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In-vitro release study of hydrophobic drug using electrospun cross-linked gelatin nanofibers

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A B S T R A C T

Delivering hydrophobic drug within hydrophilic polymer matrix as carrier is usually a challenge. Here we report the synthesis of gelatin nanofibers by electrospinning, followed by testing them as a potential carrier for oral drug delivery system for a model hydrophobic drug, piperine. Electrospun gelatin nanofibers were crosslinked by exposing to saturated glutaraldehyde (GTA) vapor, to improve their water resistive properties. An exposure of only 6 min was not only adequate to control the early degradation with intact fiber morphology, but also significantly marginalized any adverse effects associated with the use of GTA. Scanning electron microscopy imaging, Fourier transform infrared spectroscopy and thermogravimetric analysis were done to study nanofiber morphology, stability of drug and effect of crosslinking. The pH of release medium was also varied as per the gastrointestinal tract for *in-vitro* drug release study. Results illustrate good compatibility of hydrophobic drug in gelatin nanofibers with promising controlled drug release patterns by varying crosslinking time and pH of release medium.

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1. Introduction

Controlled drug delivery prevents over-dosing of drug and thus reduces the toxic effects associated with it. Efficacy of administered drug can be maintained by keeping the drug concentration in the body within its therapeutic window [1]. Due to the convenience of delivery and better patient compliance, oral route is mostly preferred [2]. To administer the drug release in the oral route, drug molecule is generally encapsulated in excipients, which protects the bioactive molecule from enzymatic degradation in gastrointestinal (GI) tract. These excipients can be in various physical forms such as micro/nanoparticles [3–5], hydrogels [6,7], thin films [8,9], micelle [10,11], micro/nanogels [12,13] *etc.* However over the past decade, nanofibers have been demonstrated as potential drug delivery systems due to their large surface area to volume ratio and controllable porosity, thereby resulting high drug loading capacity [14]. As majority of the drugs are highly hydrophobic with poor water solubility, nanofibers due to their large specific surface area facilitates their enhanced oral absorption [15]. Among the several techniques to fabricate nanofibers such as phase separation, self-assembly, electrospinning *etc.*; electrospinning is mostly preferred due to its versatility in terms of use of large number of polymers,

ease of control on fiber size with well-defined morphology and better scalability [14–17].

Gelatin is one of the most commonly used FDA approved biopolymer, as an excipient because of its biocompatibility, biodegradability, muco-adhesiveness and easy availability. It has generally been used as drug carrier in different forms such as hydrogels, microspheres, nanoparticles *etc.* [18]. As gelatin is considered to be poor in fiber processing, biomedical usage of gelatin nanofibers in tissue engineering, scaffold/bone repair, wound healing and drug delivery are based on composite fibers [19–23]. Although in recent times, only gelatin based electrospun nanofibers are also synthesized using different solvent systems, however there are very few studies available on only gelatin nanofibers used as a drug carrier [24–27]. Further to the best of our knowledge, there is no report on controlled release of hydrophobic drug using electrospun gelatin nanofiber except a recent demonstration of slow release of nystatin, an anti-fungal reagent [24]. More importantly, there is a need of systematic effort in literature to study the release of hydrophobic drug and correlate it with physiochemical conditions as well as structural properties of pure gelatin based electrospun fiber mat.

Electrospun gelatin nanofibers are water soluble, which limits their applications and long term use [28]. The crosslinking agent like formaldehyde [29], genipin [30], glutaraldehyde (GTA) *etc.* [31] have been reported in the literature, to modify gelatin *via* its amino, carboxyl or hydroxyl group respectively. GTA is most widely used

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because of its efficiency in stabilizing collagenous materials [28] and reducing biodegradation of such materials.

The objective of present work is to study the stability and release of hydrophobic drug from electrospun hydrophilic carrier. Piperine is selected as model hydrophobic drug. Piperine (1-piperoyl piperidine) is commonly known for its bio-enhancing effect on other co-administered drug [32]. It has been reported that piperine increases the bio-availability of curcumin, an anti-cancerous drug by 2000% in humans [33]. It also shows anti-depression, anti-inflammatory, anti-bacterial properties [32].

Gelatin nanofibers were prepared using electrospinning with as well as without piperine and cross-linked using saturated GTA vapor. Further, *in-vitro* release studies were performed at varying pH conditions matching human GI tract environment. Thus, we have tried to co-relate the morphology, *in-vitro* biodegradation study, stability of hydrophobic drug and effect of crosslinking with *in-vitro* release study of hydrophobic drug through hydrophilic gelatin nanofiber. This study attempts to draw a much needed attention toward exploring full potential of electrospun nanofibers as a drug delivery system, particularly for hydrophobic drugs.

2. Materials and methods

2.1. Materials

Gelatin (Type A, 175 bloom), Piperine (98%), Hydrochloric acid (ACS, 36.5–38.0%), Gluteraldehyde (25% v/v aqueous solution), Acetic acid (glacial, ACS, 99.7+%), Sodium hydroxide pallets (98%), phosphate buffer saline (pH 7.4) were purchased from Alfa Aesar, India. Deionized water (DI) (Model: Milli Q, Millipore India, resistivity 18.1 Ω) was used throughout the experiments.

