CHAPTER 1 FOOD AREA

INFLUENCE OF THE ADDITION OF ORGANIC COMPOUNDS IN OBTAINING GELATIN NANOFIBERS

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Abstract

Nanomaterials have been introduced to the food industry by developing packages and sensors containing active substances. These substances can be incorporated into nanofibers, prolonging the shelf-life of food. In this study, we developed nanofibers using a mixture of gelatin, citric acid, glycerol, and poly (ε-caprolactone) (PCL). The nanofibers were characterized using rheological, morphological, physical, and mechanical analyses. The results showed that adding citric acid increased the viscosity of the polymeric solution, but it did not reach the level of pure gelatin solution. The presence of citric acid conserved the pseudoplastic behavior required for electrospinning, decreased the fiber diameter, and provided greater tensile strength. The presence of citric acid and glycerol helped to improve the morphological characteristics of the nanofibers, resulting in continuous and homogeneous fibers with a better appearance. Fourier transform infrared spectra (FTIR) showed the presence of characteristic gelatin and PCL peaks, indicating the successful incorporation of the polymers. Nanofibers made from gelatin-citric acid and gelatin-glycerol mixtures are the most promising options for incorporating metabolites and active compounds, as they provide an accessible and biodegradable system in the food industry.

Keywords: Electrospinning, nanofiber, gelatin, poly (ε-caprolactone).

1. Introduction

The development of nanofibers has experienced a surge in recent years due to several benefits, such as higher surface area, smaller pore size, higher porosity, and tensile strength [1]. Different methods can produce these materials, including template synthesis, phase separation, self-assembly, and electrospinning [2]. Electrospinning is currently the most popular technique for nanofiber processing due to its practicality, versatility, high processing yield, low cost, and diverse morphologies [3]. This method involves applying high voltage to a polymer solution from a syringe tip. The droplet's surface becomes electrically charged, generating repulsive forces. The solvent evaporates during its trajectory to a metal collector (plate or cylinder), and the material is deposited as nanofibers [4]. Three key factors are considered when developing nanofibers: formulation factors (such as solvents and polymers used to generate the polymer solution, polymer solution concentration, concentration of a substance or active ingredient to be incorporated in the solution, etc.), processing factors (electrospinning parameters such as flow rate, applied voltage, syringe to collector distance, etc.), and environmental factors (ambient temperature and relative humidity). Electrospinning is an innovative technique that has found applications in various fields, initially in the biomedical and pharmaceutical industries, but recently expanded to areas such as food, particularly in developing smart packaging [5 – 6]. Electrospinning has proven successful in these areas as it serves as a release system for various substances, including active ingredients and/or metabolites, through an intelligent system known as "nano in a nano" [7] and is applicable in different fields. The selection of polymers plays a crucial role in this technique, as they must be suitable for electrospinning and have good mechanical properties [8]. A wide range of natural polymers, including zein [9], chitosan [10], cellulose [11], gelatin [12], pullulan [3], and even some plant and seed mucilage [13], have been used to develop nanofibers. Synthetic polymers such as ethyl cellulose [14], polyvinyl alcohol (PVA) [15], polylactic acid (PLA), and polyethylene glycol (PEG) [16] have also been used.

Gelatin is one of the most commonly used natural polymers due to its biocompatibility, accessibility, low cost, biodegradability, and high efficiency [17]. This soluble protein is obtained by breaking down the collagen structure into single chains [18]. Gelatin has been extensively studied, and electrospinning has yielded satisfactory results with this polymer [19]. Researchers have reported that fibers with a stable morphology, high water absorption capacity, and excellent

mechanical properties can be produced using gelatin [20]. Therefore, in this study, we formulated various gelatin compositions to evaluate the effect of adding a crosslinking agent, surfactant, and synthetic polymer on nanofiber production. These nanofibers will be utilized in the future for incorporating metabolites into the manufacture of smart packaging.

2. Material and methods

2.1. *Material*

Gelatin type A from porcine skin 300 bloom (Sigma Aldrich, USA) was used for nanofiber development. Other materials included glycerol, citric acid, and PCL (Mn= 800,000), also from Sigma Aldrich (USA). Glacial acetic acid, sourced from Fisher Scientific (Massachusetts, USA), was used as a solvent.

