Improved Mucoadhesive Properties of Repaglinide-Loaded Nanoparticles: Mathematical Modelling through Machine Learning-Based Approach

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Abstract: *This research work aims to develop a modified repaglinide-loaded chitosan-ethyl cellulose nanoparticles (RPG-ECSNPs) as a novel sustained-release dosage form with improved mucoadhesive properties using an emulsification solvent-evaporation technique. The RPG-ECSNPs with different particle sizes were prepared from various polymers containing ethyl cellulose (EC) as the internal phase and chitosan (CS) as the external phase, and the use of surfactants, including Tween 80 and poloxamer 188 as emulsifiers.* In vitro *drug release, drug loading amount, and entrapment efficiency have been influenced by changes in the concentrations of CS and EC. The mean droplet size and zeta potential of RPG-ECSNPs were 213 ± 8.5 nm and 16.4 ± 2.4 mV, respectively. The optimized formulation's entrapment efficiency was 66 ± 2.3%, and drug loading was 7.9 ± 1.65%. The release profile was significantly higher in PBS (90%) than in diluted hydrochloric acid (30%) during 24 h of the study. The mucoadhesive function of the particles was examined* in vitro *using part of rat intestines. The highest adhesive % was observed for the chitosan-coated NPs. No adhesive properties were noticed for chitosan-free NPs (P-value > 0.05). This indicated that ECSNPs can be successfully utilized for sustained and controlled drug delivery of RPG through the GIT.*

Keywords: *adhesive properties; chitosan; ethyl cellulose; nanoparticles*

■ INTRODUCTION

Nowadays, controlling the drug release rate is one of the interesting topics in the pharmaceutical industry. Scientists are focusing on drug release rate development and optimization through different methods. Diabetes mellitus is a worldwide health issue affecting patient quality of life [1]. Diabetes is a chronic metabolic problem that increases blood glucose because of insulin shortage. Repaglinide (RPG) is known as a fast-acting prandial glucose regulator. The action of RPG plays a role in stimulating the insulin release from pancreatic b-cells through the activation of ATP-dependent K^+ channel and can normalize the mealtime glucose excursion. The RPG is low soluble in water solution, which is about 34 µg/mL. Thus, sustained drug delivery systems have been implied to overcome many challenges associated with the adverse effects of conventional dosage forms leading to improved therapeutic outcomes. Designing a drug delivery system through synthesizing nanoparticles is a very useful method to optimize the prolonged release rate for different drugs. Surface modification of these nanocarriers through semi-synthetic polymers is able to improve drug bioavailability. Chitosan molecules (CS) are widely applied to optimize the physicochemical characteristics of the drug due to their mucoadhesive properties. They are very good candidates due to their good biocompatibility, biodegradability, and low toxicity properties [2-3]. Also, surface coating of nanocarriers using chitosan resulted in improving their stability in gastric and intestinal fluids [4]. Moreover, CS has been extensively applied in different research work to design a drug delivery system [5], artificial skin [6], artificial corneal [7], and gene therapy [8]. The role of ethyl cellulose (EC) is to control the release of RPG and gives floating characteristics. Drug release from the CS microsphere can be controlled by utilizing a naturally

existing crosslinking agent to impart a skeleton network for the pursuit of a prolonged release profile and better physiological compatibility [9]. For example, Wu and co-workers [10] reported RPG-loaded nanostructured lipid carriers with various particle sizes for enhancing oral bioavailability by solvent diffusion method.

In this study, novel nanoparticles, ethyl cellulose/chitosan (ECCS), with CS coating and EC core, was provided using the o/w emulsification procedure [11]. EC was chosen to act as a hydrophobic layer to prevent NPs from dissolving in the stomach [12]. The influence of preparation methods including CS and EC concentrations, on sustained release was studied. RPG has been chosen as the model drug that can be considered as the potential of the loaded nanoparticles in the delivery system. Further, the release profiles of RPG and mucoadhesive properties of the system were studied. Ebrahimi and co-authors [11] demonstrated the RPG drug encapsulation within solid lipid nanoparticle (SLN)-based formulations under the effect of Tween 80 and phosphatidylcholine. They discovered that the SLN formulations based on Tween 80 and phosphatidylcholine had the smallest size, the longest drug release time, and the greatest loading capacity [13]. Lokhande et al. [12] studied the encapsulation and release of RPG drug by saturated EC-ethyl acetate solution through solvent diffusion. The aim of this research work is to develop a modified repaglinide-loaded chitosan-ethyl cellulose nanoparticles (RPG-ECSNPs) as a novel sustained-release dosage form with improved mucoadhesive properties using an emulsification solvent-evaporation technique. The RPG-ECSNPs with different particle sizes were prepared from various polymers containing EC as the internal phase and CS as the external phase and the use of surfactants, including Tween 80 and poloxamer 188 as emulsifiers. *In vitro* drug release, drug loading amount, and entrapment efficiency were studied by changes in the concentrations of CS and EC.

