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Preparation and Characterization of Zein-Metformin/Gelatin Nanofibers by Coaxial Electrospinning

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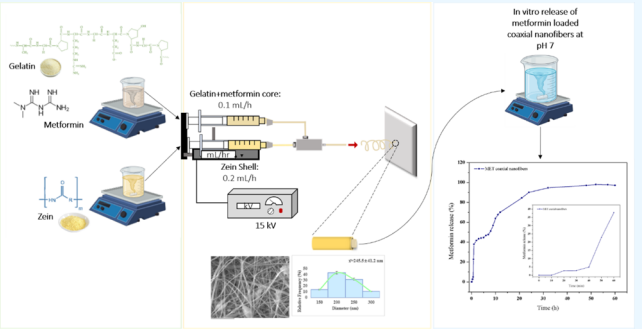
ABSTRACT: Metformin is a drug commonly used for the treatment of type 2 diabetes. However, it has been associated with damaging side effects when used over a long period of time. A potential solution to this problem is the implementation of a prolonged-release system for metformin, which would enhance the efficiency of the doses administered to patients. To achieve this, it is necessary to use materials compatible with humans. Electrospinning is an efficient technique that can be employed for this purpose, utilizing solvents that are safe for human use. Therefore, the objective of this study was to prepare and characterize a system for the prolonged release of metformin from zein and gelatin through coaxial electrospinning as well as to investigate its in vitro release. Metformin-loaded zein/gelatin coaxial nanofibers were prepared using the coaxial electrospinning technique and then characterized by morphological, structural, and thermal analysis. Morphologically, metformin-loaded zein/gelatin coaxial nanofibers were obtained with an average diameter of 322.6 ± 44.5 nm and a smooth surface. Fourier transform infrared spectroscopy (FTIR) analysis showed band shifts at a higher wavenumber due to drug−protein interactions by hydrogen bonding between N−H and C═O groups. Thermal gravimetric analysis (TGA) results suggested a possible interaction between materials due to an increase in the degradation temperatures of zein and gelatin when metformin was included. The transition of the crystallinity of metformin to the amorphous form was also confirmed by differential scanning calorimetry (DSC). Coaxial nanofibers exhibited an encapsulation efficiency of 66% and a profile release that showed an initial release of metformin (40%) in the first hour, followed by a gradual release until it reached equilibrium at 60 h and a cumulative release of 97% of metformin. It was concluded that using the coaxial electrospinning technique, it is possible to obtain nanofibers from polymeric solutions of zein and gelatin to encapsulate metformin, with a potential application as a prolonged-release system.



1. INTRODUCTION

Diabetes is known as a serious and long-term chronic disease that negatively impacts the life and well-being of many people, families, and society. It is a disease that occurs when the pancreas is not capable of producing enough insulin, or when the body does not use the insulin produced.[1] According to the IDF (International Diabetes Federation), diabetes is among the top 10 causes of death in adults, and it was estimated to have caused around 4 million deaths worldwide in the year 2017.[2] It is estimated that about 463 million people around the world, between 20 and 79 years old, are living with diabetes, with 90% of these cases attributed to type 2 diabetes (T2D).[1] In Mexico, it was estimated that 10.3% (8.6 million) of the population suffered from diabetes in 2018, of which 11.2% belonged to the state of Sonora.[3]

There are many treatments for T2D control; however, metformin is the main drug to treat and control the disease due to its ability to lower glucose on the tissues where the resistance is produced and weight control and has a low risk of hypoglycemia. The use of this drug can produce some gastrointestinal intolerance such as abdominal pain, diarrhea, vomit, and nausea, limiting its use in most patients.[4,5] These gastrointestinal symptoms limit the maximum tolerable dose in more than 50% of people, and approximately 5% probably will not tolerate any dose of it.[5] Therefore, there are many studies that have investigated and developed delivery systems of drugs

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to improve therapeutic effects and reduce side effects and toxicity of a normal dosage. Nanotechnology is a technology used to design and produce materials with sizes from 1 to 100 nm. It encompasses the application of different nanomaterials, which confer unique properties such as physical, chemical, mechanical, optical, and electronic. Currently, there are several nanotechnology applications that include its use in drug delivery, therapy, regenerative medicine, etc.[6] Regarding drug delivery, nanoencapsulation is a branch of nanotechnology that has led to a large variety of applications in the administration of drugs by enhancing their therapeutic effectiveness, supplying sustained and controlled administration to a specific site. An option for drug encapsulation is the use of polymers as they are made of biodegradable and biocompatible materials at the time the desired dose is released, through mechanisms that require changes in the pH, surface, temperature, etc.[7] Thus, nanoencapsulation of metformin can provide a prolonged release and a much higher efficiency at a lower dosage, and hence the side effects can be controlled.[8]

The use of nanofibers as potential drug carriers has been studied due to their large surface area and porosity.[9] Electrospinning is a method utilized to obtain nanofibers, providing specific properties for the material produced. In this process, a polymer solution is used, which is loaded into a syringe connected to a power source. After the electrospinning process is started, the polymeric solution is charged as a consequence of electrical forces; the surface tension increases, and the polymeric liquid slowly stretches to form a phenomenon known as "Taylor cone". With the increase of the electric field, the electric force overcomes the surface tension force, a jet of polymer solution is created from the upper part of the Taylor cone, the solvent evaporates, and the polymer fibers are collected. Coaxial electrospinning is an innovative method of conventional electrospinning and is implemented to obtain nanofibers with a core/shell system. In this method, two needles are located one inside another in order to produce that system; the size of the inner syringe is always smaller in dimension with respect to the outer needle.[10] Apart from the techniques mentioned above, there are others used in the fabrication of nanofibers for drug encapsulation such as blend electrospinning, emulsion electrospinning, suspension electrospinning, side by side electrospinning, and triaxial electrospinning. The advantage of coaxial electrospinning over other methods is that a core−shell structure of materials that are miscible and immiscible is obtained. In addition to an ability to encapsulate water-soluble and insoluble drugs dissolved in a suitable solvent, it is covered by a polymeric material as a shell.[11] Many biocompatible polymers have been widely used to prepare nanofibers such as collagen, chitosan, gelatin, and hyaluronic acid.[12]

A biopolymer commonly used to generate this type of nanomaterial is Zein. Zein is the main storage protein in corn and represents 50% or more of the total endospermic proteins. Due to its composition, zein is ideal for the formation of complex nanostructures to encapsulate different types of compounds.[13,14] Also, it has hydrophobic characteristics due to its high content of nonpolar amino acids, in addition to its high degree of polymerization, and offers advantages as a raw material in the production of biomaterials, coatings, and plastic applications since it is biodegradable and renewable.[13] On the other hand, gelatin is a biopolymer that can be easily obtained from partial hydrolysis of collagen, the most abundant structural proteins of which are found in the connective tissues of animals, such as skin, tendon, cartilage, and bones. It is very effective to generate nanostructures, and it is one of the biopolymers approved by the Food and Drug Administration (FDA) because of its high biocompatibility, biodegradability, low cost, high water retention capacity, and nontoxicity.[9] Deng[14] studied the release behavior of Allopurinol encapsulated in glucose cross-linked zein/gelatin nanofibers, which showed promising properties for drug delivery systems as it slowed the release of the allopurinol due to interactions between functional groups of polymers and drug. Furthermore, Alhakamy[15] stated that as zein is mainly hydrophobic, this could delay water penetration into nanocarriers matrix and slow down the diffusion of the drug into the medium. These remarkable materials hold great promise in the biomedical field such as being a matrix for drug delivery systems because of their amino acid composition and hydrophilicity properties, which make them potential materials for biological efficiency during their contact with blood cells, and enhancing physicochemical characteristics and thermal stability of core−shell systems.[14−20]

To our knowledge, no previous studies have aimed to determine the optimal conditions for encapsulating metformin, in conjunction with gelatin as a carrier, within a zein matrix by using coaxial electrospinning to create a sustained release system for this drug. Therefore, this research focused on utilizing coaxial electrospinning with variations in equipment parameters and polymeric solution concentrations to identify the best spinning conditions to produce core−shell nanofibers consisting of metformin, gelatin, and zein. In addition, the resultant coaxial nanofibers were characterized to analyze the compatibility and interactions between components and their effect on fiber morphology, diameter, and thermal stability. Moreover, an in vitro test was performed to evaluate the release system of the resultant coaxial nanofibers.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods. Zein, with a protein content of 86.06% (w/w), type B gelatin with a reported gel concentration of 175 g bloom, and metformin (1,1-dimethylbiguanide hydrochloride 97%) were purchased from Sigma-Aldrich (St. Louis, MO). Commercial metformin of 1000 and 850 mg (extended release) was purchased from a local drug store (Mexico). Ethanol and acetic acid were purchased from Fagalab (Mexico), and distilled water was used.

