New Nicotinic Acid-based Hydrogel: Swelling and Insulin Uptake Studies

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Received: 12.12.2021; Accepted: 10.01.2022; Published: 20.02.2022

Abstract: Designing a target-specific insulin delivery via the oral route or the non-invasive mode using the polymer-based drug delivery systems is a research area attracting the researchers' attention worldwide. In the present short communication, we report a new nano-system with a bioactive component, nicotinic acid (NA). It was conjugated via the lipase-catalyzed reaction with polyethylene glycol (PEG₄₀₀). NA and PEG were taken in a 1:1 molar ratio along with 1% of lipase and reacted at 45 °C. The PEGylated-NA was characterized for its structural aspects using different techniques. Swelling studies were carried out at pH 6.0, 7.0, and 9.0. The order of water uptake was 6.0 > 7.0 > 9.0. The PEGylated-NA was evaluated as a platform to load insulin at pH 7.4 and the physiological temperature of 37 °C. It showed a high affinity for insulin with uptake of 82.68% with an adsorption capacity of 206.7 mg/g.

Keywords: nicotinic acid; PEG-400; swelling behavior; insulin loading.

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1. Introduction

In recent times, diabetes has been a serious problem challenging health management throughout the world [1-3]. Insulin is a regulatory hormone that plays an important role in controlling glucose homeostasis. It also allows cells present in the liver and muscle to circulate sugar from the blood and store it as glycogen in the liver and muscles [4]. Decreased level of insulin in the body causes diabetes mellitus; hence, its use in treatment is recommendable through the intravenous route to the patients to avoid side effects [5]. Targeted insulin delivery is the most effective therapy to treat diabetes [6]. The use of nano-insulin has become an area of much interest [7]. Drug delivery via the oral route is the most promising mode, considering its non-invasive operation, which leads to minimum discomfort for patients. Polymeric hydrogels with a high water retention capacity are good candidates to overcome hurdles in the way of effective and site-specific drug release [8-10].

Conjugation of drugs with bioactive compounds leads to their targeted delivery directly to various organs or tissues by the controlled release that improves the pharmacokinetics of drug release and diminishes various side effects [11]. Hence, there is a need for some bioactive compounds which can decorate these drugs for target-specific delivery of drugs. For the targeted drug delivery system, conjugation of drug with polyethylene glycol (PEG) is clinically approved by FDA for humans because of its non-toxicity, non-immunogenicity, non-antigenic and amphiphilic nature with repeated ethylene oxide sub-units [12,13]. Conjugation of drug

with PEG-oligocholic acid increases circulation time, leading to sustained delivery to the target, as exemplified with PEG/poly(MAAc) reported to show resistance towards plasma [14]. There are many reports in which conjugation of PEG with various drugs resulted in controlled target-specific drug delivery in the literature. PEG attached with oligo acid and dendrimers has been reported for the targeted specific delivery of drugs [15,16]. Many other reports have been cited in the literature for sustained drug delivery by the covalent attachment of bioactive compound with PEG, which is clinically considered a potent technique [17,18]. In a study, PEGylated starch acetate nanoparticles were reported for the controlled delivery of insulin [19]. PEGylated chitosan hydrogels and cyclodextrin were also reported to enhance pharmaceutical activity and, consequently, sustain insulin release [20]. Given the above discussion, it is evident that the conjugation of some bioactive compounds with PEG is a more potent mode for sitespecific drug delivery. One study used folic acid as a bioactive component of the modified PEG-functionalized hydroxyapatite nanoparticles in paclitaxel delivery with enhanced activity [21]. The upload of insulin for oral delivery on biopolymers or bioactive materials has been reported in the literature [22-28]. In the present study, nicotinic acid (NA), a vitamin B, was PEGylated. It has lower molecular weight, higher water solubility, and a minimum side effect [29]. Its lower molecular weight is a positive feature for targeting molecules, a necessary and sufficient condition for conjugation. In a report, NA was used to investigate insulin resistance in the body [30].

Given the previous work based on drug delivery from different biocompatible polymers [31-35], we have synthesized a versatile nano-sized hydrogel to modify NA by PEGylation and utilize the resultant polymer as vehicles for the upload of insulin. The modified PEG was characterized by Fourier transform infrared (FTIR), ¹H nuclear magnetic resonance spectroscopy (¹H- NMR), X-ray diffraction (XRD), and transmission electron microscopy (TEM). The swelling behavior of PEGylated-NA was studied to justify its drug uptake and release profiles. We are submitting this report as short communication as the release profile of the insulin-loaded material has not yet been carried out. There is no similar report in the literature.

2. Materials and Methods

2.1. Materials.

Nicotinic acid (NA), triacylglycerin lipase, ethylene Glycol (EG) (Himedia Lab. Pvt. Ltd., India), polyethyleneglycol (PEG₄₀₀) (Merck, Schuchardt, Germany), insulin human mixtard (Torrent Pharmaceuticals Ltd., India), sodium hydroxide, hydrochloric acid, *di*-sodium hydrogen orthophosphate anhydrous (Na₂HPO₄), sodium dihydrogen orthophosphate dehydrate (NaH₂PO₄.2H₂O) (S.D Fine-Chemicals Ltd.), all of the analytical grade, were used as received.

