Fabrication and Sustained Release of Chlorogenic Acid from Poly(vinyl alcohol)/Poly(y-glutamic Acid) Blends Electrospun Mats

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Abstract

**Background:** Diabetic foot is a condition that is caused by high levels of glucose in the blood. Poly (γ-glutamic acid) [γ-PGA]/polyvinyl alcohol [PVA] electrospun biopolymers and their use in the release of chlorogenic acid (CGA) is a promising system, thanks to their biocompatibility and the use of (CGA) in the treatment of certain symptoms of diabetic conditions. The use of CGA was reported in 2005 as a capable substance in the treatment of lesions caused by diabetic foot ulcers, thanks to the fact that it has no toxic effects.

**Methods:** In this work, was carried out the bio-synthesis of γ-PGA producing by the *Bacillus liquefaciens* ATCC-9945a. This material was utilized in the preparation of electrospun nanofibers with solutions of polyvinyl alcohol [PVA] to 10% p/v mixed with γ-PGA to 5 and 10% p/v. These solutions were loaded with chlorogenic acid (CGA) like an active hypoglycemic agent.

**Results:** The morphological analysis shows a size decrease for the fiber with PVA/γ-PGA, compared to PVA nanofibers; this could be attributed to the hydrogen bonding interactions between γ-PGA and PVA. The use of glutaraldehyde vapors permit the nanofibers crosslinking to maintain a stable structure and allows the CGA release. The *in vitro* release analysis shows that the PVA membranes reach 28% of delivery after the first 24 hours. Instead of, for the nanofiber mat with PVA blended with γ-PGA at 5% goes up to 57% and nanofiber PVA/γ-PGA at 10% reach to 66% in the same time, the rate constant for release kinetics shows that PVA/γ-PGA at 5% it's higher than the others, being the first in reach the saturation.

**Conclusions:** Biodegradable polymer meshes were fabricated by electrospinning of PVA and PVA/γ-PGA polymer and loaded with CGA. The presence of γ-PGA into PVA solutions increases the *in vitro* drug release from electrospun fiber mats. The maximal release of CGA was reached at 10% of γ-PGA introduced in the PVA solution, which was 82% release from the electrospun polymer fiber mats. Finally, the use of Peppas and Weibull models indicates the released kinetics of CGA from PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10 electrospun fiber mats are driven by diffusion mechanism.

Background

Diabetes is a chronic degenerative disease that, in the actual, can be present both in adults and children. The world incidence it's too high, and the recent years, the risk to die be increasing1. Due to multiplying factors among which can highlight the obesity is one the most important, that can unleash cardiovascular diseases2. According to IDF (International Diabetes Federation), more than 463 million people have diabetes and be forecasts that 2045 ascent to 700 million3.

The health agencies consider diabetes that an epidemiologic problem that must be attacked. The diabetes consequence unleashes disease diverse that renal insufficiency, diabetic nephropathy, and high blood pressure. In the actual, the treatment of diabetes used various drugs, that if well every one shows efficacy, on occasions, generates side reactions or even death, it does not control the daily intake4.
Diabetic nephropathy is an ailment that is originated by high blood glucose levels; this is caused by a metabolic disorder that the patient present. Diabetic foot complications are the most cause for hospital admissions due to complications by ulcers that can come to limb amputation\(^5\). Therefore the search for a new treatment and therapeutic preventions by the control of someone ailment associates to diabetes, as well as the diabetic foot treatment, constitutes an essential field of study of an urgent need.

Chlorogenic acid (CGA) is a bioactive phenol compound found in a great variety of plant species. Several laboratories found that CGA stimulates the uptake of glucose by cultures of human adipocytes, both sensitive and resistant to insulin\(^6\).