2.2. Characterization of Polymeric Solutions. *2.2.1. Viscosity.* The viscosity of solutions was determined using a Modular Compact Rheometer (MCR-102; Anton Paar, Germany), with a concentric cylinder geometry at a constant shear rate of 50 (s^{-1}) at 25 °C.

2.2.2. Density. Density was determined using the pycnometer method described by Hernández-Tapia,[21] and the final density result was calculated using eq 1

$$\rho = \frac{M_3 - M_1}{M_2 - M_1}\ \mathrm{g/cm^3} \tag{1}$$

where M_1 was the constant weight, M_2 the reference measurement, and M_3 the measurement with the concentrations of 25% (w/v) and 30% (w/v) of both zein and gelatin.

2.2.3. Conductivity. The conductivities of the polymeric solutions of zein and gelatin at 25% (w/v) and 30% (w/v) were determined by the method described by Smeets.[22] A

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HANNA Instruments model HI 2550 conductivity meter was used for the measurements.

2.2.4. Surface Tension. For surface tension, solutions of zein and gelatin were prepared at concentrations of 25% (w/v) and 30% (w/v) and determined by the drop method using an IT Concept tensiometer.

2.3. Preparation of Zein and Gelatin Nanofibers. The polymeric solutions were prepared at concentrations of 25% (w/v) and 30% (w/v) of zein in 80% (v/v) ethanol using a heating plate and a magnetic stirrer at 500 rpm for 30 min at a temperature of 25 °C to homogenize the solution. Similarly, gelatin solutions were prepared at concentrations of 25% (w/v) and 30% (w/v) and using acetic acid at 80% (v/v) as a solvent. The homogenization of the solution was obtained using a heating plate with a magnetic stirrer at 500 rpm for 1 h at a temperature of 40 °C.

2.3.1. Electrospinning Process. To obtain zein and gelatin nanofibers, the electrospinning technique was used, which consisted of a high-voltage source (Spellman CZE 1000R), a syringe pump (Scientific, KD), and a 10 × 10 cm aluminum collector plate. Zein and gelatin polymeric solutions were charged into syringes equipped with steel needles of 19 G. To determine the optimum electrospinning conditions, the parameters of polymer concentrations of zein and gelatin, solution flow, voltage, and distance between the needle and the collector plate were varied (Table 1). The environmental conditions for the electrospinning were at a temperature of 25 °C and a humidity of 21% throughout the process.

Table 1. Electrospinning Conditions of Zein and Gelatin Solutions

zein and gelatin concentration % (w/v)	flow rate (mL/h)	needle−collector distance (cm)	voltage (kV)
25, 30	1	10	12
	1.2	12	15
	1.5	15	

2.4. Preparation of Zein−Gelatin Coaxial Nanofibers without Metformin. A solution of zein was prepared at a concentration of 25% (w/v) in 80% (v/v) ethanol (ZE = 25%). This solution was kept under constant stirring at 500 rpm for 30 min at a temperature of 25 °C. The gelatin solution was prepared at a concentration of 25% (w/v) in 80% (v/v) acetic acid. The solution was kept under magnetic stirring for 1 h at a temperature of 40 °C until a homogeneous mixture was obtained.

2.5. Preparation of Zein−Gelatin Coaxial Nanofibers with Metformin. A solution of zein was prepared at a concentration of 25% (w/v) in 80% (v/v) ethanol (ZE 25%). This solution was kept under constant stirring at 500 rpm for 30 min at a temperature of 25 °C. The gelatin solution was prepared at concentrations of 18% (w/v), 20% (w/v), and 25% (w/v) in 80% (v/v) acetic acid (GE 18%, GE 20%, and GE 25%). These solutions were kept under magnetic stirring for 1 h at a temperature of 40 °C. Subsequently, 500 mg of metformin was added to the solution and kept under magnetic stirring at 500 rpm for 30 min at a temperature of 25 °C until a homogeneous mixture was obtained.

2.5.1. Coaxial Electrospinning Process. Table 2 shows the concentrations and parameters used for the process. To obtain zein/metformin/gelatin coaxial nanofibers, the coaxial electrospinning technique was used with a syringe infusion pump (Tongli Tech TL-F6). Solutions were loaded into 10 mL plastic syringes equipped with steel needles with a 15 G needle (shell) and a 19 G needle (core). The environmental conditions for the coaxial electrospinning process were at a temperature of 25 °C and humidity of 21% throughout the process.

2.6. Characterization of Nanofibers. *2.6.1. Scanning Electron Microscopy (SEM).* Morphological characterization of the nanofibers obtained was carried out using a JEOL model 5410LV scanning electron microscope operating at 20 kV, and the samples were coated with gold before being analyzed and observed at 5000× magnification. The mean diameter was determined by measuring their diameters at about 100 different locations by using ImageJ software. The polydispersity index was determined, and the values obtained indicated whether the samples were monodisperse or polydisperse.

2.6.2. Fourier Transform Infrared Spectroscopy (FTIR). For the characterization of the molecular composition of metformin, zein, and gelatin and any chemical−structural interaction between such components in the coaxial nanofibers, their spectra were analyzed using a PerkinElmer, Spectrum Two model infrared equipment using an attenuated total reflectance (ATR) accessory, employing 16 scans at a resolution of 4 cm^{-1}.

2.6.3. Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). The behavior of degradation with respect to the temperature of the nanofibers was analyzed by thermogravimetric analysis using a PerkinElmer equipment model: Pyris 1. Samples of approximately 4 mg were subsequently heated to 600 °C, with a heating and cooling rate of 10 °C/min, under a gas flow of 20 mL/min of nitrogen. Study of the phase changes of the nanofibers with respect to temperature was determined using a PerkinElmer model 8500 calorimeter. Approximately 4 mg of sample was taken and sealed to be heated at 10 °C/min from 25 to 250 °C under a nitrogen flow of 40 mL/min.

2.7. Encapsulation Efficiency (EE) and Metformin Loading Capacity (LC). Encapsulation efficiency (EE) and loading capacity (LC) were calculated using eqs 2 and 3

Table 2. Coaxial Electrospinning Solutions and Process Parameters[a]

	flow rate (mL/h)			
	solution A (shell)	solution B (core)	needle−collector distance (cm)	voltage (kV)
ZE 25% + GEL 25%	0.5	0.5	15	15
ZE 25% + GEL 18% + MET	0.2	0.1	10	15
ZE 25% + GEL 20% + MET	0.2	0.1	10	15
ZE 25% + GEL 25% + MET	0.2	0.1	10	15

[a]ZE: zein, GEL: gelatin, MET: metformin.

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Table 3. Physicochemical Properties of Zein and Gelatin Solutions at Concentrations of 25% (w/v) and 30% (w/v)[a]

proteins	viscosity (Pa·s)	density (g/cm^3)	conductivity (μS)	surface tension (mN/m)
zein 25% (p/v)	0.0448 ± 0.0002^b	0.9526 ± 0.0014^b	943.33 ± 12.8582^b	47.2356 ± 0.0058^a
zein 30% (p/v)	0.0677 ± 0.0051^a	0.9617 ± 0.00035^a	988.33 ± 5.03322^a	31.2456 ± 0.0045^b
gelatin 30% (p/v)	0.3471 ± 0.0050^b	1.1217 ± 0.00050^b	1086 ± 11.7898^b	44.2763 ± 0.0020^a
gelatin 25% (p/v)	0.2030 ± 0.0045^a	1.1322 ± 0.00035^a	1168.66 ± 11.5036^a	29.4553 ± 0.0030^b

[a]Values correspond to the mean ± standard deviation. Different letters (a, b) indicate statistically significant differences according to the Tukey test; $p < 0.05$.