2.2. Synthesis of PEGylated-NA.

NA was modified by the PEGylation method. Lipase (1% by weight of total reaction mixture) was used as a catalyst in the esterification reaction between NA and PEG (1:1 molar ratio) at 45 °C for 8 h in Chemical Reactor (MiniblockTM, Germany). The synthesized ester, named PEGylated-NA, was washed thoroughly (4 times) to remove unreacted materials. After purification, it was refluxed by thermal treatment under temperature 70 °C in the deionized

water to remove unreacted reaction components. PEGylated-NA was separated by washing with acetone and dried in an oven (Scheme 1).



Nicotinic acid (NA)

PEGylated-NA



2.3. Characterization of synthesized products.

Synthesized product was characterized by Fourier transform infrared spectroscopy (Nicollet 5700); and transmission electron microscope images of the (Joel Stereoscan-150). Scanning electron microscopy of the synthesized sample was recorded on (SEM QUANTA 250 D9393).

2.4. Swelling study.

The swelling behavior of the PEGylated-NA was studied as a function of pH and time at 37 °C. A known weight (100 mg) of the material was immersed in the distilled water to optimize the period of swelling. Furthermore, the effect of pH on swelling was observed with respect to time (15–360 min) in buffer solutions of pH 6.0–9.0. The swollen polymer was taken out from the solution, wiped off, and then weighed immediately to calculate %swelling/water uptake (P_s) as [36]:

$$Ps (\%) = \frac{Weight of swollen hydrogel - Weight of dry hydrogel}{Weight of dry hydrogel} \times 100$$
(1)

Fick's law was applied to determine the diffusion and swelling kinetics of the polymer and is expressed as [37,38]:

$$f = \frac{M_t}{M_e} = kt^n$$
(2)

The linear form of Fick's law is represented as:

$$\ln f = \ln k + n \ln t$$
(3)

where f, M_t, M_e, k, and n are the swelling fraction, swollen mas at a time 't', mass at equilibrium, kinetic constant, and diffusion exponential, respectively. The slope of the graph lnf vs. Int gives the value of n. If n = 0.5, the solvent diffusion or transport rate is much higher than the rate of relaxation process of polymeric chains, which means swelling kinetics obey the Fickian model (Case I transport). For n = 1, the swelling kinetics obey the non-Fickian model (Case II), where solvent diffusion through the polymer is very fast compared to the relaxation rate, for 0.5 < n < 1.0, the swelling kinetics follow anomalous transport or non-Fickian model where swelling, and diffusion rate are analogous. For n > 1.0, swellings kinetics obey Super Case II transport where solvent transport occurs along with breaking of the polymeric chains.

2.5. Insulin loading study.

Accurately weighed, 100 mg of insulin was dissolved in a minimum amount of distilled water in different measuring flasks. The volume of the solution was made up to 100 mL for each insulin solution (250 ppm) using the phosphate buffer pH 7.4. After separating the insulin-PEGylated-NA from the drug solution, the optical density (OD) values of the solution containing the residual insulin were measured on a spectrophotometer at 660 nm. The hydrogels were washed thrice with the distilled water and were later dried under vacuum. The drug loading capacity (q) and entrapment/encapsulation efficiency (EE%) of the hydrogel were calculated from the following equations [39]:

$$q = \frac{T_c - R_c}{W} \times V \tag{4}$$

EE (%) =
$$\frac{T_c - R_c}{T_c} \times 100$$
 (5)

where T_c is the total concentration of the drug solution in ppm taken for loading, R_c is the concentration of rejected/unloaded drug in solution, and V is the initial volume of insulin solution. Evidence of the insulin-loaded sample was obtained by recording FTIR spectra in the 4000-500 cm⁻¹ range in KBr on Nicolet 5700 spectrophotometer.

3. Results and Discussion

3.1. Characterization of polymers by different techniques.

Conformation of synthesis of new PEGylated-NA by covalent conjugation of PEG with NA, loading of insulin on PEGylated-NA was obtained from the FTIR spectroscopy (Figure 1). FTIR spectra of PEGylated-NA shows bands at 3142 cm⁻¹ (C–H stretching vibrations), 2954 cm⁻¹ (CH₂ symmetric stretching vibrations), 1639 cm⁻¹ (C=N stretching vibrations of ring), 1580 cm⁻¹ and 1406 cm⁻¹ (C=C stretching vibrations), 1381 cm⁻¹ (CH₃ symmetric bending vibrations), 1259 cm⁻¹ and 1119 cm⁻¹ (two strong C–O stretching bands due to asymmetric and symmetric stretching C–O–C group), 1059 cm⁻¹ (C–H in-plane bending), 961 cm⁻¹ (C–O–C stretching vibrations) and 844 cm⁻¹ (C–H binding vibrations) [40,41] (Figure 1a). Insulin has a large number of functional groups, and its loading by the PEGylated-NA affects the intensity and stretching mode. After insulin uptake, all the bands remain preserved with a slight change in their position and intensity along with the appearance of the new bands at 3290 cm⁻¹, 1659 cm⁻¹, and 1570 cm⁻¹ due to N–H, amide I, and amide II bands (C=O stretching vibrations) which confirms loading of insulin onto PEGylated-NA (Figure 1b) [42].