2.2. Preparation of nanofibrous membranes

2.2.1. Preparation of electrospinning solution
Gelatin (Type A) was dissolved in acetic acid solution (20% v/v in distilled water) at 20% (w/v). The solution was stirred on a magnetic stirrer for 3 h at room temperature to get clear and homogenous solution, which was used to prepare gelatin nanofibers (GNF). In the prepared gelatin solution, piperine (2 mg/ml) was added and stirred for 2 h, to prepare piperine loaded gelatin nanofibers (G-P NF).

2.2.2. Electrospinning
Electrospun nanofibers were prepared using electrospinning set up purchased from E-Spin Nanotech Pvt. Ltd, India. The spinning solution was transferred to 3 ml plastic syringe with needle diameter of 21 gauge, by carefully avoiding air bubbles. The syringe was placed horizontally on the syringe pump. The flow rate of the feed solutions were controlled by syringe pump to make sure homogeneous flow (5 μl/min) though out the deposition. The electric potential of 12 kV was applied between tip and collector by the high voltage power supply maintained at a distance of 10 cm. The metal collector was covered by aluminum foil which was used as a substrate for deposition. Electrospinning process was carried out in the enclosed electrospinning apparatus at room temperature (27 °C) and 50% relative humidity.

2.2.3. Crosslinking electrospun membranes
Electrospun GNF and G-P NF membranes dissolve within few seconds in water, therefore, crosslinking was done by exposing it to saturated vapor of GTA (25% v/v aqueous solution). Both GNF and G-P NF, with and without substrate (*i.e.*, aluminum foil), were cut into 2 × 2 cm² sample sizes. These samples were placed inside the closed glass desiccator having 20 ml of GTA solution. Exposure to GTA vapor was done at room temperature for different time

intervals *i.e.*, 2, 4, 6, 8 and 10 min respectively. These cross linked samples now onwards are referred as GNF CX and G-P NF CX where *C* stands for crosslinking and *X* represents the time of crosslinking in minutes.

2.3. Characterization

2.3.1. Surface morphology
The morphology of the GNF and G-P NF samples, with and without crosslinking were examined by Field Emission Scanning Electron Microscopy (FESEM) (Model: SUPRA 40, Zeiss, Germany) at 10 kV with working distance 7.0 mm. The samples were sputter-coated with gold, to reduce charging effect.

2.3.2. Specific Surface Area (SSA) measurement
The Brunauer–Emmett–Teller (BET) surface area of GNF and GNF C6 was determined by N_2 physisorption using Micromeritics ASAP 2020 physisorption analyzer (USA). The sample mass was about 100 mg. All samples were degassed at room temperature for 6 h in nitrogen. The SSAs were determined by a multi-point BET measurement with nitrogen as the adsorbate.

2.3.3. Porosity measurements
To measure the porosity of nanofiber mat, samples were cut in equal pieces (1 × 1 cm²) and weighted. The thickness of the electrospun mat at minimum three different places was measured using digital micrometer (Mitutoyo, Japan). The apparent volume (V_a) was determined using the average thickness of the mat. The volume of the mat (V_g) was determined on the basis of gelatin density (1.41 g cm⁻³) and piperine (1.19 g cm⁻³) density and their mass percentage compositions adapted from [24]. Finally the porosity of the sample was determined using the following equation [24]:

$$\text{Porosity} = \left[1 - \left(\frac{V_g}{V_a}\right)\right] \times 100$$

2.3.4. FTIR spectroscopy
Electrospun non-crosslinked and crosslinked GNF and G-P NF were characterized by using Fourier Transform Infrared (FTIR) spectrometer (Model: Alpha-P, Bruker Corporation, USA). IR spectroscopy is mainly performed using the attenuated total reflection (ATR) method without any pre-treatment of the nanofabric samples. Spectra were obtained with 16 scans per sample at a resolution of 4 cm⁻¹ between 4000 and 500 cm⁻¹. All the spectra were further processed using OPUS software which was installed in the instrument system and plotted using Origin pro8.

2.3.5. Thermogravimetric analysis
Thermogravimetric analysis (TGA) of GNF, GNF C6, G-P NF and G-P NF C6 were carried out using platinum pan in helium atmosphere (Model: Pyris 1, PerkinElmer Inc., USA). Sample weight varies from 5 to 10 mg. Samples were heated from room temperature to 600 °C at a heating rate of 10 °C/min.