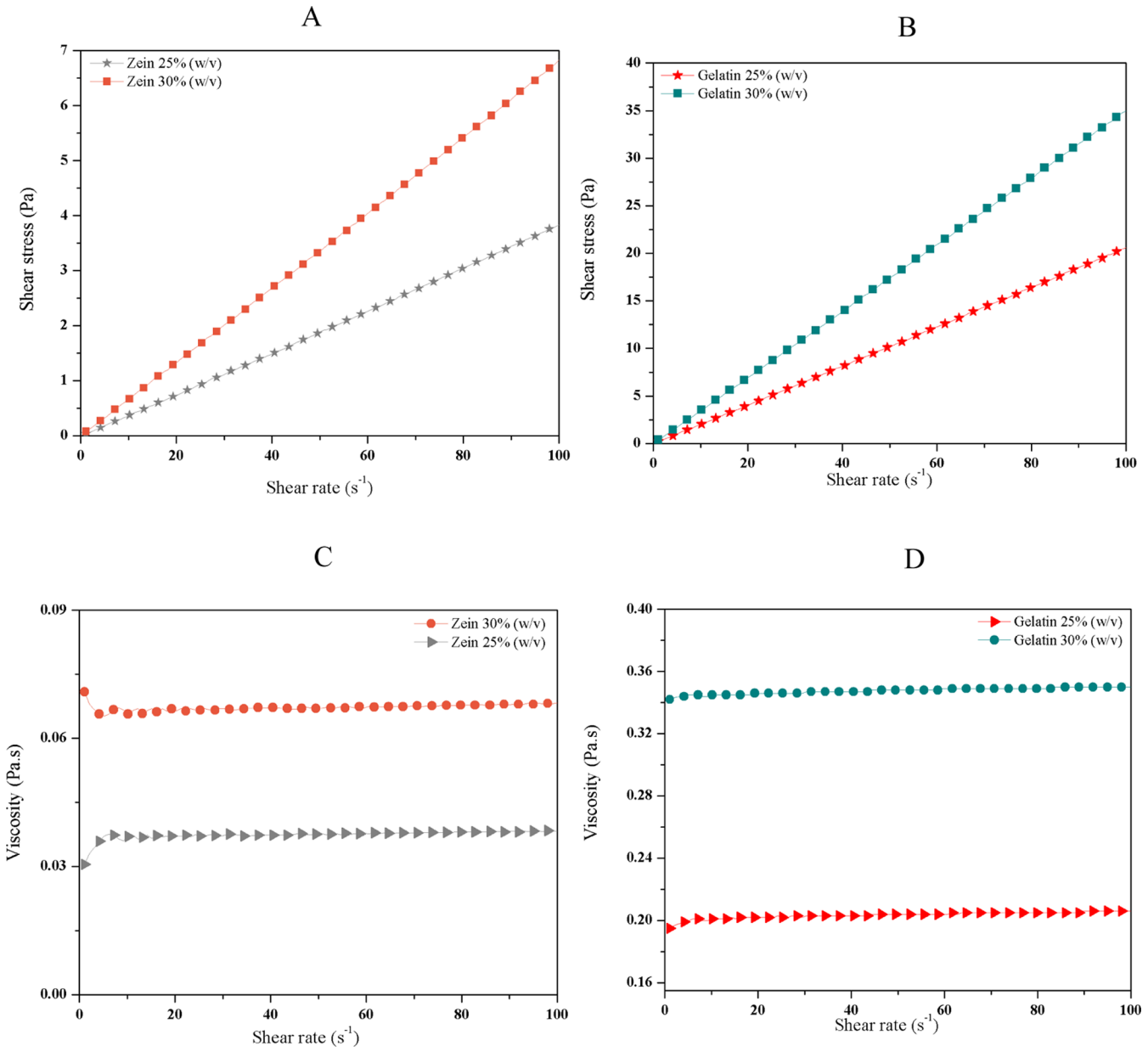

Figure 1. Rheogram of the effect of (A, B) shear stress vs shear rate and (C, D) shear rate vs viscosity of zein and gelatin solutions at 25% (w/v) and 30% (w/v).

$$\text{EE}\ (\%) = \frac{W_A}{W_T} \times 100\% \quad (2)$$

$$\text{LC}\ (\%) = \frac{W_A}{W_N} \times 100\% \quad (3)$$

where W_A is the actual weight of metformin in the coaxial nanofibers, W_T is the theoretical weight of metformin in the coaxial nanofibers, and W_N is the weight of the coaxial nanofibers.

2.8. Metformin Calibration Curve. A stock solution was prepared by dissolving 500 mg of metformin in 1 L of phosphate buffer (pH 7). From the stock solution, five concentration levels (400, 300, 200, 100, and 50 mg/dL) were prepared in phosphate buffer. Subsequently, the linear

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equation and correlation coefficient (R^2) were calculated by least-squares regression line.

2.9. *In Vitro* Release Studies. To further investigate the potential efficacy of core−shell systems for drug delivery, the release of metformin incorporated into coaxial zein−gelatin nanofibers in phosphate buffer (pH 7.0) was simulated at different times. The dissolution medium was 250 mL of phosphate buffer (pH 7) at 37 °C with magnetic stirring at 250 rpm. Samples (1 mL) were withdrawn at different intervals, replacing the sample withdrawn after each sampling. The absorbance was measured by a Varian Model Conc UV−visible spectrophotometer at a wavelength of 230 nm. Finally, the concentration was determined from the calibration curve of the standard solution with a known concentration of metformin in the same medium, and the percentage of drug release at each moment was calculated.

2.10. *In Vitro* Release Kinetics. The results obtained from the release of metformin were modeled based on the empirical model of Ritger and Peppas, which identifies the factors that can affect the rate when a solute is released from a polymeric matrix in a liquid phase. The Ritger and Peppas model is presented in eq 4

$$\frac{M_t}{M_\infty} = kt^n \tag{4}$$

where M_t corresponds to the amount of active compound released at time t and M_∞ is the amount of active compound released after infinite time. K is the kinetic constant that involves the analysis of structural and geometric characteristics of the release system, and n is the diffusion exponent that indicates the mechanism of drug release.

2.11. Statistical Analysis. All quantitative data were statistically analyzed to express them as the mean ± standard deviation (SD). Statistical significance of mean values between multiple treatment groups was accessed by analysis of variance (ANOVA) with Tukey's test. The value of $p \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Characterization of Zein and Gelatin Solutions. Viscosity, density, electrical conductivity, and surface tension were measured in the polymeric solutions (Table 3). In zein solutions, a slight increase in viscosity was observed from a concentration of 25% (w/v) to 30% (w/v). This increase may be due to interactions between polymer chains that led to a lower surface tension that often could facilitate the formation of fibers with an increase in their diameter.[23] On the other hand, a slight decrease in viscosity was observed in gelatin solutions from 25% (w/v) to 30% (w/v), which may be indicative of a reduction in interactions between molecules.[24] Figure 1C,D shows the dependence of shear rate on viscosity of zein and gelatin solutions with concentrations of 25% (w/v) and 30% (w/v). The viscosity did not show a significant change when the shear rate of the samples increased at a given temperature and concentration. This stability can be attributed to the fact that the solute had an effective dissolution in the solvent and, therefore, it is suggested that the solutions had a Newtonian behavior under the applied conditions.[25] Shear stress versus shear rate curves were fitted into the power law model, as shown in Figure 1A,B and the fitted parameters are presented in Table 4; this was to corroborate the Newtonian behavior observed in the rheograms. The results show that the

Table 4. Power Law Parameters of Zein and Gelatin Solutions at Concentrations of 25% (w/v) and 30% (w/v)

	power law parameters		
proteins	K (Pa·s^n)	n	R^2
zein 25% (p/v)	0.0355 ± 0.0027	1.0621 ± 0.0362	0.9935 ± 0.0045
zein 30% (p/v)	0.1244 ± 0.0845	1.0065 ± 0.0306	0.9984 ± 0.0012
gelatin 25% (p/v)	0.2122 ± 0.0003	1.0107 ± 0.0069	0.9998 ± 0.0
gelatin 30% (p/v)	0.3757 ± 0.0489	1.0090 ± 0.0065	0.9999 ± 0.0

flow behavior index (n) of zein and gelatin solutions at 25% (w/v) and 30% (w/v) was 1, confirming that they obey Newton's law. The density results corresponding to the 25% (w/v) and 30% (w/v) zein and gelatin solution showed a statistically significant effect ($p < 0.05$). That is, when increasing concentration, the density had an increase of 0.0091 g/cm^3 for the zein solution and 0.0105 g/cm^3 for the gelatin solution. Density is a parameter that is closely related to the formation of the Taylor cone, which can become a determining phenomenon in the formation, morphology, and diameter of the electrospun fiber.[21]

