium (pH 1.2) and basic medium (pH 7.4). The release profiles of caffeine from P_(70:30) and P_(80:20) revealed rapid caffeine release below LCST compared with that above LCST. Above LCST, caffeine release was slower, especially, in acidic medium (pH 1.2). Overall, this study demonstrates that thermo and pH-sensitive electrospun fibers may become potential systems for the encapsulation and controlled release of caffeine and similar hydrophilic models.

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REFERENCES

- [1] M. D. Pawar, G. V. N. Rathna, S. Agrawal, and B. S. Kuchekar, "Bioactive thermoresponsive polyblend nanofiber formulations for wound healing," *Mater. Sci. Eng. C*, vol. 48, pp. 126–137, 2015.
- [2] Y.-F. Goh, I. Shakir, and R. Hussain, "Electrospun fibers for tissue engineering , drug delivery , and wound dressing," *J Mater Sci*, pp. 3027–3054, 2013, doi: 10.1007/s10853-013-7145-8.
- [3] N. M. Ardila Nelson; Arkoun ,Mounia; Heuzey,Marie-Claude; Ajji,Abdellah; Panchal,Chandra J., "Chitosan–bacterial nanocellulose nanofibrous structures for potential wound dressing applications," *Cellulose*, vol. 23, pp. 3089–3104, 2016.
- [4] H.-L. ; L. Kim Jeong-Hyun; Lee, Mi Hee; Kwon,Byeong Ju; Park, Jong-Chul, "Evaluation of Electrospun (1,3)-(1,6)-b-D-Glucans/ Biodegradable Polymer as Artificial Skin for Full-Thickness Wound Healing," *TISSUE Eng. Part A*, vol. 18, pp. 2315–2322, 2012.
- [5] M. Sta, G. Aguiar, A. A. J. Forni, S. F. Medeiros, A. M. Santos, and N. R. Demarquette, "Design and characterization of PNVCL-based nanofibers and evaluation of their potential applications as scaffolds for surface drug delivery of hydrophobic drugs," *J. Appl. Polym. Sci.*, vol. 137, no. 11, 2020, doi: 10.1002/app.48472.
- [6] A. P. S. Immich, J. A. Tornero, F. C. Casas, and M. J. L. Arias, "Electrospun PLLA Membranes for Caffeine Delivery: Diffusional Approach," *J. Biomed. Sci. Eng.*, vol. 10, no. 12, pp. 563–574, 2017, doi: 10.4236/jbise.2017.1012042.
- [7] B. Ghafoor, A. Aleem, M. Najabat Ali, and M. Mir, "Review of the fabrication techniques and applications of polymeric electrospun nanofibers for drug delivery systems," *J. Drug Deliv. Sci. Technol.*, vol. 48, no. August, pp. 82–87, 2018, doi: 10.1016/j.jddst.2018.09.005.
- [8] L. Liu *et al.*, "Controlled release from thermo-sensitive PNVCL-co-MAA electrospun nanofibers: The effects of hydrophilicity/hydrophobicity of a drug," *Mater. Sci. Eng. C*, vol. 67, pp. 581–589, 2016.
- [9] S. Islam, B. Chin, A. Andri, A. Amalina, and M. Afifi, "A review on fabrication of nanofibers via electrospinning and their applications," *SN Appl. Sci.*, vol. 1, no. 10, pp. 1–16, 2019, doi: 10.1007/s42452-019-1288-4.
- [10] M. Á. R. Calderón and W. Zhao, "Applications of polymer nanofibers in bio-materials, biotechnology and biomedicine: A review," *TMS Annu. Meet.*, vol. 125, pp. 401–414, 2014, doi: 10.1002/9781118889879.ch50.
- [11] E. Rezabeigi *et al.*, "Electrospinning of porous polylactic acid fibers during nonsolvent induced phase separation," *J. Appl. Polym. Sci.*, vol. 134, no. 20, May 2017, doi: 10.1002/app.44862.
- [12] H. P. James, R. John, A. Alex, and A. K.R, "Smart polymers for the controlled delivery of drugs—a concise overview," *Acta Pharm. Sin. B*, vol. 4, pp. 120–127, 2014.
- [13] D. D. Chakraborty, L. K. Nath, and P. Chakraborty, "Recent Progress in Smart Polymers : Behavior , Mechanistic Understanding and Application," *Polym. Plast. Technol. Eng.*, vol. 57, no. 10, pp. 945–957, 2018, doi: 10.1080/03602559.2017.1364383.
- [14] N. A. Cortez-Lemus and A. Licea-Claverie, "Poly(N-vinylcaprolactam), a comprehensive review on athermoresponsive polymer becoming popular," *Prog. Polym. Sci.*, vol. 53, pp. 1–51, 2016.
- [15] M. A. Ward and T. K. Georgiou, "Thermoresponsive Polymers for Biomedical Applications," *Polymers (Basel)*, vol. 3, pp. 1215–1242, 2011.
- [16] H. L. Vihola A; Valtola, L; Tenhu, H; Hirvonen, J., "Cytotoxicity of thermosensitive polymers poly(N-isopropylacrylamide), poly(N-vinylcaprolactam) and amphiphilically