2.4. In-vitro biodegradation study

In accordance with oral delivery systems, pH of release medium was varied as per the gastrointestinal tract (GI) in human body. The pH of stomach is pH 1.5–4 due to gastric acids. The pH of small intestine (duodenum) varies from pH 6–8, where maximum absorption of nutrients takes place. Therefore, pH 1.2, 6, 7.4 and 8 are selected for further *in-vitro* biodegradation as well release study. *In-vitro* biodegradation study helps in determining the stability of the cross-linked electrospun mat in different physiological pH solutions. For this study, 5 × 5 cm² of electrospun GNF and G-P NF samples, cross-linked over different time intervals, were kept

in 25 ml solutions of pH 1.2, 6, 7.4 and 8 respectively in mechanical shaker (Model: RIS-24 plus, Remi India) for 24 h, at 37 °C and 150 RPM.

2.5. In-vitro *release study*

The release of drug *i.e.*, piperine from electrospun nanofiber mats was measured by placing 5×5 cm^2 of drug loaded fiber mat in 10 ml of release medium at different physiological pH levels (1.2, 6, 7.4 and 8). The temperature and stirring of the system were maintained at 37 °C and at 50 RPM, respectively. An aliquot sample was withdrawn, at fixed time intervals and same amount of fresh solution was added back to the release medium to maintain the sink condition. The samples were centrifuged (Model: CF-10, DAIHAN WiseSpin, Korea) for 2 min at 1300 RPM and analyzed using an UV spectrophotometer (Model: Lambda 35, PerkinElmer Inc., USA) at 342 nm as λ_{max} for piperine. The results were presented in terms of cumulative release as a function of time:

$$\text{Cumulative amount of release}\,(\%) = \left(\frac{C_t}{C_\infty} \right) \times 100$$

where C_t is the amount of piperine released at time t and C_∞ refers to total amount of drug loaded in 5×5 cm^2 sample.

2.6. Controlled drug release mechanism and mathematical modeling

To understand the drug release kinetics and the mechanism, the obtained data from *in-vitro* study was analysed using mathematical model. The most common equation to describe polymeric drug delivery system is known as Higuchi equation:

$$\frac{M_t}{M_\infty} = K_H \sqrt{t}$$

where M_t and M_∞ = absolute cumulative amount of drug released in time t and final respectively, K_H is a dissolution constant [34].

2.7. Statistical analysis

Data was analyzed with *t*-test to compare the difference between two treatment means. The null hypothesis is that the means of the measurement variable are equal for the two treatments. Results were recognized as statistically significant at the level of $p < 0.05$. The observations are presented as mean ± standard deviation (SD) of three independent experiments to confirm reproducibility of the findings. All the plots were analyzed using Origin Pro 8 software.

3. Results and discussion

3.1. In-vitro *biodegradation of the mat*

Fig. 1 summarizes the effect of crosslinking on GNF. Fig. 1a represents the non-crosslinked GNF membrane with aluminum foil. Lysine is one of the amino acids present in gelatin, which is responsible for crosslinking with aldehyde group of GTA [31]. After crosslinking, sample shrinks as shown in Fig. 1b. Therefore, membranes are not peeled from aluminum foil in order to avoid excessive shrinkage of membrane on cross linking. Fig. 1c shows the stability of crosslinked gelatin nanofiber membrane in aqueous medium even after keeping immersed. *In-vitro* biodegradation study was then done with the aim to check the stability of samples up to 24 h. GNF and G-P NF membrane with different crosslinking time are summarized in Table 1. Samples with different crosslinking time (non-cross-linked *i.e.*, 0 min and cross-linked for 2, 4, 6, 8 and 10 min) were undergone the degradation at different pH (1.2,
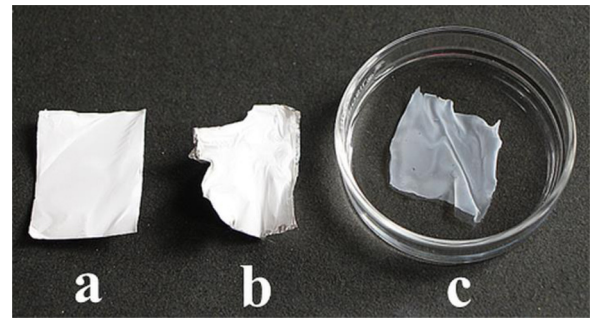

Fig. 1. Digital images representing, (a) non crosslinked GNF (b) shrinked GNF C6 and (d) GNF C6 in DI water.

Table 1
Summary of *in-vitro* biodegradation study for GNF and G-P NF crosslinked over different time interval, in dissolution medium of different pH.

pH of dissolution medium	Time of crosslinking with GTA (25% v/v) vapor											
	GNF						G-P NF					
	0	2	4	6	8	10	0	2	4	6	8	10
1.2	–	+	+	+	*	*	–	+	+	*	*	*
6	–	+	*	*	*	*	–	+	*	*	*	*
7.4	–	*	*	*	*	*	–	+	*	*	*	*
8	–	*	*	*	*	*	–	*	*	*	*	*

Where, '–' means completely degraded, '*' means not degraded and '+' refers to partial degradation in the dissolution medium after soaking for 24 h.

6, 7.4 and 8) solutions. Results of *in-vitro* biodegradation study for electrospun samples cross-linked for 6 min or above were found to be stable even after 24 h in all pH conditions. So, 6 min crosslinking time was selected for further analysis with a better comparison with 4 and 8 min crosslinked samples.