The conductivity of a polymer solution is mainly determined by the type of polymer and solvent used, and it not only affects the Taylor cone formation but also controls the morphology and diameter of the nanofibers. Thus, the polymers must be conductive for electrospinning to occur.[26] The reason is that the polymeric solution requires a sufficient charge to establish repulsive forces greater than the surface tension of the same polymeric solution for the formation of the jet.[27] According to our results, an increase in the conductivity of the solution was observed as the polymeric concentration increased, which varied from 943.33 ± 12.8582 to 988.33 ± 5.03322 μS for the concentrations of 25% (w/v) and 30% (w/v) of zein, respectively. For the gelatin solution, an increase in conductivity from 1086 ± 11.7898 to 1168.66 ± 11.5036 μS was obtained for the concentrations of 25% (w/v) and 30% (w/v), respectively, with a statistically significant difference ($p < 0.05$). This could be a phenomenon caused by the anionic nature of the polymer. A higher electrical conductivity of the polymeric solution can lead to a higher electrical repulsion force of the jet and could accelerate the formation of the Taylor cone, thus favoring the formation of the fiber.[23,28] In addition, specifically gelatin is considered a polyelectrolyte polymer; that is, it increases the charge capacity of the polymeric jet with the presence of ions in the gelatin, thus exposing it to greater tension with the electric field.[29,30] Surface tension is a parameter that has an important role in the electrospinning process since it is the force that opposes the voltage applied during the process and it is what determines electrospinning. This will change in relation to the concentration, chemical composition, and temperature of the polymer solution.[31] It is worth mentioning that bead-on string nanofibers occur when charge repulsions and viscous forces are overcome by the surface tension of the solution, affecting fiber elongation and eventually resulting in a chain of droplets. Hence, methods to avert this type of fiber morphology consist of increasing the concentration of polymeric solution to decrease surface tension.[32,33] In the solutions of zein and gelatin at different concentrations, statistically significant differences were observed ($p < 0.05$). It was shown that the

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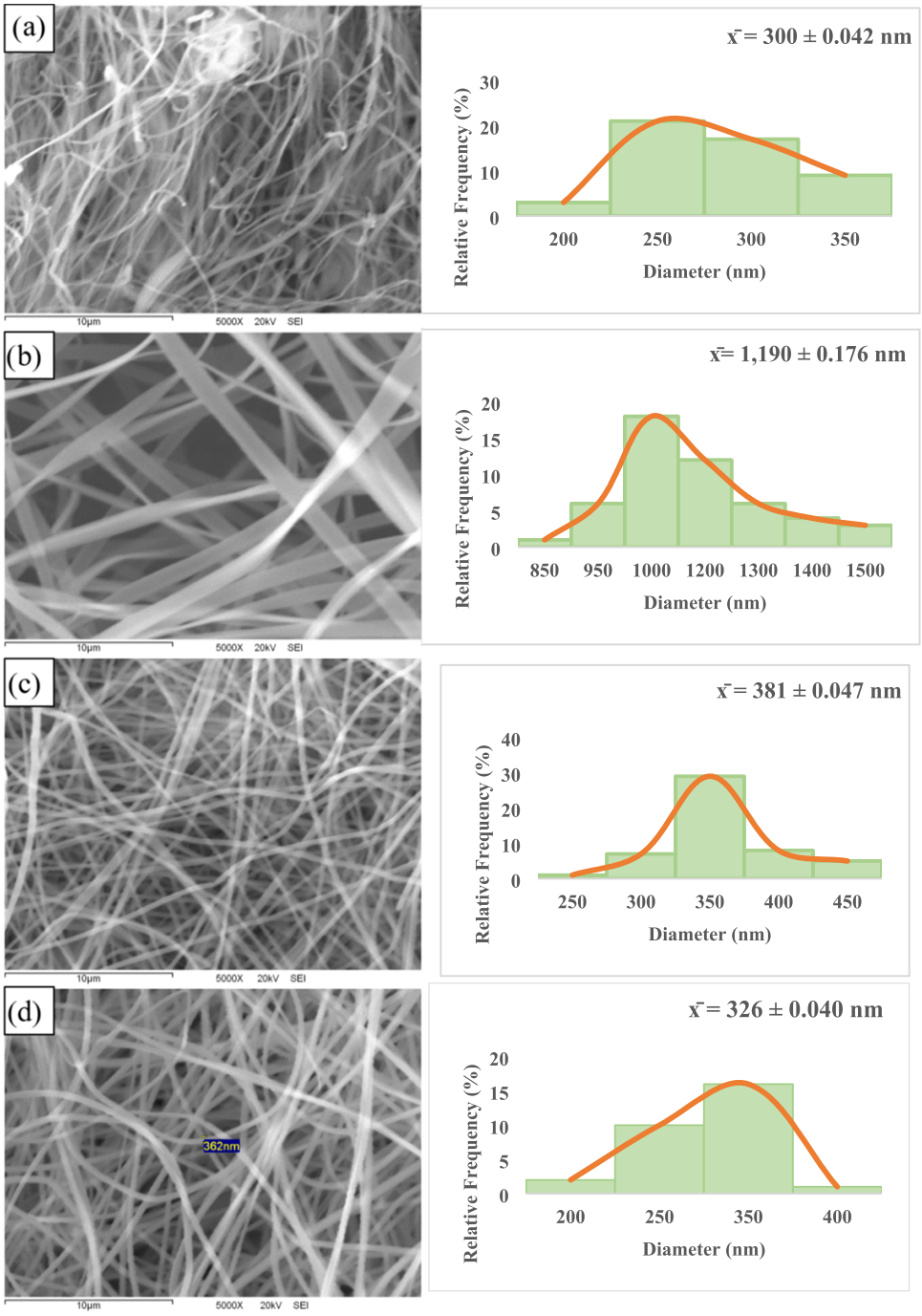

Figure 2. Micrographs of nanofibers obtained with 25% (w/v) and 30% (w/v) of (a, b) zein and (c, d) gelatin at 5000 magnifications and diameter distribution (n = 100). Electrospinning conditions: voltage of 15 kV, flow rate of 1 mL/h, and needle−plate distance of 15 cm.

surface tension decreased from 47.23 to 31.24 mN/m when increasing zein concentration from 25% (w/v) to 30% (w/v). Regarding the surface tension of the gelatin solution, it decreased from 44.27 to 29.45 mN/m as its concentration increased. This phenomenon could be caused by the increase in the repulsion force promoted by the collision of the interior molecules when the zein concentration is high. This decrease in the attractive force would promote a decrease in surface tension and time, inhibiting the electrospinning process due to the instability of the jet; that is, the formation of drops, pearls, and fibers will depend on the surface tension and its decrease.[29−34]

3.2. Obtention of Zein and Gelatin Nanofibers, and Metformin-Encapsulated Coaxial Nanofibers. The concentration of polymer solution, needle−collector distance, flow rate, and applied voltage have direct effects on the formation of coaxial nanofibers, including their mean diameter and morphology. A voltage of 12 kV, needle−collector distance of 10 and 12 cm, and flow rates of 1.2 and 1.5 mL/h, at concentrations of 20 and 25% (w/v) of both zein and gelatin, did not favor the formation of single nanofibers. As for the obtention of coaxial nanofibers, the gelatin solutions with metformin, at a concentration of 18 and 30% (p/v) of gelatin, a flow rate of 1 mL/h, and needle−collector distance of 15 cm,

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did not lead to coaxial nanofiber formation. In accordance with the literature, these behaviors may be caused by a jet instability related to a lower electrical field, an intermittent flow due to high flow rates, and strand breakages before reaching the collector plate associated with a reduced distance that could limit the duration for solvent evaporation.[35,36]