These results suggest that said pure compound could be useful in the development of new strategies for the treatment of hyperglycemia, local or systematic. Martinez-Jimenez et al. conducted a randomized clinical trial that demonstrated that the local application of insulin stimulates vascularization and tissue formation of granulation in wounds of diabetic patients\(^7\). These results strongly suggest that glycemic control in the area of the injury allows for more efficient healing even in patients with a diabetic foot. Barahui et al. demonstrated in an in vitro study that a nanocomposite drug carrier made with graphene oxide and CGA for cancer treatment show significant toxicity towards some cancer cell lines but not in regular cell lines\(^8\). Also, Martinez et al. \(^9\) in 2005 reported diabetic treatment with GCA from the extract of Mexican plants and bassoli et al.\(^10\) in 2008, analyzed their effect to reduce the glucose peak in the plasma, indicating a glycaemic index lowering role. These ethnopharmacological studies support traditional medicine. Thus, CGA can help to heal injuries caused by diabetic foot ulcers, thanks to the fact it has no toxic effects, their intake is not allergenic neither cause harm during its application\(^11\). That is why great importance count with systems that permit its release, with the final to mitigate the damage caused by this disease.

Electrospinning is a versatile and effective technique in the production of scaffolding nanostructured biomaterials with a large surface area and high porosity. Therefore, the electrospun nanofibers have become an exciting alternative in areas such as drug delivery, healing, and tissue engineering. Electrospun nanofibers have been used successfully to immobilize vitamins, enzymes, and other bioactive compounds. These nanostructures are attractive alternatives in the release of these compounds, as they provide advantages such as a large surface area that increases the contact area between the mixture encapsulated and the release medium\(^12\).

Moreover, electrospun nanofibers in the pharmacologic field have had significant interest thanks to the be a bio-functional system. That allows the direct interaction between polymers and drugs, for that reason is of paramount importance count with compatible polymer with the drug and as well to be biocompatible materials.

In this work, we use CGA encapsulated in Poly (vinyl alcohol) (PVA), and PVA blended with γ-PGA electrospun nanofibers mats, as a model of sustained release of an anti-diabetic and healing compound with potential medical application in the diabetic foot treatment. We demonstrated that CGA was
successfully incorporated into PVA or PVA/γ-PGA nanofiber mats through electrospinning and released to a buffer phosphate media in a sustained manner for more than 200 h. Finally, In vitro, drug release profiles were obtained by analyzing the effect of the incorporation γ-PGA on controlling the kinetics of CGA release.

**Methods**

**2.1 Materials**

Poly (vinyl alcohol) (98% hydrolyzed) (average Mw 126 kg/mol), poly (vinyl alcohol); 87–89% hydrolyzed and average Mw 13–23 kg/mol) were purchased from Aldrich. CGA was obtained from Sigma. All other reactives used in this work were of analytical grade.

**2.2 Biosynthesis of Poly (γ-glutamic acid)**

The biosynthesis of γ-PGA was carried out by growing *Bacillus licheniformis* ATCC-9945a bacteria, using “E” medium, adjusted to pH 7, as was previously described in the literature. The microorganism was incubated at 37 °C and 250 rpm for 24-48 h. The γ-PGA was purified by centrifugation, and the supernatant was washed twice with acetone and deionized water (1:1). Then, the precipitated product was lyophilized and stored in the refrigerator.

**2.3 Preparation of nanofbers of PVA and PVA/γ-PGA by electrospinning**

The nanofbers mats were prepared by using a 10 % solution of PVA (high and low molecular weight) and also PVA blended with 5 or 10 % of γ-PGA, the different nanofbers will be referred to as like PVA, PVA/γ-PGA 5 and PVA/γ-PGA 10, respectively. CGA of 0.6 mg/ml concentration was added to these solutions. Before electrospinning, the solutions were loaded in a 5 mL plastic syringe. After that, samples were injected at the flow rate of 0.2 ml/h, maintaining a syringe-collector distance of 15 cm, and an applied voltage of 20 kV. Once, the nanofber mats were electrospun; they were crosslink using glutaraldehyde. Typically, PVA and PVA/γ-PGA nanofber mats were kept in contact with glutaraldehyde vapor for various periods inside a desiccator. After that, the samples were withdrawn from the desiccator and heat-treated at 40 °C in an oven for 20 min. Finally, the samples were stored in zip-lock plastic bags and kept in the refrigerator until their further characterization.

**2.4 Chlorogenic acid release.**

The release of CGA from the polymeric electrospun mats was quantified by UV/Vis spectroscopy. To that purpose, a calibration curve was built using a buffer solution of phosphate (1M to pH 4.8), using CGA standards in the concentration interval of 0.5-160 μg/mL, and taken a lecture of maximum absorbance at 324 nm.