2.2. *Preparation of polymeric solutions*

Various formulations were created with a gelatin concentration of 20 % (w/v) and a water-acetic acid mixture in a 50:50 ratio. The ratio of the polymers and organic compounds utilized in each formulation is presented in Table 1. Each formulation was stirred at 350 rpm for 1 h at 60 °C. The GePCL combination was the only formulation prepared with a total polymer concentration of 10 % (w/v), using acetic acid as a solvent, and was stirred for 3 h to improve the incorporation between the two polymers. Finally, all solutions were cooled to 30 °C, loaded into a syringe, and utilized for electrospinning.

Table 1. Formulations of polymeric solutions.

Mixture	Ge (%)	GeCA (%)	GeGL (%)	GePCL (%)
Gelatin	100	99	100	70
Citric acid	-	1	-	-
PCL	-	-	-	30
Solvents				
Water	50	50	49.7	-
Acetic acid	50	50	50	100
Glycerol	-	-	0.3	-

2.3. *Electrospinning process*

Nanofibers were produced using electrospinning equipment from Bioinica & Fluidnatek LE-100 (Spain). The polymeric solution was loaded into a disposable syringe (5 mL) with an 18-gauge stainless steel needle tip. Each mixture was introduced into the injection pump, and a flow rate of 0.8-1.0 mL/h was used. Two electrodes (positive and negative) were placed near a high-voltage source. A voltage of +18 kV and -5 kV was applied between the syringe tip and the metallic collector plate, which was positioned 10 cm away, creating an electric field and facilitating the formation of nanofibers. Nanofibers were collected on wax paper (10 × 10 cm) previously placed on the collector. Finally, the nanofibers were stored in a desiccator for 24 h.

2.4. *Scanning electron microscopy (SEM)*

A small quantity of nanofibers was obtained and attached to a metal sample holder using double-sided tape. The samples were subsequently sputter-coated with a gold layer for 120 seconds (Desk IV, Denton Vacuum, USA) and imaged at an acceleration voltage of 20 kV. Micrographs were captured at various magnifications using SEM equipment from JEOL (JMS-6390LV, Japan).

2.5. *Fourier transform infrared (FTIR) spectra*

FTIR analysis was performed using a spectrophotometer (Cary 630, Agilent Technologies, USA). The analysis involved the identification of characteristic signals (peaks) present in an infrared spectrum. These signals arise from interatomic vibrations between functional groups present in the nanofibers, and peaks can be observed at different wave numbers within a range of 4000 to 500 cm^{-1}. An attenuated total reflectance cell was utilized with a resolution of 4 cm^{-1} and 32 scans.

2.6. *Rheological Characterization*

The viscosity behavior of the polymeric solutions was determined according to the method described by Kazemianrad [17], with some modifications. A rheometer (Anton Paar MCR 302, Austria) was utilized to measure the viscosity as a function of the shear rate. The required geometry was a cone-plate PP25-1 (24.981 mm diameter), and measurements were taken at shear rates ranging from 1 to 100 s^{-1} with 10 s^{-1} intervals, resulting in a total of 30 data points. The samples were measured at room temperature (25 °C) and with a 0.5 mm gap.

2.7. *Physical and mechanical test*

The thickness of the nanofibers was measured using a Mitutoyo 547-500S micrometer (Digimatic, Japan). Similarly, mechanical property characterization was conducted using the methodology described by Nilsuwan [21], with some modifications. A Texture Analyzer (TA-XT2i) software was employed using the ASTM D882-02 standard method. Nanofiber samples were cut into strips (50 × 20 mm) for analysis. The strips were positioned between equipment tongs spaced 30 mm apart. The software was configured to use a stretching speed of 1 mm/min, a test speed of 3 mm/s, and a force of 1 N. Tensile strength (TS) was then calculated.

2.8. *Statistical Analysis*

The results were presented as the mean ± standard deviation (SD). Data were analyzed using a one-way ANOVA, followed by mean comparisons using the Least Significant Difference (LSD) method, using OriginPro 8.5 software. Statistical significance was tested at $p < 0.05$.