3.3. Morphological Characterization of Zein and Gelatin Nanofibers. Figure 2 shows the SEM micrographs of electrospun nanofibers of zein from 80% (v/v) ethanol solutions with concentrations of 25% (w/v) (a) and 30% (b) (w/v) and the diameter distribution of the samples. Likewise, gelatin nanofibers in acetic acid at 80% (v/v) with concentrations of 25% (w/v) (c) and 30% (w/v) (d) and the diameter distribution of the samples are shown. Analyzing the micrographs, it was observed that at a concentration of 25% (w/v) of zein (Figure 2a), smooth and randomly oriented nanofibers were obtained with a mean diameter of 300 ± 0.042 nm. On the other hand, as the zein concentration increased to 30% (w/v) (Figure 2b), nanofibers with ribbon or flattened morphology were obtained, with a larger diameter of 1190 ± 0.176 nm.[37,38] It has been reported that this phenomenon most likely occurs due to the lack of solvent evaporation during the electrospinning process, resulting in wet fibers that collapse to flat nanofibers into the collector.[39] As for the gelatin nanofibers at a concentration of 25% (w/v) (Figure 2c), smooth nanofibers without defects were observed, randomly oriented, and with a mean diameter of 381 ± 0.047 nm. As the gelatin concentration increased to 30% (w/v) (Figure 2d), the same morphology was maintained; however, a decrease in the mean diameter was observed at 326 ± 0.040 nm. According to the polydispersity index, the values of both the zein and gelatin nanofibers were close to 0, which indicated that the sample was monodisperse (Table 5).[40,41] In accordance with the results obtained in physicochemical analyses and scanning electron microscopy (SEM), zein solution at 25% (w/v) was the most suitable concentration to be part of the shell and, consequently, was selected for further analyses.

Table 5. Mean Diameter and Polydispersity Index of Zein and Gelatin Nanofibers at 25% (w/v) and 30% (w/v)[a]

protein	diameter (nm)	PDI
zein 25% (w/v)	300.0 ± 0.042[b]	0.140
zein 30% (w/v)	1,190.0 ± 0.176[a]	0.147
gelatin 25% (w/v)	381.0 ± 0.047[b]	0.123
gelatin 30% (w/v)	326.0 ± 0.040[a]	0.122

[a]The values correspond to the mean ± standard deviation. Different letters (a, b) between treatments are significantly different; $p < 0.05$.

3.4. Morphological Characterization of Zein−Gelatin Coaxial Nanofibers with Metformin. The coaxial zein/metformin/gelatin nanofibers were obtained by a coaxial electrospinning technique and analyzed by SEM (Figure 3). SEM micrographs of coaxial nanofibers with 25% (w/v) zein concentration in shell and gelatin at concentrations of 18% (w/v), 20% (w/v), and 25% (w/v) with 500 mg of metformin in the core were obtained to determine differences between gelatin concentrations. The mean diameters of the coaxial nanofibers were 245.5 ± 41.2 nm, 290.5 ± 38.9 nm, and 322.6 ± 44.5 nm for the concentrations of 18% (w/v), 20% (w/v), and 25% (w/v) of gelatin, respectively. Polydispersity index values close to 0 were obtained, thus showing monodispersity (Table 6). Morphologically, no residues of metformin crystals were observed on the surface, confirming the solubility of the drug in acetic acid.[42] The coaxial nanofibers with a gelatin concentration of 18% (w/v) presented surface defects such as beads, a random distribution, and some of them flattened.[29] Contrary to the gelatin concentrations of 20% (w/v) and 25% (w/v), smooth morphologies were observed. Likewise, it is shown that, at a higher concentration of gelatin, the diameter increased from 245 to 322 nm. It is easily deducted that the higher the polymeric concentration in the core with metformin, the higher the viscosity and the greater the difficulty in stretching the polymer solution in the electrospinning process, resulting in a larger fiber diameter.[42]

3.5. Fourier Transform Infrared Spectroscopy (FTIR). Spectra of zein, gelatin, metformin, and coaxial samples were analyzed (Figure 4). Metformin exhibits characteristic vibrations in the primary, secondary, and imine amine bands.[43] Similar results were shown in the spectrum of metformin with vibrations characteristic of asymmetric primary amine N−H stretching (3368 cm^{-1}), symmetric secondary amine N−H stretching (3290 cm^{-1}), imine N−H bending (1622 cm^{-1}), and CH_3 (1444 cm^{-1}). As for proteins, they have nine characteristic absorption bands: Amides A, B, and I−VII.[44] The spectra of zein and gelatin obtained showed stretching vibrations of amide A band in N−H and O−H (3282 cm^{-1} for zein and 3280 cm^{-1} for gelatin), stretching of amide I in C−O (1630 cm^{-1} for zein and 1629 cm^{-1} for gelatin), amide II bending in N−H (1515 cm^{-1} for zein and 1520 cm^{-1} for gelatin), and amide III deformation in C−H and N−H (1237 cm^{-1} for zein and 1231 cm^{-1} for gelatin). In the spectra of the coaxial zein/gelatin (Z/G) samples, the characteristic protein bands were also reflected in both conventional and coaxial nanofibers. However, the intensities in the absorption bands in the coaxial nanofibers were stronger than the plane samples, suggesting a formation of hydrogen bonds between proteins.[43] In the coaxial samples of zein/gelatin/metformin (Z/G18%−20%−25%/M), decreases in the intensities of the characteristic bands Amide A, I, II, and III were observed in the plane samples. Likewise, vibrations present in the region > 1500 cm^{-1} in the spectrum of metformin completely disappear in the coaxial samples, attributed to a possible interaction of the drug with the proteins and therefore improve the compatibility between them.[45,46]

3.6. Thermal Analysis of Nanofibers. *3.6.1. Thermogravimetric Analysis (TGA) and First Derivative (DTG).* The degradation behaviors of the nanofiber components with respect to temperature were obtained by thermogravimetric analysis (TGA) and the first derivative (DTG). Thermograms of zein, gelatin, and metformin powders, as well as the coaxial samples, were obtained (Figure 5). Regarding zein, it presented weight loss in two stages; The first stage occurred from 150 to 250 °C, losing 6% of its total weight, mainly due to losses of water and volatile compounds. The second stage was from 239 to 420 °C, losing around 78% of its initial weight, which represents the thermal degradation of the material.[47,48] The gelatin presented weight losses in two stages as well; the first stage was at <100 °C, losing 10% of its weight, attributed to water evaporation. The second stage was observed from 241 to 430 °C, losing 72% of its total weight, attributed to degradation by hydrolysis and oxidation.[49,50] The metformin thermogram showed that it is thermally stable up to 210 °C, followed by its degradation in four stages, at 220, 327, 350, and 370 °C until 92% of its weight has been lost.[51] In the zein,