■ EXPERIMENTAL SECTION

Materials

CS (Mw 250 kD, degree of deacetylation (DD) 90%) was provided from Aladdin Chemistry Co. Ltd. EC (200 cPa·s) was provided from Sinopharm Chemical Reagent Co. Ltd. Anhydrous ethanol, dichloromethane, sodium dihydrogen phosphate, sodium hydrogen phosphate, Tween 80, and acetic acid were obtained from Tianjin Feng Chuan Chemical Reagent. The provided materials and solvents were analytical grade. RPG was provided by Shanghai purple reagent factory. A dialysis bag was provided by Spectrum Chemical MFG.

Instrumentation

A transmission electron microscope (TEM, Tecnai G2−12−Spirit Biotwin-120 kV from FEI) has been used to characterize the morphology of nanoparticles. The particle size distribution (PSD) of the microsphere was measured using photon correlation spectroscopy. A vibrating incubator (HZQ-F160, Harbin Donglian Electronic & Technology Development, China) was used to extract the entrapped RPG.

Procedure

Preparation of microspheres

As much as 1 g EC was dissolved in 10 mL of a mixture of dichloromethane (DCM) and ethanol (3:1, v/v). RPG was added in EC solution at a concentration of 2% w/v, which constitutes the oil phase. Then 0.2 g CS was dissolved in 40 mL acetic aqueous solution (1%, v/v) at 25 °C, which constitutes the aqueous phase. Tween 80 (1%, v/v) was added into the CS solution under stirring [14]. The EC solution was poured into the CS solution dropwise under vigorous stirring at 2000 rpm/min for 60 min to form the primary o/w emulsified solution [15-16]. The obtained emulsified solution was put under moderate stirring for another three hours to allow the removal of the organic solvent. In the end, the obtained nanoparticles were filtered and dried at room temperature.

Characterization of nanoparticles

Particle size and zeta potential determination. The sample was diluted 100 times with pure nano water and all measurements were performed at 25 °C at a fixed scattering angle of 90 utilizing a He–Ne laser at 633 nm using PSD. The same instrument was applied to measure the surface charge of the microsphere diluted sample. All measurements were done in triplicate.

Surface morphology. TEM has been used to characterize the morphology of nanoparticles. First, nanoparticles were freeze-dried under a vacuum at −40 °C utilizing a ScanVac CoolSafe freeze dryer (LaboGene ApS, Denmark). In the next step, 1 mg of the dried nanoparticles was loaded on the copper sample stub utilizing a double-sided carbon adhesive, and the extra number of particles were deleted. In order to analyze the images, image software "Soft-Imaging Software GmbH CM-Prof 2.11.002" has been used.

Determination of loading amount and entrapment efficiency. An amount of 50 mg of the lyophilized nanoparticles were triturated until a fine powder was obtained. A mixture of 50 mL of acetone and distilled water (1:3, v/v) was added to get a homogeneous dispersion. The obtained suspension was stirred continuously at 150 rpm/min in a vibrating incubator at 37.5 °C (HZQ-F160, Harbin Donglian Electronic & Technology Development, China) to extract the entrapped RPG. After filtrating the suspension, the filtrate was collected to analyze the content of RPG spectrophotometrically at 243 nm. The drug loading (DL) was measured through Eq. (1):

$$DL\% = \frac{Wt_{dl}}{Wt_{td}} \times 100 \quad (1)$$

where Wt_{td} denotes the total value of the drug included and Wt_{dl} denotes the value of drug in the microspheres. Then the entrapment efficiency (EE) was calculated from Eq. (2):

$$EE\% = \frac{Wt_{dl}}{Wt_{tm}} \times 100 \quad (2)$$

where Wt_{tm} denotes the total amount of microspheres.