These results are quite significant while compared to previous reports on crosslinking of gelatin nanofibers [25,26,28]. Although GTA is very effective in cross linking gelatin and therefore widely used, however its prolonged exposure up to 24 h as reported in literature [26,28] may have adverse cytotoxic effects. Here in this work, we expose only for 6 min to saturated GTA vapor for crosslinking to achieve the desired stability of the fabric.

3.2. Surface morphology

The surface morphology of electrospun GNF and G-P NF membrane with and without crosslinking are represented in Fig. 2. SEM micrographs shows continuous, long nanofibers with fiber diameter in the range of 50–200 nm for both GNF and G-P NF as shown in Fig. 2a and c respectively.

Due to the hydrophilic nature of gelatin, it allows the water molecules along with GTA molecules from the saturated vapor, leading to changes in morphology on crosslinking even for only 6 min. It can be observed that the fibers fuse with one another at contact points (Fig. 2b), as a result of the partial dissolution of the fiber segments when they come in contact with moisture rich GTA vapor [28,35]. However, in case of G-P NF, presence of hydrophobic piperine discourages the interaction of water molecules in GTA vapor with fibers. It leads to relatively less fusing and minimal effect on fiber morphology at the point of contact of fibers (Fig. 2d).

3.3. Specific surface area and porosity

BET surface area of electrospun GNF was found to be 23.4 ± 1.2 m^2/g. On exposing for 6 min with saturated GTA vapor, the BET surface area decreased to 18.2 ± 1.8 m^2/g. A similar change in total pore volume was also observed
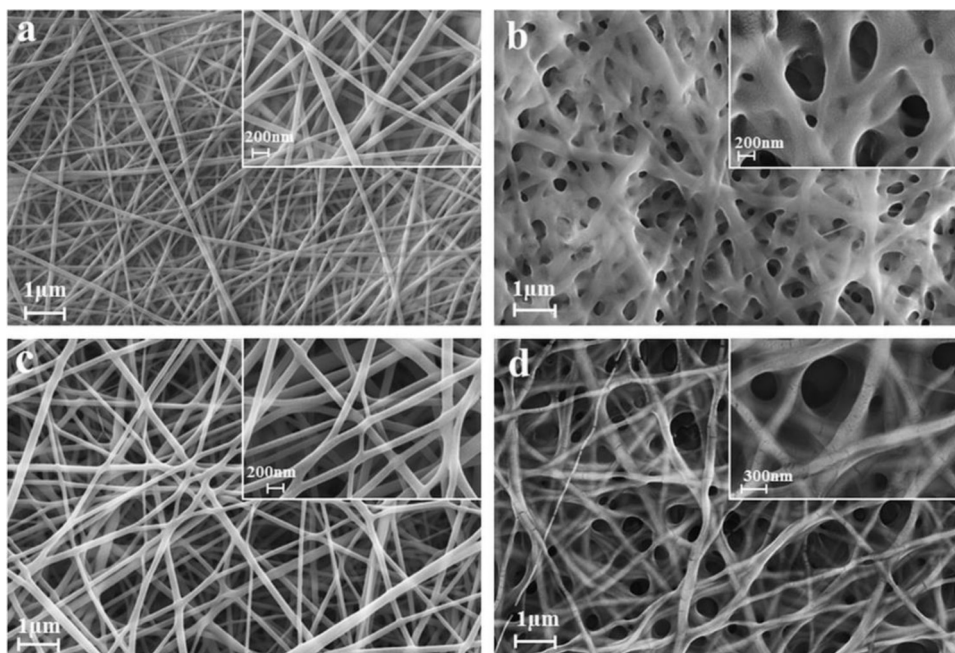
Fig. 2. FESEM images of electrospun (a) GNF; (b) GNF C6; (c) G-P NF; and (d) G-P NF C6 samples.

$(0.063 \pm 0.001$ and 0.05 ± 0.001 cm^3/g for electrospun GNF and GNF C6 respectively). However average pore diameter as measured by Barrett–Joyner–Halenda (BJH) method remains almost unchanged to 10.8 ± 0.9 and 10.9 ± 0.6 nm for electrospun GNF and GNF C6 fiber samples respectively. These results are also reflected in the porosity measurements. For GNF, porosity was measured to be $89.9 \pm 0.3\%$ which reduced to $83.3 \pm 1.0\%$ after 6 min cross linking. This decrease in surface area, porosity and total pore volume can be explained due to fusion of fibers in contact with water molecules present along with GTA vapor, as illustrated in Fig. 2. Similarly as expected for G-P NF, reduction in porosity after crosslinking (6 min) was significantly less (90.2 ± 0.8–$87.9 \pm 0.8\%$) which was also evident from FESEM images in which fiber morphology remain almost intact even after crosslinking. Therefore, the electrospun G-P NF membrane fabricated and used as carrier has sufficiently large surface area, even after crosslinking with GTA vapor.

