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A Hybrid Modeling Approach for Optimization of PMAA– Chitosan–PEG Nanoparticles for Oral Insulin Delivery

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This study was to develop pH sensitive polymethacrylic acid–chitosan–polyethylene glycol (PCP) nanoparticles for oral insulin delivery. This was achieved by dispersion polymerization of methacrylic acid (MAA), polyethylene glycol (PEG) and chitosan (CS) in the presence of cross linking agent, ethylene glycoldimethacrylate (EGDMA), and polymer initiator, potassium persulphate. Method development was carried out based on fractional factorial design by varying process parameters such as ratio of MAA to CS, ratio of MAA to EGDMA and the initial amount of insulin used to prepare PCP nanoparticles. PCP nanoparticles were characterized with different techniques including FTIR, DLS, and scanning electron microscopy (SEM). Insulin was incorporated into the nanoparticles by diffusion filling method. It was found that the PCP nanoparticles exhibited good protein encapsulation efficiency (up to 99.9%). The findings revealed that the nanoparticles were spherical with smooth surfaces. The particles size average was determined to be 172 nm by DLS and 86 nm by SEM. The in vitro release profile of PCP nanoparticles were investigated both in acidic (simulated gastric fluids, pH:1.2) and neutral buffered solutions (simulated intestinal fluids, pH: 7.4). In order to have the best performance of nanoparticles, the process parameters was optimized using support vector regression (SVR) method in combination with genetic algorithm (GA). The results revealed that the optimum settings were as follows: MAA/ Cs mole ratio (%): 297.35, Cs/TPP mole ratio (%): 51.4, and the initial insulin amount (mg): 50.3. The findings showed that nanoparticles exhibited pH responsive release profile where the extent of drug release in simulated intestinal medium was almost two folds more than the simulated gastric media. Global sensitivity analysis was also used to identify the impact of different variables on the PCP nanoparticles characteristics. This study introduces new approach to rational design of nanoparticles according to the properties of interest.

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1. Introduction

Nowadays, diabetes is one of the most important concerns for human health which has involved more than 2.8% of human being in 2000 [1]. Great efforts have been focused on the development of efficient insulin delivery such as glucose-sensitive multilayer films [2,3]. Due to some issues associated with insulin injection, research has focused on alternate ways of delivering insulin e.g. oral delivery

[4]. Polymeric nanoparticlulate delivery systems, especially complexation hydrogels are suitable candidate for oral insulin delivery due to their abilities to respond to changes in pH in the GI tract and provide protection to the drugs from the harsh environment of the GI tract. Polymethacrylic acid–chitosan–polyethylene glycol (PCP) nanoparticles are complexation hydrogels intended for oral insulin delivery [5-7]. It has been shown that the oral administration of insulin loaded complexation hydrogels result in significant increase in insulin absorption and consequently lead to considerable reduction of the blood glucose levels in healthy and diabetic rats [8].

It is believed that PCP characteristics in terms of drug encapsulation, release profile, drug stability, etc can be modulated by altering the nanoparticles composition or by changing the process parameters. Besides, identifying the relationship between the process parameters and the resultant PCP properties would help understand the impact of various factors, which consequently will lead to the accurate prediction of PCP properties and the rational design of PCP-based drug carrier systems.

Several approaches to understand and model the polymeric based nanoparticles have been proposed in the literature [9-11]. Motwani et al. [12] optimized mucoadhesive chitosan (CS)-sodium alginate (ALG) nanoparticles as a new vehicle for ophthalmic delivery of gatifloxacin by employing a 3-factor, 3-level Box-Behnken statistical design and response surface methodology of the corresponding polynomials. Similarly, some numerical modeling techniques have been proposed for modeling the size of nanoparticles [13, 14]. Asadi et al. [14] used an artificial neural network (ANN) to model the size of nanoparticles as a function of process parameters. However, the commonly used modeling techniques such as regression analyses and neural networks were not successful in precise prediction of nanoparticles characteristics due to the nonlinearity and non-stationarity of the nanoparticles properties. To overcome this shortcoming, a prediction model based on support vector regression (SVR) is proposed in this paper. In SVR, the flexibility to choose a sensitivity function (epsilon) and vary the training as per the user's needs plays an important role in capturing the trend and better development of model.