Release kinetics

The release kinetic of RPG from the microsphere was studied by fitting the release data within gastric and intestinal fluids using the following mathematical models. Zero-order release method was described in Eq. (3) [18-19]:

$$Q_t = k_0 t \quad (3)$$

where Q_t denotes the percentage of drug release rate at time t, k_0 is the release rate constant, and k_1 denotes the release rate constant for the first-order kinetics. First order release model was described in Eq. (4) as follows [18-19]:

$$\ln(100 - Q_t) = \ln 100 k_1 t \quad (4)$$

while Higuchi's equation (Eq. (5)) is as follows:

$$Q_t = k_H t^{0.5} \quad (5)$$

where k_H denotes the Higuchi release rate constant [20].

HPLC analysis

The mobile phase contains acetonitrile (40%), and phosphate buffer (60%, pH 2.5, 10 mM) was delivered at a flow rate of 1.0 mL/min. In order to characterize the results, a UV detector was used at a wavelength of 245 nm. The NPs were diluted using a chloroform:methanol (1:1, v/v) mixture, and the drug content was determined by the HPLC.

Physical stability of NPs

The storage stability of optimized NPs was analyzed at three various temperatures, i.e., 4, 25, and 45 °C for 2 months. Then, the changes in particle size, ZP, entrapment efficiency, and drug content are measured as well. In order to investigate the stability of NEs at pH 1.2 at a range of time including 0, 0.5, 1, 2, 5, 12, and 24 h, then the NEs samples were studied for mean droplet size, polydispersity index, and zeta potential.

Mucoadhesive properties

To evaluate the mucoadhesive function of the NPs *in vitro*, a particle counting method (Coulter counter) was used after confirming the relationship between the NPs concentration (mg/mL) and the number of NPs measured. This test was carried out using an intestinal tube (10 cm) isolated from a Wistar rat. After washing the intestine tube with saline solution, the tube was filled with the NPs solution and diluted 100 times with a phosphate buffer solution (pH 7.4), and then sealed with closers. The tube was then incubated in water at 37 °C for 2 h. The number of NPs was measured before and after incubation and the mucoadhesive % was calculated by the following equation (Eq. (6)):

$$Mucoadhesive\% = \frac{N_0 - N_s}{N_0} \times 100 \quad (6)$$

where N_0 and N_s are the number of NPs before and after incubation, respectively.

In vitro *release study*

"Dialysis sac" procedure was applied consisting of a dialysis membrane (cut-off 12 kDa) collected as a closed sac, including 3 mL of RPG-NPs or Cs-RPG-NE, in 100 mL of dissolution test medium with stirring at 70 rpm for 60 min at 37 °C. As much as 0.5 mL of the samples were withdrawn after 12 h, and the amount of RPG was determined by HPLC. In order to illustrate the influence of pH on the drug release rate, the test was carried out at different pH (1.2 and 7.4) as gastric and intestinal fluids, respectively.

Statistical analysis

All analyses were repeated three times. Statistical analyses were carried out utilizing some statistical software such as Prism-5 (GraphPad Software Inc., San Diego, CA).

■ RESULTS AND DISCUSSION

Preparation and Characterization of NPs

Size characterization of ECSNPs in different buffer media shows the stability of the formulation. From the TEM micrographs, it is shown that the nanoparticles prepared by emulsification solvent-evaporation are generally with uniform particle size distribution and spherical in shape. TEM micrograph of ECSNPs illustrated that the range of dimensions was about 150–250 nm in length and 7–10 nm in width (Fig. 1), while RPG-loaded ECSNPs had size ranges from 50 to 100 nm in length and 4–6 nm in width (Fig. 2). The outputs are in good agreement with DLS results. González et al. [17] have shown the morphology of CS NPs through low molecular weight by TEM analysis that has the size of individual particles was 5 to 10 nm.