3. Results and discussion

3.1. *Scanning electron microscopy (SEM)*

Figure 1 depicts SEM micrographs of four types of nanofibers: gelatin (Ge), gelatin-citric acid (GeCA), gelatin-glycerol (GeGL), and gelatin-polycaprolactone (GePCL). In most of the images, the nanofibers appear smooth, uniform, linear, and free of defects such as beading, dripping, and broken fibers. The desirable properties of gelatin, such as being a highly natural polymer, hydrophilic, with a low melting point, and good dispersibility, make it suitable for creating high-quality nanofibers with thickening, dispersing, and emulsifying properties [22, 23]. The smooth, uniform, and bead-free nanofibers in these micrographs are mainly due to the high concentration of Ge used in the polymer solution, which was 20 % (m/v). Okutan [24] has shown that a concentration greater than 14 % w/v is required to create nanofibers with uniform and defect-free structures. By increasing the polymer solution concentration, a stable solution stream is created that does not break, resulting in a uniform nanofiber deposit [25, 26]. In contrast, low-concentration gelatin polymer solutions have been reported to produce beads and droplets instead of nanofibers [27, 28].

Moreover, it has been observed that the viscosity of a polymer solution increases proportionally with its concentration and the molecular weight of its components [29]. Thus, the GePCL solution was expected to have the highest viscosity; however, it had a low viscosity because it was prepared at a lower concentration (10 %) than the other formulations (20 %). As a result, it showed a rheological behavior very close to Newtonian. The low concentration and viscosity of the solution, along with the partial phase separation between Ge and PCL, caused an unstable solution jet, resulting in fibers of large size and heterogeneous diameters, as well as solution droplets when deposited on the collector.

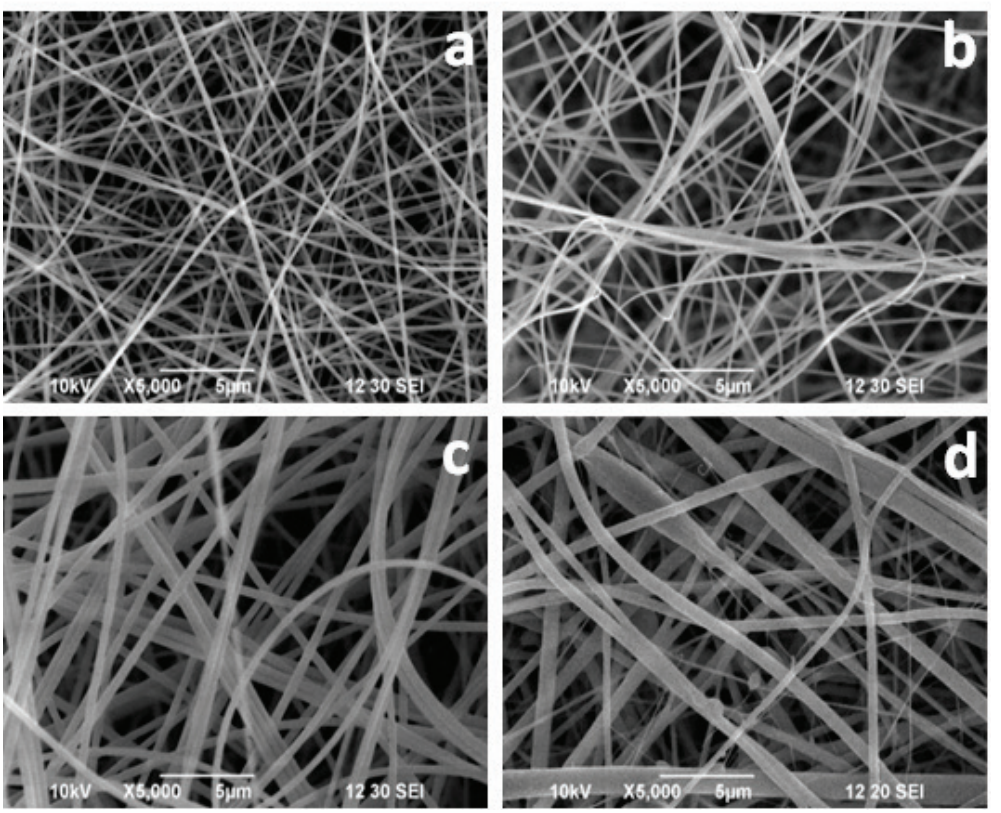

Figure 1. SEM images of nanofibers formulated with Ge (a), GeCA (b), GeGL (c) and GePCL (d).