3.4. Thermal properties

TGA analysis of electrospun GNF, GNF C6, G-P NF and G-P NF C6 fabric are shown in Fig. 3. Initial weight loss up to 100 °C is found to be 6.6, 7.5, 7.5 and 7.7% for GNF, GNF C6, G-P NF and G-P NF C6 respectively, due to the elimination of absorbed and bounded water molecules in the membrane. In case of pure gelatin fibers, weight loss after cross linking increases from 6.6 to 7.5% as compared to non-crosslinked samples. Similar observation is made for piperine loaded gelatin nanofibers before and after crosslinking (7.5–7.7%). This increase may be due to more adsorption of water molecules present along with GTA molecules while crosslinking with saturated vapor. However more interestingly, we observe that for drug loaded nanofiber samples, this increase in weight loss after crosslinking was less (0.2%) as compared to pure gelatin nanofibers (0.9%). As discussed earlier in Section 3.2, this is due to hydrophobic nature of drug (piperine) which restricts the adsorption of water molecules during crosslinking which is also evident from FESEM images of G-P NF C6 samples (Fig. 2d) showing intact fiber morphology even after crosslinking. Further second stage of weight loss as observed from 250 to 450 °C corresponds to thermal degradation of gelatin due to the breakage of protein chain. For GNF and G-P NF,
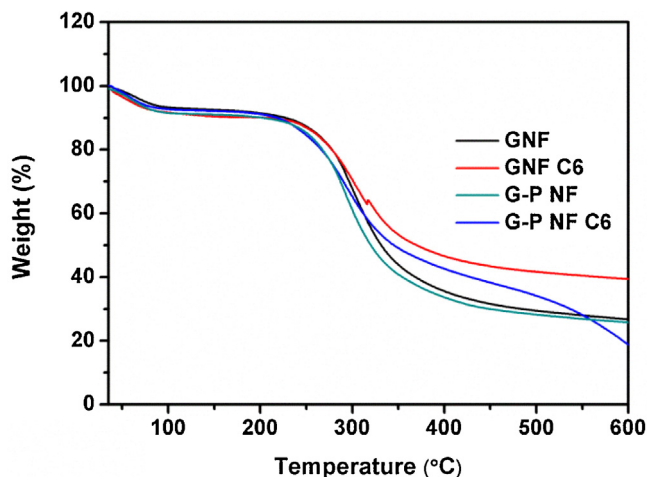

Fig. 3. Thermogram of GNF, GNF C6, G-P NF, G-P NF C6 samples.

this weight loss was found to be 56.3 and 55.5% respectively that was reduced to 43.9 and 46.3% for GNF C6 and G-P NF C6 sample respectively. Thus we observe that crosslinking with GTA vapor for 6 min (GNF C6 and G-P NF C6), increased the thermal stability of the fabric which was further improved by adding the hydrophobic drug (piperine).

3.5. Drug–polymer interaction: FTIR analysis

To know the chemical composition, effects of crosslinking and the interactions between the drug and polymer matrix FTIR analysis were attempted. The absorption bands at 3273.10 cm^{-1} (N—H stretch), 1631.66 cm^{-1} (amide I, C=O and C—N stretch), 1536.31 cm^{-1} (amide II, N—H bend and C—H stretch) and 1237.88 cm^{-1} (amide III) are the characteristic bands of GNF (Fig. 4a) [35]. On crosslinking, aldehyde group (—CHO) of GTA reacts with the amino group of the lysine which is present in gelatin and amino (—NH$_2$) groups interact with the carbonyl groups of GTA to form new covalent (—C=N—) bonds [31]. During crosslinking, first
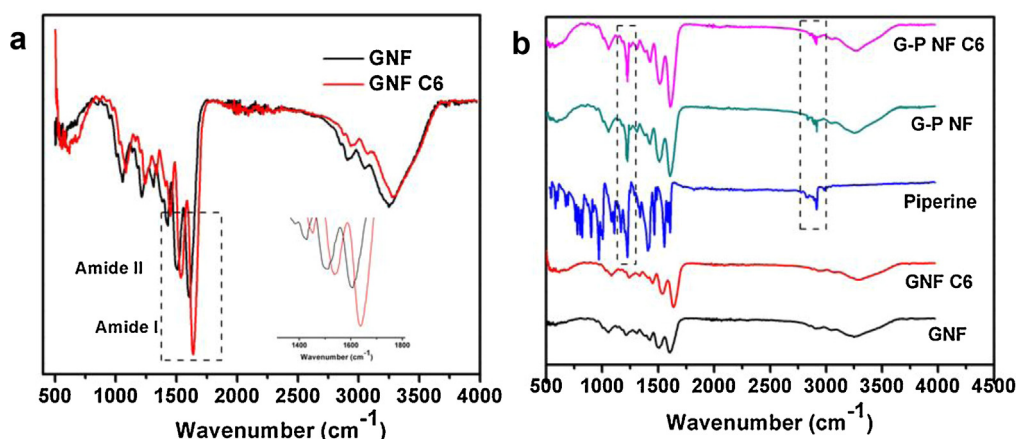
Fig. 4. FTIR spectra of (a) GNF and GNF C6 showing the effect of crosslinking; (b) GNF, GNF C6, piperine, G-P NF and G-P NF C6 respectively to show the stabiltiy of drug (piperine) in electrospun gelatin fiber matrix.

amide I (C═O and C─N stretching) peak shifts from 1605.30 cm^{-1} to 1631.87 cm^{-1} indicating its interaction during crosslinking. Similar trends are also observed in amide II and III peaks of gelatin, which confirm the hydrogen bonding with aldehyde groups of GTA.