To follow the kinetic release of CGA from electrospun nanofiber, these were introduced in a closed dialysis tubing cellulose membranes, containing 80 mL of phosphate buffer and dialyzed against a
solution of the same buffer. The different electrospun mats were incubated in a shaker at 37 °C and 250 rpm. At certain intervals of time, the amount of CGA release of each system was monitoring by taken out 2 mL from the buffer solution outside the dialysis membranes. The amount of CGA released at that time was registered by Uv/Vis spectroscopy, as described previously. The withdrawn volume from the outer phase of dialysis bag was replaced with fresh phosphate-buffered at each predetermined time intervals.

### 2.5 Characterization techniques.

The NMR spectra were recollected in a DELTA 300 MHz equipment for 1H. For this, 10 mg of γ-PGA was dissolved in D$_2$O under an inert atmosphere. The molecular weight was determined in APC from Waters model UPLC with a UV detector operate at 215 nm with a silicon column and eluent aqueous solution NaHPO$_4$ : Acetonitrile in a 4:1 relationship. ATR Fourier transformed infrared (FTIR) analysis was carried out in Nicolet iS5 Thermo scientific iD7ATR, in the range from 4000-600 cm$^{-1}$, 16 resolutions, 64 scans, using germanium as reference standard material. SEM characterization was realized with a JEOL JSM-7041F. The samples were gold-palladium sputter-coated, and the size distribution was achieved with image J software. The absorptions spectrums were accomplished with a Shimadzu 2401PC spectrophotometer.

### Results

#### 3.1 γ-PGA characterization

Previous to use the γ-PGA to prepare the nanofibers mats, the polymer was characterized by RMN-$^1$H spectra to assure its purity. The RMN-$^1$H spectra show the chemicals displacements that correspond to γ-PGA (Fig. 1), the proton for α-CH at 4.2 ppm, and β-CH$_2$ has two signals at 2.16, 1.9 ppm, and γ-CH$_2$ at 2.37 ppm.$^{15}$

#### 3.2 Presence of γ-PGA in the PVA nanofibers

The presence of γ-PGA in nanofiber mats was determinate by FT-IR spectra (Fig. 2). In the PVA case, the existence of O-H stretching in the region at 3298 cm$^{-1}$ and the methyl groups at 2940 y 2898 cm$^{-1}$, they overlap with the γ-PGA presence and the acetyl group C-O characteristic to this polymer at 1090 cm$^{-1}$. The increase of the γ-PGA can observe the corresponding band for amine group N-H stretching at 1590 cm$^{-1}$, and 1640 cm$^{-1}$ for PVA/g-PGA 5 y PVA/g-PGA 10 , In 3307 cm$^{-1}$ found the band attributed to the OH stretching mode. The C-O stretching vibration at 1222 cm$^{-1}$ and the corresponding C-N stretching to 1132 cm$^{-1}$, the peak to corresponding to the aliphatic side for γ-PGA are at 3073 and 2932 cm$^{-1}$. The molecular weight of γ-PGA obtains through to the biosynthesis with *Bacillus licheniformis* ATTC9945a strain shows an Mw=243,023 g/mol (Fig S1).

#### 3.3 Morphology
Highly hydrolyzed PVA is difficult to electrospun due to its high surface tension. Previously, we have overcome the problem by blending, partially hydrolyzed, and low molecular weight PVA with highly hydrolyzed and larger molecular weight PVA. We think that the first polymer provides better mechanical properties and stability to the solvent. In contrast, the second polymer improves the electrospinnability of the blended polymer solution\textsuperscript{12a}.

The morphology and diameter of the electrospun nanofibers of PVA were analyzed before and after the crosslink, CGA encapsulation processes (Fig 2S). SEM morphological characterization of nanofibers shows that using a 10 % solution of PVA can form fibers easily, at the nanometric size order. The obtained nanofiber had an average diameter of 277 nm, and they are flawless and smooth, verifying that the elaboration conditions were optimum. Once, the nanofiber mats were submitted to crosslink by a glutaraldehyde vapor process, and the fiber diameter was increased until 298 nm. However, this increase in the fiber diameter was not appreciative, conserving the particular large surface area of the nanofibers (Fig. 3).

