



Development of a nanostructured double-layer coated tablet based on polyethylene glycol/gelatin as a platform for hydrophobic molecules delivery

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Abstract

The aim of the current study was to develop a nanostructured double-layer for hydrophobic molecules delivery system. The developed double-layer consisted of polyethylene glycol-based polymeric (PEG) followed by gelatin sub coating of the core hydrophobic molecules containing sodium citrate. The polymeric composition ratio of PEG and the amount of the sub coating gelatin were optimized using the two-level fractional method. The nanoparticles were characterized using AFM and FT-IR techniques. The size of these nano capsules was in the range of 39-76 nm depending on drug loading concentration. The drug was effectively loaded into PEG-Gelatin nanoparticles ($\approx 47\%$). The hydrophobic molecules-release characteristics in terms of controlled-release duration and dissolution efficiency were examined in various dissolution media, such as physiological pH (7.4) and simulated stomach fluid (3.4). Consequently, the optimized double-layer for hydrophobic molecules delivery system showed a gradual release of hydrophobic molecules in the and in physiological pH, indicating its novelty for using as a platform for hydrophobic molecules delivery.

Keywords: Gelatin, Poly ethylene glycol; Polymeric nanoparticles; Cationic polymer; Gelation ionic technology

1. Introduction

Using the nanotechnology techniques in drug delivery has a widely applications and are expected to change the landscape of the biotechnology and pharmaceutical industries for the next coming years. The development in the nanotechnology products allow to achieve lots of improvement regarding to the drug delivery for pharmaceutical companies such as delivering of poorly water-soluble drugs, designing of drugs for specific targeting in a tissue or cell, delivering of the large molecules, delivering of two or more of drugs at the same time and transcytosis of drugs across endothelial barriers and tight epithelial [1-3]. Therapeutic strategies that are focusing on the targeting of the infected areas have been greatly challenged by drug solubility and its tolerable level for prolonged time intervals. Nanotechnology has currently gained considerable attention to be the most promising technique for designing ideal carrier systems which solves these problems. Polymeric nanoparticles are one such example of these systems. Polymeric nanoparticles have recently been developed to improve the therapeutic efficacy of various bioactive molecules and drugs through enhancing their

solubility, tolerability, preventing immediate drug degradation and improving its absorption into the targeted tissue [4, 5]. They have been also exploited to design drug delivery systems that are carrying therapeutic agents to the targeting cells without affecting the collateral tissues [4, 6-8]. Drug delivery which utilizes polymeric nanoparticles have demonstrated many advantages compared to other therapeutics including efficacy improvement, controlled drug release, and reducing side effects [9-11].

During the last years, cationic polymer nanoparticles demonstrated distinctive properties involving provide of oriented interactions with proteins and strong bonding with DNA, which offer an ideal choice in emerging biomedical applications. Conversely, pH-sensitive anionic polymeric nanoparticles which have been synthesized as drug delivery carriers show a swelling behavior at high pH which is not appropriate for transport in the acidic environment around tumor tissues [12, 13]. Cationic polymers overcome this challenge by a showing of acid swell capability which makes them a good choice for drug delivery. Cationic polymers which are originated from natural sources have substantial positive charges and their structure

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characterized by biodegradable, low toxicity, and low immunogenicity properties [4].