Figure 2 displays the frequency histograms of nanofiber diameters. The results indicate that the average diameter of nanofibers produced solely from gelatin is the smallest, at 224±5nm. In contrast, incorporating additional components such as citric acid, glycerol, and PCL increases diameters of 250±6, 468±13, and 533±5 nm, respectively. The observed increase in nanofiber diameter follows the order of increasing molar mass of the components, which can explain the trend.

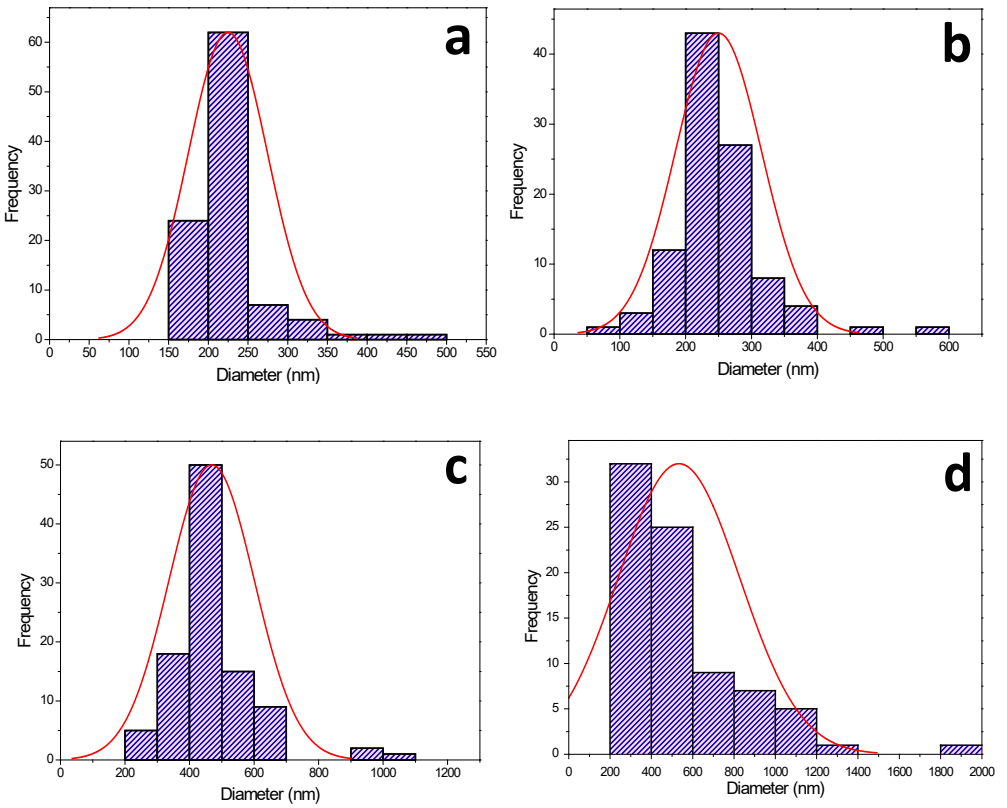

Figure 2. Frequency histograms of the distribution of diameters of the nanofibers obtained with Ge (a), GeCA (b), GeGL (c) and GePCL (d).

3.2. *FTIR Analysis*

Figure 3 displays the FTIR spectra of the nanofibers produced using gelatin (Ge), gelatin-citric acid (GeCA), gelatin-glycerol (GeGL), and gelatin-polycaprolactone (GePCL). Each spectrum shows characteristic signals that can be used for practical analysis, and the molecular structures of the nanofiber components were also included. Gelatin is a natural polymer and protein that produces well-defined signals, such as the peak at 3294 cm^{-1}, corresponding to the symmetric stretching vibrations of N-H amino groups belonging to primary amides. The signals at 2930 and 2850 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of CH_2 groups, respectively. The intense PCL peak at 1724 cm^{-1} is a characteristic signal of carbonyl groups (C=O) in the ester-containing PCL in its chemical structure [30]. Likewise, a characteristic Ge signal can be observed at 1640 cm^{-1}, corresponding to the stretching vibrations of C=O groups belonging to primary