The inclusion of $\gamma$-PGA in the PVA solution has a critical effect on nanofibers average diameter in both regular and after crosslinked. The addition of $\gamma$-PGA at 5 reduces the average size diameter of the nanofibers at 163 nm and, after crosslinked them, suffer an increase up to 221 nm (Fig. S3). Once, the nanofibers were crosslinked shows an entanglement. Both the fiber diameter increment and entanglement could be related to the absorption of glutaraldehyde vapor inside the nanofiber structure. Nevertheless, the diameter increment was not apparent in comparison to the diameter without any crosslinking treatment. Hence, we can conclude that glutaraldehyde vapor treatment is an effective method to crosslink PVA blended with other polymers.

Figure 3 shows the SEM micrograph for nanofibers PVA /$\gamma$-PGA 10. These nanofibers have a size average of 119 nm. The fibers have a homogenous surface, and they are defect-free. The rise of $\gamma$-PGA into the PVA solution had a notorious effect on the average diameter of the electrospun nanofibers. It was observed as the concentration of $\gamma$-PGA increase; there was a reduction in the average diameter. These reductions could be attributed to the strong hydrogen bonding among OH groups of PVA and NH\textsubscript{2} groups of $\gamma$-PGA. However, the charge density of the polymer solution is a crucial factor in obtaining defect-free and thinner diameter fibers\textsuperscript{17}. $\gamma$-PGA is a natural polyelectrolyte, and as such, its density charge is high. So, increasing the concentration of $\gamma$-PGA in the PVA solution is expected to obtain thinner fiber. As well, because of the increase in the conductivity of the polymer solution\textsuperscript{18}.

After crosslinked PVA /$\gamma$-PGA 10 with glutaraldehyde, again, there is an increase of average diameter up to 148 nm, which confirms the bonding among polymer chains. Likely, the absorption of glutaraldehyde vapors during the polymer electrospun.

### 3.4 *In vitro* release of ACG from fibers of PVA and PVA /$\gamma$-PGA.

In this work, the cumulative release in vitro of GCA nanofibers phosphate buffer, pH 4.8, was studied, and the percentage of the accumulative GCA release against time was plotted. As it is shown for many other
systems, typically consisted of biphasic release profiles, beginning with a burst release stage and then follow by sustained release phase in the time (Fig. 4). The presence of the burst phase could be due to the release of free GCA molecules and GCA incorporated into the network nanofibers through non-inclusion interactions. Moreover, the release of CGA inside of the nanofibers occurs through slower dissociation, and diffusion processes should be related to the sustained release phase. As can see in Fig. 4, after the one h, the release of CGA from PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10 electrospun mats reached 6, 28, and 66%, respectively. Similarly, the maximal CGA diffused out to the three systems’ buffer solution is achieved after 72 h with values of 36, 65, and 82 %, respectively. The presence of the γ-PGA has a positive effect by facilitating the CGA release from the polymer spun mats. Such influence has explained by the existence of repulsive negative charges, which at pH 4.8 are located in the carboxylic acid groups of both γ-PGA and CGA\textsuperscript{18b}. A second possibility could be attributed to strong hydrogen bonding among inter and intra-chain OH groups of PVA that limit the diffusion of small molecules, as is the CGA. On the other hand, γ-PGA is a linear natural polyelectrolyte that acts as porogenic material. Which, likely form small micrometric pores by increasing the surface area to facilitate the CGA release from the fiber mats (Table S1)\textsuperscript{19}.

Drug release kinetics and mechanism is a fundamental aspect to describe the main properties and characteristics of a carrier system. Several kinetic models have applied to study drug release from different systems. Usually, the release process of drug molecules from electrospun fibers may be described with zero-order, pseudo-first-order kinetic, or pseudo-second-order kinetic equations\textsuperscript{20}.

Herein, the \textit{in vitro} release of CGA from the electrospun PVA and PVA/γ-PGA) nanofiber mats was fitted with pseudo-first kinetics order\textsuperscript{21}.

\begin{equation}
\ln(q_e - q_t) = \ln q_e - k_1 t \quad \text{Pseudo-first order}
\end{equation}

Where $K_1$ Is the rate constant obtained by the pseudo-first-order equation, $t$ is the time, $q_e$ and $q_t$ are amount release at the equilibrium time and amount release at any time, respectively. The $k_1$ value can be obtained from the slope of the linear plot.