Researchers have illustrated the application of SVR in modeling and optimization in different fields [15, 16]. Recently, hybrid techniques of SVR integrated with genetic algorithm (GA) was also introduced to identify optimal process parameters [17]. Yang et al [15] reported SVR to control and optimize the structure of core polymer particle. This methodology was also used for different purposes including discovery of antibiotics-derived polymers for gene delivery [18], and modeling drug interactions [19]. Although, hybrid SVR/GA technique is a promising candidate for process modeling and optimization, especially in the case of complex process models, but it has not been used in optimization nanoparticles characteristics for drug delivery purpose.

The goal of this research was to optimize a set of formulation of PCP in terms of their beneficial properties as oral insulin delivery and to determine which of parameters exhibited the greatest impact on PCP characteristics as an oral insulin delivery device. To achieve this goal, a numerical modeling method based on combining nonlinear machine learning algorithms (SVR) and evolutionary calculation algorithms (GA) was considered for predicting and optimization of PCP characteristics.

2. Experimental

2.1. Material and method

Chitosan (CS) by degree of deacetylation ≥75% was provided from Sigma. Human insulin 100 IU/mL was obtained from Ronak compony (Iran). Methacrylic acid (MAA), polyethylene glycol (PEG, Mw 20,000 Da), ethylene glycol dimethacrylate (EGDMA), potassium persulphate and solvents all were from Merck and purchased locally and used as received.

2.2. Preparation of nanoparticles

Preparations of PCP nanoparticles were accomplished based on dispersion polymerization adapted from the process described by Sajeesh and Sharma [4]. In brief, all monomers were mixed together in 30 ml of distilled water and stirred on a magnetic stirrer for 15 min. Potassium persulphate was added into the solution to initiate polymerization. The temperature of the mixture was increased to 60 °C for 6 h and resulting suspension was filtered and washed several times with distilled water to remove residual monomers. Finally, the resultant nanoparticles were filtered and dried under vacuum at room temperature.

2.3 Insulin loading

Insulin loading was conducted by equilibrium partitioning of insulin into the nanoparticles. Briefly, the pH of the insulin solution (concentration 100 I.U. /ml) was adjusted to 7.4 by adding 1N NaOH solution. Next, 0.05 g of PCP nanoparticles was added to the insulin

solution at 37°C for 6 h at pH 7.4 to allow maximum loading by swelling nanoparticle and then pH of the insulin solution was lowered gradually to 2.5 by adding 1N HCl, to collapse the polymers in order to trap the insulin inside the nanoparticles by deswelling. Insulin loaded nanoparticles was centrifuged, freeze dried and stored at 4 °C for further studies.

2.4. Physicochemical characterization of PCP nanoparticles

2.4.1 Determination of drug Encapsulation efficiency (EE %)

The amount of insulin incorporated into or onto the nanoparticles was determined from the concentration of insulin remaining in the dispersion medium after separation of the particles. Briefly, insulin-loaded PCP nanoparticles were carefully were separated from the solution by filtration and underneath from the filtration was carefully decanted and insulin content in the underneath was analyzed with reverse phase high performance liquid chromatography (RP-HPLC). Encapsulation efficiency (EE %) of insulin was calculated using Eq (1).

$$\text{EE (\%)} = \frac{\text{total amount of insulin-amount of free insulin}}{\text{total amount drug}} \times 100\% \quad (1)$$

2.4.2 Particle size determination

The nanoparticles were dispersed in phosphate buffer solution at pH 7.4 and sonicated for 30 min at 20 °C before size measurement. The obtained homogeneous suspension was examined for the average particle size and size distribution. The zeta potential and particle size distribution of the prepared nanoparticles were determined by photon correlation spectroscopy (PCS) using a Nano/zetasizer (Malvern Instruments, Nano ZS, ZEN 3600, UK) working on the dynamic light scattering (DLS) platform The measurement was carried out at room temperature.

2.4.3 Scanning electron microscopy (SEM)

SEM images were obtained using a Philips XL30 electron microscope operating at 20 kV, technology with 3.5 nm Resolution.

2.4.4 FT-IR analysis of nanoparticles

The FTIR spectra of nanoparticles were performed on FT-IR instrument (BRUKER, Germany). Nanopowders were pressed and the FTIR spectra of the KBr disks were recorded for the wavenumber range of 400 to 4000 cm^{-1}.

2.4.5 Determination of the swelling behavior of the nanoparticles

The swelling behavior of the nanoparticles was studied in 0.2 M phosphate buffer solution (pH=7.4, and 1.2). PCP nanoparticles (5 mg) were suspended in 1 ml of buffer solution in a preweighed mirocentrifuge tube. At each predetermined time interval excess buffer solution was carefully removed and blotted with a piece of paper to absorb excess water on surface and then weight of swollen particles was determined. The degree of swelling was calculated as follows:

$$\text{Degree of swelling} = \frac{\text{Ws}-\text{Wd}}{\text{Wd}} \quad (2)$$

Where W_d is the weight of the dry particles and Ws is the weight of the swollen particles.