Fig. 4b represents the effect of crosslinking in presence of piperine. The absorption bands at 2920.71 cm^{-1} (aliphatic C─H stretching), 1567.03 cm^{-1} (aromatic stretching of C═C, benzene ring) and 1231.62 cm^{-1} (asymmetrical stretching of ═C─O─C) are the characteristic bands of piperine. The presence of absorption peak due to C─H stretching around 2909.59 cm^{-1} (G-P NF) and 2919.71 cm^{-1} (G-P NF C6) are attributed to the presence of piperine in the matrix. Similar peaks are observed due to asymmetric stretching of ═C─O─C in G-P NF (1220.35 cm^{-1}) and G-P NF C6 (1229.34 cm^{-1}) samples respectively. This confirms the successfully loading piperine in the nanofibers. Absence of any new peak further and no significant shift in the peak position in piperine loaded nanofibers also shows the stability of the drug in both non-crosslinked and crosslinked samples confirming no physical or chemical interaction of hydrophobic drug with hydrophilic polymer matrix.

3.6. In-vitro drug release study

The release of drug from a polymer matrix is modulated by diffusion of drug and/or degradation of the polymer matrix. Insufficient physical and chemical interactions (as evident in the FTIR study) between the hydrophobic drug molecules and the hydrophilic polymer matrix led to sudden release of drug molecules within few hours from the surface. As the crosslinked G-P NF membrane swells, due to presence of water molecules, the osmotic pressure provides the driving force for release of drug in the release medium. Therefore, after 2 h, there is sustained release of drug as drug diffuses to the release medium through the carrier gradually.

3.6.1. Effect of pH value of release medium

Studying and controlling the drug release at different pH is an important consideration for designing a vehicle for oral route. As drug molecules need to follow the GI tract and should be absorbed in small intestine, we need to examine release profile from harsh acidic conditions to slight basic environment. In this work, in-vitro drug release studies were performed in different pH conditions as per the human GI tract environment i.e., pH 1.2 (stomach), pH 6 (duodenum), pH 7.4 (small intestine) and pH 8 (large intestine) as shown in Fig. 5.

For crosslinking time of 4 min (G-P NF C4), piperine release percentage was 95.7 ± 3.6, 90.5 ± 3.1, 82.8 ± 6.0, 77.8 ± 3.0% (Fig. 5a)

Table 2
List of drug release co-efficient as fitted in Higuchi Model.

Release model	Samples (6 min)				
		pH 1.2	pH 6	pH 7.4	pH 8
Higuchi model	K_H	0.036	0.037	0.045	0.0588
	R^2	0.9776	0.9846	0.9949	0.9892

while for crosslinking time of 6 min (G-P NF C6), drug release percentage was significantly decrease 87.7 ± 2.1, 85.6 ± 2.9, 77.6 ± 5.8 and 72.6 ± 3.4% for pH values 8, 7.4, 6 and 1.2 respectively (Fig. 5b). We observe that the total amount of drug release is less in the solution with pH 1.2, compared to higher pH, irrespective of crosslinking time. This may be due to protonation of hydrophilic groups of the polymer matrix in acidic pH, which discourages formation of H-bonds with water molecules resulting in less swelling of the membrane [36]. If the matrix is not swelling much, drug molecules will not get enough osmotic pressure, helping in reducing drug release amount. However in alkaline pH, hydrophilic groups form more H-bonds with release medium which invites more water molecules inside the carrier leading to better swelling and more drug release in the dissolution medium. Similarly, with compare to G-P NF C6 and G-P NF C8 (Fig. 5c), after 24 h, piperine release was decreased significantly 72.7 ± 8.1, 65.5 ± 4.6, 62.6 ± 0.4 and 58.6 ± 3.6% for pH values 8, 7.4, 6 and 1.2 respectively, demonstrating the above explanation.

3.6.2. Effect of crosslinking time

With increasing the crosslinking time from 6 to 8 min, the large amount of drug release in pH 1.2 can be controlled. Our main objective is to release maximum drug in higher pH (7.4, 8) i.e., pH of small intestine, where drug will be absorbed. In release medium of pH 1.2, the amount of drug release, within 2 h, for G-P NF C4, G-P NF C6 and G-P NF C8 are approximately 48.5 ± 2.9, 45.5 ± 2.7 and 30.5 ± 2.6% of total drug respectively (Fig. 6a). Further, the drug release amount decreases significantly from 72.6 ± 3.4% in G-P NF C6 to 58.5 ± 3.6% in G-P NF C8, after 24 h release in pH 1.2. Therefore, increase in crosslinking time decreased the release percentage.