Figure 1. FTIR spectra of (a) PEGylated-NA and (b) insulin-loaded PEGylated-NA.

TEM/SEM images were used to study and reveal the polymeric structures' shape, size, or surface morphology. It provides evidence of changes affected in the surface morphology after derivatization. SEM image of the PEGylated-NA shows a long porous space between the molecules (Figure 2a). After insulin adsorption, the surface becomes less porous and denser, revealing the absorption of insulin on the PEGylated-NA matrix (Figure 2b).



Figure 2. SEM images of (a) PEGylated-NA and (b) insulin-loaded PEGylated-NA.



Figure 3. TEM images of (a) PEGylated-NA and (b) insulin-loaded PEGylated-NA.

TEM images were recorded to study and reveal the shape and size of the polymeric structures pre- and post-drug loading. The TEM image of the PEGylated-NA shows a long smooth nanorod-type structure (Figure 3a). The size of these rods is < 100 nm. Nanorod shape or structure and smooth surface result from the self-assembly of the PEG chains on the one end and residual NA moiety at the other end. Later, in insulin-loaded PEGylated-NA, insulin molecules get deposited on the PEGylated-NA surface, which is evident from Figure 3b.

3.2. Swelling study of PEGylated-NA.

The PEGylated-NA behaves as hydrogel by absorbing a large amount of water (Figure 4). Such property augurs well for its use as a drug delivery device—the appreciable high water uptake results from the self-assembled structure of the amphiphilic PEG chains. In the case of PEGylated-NA, water molecules interact with the PEG chains.



Figure 4. P_s of PEGylated-NA at different pH as a function of time at 37 °C.



Figure 5. Swelling kinetic plots at different pHs.

The maximum equilibrium swelling of 1295% was attained within 300 min at 37 °C. Structure and environment-dependent swelling has been reported in the literature for PEG microspheres [43]. Along with the high-water uptake by the PEGylated-NA, another important attribute of this nanomaterial is its stimuli-responsive swelling. These two features are related https://biointerfaceresearch.com/

to the high drug loading and the site-specific drug release. PEGylated-NA can partition a large amount of drug or bioactive molecules from water and release that at an appropriate site depending on the stimuli-responsive nature, or pH in this case. The maximum swelling was observed with the order of pH responsiveness to water uptake as the swelling order was found to be 6.0 > 7.0 > 9.0 at 37 °C (Figure 4). From Fick's law, the value of *n* was found to be 1.193, 1.28, and 1.18 for pH 6, 7, and 9, respectively, that swelling kinetics follows Super Case II transport of solvent (Figure 5).

3.3. Preliminary investigation of insulin uptake.

Loading insulin onto the PEGylated was carried out at the maximum solubility of the drug at pH 7.4 under the phosphate buffer saline (PBS) medium and at the physiological temperature of 37 °C. It has uptake up to 82.68% with q value up to 206.7 mg/g. These values are far higher than reported in an earlier study [34]. High loading of insulin has many pharmaceutical applications as a single dose large amount of drug for a large therapeutic range can be used for the localized use at a part of the body (Figure 6).



Figure 6. Insulin uptake study by PEGylated-NA.

A comparative study of the insulin uptake capacity and entrapment efficiency of the synthesized hydrogel with other polymeric systems is shown in Table 1, revealing that the synthesized hydrogel is an efficient drug delivery device for insulin delivery.

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S. No.	Polymeric materials	EE (%)	References
1.	Chitosan/TPP nanoparticles	69.37 ± 4.71	[42]
2.	mPEG10%-CS-GMC10%	71.3 ± 1.02	[44]
3.	Bipolymer lipid hybrid nanocarrier	50.94	[45]
4.	Chitosan hydrogels	78.0 ± 0.7	[46]
5.	Polycation complexes	> 50.0	[47]
6.	Carboxymethyl chitosan nanocarriers	83.78 ± 3.73	[48]
7.	Polyelectrolyte complex (Ins/NGs-PEC)	85.2	[49]
8.	PEGylated-NA	82.68	Present study

Table 1. Comparison of insulin uptake capacity (q) and encapsulation efficiency EE (%) of the synthesized hydrogel with some polymeric systems.

4. Conclusions

The present work aimed to design a versatile hydrogel by PEGylation of nicotinic acid (NA), without using a crosslinker via the lipase-catalysis. The PEGylated-NA has nanorod shaped structure with smooth surface morphology. These undergo high swelling when studied under media of different pH, from 6.0-9.0. The PEGylated-NA absorbs 82.68% of insulin at pH 7.4 and 37 °C. These NA-based nanorods have attractive applications in insulin release studies.

Funding

This research has no external funding.

Acknowledgments

The authors are grateful to the Department of Chemistry, Himachal Pradesh University, for providing facilities to carry out this work.

Conflicts of Interest

There is no conflict of interest for this work.

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