It was found that with the above kinetic model is appropriate for describing the kinetic release process of CGA from the electrospun fiber mats. Figure 5 shows the plots of $\ln(q_e - q_t)$ vs. $t^{0.65}$ for the release of CGA at pH 4.8 and 37 °C conditions. As can see in all the graphics, a clean straight line was obtained.

Table 1 shows the calculated values of $K_1$, been 0.29, 0.33, and 0.11 for PVA/γ-PGA 10, PVA/γ-PGA 5, respectively. In the three cases, the lineal correlation coefficient ($R^2$) is very high and quite similar.

\textbf{Table 1.} Rates constants ($K_1$) and correlation coefficient $R^2$ of PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10.
From the above information values of the constant velocity $K_1$, several observations can be drawn. The lowest value of this constant is for the PVA fiber mats, which could indicate that the release of CGA takes a longer time to reach saturation equilibrium, showing the burst effect. Meanwhile, the value of $K_1$ for the PVA/γ-PGA 10 and PVA/γ-PGA 5 fiber mats are very similar, meaning that the presence of γ-PGA influences the release of CGA by a diffusion effect and both cases reach the saturation in less time than of PVA fiber mats.

Alternatively, the Peppas\textsuperscript{22} and Weibull\textsuperscript{23} models were applied to obtain more information regarding the type of diffusion mechanism.

\[
\frac{q_t}{q_T} = k t^n \tag{2}
\]

\[
\frac{q_t}{q_T} = 1 - e^{-\alpha t^\beta} \tag{3}
\]

Where: $q_t$ and $q_T$ represent the concentration of ACG released at any time and the total amount of this compound loaded in the fibers, $t$ is the release time, $k$ represents a constant kinetic, $n$ is the exponent that shows the mechanism of the liberation (equation 2), and finally $\alpha$ corresponds to a scale factor, and $\beta$ is a form factor in equation (3). The exponent $n$ below 0.5 in the Peppas model represents a drug control release driven by a diffusion effect, and for the exponent $\beta$ lower than 0.75 in the Wiebull model (Fig. 6).

Figure 6 shows the plots of $q_t/q_T$ vs. $t$ for the release of CGA in the fiber mats at pH 4.8 for Peppas and Weibull models. The simulation result indicates that PVA, PVA/γ-PGA 10, and PVA/γ-PGA 5 have a good fitting to the drug control release of Peppas model with a fair regression coefficient ($R^2$), being the best for the PVA system and slightly worst for the PVA/γ-PGA system. Otherwise occurs with the Weibull model, it found that simulation results of the PVA system unfit, but an excellent fitting was observed for PVA/γ-PGA 10 and suitable for PVA/γ-PGA 5. Again these data indicate a drug release mechanism to be predominantly diffusion-controlled with exponent $n$ ranging from 0.19 to 0.21\textsuperscript{20b,24}, where the investigated formulation and processing variables did not alter the drug release mechanism (Table 2). The unfit data of the release of CGA from the PVA electrospun fiber mats in the Weibull model can be attributed to the percentage of the released drug, which is lower than 50%. Introducing a small
modification of 0.5 for 1 in the equation of this model shows an excellent fit to the experimental data ($R^2$, 99). Therefore, this model limits its application in systems with a drug release percentage of less than 50% (Fig. S4).

**Table 2.** Kinetics models parameters of CGA released from loaded PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10 fiber mats.

| Sample               | Peppas Model | Weibull Model |
|----------------------|--------------|--------------|
|                      | $d$          | $K$          | $R^2$ | $\alpha$ | $\beta$ | $R^2$ |
| PVA/γ-PGA 10         | 0.21±0.04    | 0.28±0.05    | 0.84  | 0.22±0.05 | 0.42±0.05 | 0.92  |
| PVA/γ-PGA 5          | 0.19±0.04    | 0.24±0.05    | 0.78  | 0.23±0.06 | 0.30±0.06 | 0.83  |
| PVA                  | 0.21±0.02    | 0.13±0.01    | 0.92  | -         | -         | -     |

**Discussion**

The γ-PGA was satisfactory obtained by biosynthesis process, and the mixed with PVA permit be a great candidate to drug release, for the biocompatibility and biodegradability and the easy formation to electrospun mats.