Gelatin is an example of such cationic polymers which has been used in this study as a carrier system through encapsulation of the previously prepared imidazo pyrimidine derivative compound. Gelatin is a low-cost, biocompatible and biodegradable natural polymer which is derived from collagen and broadly employed for biomedical and pharmaceutical applications [14-17]. It contains 18 non-uniformly amino acids with both positive and negative charges. Lysine and arginine residues provide the essential cationic property for gelatin. It has been reported that the crosslinking degree of gelatin regulates its swelling behavior, thermal and mechanical properties. Gelatin as a safe material has been approved by the US Food and Drug Administration (FDA), which is widely used in the synthesis of different bio-materials [4, 18]. Also, because of its ability to improve release process, anti-tumor efficiency and inconsiderable side effects, gelatin has been widely applied as a nano-carrier for the delivery of both hydrophobic and hydrophilic anti-tumor therapy such as cisplatin [19], doxorubicin [20], methotrexate [21] and paclitaxel [22]. It was already employed as drug delivery, gene delivery, and in tissue engineering [23-26]. In this work, polyethylene glycol (PEG) was employed as an additional coating polymer [4]. PEG is considered a hydrophilic natural charged polymer [20] which has been characterized as a biocompatible, non-immunogenic, non-toxic and water-soluble polymer. It is often utilized in cryo-protection, preparation of pharmaceuticals, protection of tissue culture and organs [9]. The poor lymphatic drainage of tumors, which is known as the enhanced permeability and retention effect (EPR), enhanced retention of macromolecular drugs within the tumor mass [27-29]. Also, circulating drug delivery devices can be removed from circulation by opsonins (plasma proteins) within seconds to minutes through the reticulo-endothelial system [30, 31]. A general strategy to prolong circulation kinetics includes encapsulation of PEG to the active pharmaceutical ingredient, thereby enhancing EPR and reducing attachment by opsonins and proteins and minimizing immunogenicity [32, 33].

A PEGylated of adenosine deaminase was the first PEGylated biopharmaceuticals which has been approved by the FDA and available in the markets in 1990 as a therapy for severe combined immunodeficiency disease (SCID)[34]. Since then, PEGylation become a backbone in the production of biopharmaceutical: where interventions studies show that at this time there are 75 finished recruiting (active trials) including a PEGylated therapeutic, and an extra 219 either recruiting, not yet recruiting or available for expanded access (open studies). Unaccompanied PEG has also been under development to use as a low-dose

resuscitation solution for intravenous administration in the treatment of hemorrhagic shock.[32, 35, 36].

In the present study, a double-layer coated hydrophobic molecules delivery as a novel platform was developed. The layer contains dispersed polyethylene glycol-based polymeric (PEG) and gelatin sub coating layer for the hydrophobic molecule core. The core hydrophobic molecule consisting of sodium citrate was used as the basicity agent. A 6-[2-(biphenyl) imidazo (1,2a) pyrimidine-3-yl]-4-nitro pyrimidine-2 (1H)-one compound (IP) (Figure 1) was used as a model of a hydrophobic molecule, the organic compound was synthesized and fully characterized [37]. The IP compound is a derivative of imidazo [1,2-a] pyridine compounds which are characterized by many biological actions such as antimicrobial, antifungal, antibacterial, antiviral and antitumor activities [38-40]. These compounds have received much interest from the pharmaceutical industries [39, 41-45]. The biological activity of the IP compound was successfully *in vivo* examined to be a promising antioxidant and hepatoprotective agent [37]. All of the previous features have been taken in the consideration during choosing the compound in the current study as the hydrophobic molecule model. Both the PEG and gelatin polymeric compositions for coating and sub coating layers were optimized. Figure 2 illustrates the stepwise of the coating mechanism. The molecule-release characteristics were examined in various dissolution media, such as physiological pH (7.4) and simulated stomach pH fluid (3.4). The present approach represents a novel platform, never reported yet for hydrophobic drug release and can be effectively applied.

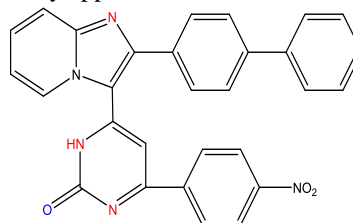


Figure 1. The chemical structure of the hydrophobic molecule (IP)

2. Materials and methods

2.1. Materials

PEG 6000, gelatin, sodium citrate, lactose hydrate, L-HPC, polyvinylpyrrolidone, magnesium stearate, croscarmellose sodium, sodium citrate and colloidal silicon dioxide were of high purity (>98%) and purchased from Sigma-Aldrich. To avoid the interference's effect, all the dilution processes were conducted using deionized water including preparation of the buffer solutions. IP was kindly gifted by Dr. Naeemah Jabbar Owaid.