Table 1 indicates the results of RPG-ECSNPs with different particle sizes, which are prepared from EC, Tween 80, poloxamer 188, and CS using an emulsification solvent-evaporation technique. Table 1 illustrates that all five formulations were stable and showed changes in particle size growth when the ratio of CS in comparison with EC increased from 117 ± 5.09 to

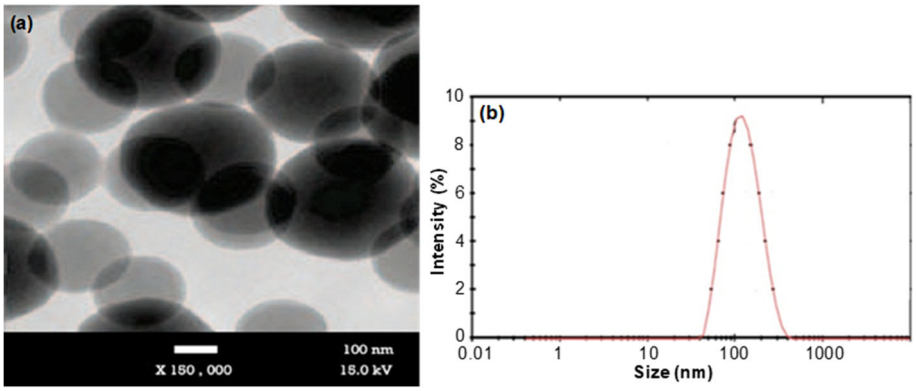

Fig 1. (a) TEM monographs and (b) DLS techniques of the ECSNPs nano-formulation

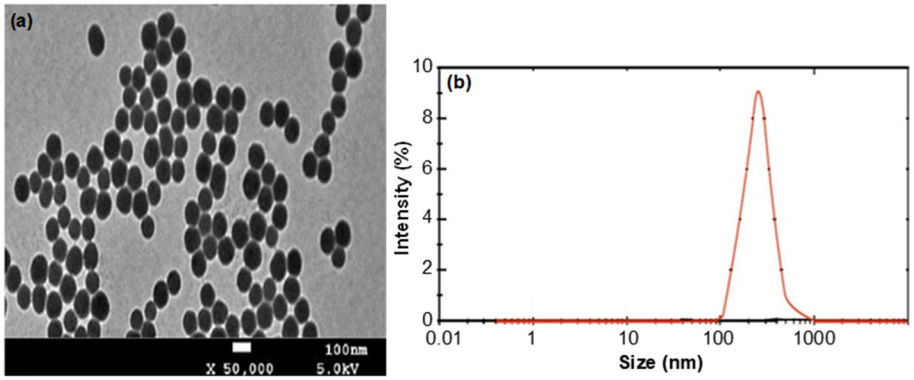

Fig 2. (a) TEM monographs and (b) DLS techniques of the RPG-ECSNPs nano-formulation

Table 1. Results of optimized formulations

Batch	EC/CS Ratio	PDI (Mean ±SD), n = 3	*In vitro* drug release (%) (Mean ±SD), n = 3	Particle size (nm) (Mean ±SD), n = 3	Zeta potential (mV) (Mean ±SD), n = 3
A	1:1	0.510 ± 0.12	2.88 ± 0.98	117.00 ± 5.09	−22.90 ± 0.48
B	1:2	0.267 ± 0.06	44.69 ± 1.21	213.50 ± 8.68	16.40 ± 2.43
C	1:3	0.248 ± 0.04	52.71 ± 1.29	386.70 ± 10.88	15.69 ± 0.89
D	1:4	0.355 ± 0.05	59.64 ± 1.10	443.70 ± 7.56	29.40 ± 1.00
E	1:5	0.478 ± 0.07	78.26 ± 2.08	509.40 ± 11.56	28.00 ± 2.56

509.4 ± 11.56 in batches A-E. The PDI values with a higher amount of CS for all batches reduced from 0.510 ± 0.12 to 0.248 ± 0.04, while the percent drug release is increased from 2.88 ± 0.98% to 78.26 ± 2.08%. Therefore, the addition of CS caused an enhancement in drug release. Vaghani et al. [21] showed the network of CS and polyvinyl pyrrolidone hydrogels improved the loading of RPG by up to 95%. The RPG-NPs had various zeta potentials and particle sizes with corresponding blank NPs, indicating that the participation of RPG had an influence on the zeta potential and particle size. However, the RPG-ECSNPs with the different batches had variant zeta potential values, indicating various surface charges between batch A and other batches. For example, Wu et al. [8] reported the RPG-loaded nanostructured lipid carriers (RPG-NLCs) had similar zeta potential and particle size compared to NLCs, showing that the taking part of REP had no significant influence on the zeta potential and particle size.