2.5 In Vitro drug Release Study

The drug release behavior of the PCP nanoparticles was studied in simulated intestinal pH (PBS, pH=7.4) as well as in the simulated gastric media with the pH value of 1.2. Typically, 0.015g of the freeze-dried nanoparticles were placed into a dialysis bag (cut-off 100 kDa) and dialyzed against 12 mL of simulated gastric buffer at 37 °C at 100 rpm for 120 min. Then, in order to determine the drug concentration, and thereby time dependent drug release profile, 0.5 mL of solution was taken out and replaced with 0.5 mL of fresh buffer solution maintained and evaluated for drug content by RP-HPLC. Next, the buffer solution of sink was replaced by the simulated intestinal buffer solution and a sample was taken after 180 min following the same procedure described earlier.

In order to determine the protective ability of the PCP nanoparticles for insulin under human stomach simulated environment, the release study of insulin in pepsin solution was also assessed. The gastric simulated solution was prepared by dissolving 3.2 g of pepsin in 7.0 ml of HCl and 2.0 g of sodium chloride and adding water to reach 1000 ml. The pH value of solution was adjusted to1.5 by HCl. Subsequently, the same protocol as described for PBS buffer solution was followed for the release study.

2.6 Insulin assay

Insulin concentration was analyzed by RP-HPLC using a Waters 1525 binary HPLC pump separations module, with a Waters 2487 dual absorbance detector (Waters, KNAURE, American) and a C18 column (250mm×4.6mm, particle size 5μm; Perfectsill, MZ-Analysen technik, Germany). The mobile phase consisted of two solutions; solution A was water with 0.1 vol. % Trifluoroacetic acid (TFA) and solution B was acetonitrile with 0.1 vol. % TFA. Solution A and B premixed with together and sonicated for 10 min and introduced with a flow rate of 1 ml/min. UV detection was performed at a wavelength of 220 nm and 50 μl of sample was

injected for each analysis. To determine the linearity of the method, different concentrations of insulin in the range 0.31–0.038 mg/ml were prepared and by plotting a calibration curve, a correlation coefficient of 0.997 was obtained.

Insulin was also analyzed with enzyme-linked immunosorbent assay method (ELISA-Monobind Insulin AccuBind, Lake Forest, CA) by reading the optical density with microplate reader at 450 and 650nm as a reference.

2.7 Experimental design

To optimize the characteristics of PCP nanoparticle for oral insulin delivery, fractional factorial design was applied. Independent variables (Xi) studied were MAA/Cs ratio (%), CS/TPP ratio (%), and initial insulin amount (mg) (table 1). The dependent variables (Yi) were particle size (nm), Polydispersity index, zeta Potential (mV), entrapment Efficiency (%), the extent of insulin released in simulated gastric fluid (% at 120 min), and the extent of insulin released in simulated intestinal fluid (% at 180 min). The design was composed of 3 variables set at 3-levels each. The design required a total of 15 experiments. The different values for the factor were chosen on the basis of previous preliminary experiments.

Table 1. Parameters used for fractional factorial design and their corresponding level

Factor	Levels used		
Independent variables	-1	0	1
X1 = MAA / Cs ratio (%)	100	250	500
X2 = Cs /TPP ratio (%)	10	30	60
X3 = insulin amount (mg)	20	40	70

2.8 Support vector regression (SVR)

Support vector machine (SVM) is a new non-linearity machine learning method based on the statistical learning theory. The most attractive feature of SVM is its excellent generalization ability particularly in the case of small sample. Besides, it could overcome the problem of local optimal solution, over-fitting, and low convergence rate which are associated with common artificial intelligence methods like artificial neural network (ANN). When SVM is used in regression, it is called support vector regression (SVR) which is a kernel-based supervised learning algorithm. In fact, kernels algorithm map the data onto a higher dimensional space to account for nonlinear data. SVR has been applied successfully to solve problems in numerous fields and proved to be a better prediction model [16]. Generally, to develop the model, SVR use a number of data set as training data, while the other samples are used as the test samples. The goal of SVR is to find the linear functional f, which maps variables of the input space in the variable output space. Then, the model is used to predict the selected sample.