2.2. Methods

2.2.1. The preparation of gelatin nanoparticles /dispersed sub coating layer

A modified protocol was used for the preparation of gelatin nanoparticles based on an ionic interaction between negatively charged sodium citrate and positively charged gelatin solution [9]. A compound IP was directly dissolved in (2 mg/ml) of sodium citrate solution to get a drug concentration of (0.26, 0.52, 0.78, 1 and 1.3 mg/ml). Gelatin was dissolved in 1% acetic acid solution at a concentration of 4 mg/ml. Then, 4ml of IP solution in sodium citrate (2mg/ml) was dropped into 10ml of gelatin solution under magnetic stirring (1000 rpm) at room temperature. Gelatin nanoparticles suspension was stirred for 90 minutes at 25°C for further crosslinking of nanoparticles. Finally, gelatin nanoparticles were collected by centrifugation at 10,000 g and kept at -20 °C for the further steps. IP-Gelatin nanoparticles were characterized using Atomic Force Microscopy (AFM) and Shimadzu FT-IR (FTIR-8400) spectrophotometer.

2.2.2. The PEG coating (The outer layer)

IP-gelatin nanoparticles with different drug concentrations were encapsulated with PEG solution as following; first, PEG was dissolved in distilled water to form a 10 % solution. Then, PEG solution was slowly added to the IP-gelatin solution at 1:1 ratio, under magnetic agitation at 25 °C for 90 minutes. Finally, different concentrations of encapsulated IP-gelatin-PEG nanoparticles were collected by centrifugation at 10,000 g and kept at -20 °C for the characterization. All of synthesis steps are illustrated in Figure 2. The morphology of IP-gelatin-PEG nanoparticles was studied using AFM and their FT-IR spectra were also determined using of potassium bromide discs.

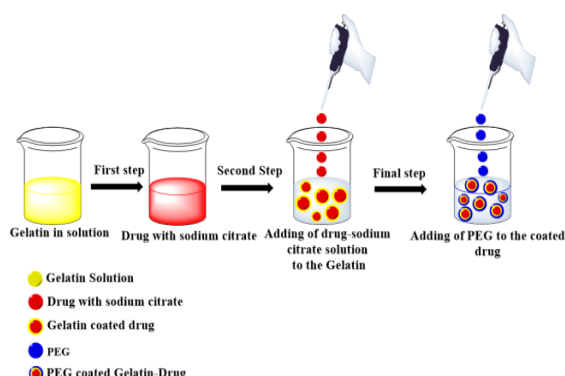


Figure 2. The steps wise mechanisms of the double coating process to the hydrophobic molecule (IP)

2.2.3. Tablets' core preparation

The direct compression method and a conventional wet-granulation were used for the preparation of the tablet's core. For wet granulation, 85 °C was used for drying the PEG: gelatin sub-coating IP nanoparticles powder using a Thermo Fisher Scientific desiccator (USA), and both a diluent (lactose hydrate) and a lubricant Low-substituted Hydroxy Propyl Cellulose (L-HPC) were premixed with the IP granules using a V-mixer (Type VB3, Germany) for 10 minutes. The formed powder mixture was mixed with 20% w/w, in ethanol of polyvinylpyrrolidone solution as a binder agent and using a high-speed mixer (Type Xena-II, South Korea) for damping the granulates. An oven at 70 °C for 4 hours was applied for drying the granules which were passed through a 20-mesh sieve. While for the direct compression method, the obtained granules from the wet granulation process were mixed with magnesium stearate, croscarmellose sodium, sodium citrate and colloidal silicon dioxide using a cube mixer for 10 minutes. Finally, the obtained granules powder was compressed into the shapes of tablets using on a single-punch tablet machine. The prepared tablets were used for further experiments.

2.2.4. Physicochemical characteristics of the prepared coated tablets

After 24 hours of relaxation time, the Physicochemical properties like weight-variation, crushing strength, friability and tablets content of the prepared tablets were performed. The Weight-variation experiments were conducted on 10 individually weighed tablets using Mettler balance (analytical balance, USA). A hardness tester (type PTB111E; Pharma, Germany) was used for crushing strength tests. The friability of the prepared tablets was calculated based on losing in the percentage weight of tablets (4 minutes, 20 tablets and 25 rpm). Finally, the tablet content determination was performed by measuring the absorption at 450 nm using PD-303 APEL spectrophotometer. An individually 10 tablets were weighted, crushed into a fine powder and a suitable weight of it was taken to obtain 5 mg of IP. The powder was dissolved in ethanol and determine using PD-303 APEL spectrophotometer (See Table 2).