Similar control over the release percentage was obtained for pH 6, 7.4 and 8 for both initial fast release and prolonged sustained release as shown in Fig. 6b–d. Therefore, manipulating the crosslinking exposure time from 4 to 8 min, we can engineer the inter-fibrous porosity, which may result in sustained release of drug molecules. Also, from release study we can conclude that, the vehicle (G-P NF C6) is capable of protecting the drug from the harsh
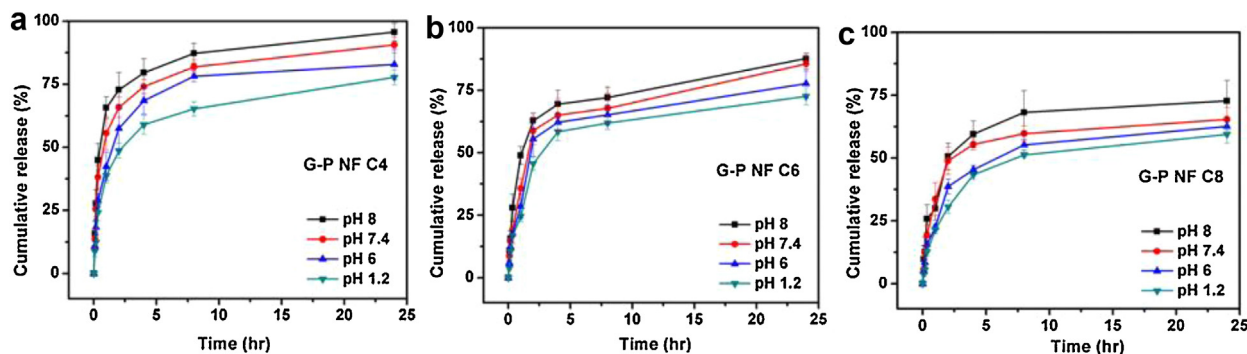
Fig. 5. Cumulative *in-vitro* release profiles of piperine for (a) G-P NF C4, (b) G-P NF C6 and (c) G-P NF C8 in different pH (1.2, 6, 7.4 and 8) (significance value, $p < 0.05$).
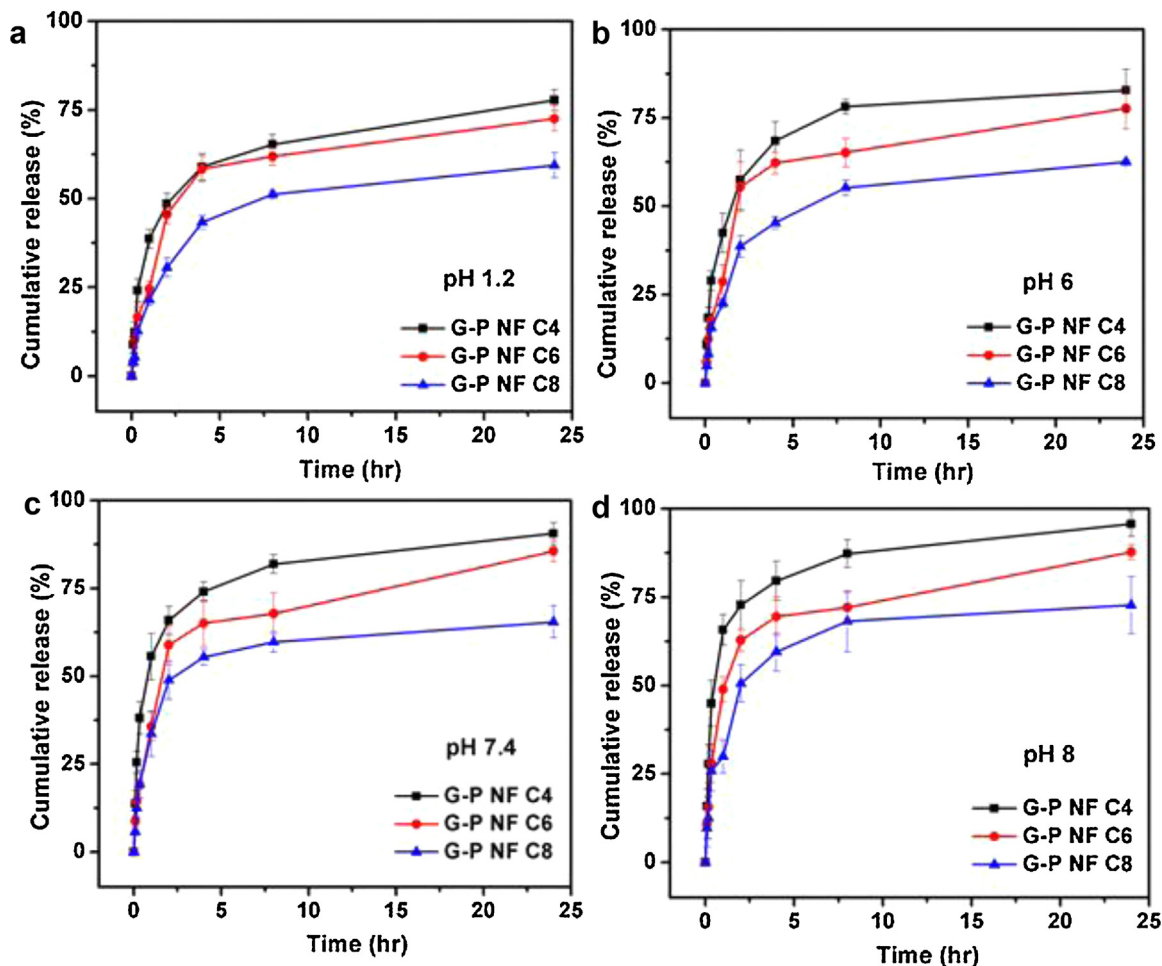