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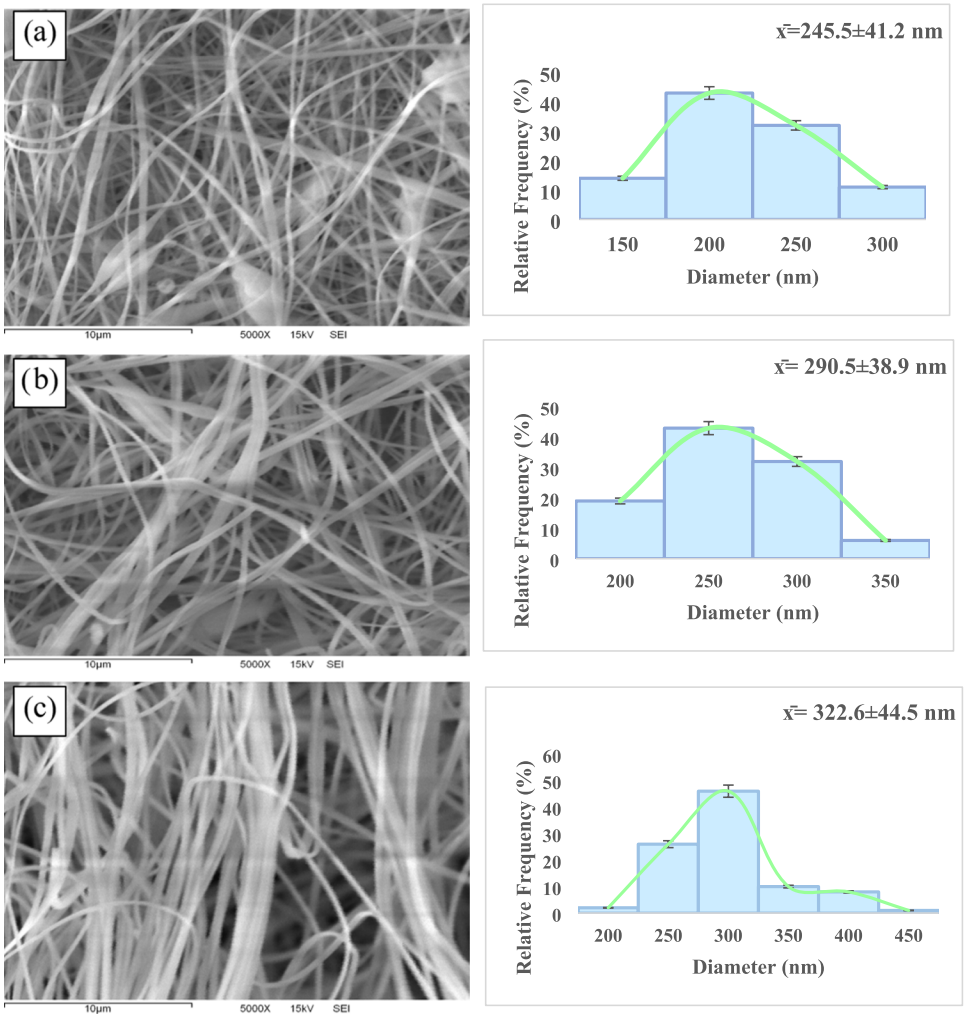

Figure 3. Micrographs of coaxial nanofibers obtained with zein at 25% (w/v), (a) 18%, (b) 20%, and (c) 25% (w/v) of gelatin and metformin at 5,000 magnifications and diameter distribution (n = 100). Electrospinning conditions: voltage: 15 kV, shell flux: 0.2 mL/h, core flux: 0.1 mL/h, and needle−plate distance: 10 cm.

Table 6. Mean Diameter and Polydispersity Index (PDI) of Coaxial Nanofibers[a]

concentration	diameter (nm)	PDI
ZE 25% + GEL 18% + MET	245.5 ± 41.2^{c}	0.167
ZE 25% + GEL 20% + MET	290.5 ± 38.9^{b}	0.134
ZE 25% + GEL 25% + MET	322.6 ± 44.5^{a}	0.137

[a]The values correspond to the mean ± standard deviation. Different letters (a, b, c) between treatments are significantly different; $p < 0.05$.

gelatin, and Z/G coaxial nanofibers, the degradation temperature of the materials increased in all three samples, since in the electrospinning process, the structures are more ordered and, therefore, confer greater thermal stability to the material.[52] Finally, according to the thermograms of the coaxial nanofibers (Z/G18%−G20%−G25%/M), significant weight losses were shown in three stages. In the first stage, there was a loss of 5% of its weight from 135 to 175 °C, which corresponds to a dehydration of the nanofibers. In the second stage, there was a loss of 73% of its weight at an initial temperature of 200 °C and continued up to 400 °C. These temperatures were lower with respect to the zein and gelatin targets, which could be related to interactions between the materials.[52] In the third stage, there was a loss of 22% of its value at a temperature > 400 °C. This can be attributed to the decomposition of stable structures formed during the electrospinning process. Likewise, it seemed that the degradation temperatures of the coaxial nanofibers decreased; these results suggest a possible drug−protein interaction.[52]

3.6.2. Differential Scanning Calorimetry (DSC). The behavior related to phase changes, such as glass transition temperature and melting points of metformin, 25% (w/v) zein−gelatin nanofibers, and coaxial 25% (w/v) zein/gelatin nanofibers with metformin, was analyzed by DSC, and their thermograms are shown in Figure 6. The metformin thermogram shows an initial flat profile followed by a sharp endothermic peak at approximately 234 °C, corresponding to the melting point and crystalline nature of the drug.[53] On the contrary, the thermograms of zein and gelatin nanofibers at 25% (w/v) do not show any characteristic peaks because zein and gelatin have an amorphous structure.[54] However, in the zein nanofibers, an endothermic peak occurred at approximately 100 °C, corresponding to the glass transition (T_g).[55,56] The gelatin nanofibers at 25% (w/v) showed the same behavior, with an endothermic peak at 115 °C, attributing it to its T_g, and with water loss along the process of electrospinning.[57,58] Coaxially, the zein/gelatin nanofibers showed a single endothermic peak at 98 °C corresponding to the glass

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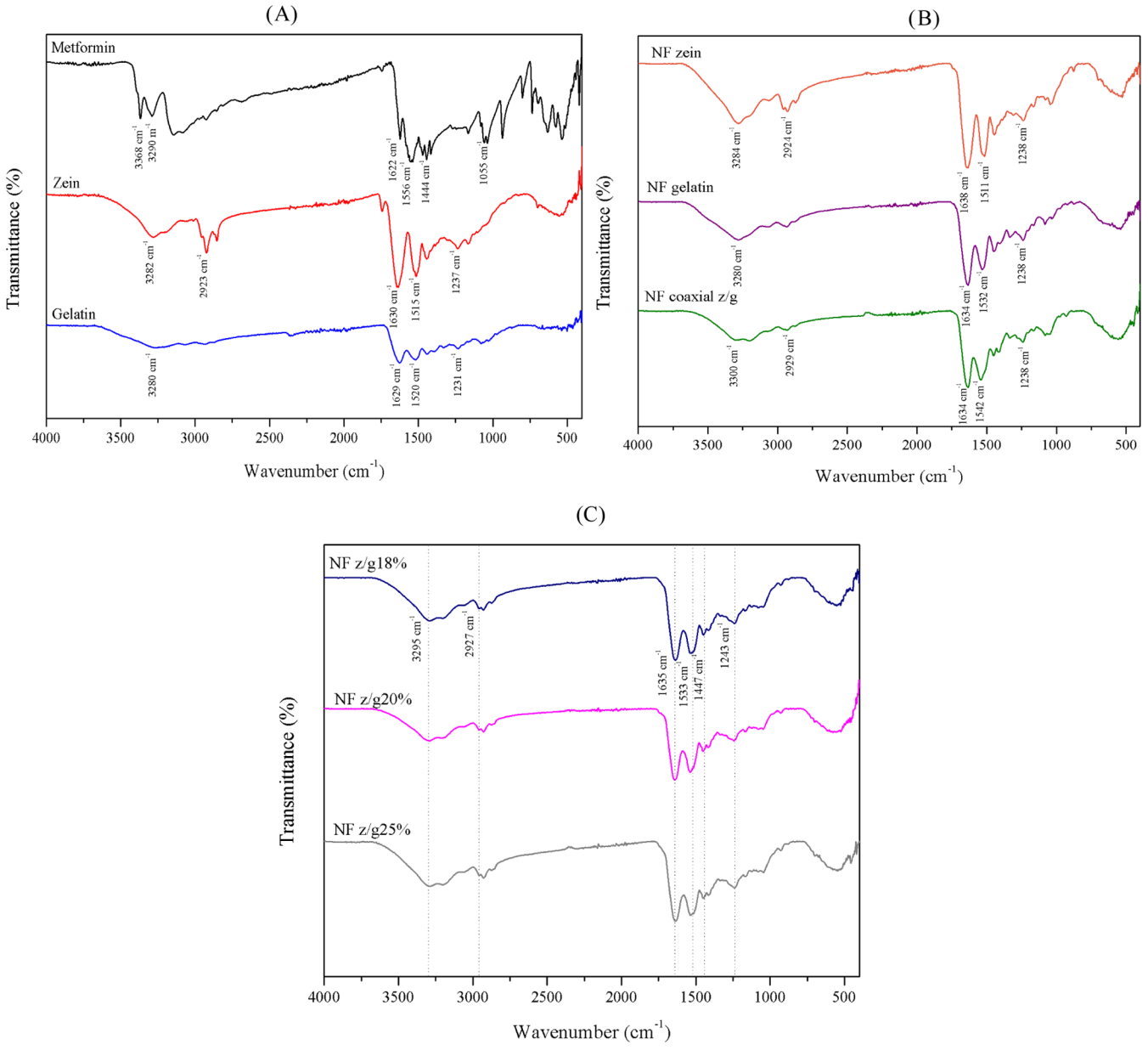

Figure 4. ATR-FTIR of (A) pure metformin, zein, and gelatin, (B) zein and gelatin nanofiber, z/g coaxial nanofiber, and (C) coaxial nanofibers of zein 25% (w/v)/metformin/gelatin 18−20−25% (w/v).

transition temperature, which had a small decrease compared with the nanofibers separately.