Table 1. The physical and chemical characteristics of prepared tablets

Friability %	Weight (mg)	Hardness (Kg/cm ²)	Drug content
0.02±0.004	0.31±0.02	8.05±0.09	99.95±0.1

2.2.5. Estimation of IP encapsulation efficiency

A suitable weight equivalent to 5 mg from the prepared tablets were taken, powdered and treated by the simulation buffers in order to determine the

encapsulation efficiency (EE%) by measuring the absorption of the supernatant at 450 nm using a PD-303 APEL spectrophotometer. The corresponding calibration curve was made by testing the supernatant of IP nanoparticles. Encapsulation efficiency was calculated using the following equations [9].

$$EE = \frac{W_t - W_f}{W_t}$$

Where W_t represents the total amount of IP; W_f is the amount of free IP in the supernatant.

2.2.6. The dissolution experiment of the prepared tablets *in vitro* (IP releasing)

The dissolution experiments of the encapsulated molecule were conducted using the dissolution tester (Agilent, VK7025 model, USA) for 300 minutes. The revolution speed of paddles, temperatures and volume of dissolution medium (phosphate buffer) were 50 rpm, 37 °C and 1000 ml respectively. In the beginning, an equivalent of 0.782 mg/ml IP is immediately transferred into the dissolution medium as soon as the starting of the dissolution test. The mentioned procedure was repeated at each measuring time. The molecule-release studies were primarily performed in the simulated physiological pH of blood fluid 7.4 for 300 minutes, which was then replaced by pH 3.4 (which is the simulated stomach fluid) for 300 minutes as well. At preselected sampling points, 10 ml of the sample was then transported and filtered through a membrane (whatman filter paper 0.45 μM). Finally, the filtrate was further diluted with the buffer solution proper the analysis test. In each sample, the amount of IP was determined by measuring the absorption at 450 nm using PD-303 APEL spectrophotometer. The corresponding calibration curve was made by testing the supernatant of IP nanoparticles.

Table 2. Effect of IP concentration into EE and size of gelatin-PEG nanocapsule

IP conc. (mg/ml)	IP-gelatin-PEG	
	EE (%)	size (nm)
0.261	29.62	51.19
0.521	39.26	44.93
0.782	46.73	39.43
1.043	47.28	55.04
1.304	37.37	76.15

3. Results and discussion

Dual drug encapsulation was achieved through coated of a hydrophobic IP compound with gelatin and PEG to synthesize of a nanocapsule with unique properties that enable it to deliver IP to the targeting tissues. A compound IP, which has been previously prepared in our laboratory, exhibited hepatoprotective and antioxidant activities and this was reported by S. Ahmed and Mahmoud (2020) as a potential therapeutic agent [37]. The activity of this compound was *in vivo* tested and the study was carried out with a mice model using Albino male mice after inducing hepatic damage with CCl₄. The results of liver histology demonstrate the ability of this compound to recover and repair the damage that happened in liver tissues [37]. In the current study, IP has been initially encapsulated with gelatin to produce nanoparticles based on the ionic interaction of negatively charged sodium citrate and positively charged gelatin. The ionic interaction extremely affected by the charge density of both gelatin and sodium citrate. It has been reported that cationic nanoparticles with a size of 100–200nm show a rapid removal process than those with negative charge [4]. Therefore, the surface of the gelatin nanoparticles was modified with PEG to improve its biocompatibility, dispersibility, non-immunogenicity, and low protein absorption [46, 47]. Furthermore, PEGylation can enhance the blood circulation time and consequently improves the cellular uptake through protection of nanoparticles from elimination by phagocytosis [48]. FT-IR spectra show the following changes after the encapsulation process. Firstly, after encapsulation with gelatin a strong peak for amide I has appeared at 1633cm⁻¹ and we speculate that the peak for amide II was overlapping with the peak for the IP in the same position 1589 cm⁻¹ which indicates the formation of an amide bond between amine groups of gelatin and carboxyl groups of acetic acid and sodium citrate. Furthermore, peaks for amine at 3463, 3460 cm⁻¹ for NH₂ group and for OH of COOH group at 3434, 3398 cm⁻¹ have also appeared which belong to gelatin. Secondly, the coating of IP-gelatin nanoparticles with PEG was observed through the appearance of OH peak at 3490 cm⁻¹. It was also observed that there was slightly shifting in the active group's peaks of IP after encapsulation with gelatin and PEG which indicates that the IP did not chemically interact with a copolymer, see Figure 3. The EE of IP was also studied where the EE was significantly affected by the IP concentrations. Table 1 shows that EE is directly proportional to IP concentrations. It was noticed that EE was increased by ≈10% when we duplicate the initial concentration of IP and by ≈17% when the