Fig. 6. Cumulative *in-vitro* release patterns of piperine for different crosslinking time (G-P NF C4, G-P NF C6, G-P NF C8) in (a) pH 1.2; (b) pH 6; (c) pH 7.4; and (d) pH 8 release medium (significance value, $p < 0.05$).

condition (pH 1.2) of GI tract and is able to release in the absorption site, *i.e.*, small intestine, in a sustained manner.

3.6.3. Drug release mechanism:

To understand the release mechanism, the *in-vitro* release data was analyzed using the classical Higuchi model and respective Higuchi dissolution constants are presented in Table 2. It is a very clear indication that, diffusional force is playing the major role for drug delivery. The decrease in K_H values were found for all the cases which indicates the increase in diffusional barrier. The probable reasons behind the increased diffusional barrier are the diffused and packed fiber structure which also reduces drug molecule pen-

etrability through the matrix. With increase of crosslinking time, K_H value has decreased. Porosity, morphological images, release study data also support these observations. The K_H value can be a good indicator for required drug release profile.

As compared to the previous reports where electrospun gelatin nanofibers are used for drug delivery [24–27], these results suggest to provide a tighter control over sustained drug release and that too at different pH of the release media, as summarized in Table 3.

As mentioned earlier, there is only one report on release of hydrophobic drug from electrospun gelatin nanofibers. Even in that report [24], release study was done only at a given pH 7.4. Additionally, either there was an initial burst release (75% release in

Table 3
A comparison of the present work with drug release profile from electrospun gelatin nanofibers reported in literature.

S. No	Crosslinker/time of cross linking	Drug/nature	Solvent	Remarks
1	Polyethyleneglycol-diacrylate/30 min	Nystatin/hydrophobic	HFP	(i) Drug release study only at pH 7.4 (ii) Fiber diameter: few microns (iii) Initial burst release (75% in 24 h)
2	Proanthocyanidin, GTA/45 min	MAP/ hydrophilic	Formic acid	(i) PVA is added in gelatin for producing nanofibers (ii) Prolonged exposure to GTA (iii) Initial burst release (65% in 1 h)
3	GTA/24 h	Heparin/ hydrophilic	Aqueous acetic acid	(i) Prolonged exposure to GTA (ii) Drug release only at pH 7.0 (iii) Slow release
4	NHS, EDC/24 h	Cefradine/hydrophilic	Water and ethanol	(i) Use of ethanol as solvent (ii) Prolonged crosslinking (iii) Initial burst release (50% in 4 h)

first 24 h) or very slow release (35% release in five days with 22% release in first 24 h). Further as we see in Table 3, all previous reports based on only electrospun gelatin nanofibers focus either to deliver the hydrophilic drug [25–27] or cross linking is done for prolonged time (up to 24 h) [24–27] or there is a unwanted signature of initial burst release [24,25,27]. Clearly this work addresses most of these challenges as confirmed by *in-vitro* drug release studies discussed above and suggests that controlled crosslinking plays a very important role in porosity of the matrix with minimal effect on fiber morphology. This in turn essentially helps to get stable, sustained and control release of hydrophobic drug with highly porous electrospun gelatin nanofiber matrix as a delivery vehicle.

4. Conclusions

Electrospun gelatin nanofibers were fabricated and exposed to saturated GTA (25% v/v) vapor for crosslinking. Interestingly, only 6 min exposure was sufficient to control the degradation. Besides increasing water resistivity, crosslinking also improved the thermal stability of membrane. These electrospun gelatin fibers were then successfully demonstrated as a carrier for a model hydrophobic drug *i.e.*, piperine. This system have the potential in drug delivery system due to following observations: (i) piperine was found to be stable in hydrophilic electrospun gelatin nanofiber carrier; (ii) from *in-vitro* release study, piperine was effectively delivered over prolonged duration of release; (iii) piperine release rate can be modulated by pH of the release medium at the site of release and the degree of cross-linking of the carrier.

Acknowledgments

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