For the coaxial zein/gelatin nanofibers loaded with metformin, endothermic peaks were shown at around 94 °C, which could be attributed to the presence of zein and gelatin proteins. However, there was a migration of the endothermic peak at a lower temperature with respect to the target nanofibers. This decrease can be attributed to the fact that the proteins had a lower affinity for water molecules because the hydrophobic molecules are encapsulated.[59] Likewise, the characteristic endothermic peak of metformin was not observed in the 25% (w/v) zein/gelatin coaxial nanofibers, which may have occurred because metformin was not found in its crystalline form but rather in an amorphous form in the nanofibers or else, it is molecularly dispersed in the polymeric matrix. They also suggest that metformin was converted into an amorphous form by the rapid evaporation of the solvent during the formation of the nanofibers in the electrospinning process.[53,56,−6062]

3.7. *In Vitro* Release Studies. According to the metformin calibration curve (shown in Figure S1), a linear regression equation of $Y = 0.0005x + 0.1646$ with $R^2 = 0.9929$ was obtained, where "Y" corresponds to the absorbance and "x" to the concentration in mg/dL. Using this equation, the percentage of metformin released was calculated (Figure 7). The release of metformin from the coaxial zein/gelatin nanofibers, which used concentrations of 25% (w/v) for both core and shell solutions, was carried out at a pH of 7 at 37 °C with magnetic stirring. Likewise, releases of commercial formulations of Metformin 1000 mg and Metformin 850 mg (prolonged release) were performed under the same conditions to compare their release profiles (Figure 8). The encapsulation efficiency (EE) and loading capacity (LC) of metformin within these coaxial zein−gelatin nanofibers were

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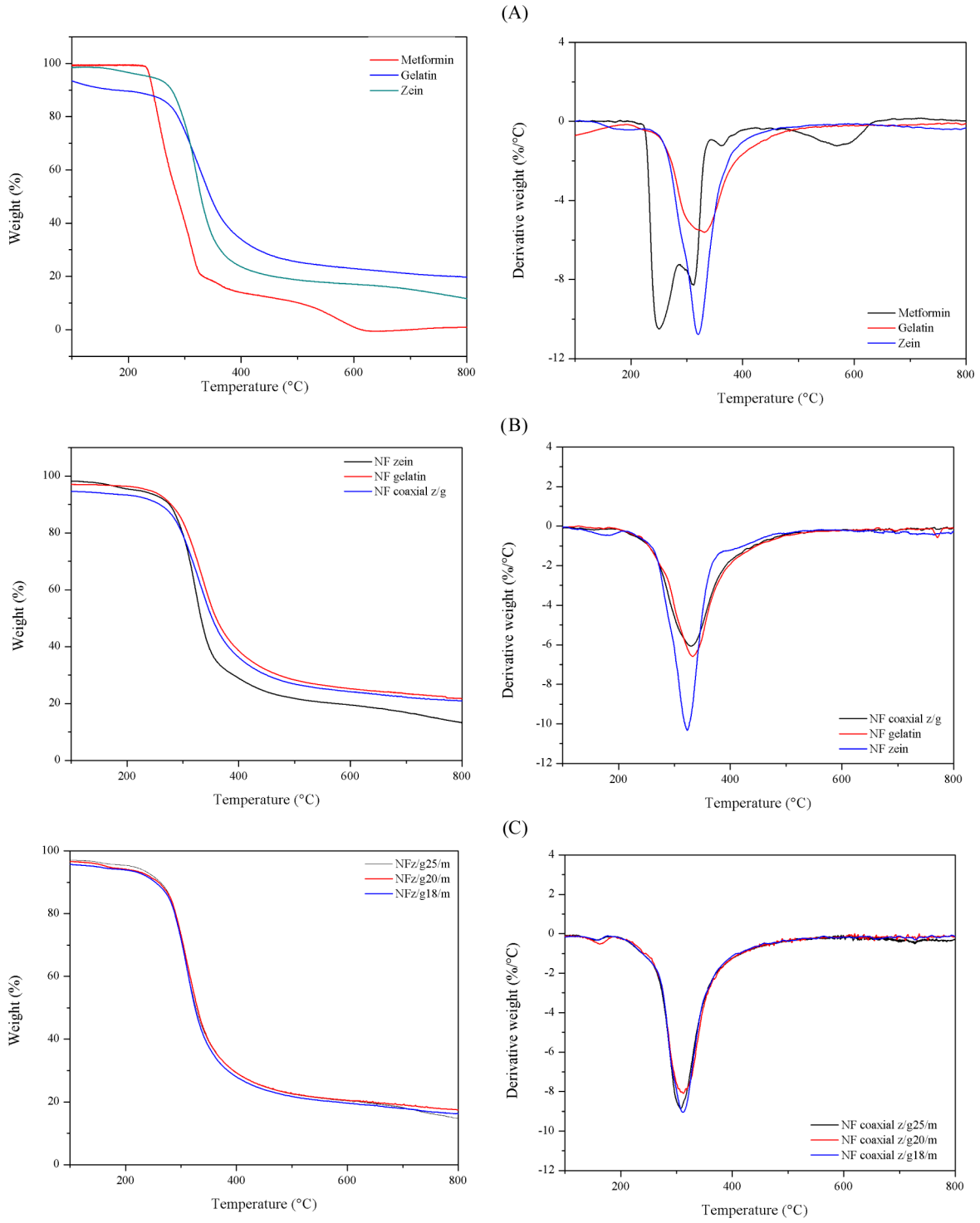

Figure 5. TGA and DTG of (A) pure metformin, zein, and gelatin, (B) zein and gelatin nanofiber, z/g coaxial nanofiber, and (C) coaxial nanofibers of zein 25% (w/v)/metformin/gelatin 18−20−25% (w/v).

calculated and found to be 66 and 19.94%, respectively. Regarding the release profile of the metformin-loaded zein−gelatin coaxial nanofibers, they showed a considerably high initial release percentage of metformin (40%) in the first hour, similar to the comparative formulations. This could be explained by the lack of binding of the drug with the polymeric matrix, where the fibers act as a physical vehicle rather than being chemically bonded, or by the presence of the active component in regions close to the internal surface. This was followed by a gradual release to equilibrium at 60 h, with a cumulative release of 97% of metformin, as opposed to the commercial metformin 850 mg formulation, which reached equilibrium at 12 h with a cumulative release of approximately 87%. As an amphiphilic biopolymer (mainly hydrophobic), zein provides a stable matrix for water-soluble drugs such as metformin, helping to prevent degradation of the drug and control its release rate. Therefore, a more prolonged release of metformin contained in coaxial zein−gelatin nanofibers was