initial concentration was triplicated. This surge in the EE was slightly changed when using four times of initial concentration $\approx 18\%$. However, the EE was

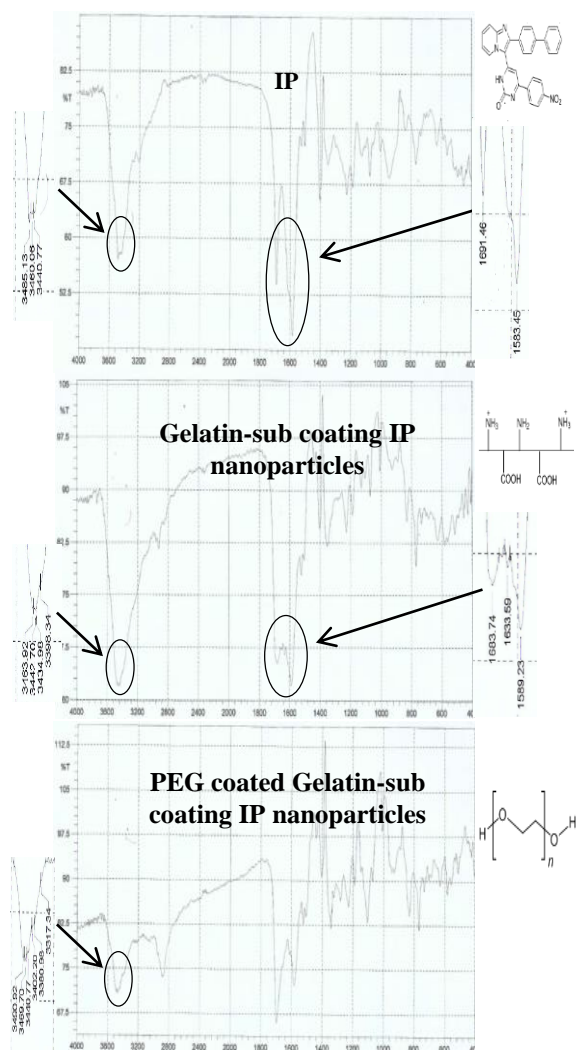


Figure 3. A schematic illustrates the FT-IR spectra for IP, IP-gelatin and IP-gelatin-PEG nanoparticles under experimental conditions.

dropped down to only $\approx 8\%$ using 5 times of initial concentration which was less than the EE of the initial concentration. We speculate that IP encapsulation reached the saturation (maximum loading) when using three times of initial concentration and the surge in IP above that concentration has a negative effect on the encapsulation process, Table 2. AFM was used to estimate the morphology of synthesized nanoparticles. Because of the high resolution, the AFM technique has been widely applied to study the structure of biological samples with a size of less than a few nanometers [49]. Our results show that IP-gelatin-PEG nanoparticles were prepared in size between 39 and 76 nm, Table 2.