- modified poly(N-vinylcaprolactam),” *Biomaterials*, pp. 3055–3065, 2005.
- [17] S. F. Medeiros, J. C. S. Barboza, M. I. Ré, R. Giudici, and A. M. Santos, “Solution Polymerization of N-vinylcaprolactam in 1,4-dioxane. Kinetic Dependence on Temperature, Monomer, and Initiator Concentrations,” *J. Appl. Polym. Sci.*, vol. 118, pp. 229–240, 2010.
- [18] S. F. Medeiros, A. M. Santos, H. Fessi, and A. Elaissari, “Synthesis of biocompatible and thermally sensitive poly(N-vinylcaprolactam) nanogels via inverse miniemulsion polymerization: Effect of the surfactant concentration,” *J. Polym. Sci. Part A Polym. Chem.*, vol. 48, no. 18, pp. 3932–3941, 2010, doi: 10.1002/pola.24165.
- [19] M. Sta, D. B. Tada, S. F. Medeiros, A. M. Santos, and N. R. Demarquette, “Materials Science & Engineering B Electrospun poly (NVCL- co -AA) fibers as potential thermo- and pH-sensitive agents for controlled release of hydrophobic drugs,” *Mater. Sci. Eng. B*, vol. 276, no. October 2021, p. 115531, 2022, doi: 10.1016/j.mseb.2021.115531.
- [20] L. M. Bronstein *et al.*, “Core - Shell Nanostructures from Single Poly (N - vinylcaprolactam) Macromolecules : Stabilization and Visualization,” no. 13, pp. 2652–2655, 2005.
- [21] W. Lequeieu, N. I. Shtanko, and F. E. Du Prez, “Track etched membranes with thermo-adjustable porosity and separation properties by surface immobilization of poly(N-vinylcaprolactam),” *J. Memb. Sci.*, vol. 256, no. 1–2, pp. 64–71, 2005, doi: 10.1016/j.memsci.2005.02.007.
- [22] S. F. Medeiros, P. F. M. Oliveira, T. M. Silva, B. R. Lara, A. Elaissari, and A. M. Santos, “Biocompatible and multi-responsive poly(N-vinylcaprolactam)-based microgels.pdf,” pp. 191–201, 2015.
- [23] A. Bitar, Ahmad; Fessi, Hatem; Elaissari, “Synthesis and Characterization of Thermally and Glucose-Sensitive Poly N-Vinylcaprolactam-Based Microgels.” pp. 709–719, 2012.
- [24] A. Imaz and J. Forcada, “N-vinylcaprolactam-based microgels: Synthesis and characterization,” *J. Polym. Sci. Part A Polym. Chem.*, vol. 46, no. 7, pp. 2510–2524, 2008, doi: 10.1002/pola.22583.
- [25] S. M. Ponce-Vargas, N. A. Cortez-Lemus, and A. Licea-Claverie, “Preparation of poly(N-Vinylcaprolactam) (NVCL) and statistical copolymers of NVCL with variable cloud point temperature by using a trithiocarbonate RAFT agent,” *Macromol. Symp.*, vol. 325–326, no. 1, pp. 56–70, 2013, doi: 10.1002/masy.201200045.
- [26] S. F. Medeiros, M. V. Lopes, B. Rossi-Bergmann, M. I. Ré, and A. M. Santos, “Synthesis and characterization of poly(N- vinylcaprolactam)-based spray-dried microparticles exhibiting temperature and pH- sensitive properties for controlled release of ketoprofen,” *Drug Dev. Ind. Pharm.*, vol. 43, no. 9, pp. 1520–1576, 2017, doi: 10.1080/03639045.2017.1321660.
- [27] H. Li *et al.*, “Dual temperature and pH responsive nanofiber formulations prepared by electrospinning,” *Colloids Surfaces B Biointerfaces*, vol. 171, pp. 142–149, 2018.
- [28] S. Dai, P. Ravi, and K. C. Tam, “pH-Responsive polymers: synthesis, properties and applications,” *Soft Matter*, vol. 4, pp. 435–449, 2008.
- [29] S. F. Medeiros, M. V. Lopes, B. Rossi-Bergmann, M. I. Ré, and A. M. Santos, “Synthesis and characterization of poly(N-vinylcaprolactam)-based spray-dried microparticles exhibiting temperature and pH-sensitive properties for controlled release of ketoprofen,” *Drug Dev. Ind. Pharm.*, vol. 43, no. 9, pp. 1519–1529, 2017, doi: 10.1080/03639045.2017.1321660.
- [30] S. Gaspar and F. Ramos, “Caffeine: Consumption and Health Effects,” *Encycl. Food Heal.*, pp. 573–578, 2016.
- [31] S. Ullrich, Y. C. de Vries, S. Kühn, D. Repantis, M. Dresler, and K. Ohla, “Feeling smart: Effects of caffeine and glucose on cognition, mood and self-judgment,” *Physiol. Behav.*, vol. 151, pp. 629–637, 2015, doi: 10.1016/j.physbeh.2015.08.028.