In this study, the linear model f(x,θ) in the feature space was given by the following equation:

$$\mathbf{y}_i = f(\mathbf{x}, \boldsymbol{\theta}_i) \quad \mathrm{i} = 1,...,6$$
$$\mathbf{x} = \{\mathbf{x}_1, \mathbf{x}_2, \mathbf{x}_3\} \tag{3}$$

Where xi, yi, $\boldsymbol{\theta}_i = \{\boldsymbol{\alpha}, \boldsymbol{\omega}, \mathbf{b}\}$ mean the sample vector, the response and the learning parameters, respectively. In this study, SVR was performed using radial basis function (RBF) as the kernel function (eq. 4).

$$f(\mathbf{x}, \boldsymbol{\theta}_i) = \sum_{j=1}^{\ell} \omega_{ij} \sqrt{0.1 + \left(\eta \cdot \left\| \bar{\mathbf{x}} - \alpha_{ij} \right\|_2\right)^2} + b_i \tag{4}$$

Where *l* stands for supporting vectors and η indicates the flattest function which was set to 0.125. The overall performance of the model was evaluated by measuring the mean absolute error (MAE).

In order to eliminate dimension differences, the following formula was used for data normalization, and then all input and output data were normalized to the range [0, 1].

$$\bar{\mathbf{x}}_k = \frac{\mathbf{x}_k - m_k}{\sigma^2_k} \quad \mathrm{k} = 1,2,3 \tag{5}$$

Here, x denotes the original data point, and m and σ are the mean and standard deviation values in the data set, respectively (table 2).

Table 2. The mean and standard deviation values in the data set

	MAA / Cs mole ratio (%)	Cs /TPP mole ratio (%)	insulin amount (mg)
m	280.769	33.076	43.076
σ^2	165.250	20.568	20.568

2.9 Genetic algorithm (GA)

Genetic algorithm (GA), derived from Darwin's theory of natural selection and evolution, was first proposed by Holland [20]. A genetic algorithm starts with an initial population (chromosomes), representing a possible solution to a given problem. Each chromosome is assigned a fitness value according to the result of the fitness function. Highly fit chromosomes are given more opportunities to reproduce and the offspring share features taken

from their parents. GAs find a best set of values of parameters that maximize (or minimize) a function by generating a population of solutions at one generation, and then calculating the value of the goal function for each solution. In this study the cost function was defined as follows:

$$Cost(\mathbf{x}) = K \cdot \sum_{i=1}^{6} \mu_i \cdot f(\mathbf{x}, \boldsymbol{\theta}_i) \quad (6)$$

Where the constant K was set to 1000 and μi indicates the weight coefficient for each variable. The weight coefficient for each given dependent variable determines the corresponding variable importance in the optimization process.

Considering the excellent feature selection capability of GA and the robust modeling performance of SVR, in this study a hybrid modeling based on SVR and GA was constructed and used to optimize PCP nanoparticles for oral insulin delivery, expecting to obtain model with better prediction performances.

3. Results and discussion

In this study, we attempted to optimize the characteristic of pH sensitive hydrophilic nanoparticles for oral insulin delivery. For this purpose, we used inter-ionic method for the preparation of pH sensitive PCP nanoparticles by complexion between MAA, CS, PEG and EGDMA. Crosslinking of PMAA brings about stability to the system and renders a pH dependent swelling/deswelling behavior, and making the system more appropriate for the application. PEG was used to stabilize insulin and to form interpolymer for better insulin retention capacity. This study was performed to investigate the impact of different process parameters such as the weight ratio of MAA to CS, the weight ratio of CS to TPP and initial amount of insulin fed into the nanoparticles on their characteristics for oral insulin delivery, while concentration of some various formulation parameters was constant during the optimizing. MAA could overcome poorly solubility of CS in neutral or alkaline solution by imparting acidity to the solution. EGDMA as cross linking agent determines morphology and solidity socket of nanoparticles. Amount of EGDMA should be optimized in order to avoid socket of nanoparticle from being very firm to allow insulin release and not very soft to deform it. Also EGDMA renders a pH dependent swelling/deswelling behavior to making the system more appropriate for the application.

3.1. Physiochemical characterization

Fig. 1 presents the FTIR spectra of the prepared PCP particles. Four main absorption bands at 3447, 2949, 1722 cm^{-1} appeared in these spectra which may be attributed to the stretching of O-H/N-H, C-H, C=O, respectively [21]. For comparison, the spectra of insulin and insulin loaded PCP nanoparticles were also included in Fig.1. The insulin loaded PCP nanoparticles precursor displayed the absorption band of insulin at 1637 cm^{-1}, which was probably attributed to the Amide I bond of insulin, which is characteristic of protein spectrum.