It has also been revealed that there was an inverse correlation between EE and nanoparticles size and the

best result was obtained when loading 0.782 mg/ml of IP, where it gave nanoparticles with lowest size and highest EE, Figure 5. Therefore, the use of higher IP concentrations can negatively affect EE and the size of nanoparticles. This might be due to the use of high IP concentration leading to nanocapsules aggregation and consequently increase of their size. Furthermore, physiological pH 7.4 and stomach pH 3.4 were chosen to investigate the behavior of synthesized nanocapsules in various regions of the human body. The effect of these pHs on the releasing of IP from IP-gelatin-PEG capsules were *in vitro* monitored under controlled time and temperature 37°C . Figure 6 shows that the IP was slowly released after 30 minutes of treating with phosphate buffer solutions at pHs 7.4 and

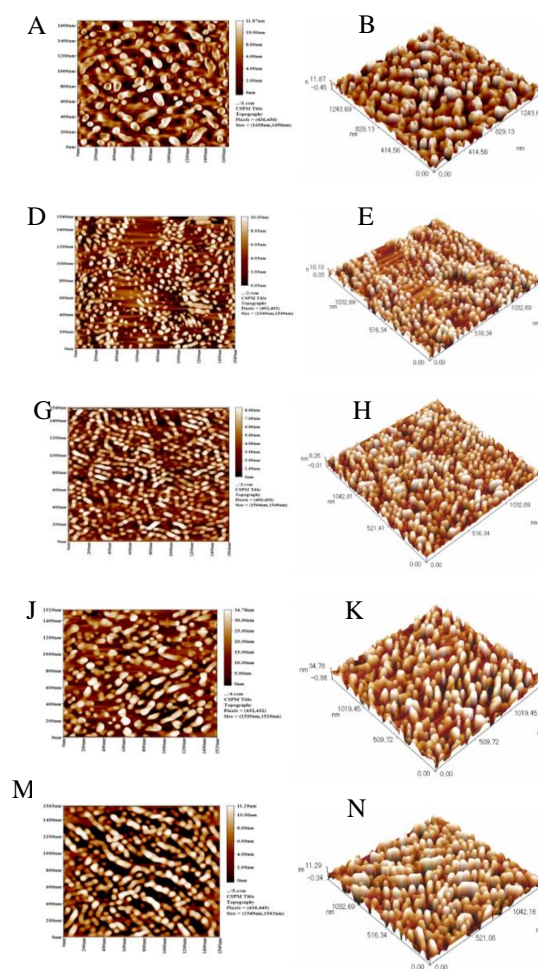


Figure 4. AFM images for IP-gelatin-PEG nanoparticles, where the first column shows 2D image, the second column shows 3D image while the third column shows size granulated distribution for IP-gelatin-PEG nanoparticles in different drug loading concentrations. A and B represent the first loading concentration (0.261mg/ml), D and E represent the second loading concentration (0.521mg/ml), while G and H represent the third loading concentration (0.782mg/ml), J and K represent the fourth loading concentration (1.043mg/ml). Finally, M and N represent the fifth loading concentration (1.304mg/ml).

3.4. The double-layer for hydrophobic molecules delivery system showed a gradual release of hydrophobic molecules in the stomach and in physiological pH to approximately reach 100% at 300 minutes.

This result also showed that IP-gelatin-PEG nanocapsules were easily hydrolyzed at pH 7.4 than pH 3.4 which led to the release of IP at pH 7.4 faster than pH 3.4. This might be due to the electrostatic interactions of polymer capsules were easily disrupted at physiological pH than the acidic pH, Figure 6. This result comes to agree with the previous study carried on the rifampicin–chitosan–poly PEG nanoparticles which revealed that these nanoparticles were also easily hydrolyzed at physiological pH than the acidic pH [9]. The relatively long time of drug-releasing can improve the drug activity through increasing time-acting therapy.

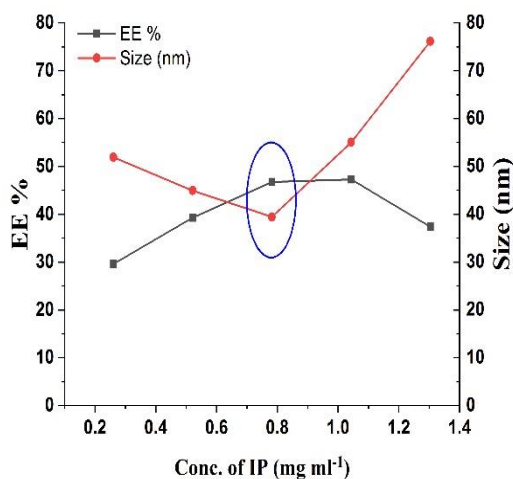


Figure 5. The correlation between EE% and size of IP-gelatin-PEG nanoparticles, where the best EE and size were surrounded by a blue circle.