The addition of γ-PGA in the PVA solution has a critical effect on nanofibers average diameter in both regular and after crosslinked. The addition of γ-PGA reduces the average size diameter of the nanofibers, and after crosslinked with glutaraldehyde, suffer an increase up, which confirms the bonding among polymer chains.

The comportment of release shows a burst release stage. The release of CGA inside of the nanofibers occurs through slower dissociation and diffusion processes, the release behavior for PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10 electrospun mats reached 6, 28, and 66%. Such a tendency is attributed to the γ-PGA presence, facilitating the CGA release for the strong hydrogen bonding among inter and intra-chain OH groups of PVA that limit the diffusion of small molecules.

The analytical models confirm that the mechanism involucrè in the release to CGA carries out by diffusion effect. The constant velocity $K_1$ values for PVA/γ-PGA 5, and PVA/γ-PGA 10 are very similar, and the PVA mats are lower, which could indicate that the release of CGA takes a longer time to reach a saturation equilibrium by intra-chain OH groups of PVA.

In this work, it is demonstrated that the PVA and PVA/γ-PGA electrospun fiber mats were successfully loaded with CGA, and such systems have a significant advantage as system release of this natural anti-diabetic drug.

**Conclusions**
Biodegradable polymer meshes were fabricated by electrospinning of PVA and PVA/γ-PGA polymer solutions, loaded with CGA for the assessment of the effect of γ-PGA on the drug delivery systems. Our results indicate that crosslinking with glutaraldehyde is an effective method to keep the stability of polymer mats. The presence of γ-PGA into PVA solutions increase notoriously the in vitro drug release from electrospun fiber mats, which is concentration-dependent. Although, it was observed that for the studied systems, a release profile is practically the same for all of them. An initial phase that corresponds to a burst release stage and then follows by a sustained release phase in the time. The maximal release of CGA was reached at 10% of γ-PGA introduced in the PVA solution, which was 82% release from the electrospun polymer fiber mats. The γ-PGA could have a positive effect on the drug release due to the partial repelling negative charges of carboxylic groups present in both CGA and γ-PGA at pH 7. The strong hydrogen bonding of PVA could be a second alternative to explain this release behavior. Finally, the application of the pseudo-first-order model is applicable and fits into releasing kinetics of the CGA from the polymer fiber mats. The use of Peppas and Weibull models indicates the released kinetics of CGA from PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10 electrospun fiber mats are driven by diffusion mechanism. Apparently, the Weibull model does not apply to those systems when the drug released is under 50%.

Declarations

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Authors contributions

I.E.S. Conceptualization, Methodology, C.C Methodology, A.D.L. Formal analysis A.L.P. Visualization, Investigation, R.T Supervision and visualization and J.R.G. Project administration, Writing- Original draft preparation Writing - Review & Editing.

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Availability of data and materials.

The current study are available from the corresponding author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have consented to the submission of this manuscript for publication.
Competing interest

There are not conflict to declare.

Abbreviations

CGA: Chlorogenic acid; γ-PGA: Poly(γ-glutamic acid); PVA: polyvinyl alcohol; PVA/γ-PGA 5: PVA with 5 % of γ-PGA; PVA/γ-PGA 10: PVA with 10 % of γ-PGA

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**Figures**

**Figure 1**

RMN 1H spectra of γ-PGA synthesized by Bacillus licheniformis ATTC9945a.
Figure 2

FT-IR spectra of nanofibers a) PVA, b) PVA/$\gamma$-PGA 5 and c) PVA/$\gamma$-PGA 10.
Figure 3

SEM micrographs of The PVA/γ-PGA, 10 nanofiber, obtains by electrospun with and without crosslinking (a) and c), respectively. Diameter size distribution for nanofiber with and without crosslinking (b), d), respectively.
Figure 4

The release profiles of chlorogenic Acid from PVA, PVA/γ-PGA 5, and PVA/γ-PGA 10.

Figure 5

Release of CGA from electrospun fiber mats as a function of t0.65 at pH 4.8 and 37°C.
Peppas Model for PVA, PVA/γ-PGA 5, PVA/γ-PGA 10, and Weibull model for PVA/γ-PGA 5, and PVA/γ-PGA 10.

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