amide groups [31]. The amide signal confirms the presence of Ge in the GePCL nanofibers, which are highly hygroscopic due to their interaction with hydrogens [32]. This same signal is attributed to the random helix-like spiral conformation of the gelatin structure. In the GeCA and GeGL spectra, peaks are observed at the same wavenumbers as in the Ge spectrum, but with decreased intensities, likely due to the formation of fiber component interactions, which was corroborated by the high solubility of Ge in the solutions with CA and GL. The signal at 1526 cm^{-1} refers to bending vibrations of N-H amino groups presented by secondary amides and stretching vibrations of the C-N bond of the Ge structure. The signals at 1240 and 1160 cm^{-1} correspond to asymmetric stretching vibrations of the C-O-C bond of PCL, which are characteristic peaks of PCL, thereby confirming the incorporation of this polymer with Ge [33].

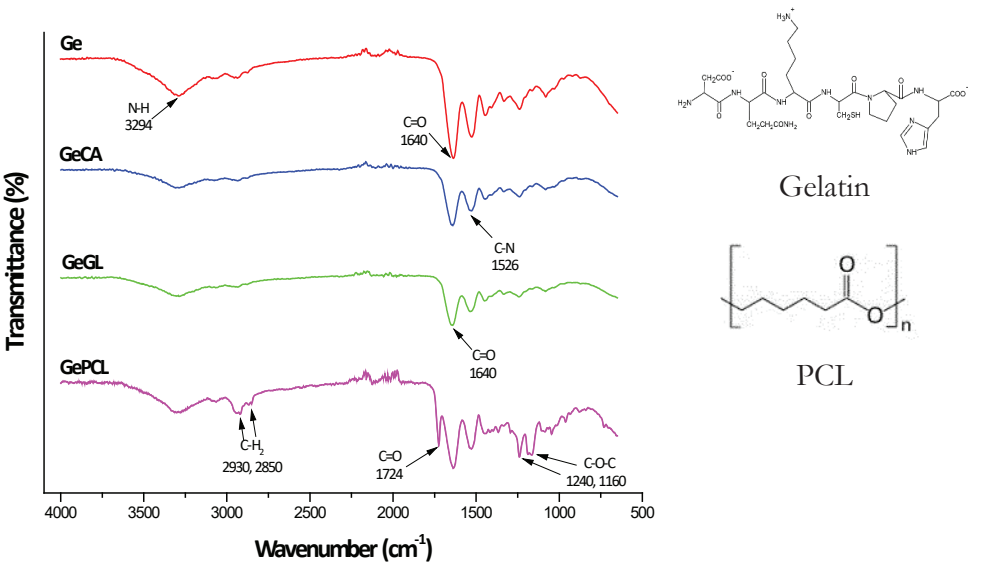

Figure 3. FTIR spectra corresponding to nanofibers of Ge, GeCA, GeGL and GePCL.

3.3. *Rheological Properties*

Viscosity is a crucial parameter in the electrospinning process, as it determines the formation of continuous and uniform fibers [8]. Figure 4 illustrates the viscosity behavior concerning a shear rate of 1–100 s^{-1}. The gelatin solution displayed behavior similar to that of a Newtonian fluid within the 1–60 s^{-1} range. However, at higher shear rates, the viscosity increased to 496 mPa.s, indicating a thickening shear behavior (rheopectic). GeCA and GeGL formulations showed similar behavior with the

same tendency but with a lower ratio than Ge. The GePCL mixture demonstrated almost Newtonian behavior throughout the shear range. The maximum viscosity – reached at a shear rate of 100 s^{-1} – was significantly higher in the Ge solution ($p<0.05$) than in GeCA and GeGL, which exhibited the lowest values.

The obtained flow curves were fitted to the Power Law model, and rheological parameters, including consistency coefficient (K) and flow behavior index (n), were calculated (Table 2). The Ge solution exhibited the highest value of K, while GePCL had the lowest value, as confirmed by the apparent viscosity results. In contrast, the n value of Ge was almost 1 within the 1–60 s^{-1} range, indicating similar Newtonian behavior. GeCA, GeGL, and GePCL solutions had values less than 1, indicating a shear-thinning behavior (pseudoplastic). This is favorable for nanofiber formation because, according to Mosayebi [34], polymers' arrangement during electrospinning is directly related to pseudoplastic behavior.