Drug Loading and Entrapment Efficiency

Poovi et al. [22] illustrated the alteration of the polymer and RPG ratios could affect the drug loading and entrapment efficiency. In this work, RPG was loaded onto polymeric nanoparticles by emulsification solvent-evaporation technique. Drug loading, particularly in the case of polymeric nanoparticles (PCENs), is based on adsorption and calculated using Eq. (1) [23]. The DL% and EE% values in order were 6.5 and 51.7% in batch A and 7.9 and 66.0% in batch B. The highest DL found in batch B may be because of the larger surface area and pore volume than that of batch A. It could be noticed that the drug loading amount was enhanced with an increase in the EC concentration in the formulation. This is attributed to the drug adsorption onto EC due to its non-ionic cellulose properties (binding properties). The more EC amount used, the more RPG loading amount. Increasing the ratio of CS/EC produced less or unpredictable results of drug content inside the nanoparticle (Table 2).

In Vitro Drug Release

Different mathematical models have been utilized to explain the kinetics of the drug release from the ECSNPs. In this study, the drug release rate has been analyzed at different pH, which is responsible for the ionization of the existing functional group [18-19]. It has been shown that the structure of the polymer does not change in acidic media, and the release rate can be controlled through diffusion. In less acidic media, the release is increased due to the relaxing of the polymers' chain owing to their swelling properties. It has been found that the drug release rate was increased through the loading of the drug in the ECSNPs. The designed formulation (B) has been applied to analyze the influence of various media pH. An appropriate model has been chosen to illustrate drug-release behavior. The kinetic rate constant (k) and the correlation coefficient (R^2) are illustrated in Table 3. The release behavior from this formulation (B) did not follow the Higuchi model, indicating high drug content and related to drug content.

Table 2. The amount of drug loading efficiency (DL) and entrapment efficiency (EE) of RPG-ECSNPs

Batch	CS (w/v%)	EC (w/v%)	DL (%)	EE (%)
A	1.0	1.0	6.5	51.7
B	1.0	2.0	7.9	66.0
C	2.0	1.0	5.3	54.7
D	3.0	1.0	4.4	52.4
E	4.0	1.0	4.1	50.4

Table 3. Parameters of *in vitro* release evaluation through different pH

Fitting Model	pH	Equation	Relative parameters	
			k value	R^2
Zero-order	2.0	1	0.339	0.958
	6.8		0.230	0.942
First-order	2.0	2	0.002	0.973
	6.8		0.007	0.990
Higuchi	2.0	3	4.309	0.978
	6.8		3.155	0.983

The release data of all examined models illustrated proper fitting to the Higuchi model, which is shown through the higher R^2 values (0.968–1.000) in comparison with the other applied models such as zero-order and first-order kinetics equations. Hence, the drug release kinetics illustrate a correlation between concentration and drug release rate.

Mucoadhesive Properties

The polymer layer on the platform was shown using the zeta potential. The mucoadhesive function of the ECSNPs was examined *in vitro* using part of rat intestines. The number of particles was adopted to evaluate the adhesive % of the ECSNPs. The highest adhesive % was observed for the (CS-Poloxamer)-coated NPs. No adhesive properties were noticed for CS-free NPs. The amount of CS polymer in the coating layer described the mucoadhesion function. It could be concluded that the more effective coating leads to higher adhesiveness [20].

■ CONCLUSION

The aim of this research work is to develop a modified RPG-ECSNPs as a novel sustained-release dosage form with improved mucoadhesive properties using an emulsification solvent-evaporation technique. The results demonstrated that the alteration in the concentrations of CS and EC has a great impact on drug loading amount, entrapment efficiency, and drug release. The drug release from ECSNPs was remarkably higher in PBS (90%) than in diluted 30% HCl during 24 h of the study. The highest adhesive % was observed for the chitosan-coated NPs. The output of this study illustrated that the saturated EC-ethyl acetate solution enhanced the efficiency of RPG encapsulation at 0.5% PVA. The RPG-EC nanoparticle's effect on control of the drug release rate prolongs it with no chemical interaction between them.

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