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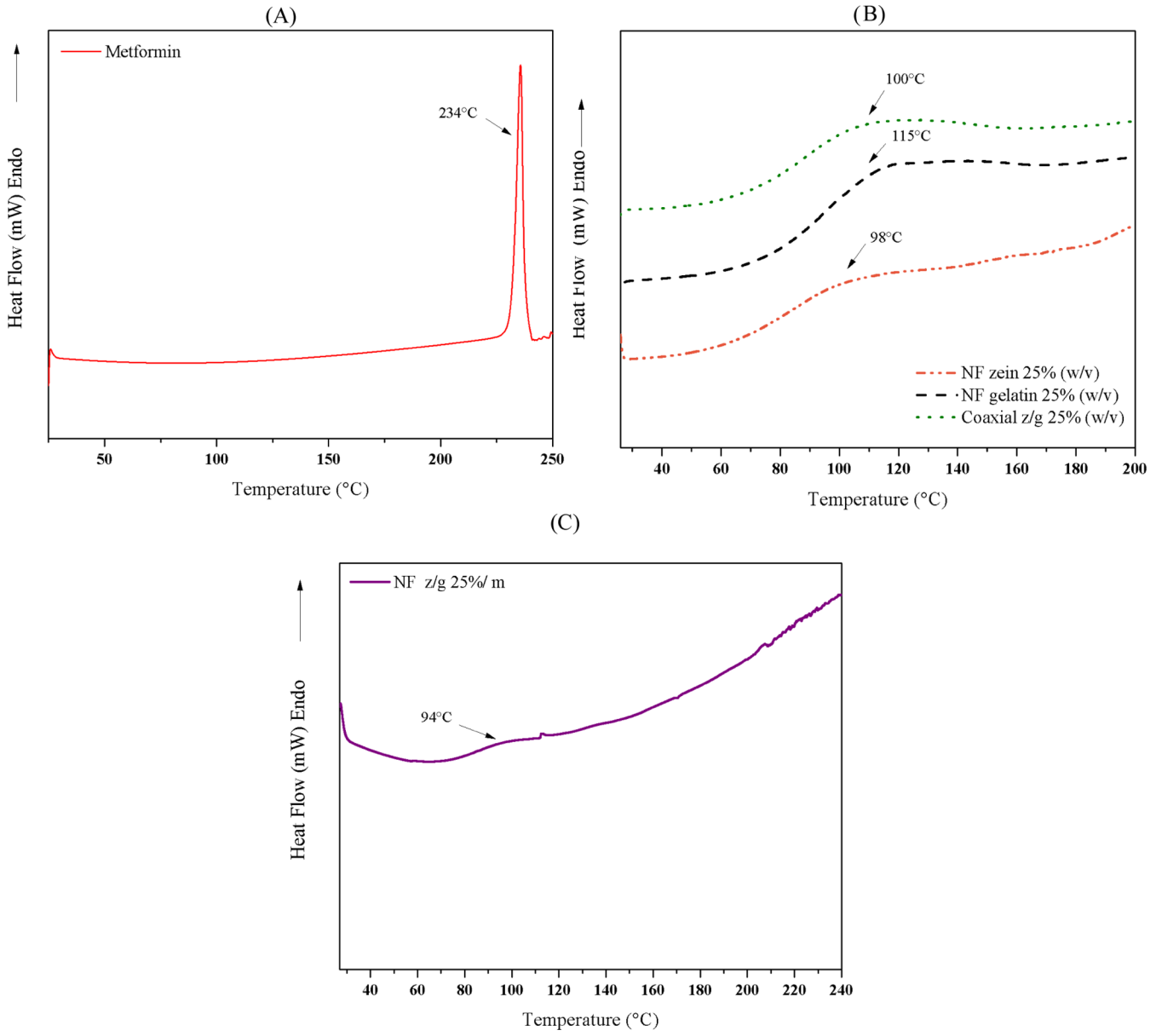

Figure 6. DSC thermograms of (A) pure metformin, (B) zein and gelatin nanofibers, z/g coaxial nanofiber, and (C) coaxial nanofiber of zein/gelatin 25% (w/v) with metformin.

achieved compared with the commercial prolonged-release formulation of metformin. For achieving the best results, coaxial nanofibers with a concentration of 25% (w/v) for both the core and shell solutions were utilized.

3.8. *In Vitro* Release Kinetics. The semiempirical Ritger and Peppas equation was fitted for the first 60% of metformin release in coaxial zein−gelatin nanofibers. A value of $n = 0.645$, $k = 0.175$, and $R^2 = 0.846$ was obtained, which indicates that the release of metformin exhibited non-Fickian kinetics and that it was due to anomalous diffusion and represents a release mechanism driven by a combination of diffusion and relaxation of the chains.[63] Likewise, low values of k indicate that the core−shell structure greatly influenced the release constant of coaxial zein−gelatin nanofibers, indicating a slower release.[64] Regarding the release kinetics by anomalous diffusion, this behavior could be attributed to the diffusion and relaxation of the polymer (zein).[65,66]

4. CONCLUSIONS

Using the coaxial electrospinning technique, it is possible to obtain nanofibers from polymeric solutions of zein and gelatin to encapsulate metformin. The morphology of the nanofibers is influenced by the variation in the concentrations of zein and gelatin; the concentrations of 25% (w/v) for both polymeric solutions were adequate to obtain nanofibers, resulting in nanofibers with a smooth morphology, without defects, with a tendency to monodispersity, an average particle diameter of <400 nm, and an encapsulation efficiency of 66%. Thermal analysis showed that by including zein and gelatin in the nanofibers, they provided stability to metformin by increasing its maximum degradation temperature, suggesting a possible drug−protein interaction; these results were complementary by FTIR analysis, which demonstrated band shifts at a higher wavenumber (cm^{-1}), due to drug−protein interactions via hydrogen bonding between N−H and C═O groups of proteins and the drug. The release of metformin from coaxial

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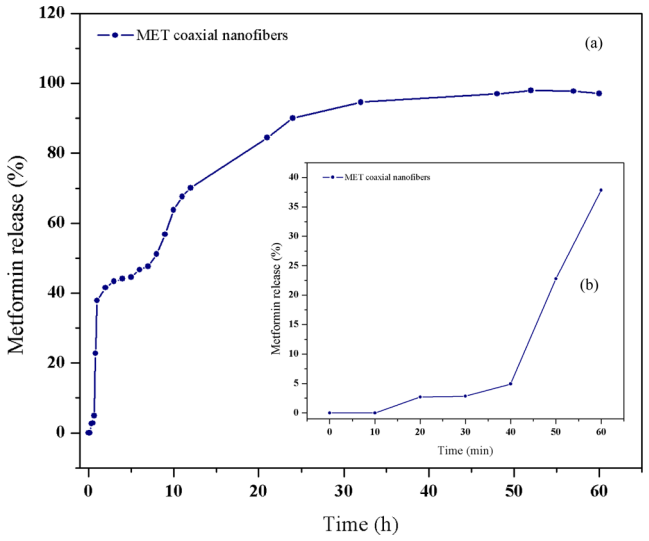

Figure 7. (a) Release profile of metformin from coaxial zein−gelatin nanofibers at pH 7 for 60 h. (b) Inset: release profile of metformin for the first 60 min.

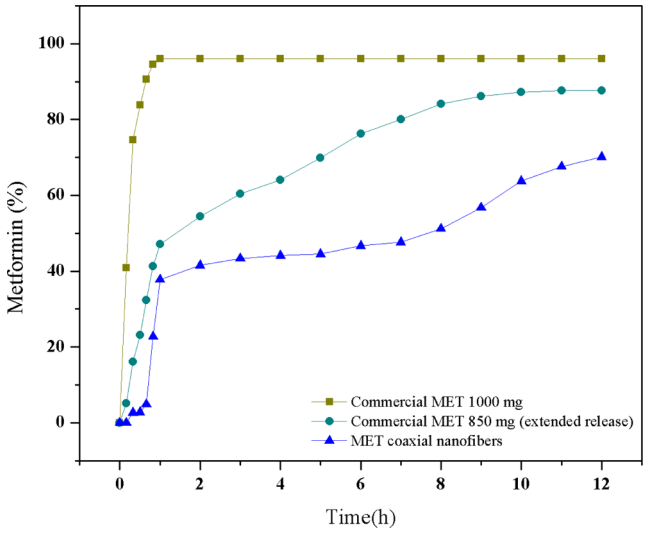

Figure 8. Release profile of commercial Metformin 1000 mg, Metformin 850 mg, and a metformin-loaded zein−gelatin coaxial nanofiber.

nanofibers at pH 7 showed a more sustained release >24 h with a cumulative percentage release of 97%. Zein and gelatin are mainly responsible for the sustained release due to their structures composed of nonpolar amino acids, which provide them the property of being a barrier against water and thus be used to obtain nanomaterials destined for sustained drug release. Therefore, the results of this research provide evidence that supports the implementation of biodegradable polymeric matrices in a core−shell system obtained by coaxial electrospinning for prolonged drug release.

■ ASSOCIATED CONTENT

Supporting Information

Metformin calibration curve. The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02016.

Metformin calibration curve (Figure S1) (PDF)

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Notes

The authors declare no competing financial interest.

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