Conclusion

A nanostructured double-layer for hydrophobic molecules delivery system as a novel platform was successfully designed and applied for controlling hydrophobic molecular delivery in the simulated stomach and physiological blood fluids. The microclimate-basicity medium technique of the hydrophobic molecular core was employed by introducing sodium citrate. The positive sub coating gelatin layer has attracted the hydrophobic molecules by employing of negatively charged of sodium citrate. The amounts of coating and sub coating polymeric compositions ratio PEG:Gelatin were optimized. The

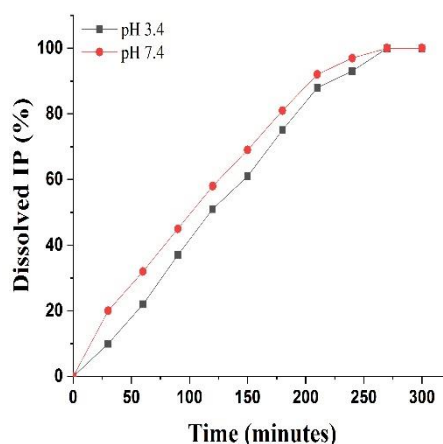


Figure 6. The dissolution of IP nanoparticles at pHs 7.4 and 3.4 *in vitro*. A 2-mL aliquot of sample was withdrawn at regular time intervals (30, 60, 90, 180, 210, 270 to 300 minutes) and replaced with the same amount of fresh buffer solutions (7.4 and 3.4). The samples collected were analyzed using PD-303 APEL spectrophotometer at 450 nm. The dissolution experiments were carried out in triplicate.

IP compound, which previously synthesized in our laboratory, was successfully loaded into gelatin-PEG polymeric micelles to form IP-gelatin-PEG nanocapsules. The characterization of IP-gelatin-PEG indicates that these nanoparticles were in size between 39 and 76 nm depending on the IP loading concentration. Also, IP-gelatin-PEG displays relatively long time and pH depending drug releasing which can improve its time-acting therapy through slow release of IP drug from nanoparticles. Consequently, the optimized double-layer for hydrophobic molecules delivery system has shown a controlled manner for hydrophobic molecule-release in the simulated stomach and physiological blood fluids. Taken together, it can be concluded that these IP-gelatin-PEG nanocapsules offer a potential way for the treatment of several human diseases.

Conflicts of interest

There are no conflicts to declare.

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الخلاصة:

كان الهدف من الدراسة الحالية هو تطوير طبقة مزدوجة نانوية لنظام توصيل الجزيئات الكارهة للماء. تتكون الطبقة المزدوجة المطورة من بوليمر قائم على البولي إيثيلين جلايكول (PEG) متبوعاً بطبقة فرعية من الجيلاتين للجزيئات الكارهة للماء التي تحتوي على سترات الصوديوم. تم تحسين نسبة التركيب البوليمري لـ PEG وكمية الجيلاتين المطلي الفرعي باستخدام طريقة الكسور ثنائية المستوى. تم تمييز الجسيمات النانوية باستخدام تقنيات AFM و FT-IR. كان حجم هذه الكبسولات النانوية في حدود 39-76 نانومتر اعتماداً على تركيز تحميل الدواء. تم تحميل الدواء بشكل فعال في الجسيمات النانوية-PEG-Gelatin (~47%). تم فحص خصائص إطلاق الجزيئات الكارهة للماء من حيث مدة الإطلاق الخاضعة للرقابة وكفاءة الذوبان في وسائط الذوبان المختلفة، مثل درجة الحموضة الفسيولوجية (7.4) ومحاكاة سائل المعدة (3.4). وبالتالي، أظهرت الطبقة المزدوجة المُحسَّنة لنظام توصيل الجزيئات الكارهة للماء إطلاقاً تدريجياً للجزيئات الكارهة للماء في درجة الحموضة الفسيولوجية وفي درجة الحموضة الفسيولوجية، مما يشير إلى حداثة استخدامها كمنصة لتوصيل الجزيئات الكارهة للماء.