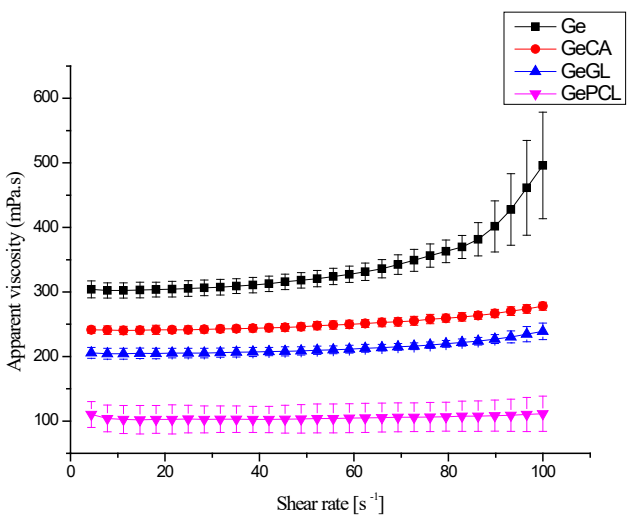

Figure 4. Flow curves of polymeric solutions: Ge, GeCA, GeGL and GePCL.

Table 2. Physical, mechanical and rheological parameters of nanofibers and Ge, GeCA, GL and GePCL solutions.

Formulation	Thickness (mm)	TS (MPa)	μ (mPa.s) [100 s^{-1}]	K (mPa.sn)	n
Ge	0.20 ± 0.02 [a]	0.96 ± 0.13 [b]	496.0 ± 0.08 [a]	279.15 ± 10.28 [a]	0.9616 ± 0.09 [a]
GeCA	0.10 ± 0.01 [b]	1.64 ± 0.16 [a]	278.0 ± 0.01 [b]	233.59 ± 6.71 [b]	0.3290 ± 0.09 [b]
GeGL	0.23 ± 0.01 [a]	1.38 ± 0.03 [a]	239.0 ± 0.01 [b]	198.24 ± 10.33 [c]	0.2686 ± 0.10 [b]
GePCL	0.06 ± 0.02 [c]	1.50 ± 0.11 [a]	111.3 ± 0.03 [c]	100.15 ± 20.23 [d]	0.0846 ± 0.04 [c]

TS=Tensile strength; K= Consistency coefficient; n= Flow behavior. Different letters in the same column indicate a significant difference ($p < 0.05$).

3.4. *Physical and mechanical tests*

Table 2 shows the thickness and TS values. Ge and GeGL formulations exhibited the highest thickness values, followed by GeCA and GePCL ($p < 0.05$), indicating that pure gelatin and its combination with glycerol promote the development of a thicker film. However, these formulations showed low TS values, possibly due to gelatin's high viscosity and rheological behavior. Furthermore, adding glycerol increases the nanofibers' plasticity and decreases the elastic modulus [35]. GePCL sample demonstrated a similar TS value to GeGL and the lowest thickness. GeCA had an intermediate thickness value (0.1 mm) and the highest TS value (1.86 MPa). Hence, in addition to the SEM images, GeCA may be considered the best nanofiber formulation due to citric acid facilitating better interaction between the molecules and increasing the hydrogen bond interactions between the carboxyl and amino groups of gelatin protein.

Citric acid has been investigated as a cross-linker, and successful results have been achieved in improving mechanical properties, increasing TS values, and decreasing the breaking point [36]. Although a low concentration of citric acid was used in this study, it likely enhanced these properties. However, a statistical difference was observed only in the Ge formulation.

4. Conclusions

Adding CA, GL, and PCL improved the final properties of the gelatin nanofibers. Gelatin nanofibers displayed good morphological characteristics, but their fiber diameters were the smallest, and their TS values were the lowest. On the other hand, the GeCA mixture presented the best physical characteristics by obtaining fibers close to those of Ge but with improved mechanical properties while maintaining pseudoplastic behavior. Similarly, the diameters of GeGL nanofibers were the most homogeneous and exhibited a similar rheological and physical behavior to GeCA. In conclusion, GeCA and GeGL formulations could be the best options for incorporating metabolites or active compounds into food.

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