- [32] S. Ullrich, Y. C. de Vries, S. Kühn, D. Repantis, M. Dresler, and K. Ohla, "Feeling smart: Effects of caffeine and glucose on cognition, mood and self-judgment," *Physiol. Behav.*, vol. 151, pp. 629–637, 2015, doi: 10.1016/j.physbeh.2015.08.028.
- [33] S. Cappelletti, D. Piacentino, G. Sani, and M. Aromatario, "Caffeine: Cognitive and physical performance enhancer or psychoactive drug," *Curr. Neuropharmacol.*, p. 554, 2015.
- [34] N. Ojeh, O. Stojadinovic, I. Pastar, A. Sawaya, N. Yin, and M. Tomic-Canic, "The effects of caffeine on wound healing," *Int. Wound J.*, vol. 13, no. 5, pp. 605–613, 2016, doi: 10.1111/iwj.12327.
- [35] G. Singhvi and M. Singh, "REVIEW: IN-VITRO DRUG RELEASE CHARACTERIZATION MODELS," *Int. J. Pharm. Stud. Res.*, vol. II, no. I, pp. 77–84, 2011.
- [36] A. Mendyk, R. Jachowicz, K. Fijorek, P. Dorożyński, P. Kulinowski, and S. Polak, "KinetDS : An Open Source Software for Dissolution Test Data Analysis," *Dissolution Technol.*, no. 778051, pp. 5–11, 2012, doi: 10.14227/DT190112P6.
- [37] P. L. Söti *et al.*, "Preparation and comparison of spray dried and electrospun bioresorbable drug delivery systems," *Eur. Polym. J.*, vol. 68, pp. 671–679, 2015, doi: 10.1016/j.eurpolymj.2015.03.035.
- [38] N. DAS LAKSHMI V., "Biodegradation of Caffeine by *Trichosporon asahii* Isolated from Caffeine Contaminated Soil," *Int. J. Eng. Sci. Technol.*, vol. 3, no. 11, pp. 7988–7997, 2011.
- [39] M. M. Paradkar and J. Irudayaraj, "A rapid FTIR spectroscopic method for estimation of caffeine in soft drinks and total methylxanthines in tea and coffee," *J. Food Sci.*, vol. 67, no. 7, pp. 2507–2511, 2002, doi: 10.1111/j.1365-2621.2002.tb08767.x.
- [40] U. E. Illangakoon *et al.*, "Fast dissolving paracetamol/caffeine nanofibers prepared by electrospinning," *Int. J. Pharm.*, vol. 477, no. 1–2, pp. 369–379, 2014, doi: 10.1016/j.ijpharm.2014.10.036.
- [41] J. Park and I. Lee, "Controlled release of ketoprofen from electrospun porous polylactic acid (PLA) nanofibers," *J Polym Res*, pp. 1287–1291, 2011, doi: 10.1007/s10965-010-9531-0.
- [42] S. Hubert *et al.*, "Process induced transformations during tablet manufacturing: Phase transition analysis of caffeine using DSC and low frequency micro-Raman spectroscopy," *Int. J. Pharm.*, vol. 420, no. 1, pp. 76–83, 2011, doi: 10.1016/j.ijpharm.2011.08.028.
- [43] D. Romano, C. L. Romano, F. Dell, and O. Nicola, "Healing of surgical site after total hip and knee replacements show similar telethermographic patterns," pp. 81–86, 2011, doi: 10.1007/s10195-011-0135-1.
- [44] J. J. Sheng, N. A. Kasim, R. Chandrasekharan, and G. L. Amidon, "Solubilization and dissolution of insoluble weak acid , ketoprofen : Effects of pH combined with surfactant," *Eur. J. Pharm. Sci.*, vol. 29, pp. 306–314, 2006, doi: 10.1016/j.ejps.2006.06.006.

Highlights

Thermo-and-pH-sensitive scaffolds developed by electrospinning

Controlled release of hydrophilic drug model

Drug release depends on the copolymers used in scaffolds

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Natural Sciences and Engineering Research Council of Canada (NSERC),
The University Mission of Tunisia in North America (MUTAN)
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