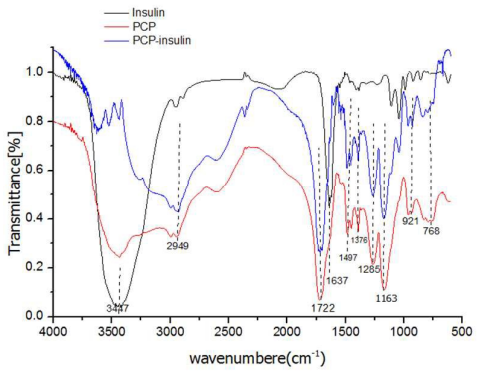

Fig. 1. FT-IR spectra of insulin, PCP and insulin loaded PCP nanoparticles

Analysis of the size of PCP particles revealed that the average diameter of particles were in the range 172-800 nm for different formulations. The histogram of typical particle size is shown in fig 2. The zeta potential of insulin loaded nanoparticle was determined to be about -26 mV. The negative charge of nanoparticles can be related to the presence of COO^- on the surface of hydrogel nanoparticles.

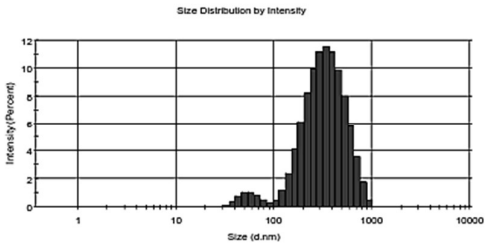

Fig 2: Size distribution of PCP nanoparticle by intensity

Fig. 3 a, b illustrates the SEM image of empty and insulin loaded PCP nanoparticle. As shown, nanoparticles prepared were uniform and spherical in shape with the average size of 86 nm. As it can be seen, there is no significant change in morphology of nanoparticles after insulin loading, however morphology of nanoparticles obviously changed after drug release presumably due to the nanoparticles swelling.

As it can be seen, the particle size obtained by the SEM technique is much smaller than that of DLS technique. It can be explained by the fact that DLS reports the hydrodynamic diameter of particles while SEM indicates the size of nanoparticles in the solid state. So it is not surprising that the size of particles determined by DLS technique to be bigger than that of SEM. Similar results have been reported in the literature [22]. Thereby, in the case of PCP nanoparticles considering high extent of swelling, it is not surprising to have such high difference.

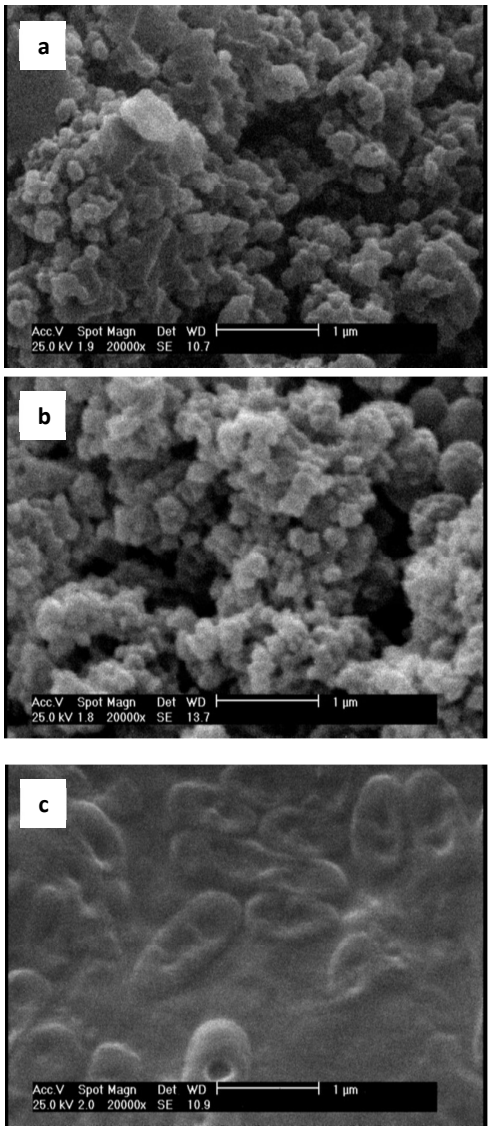

Fig.3. SEM image of (a) empty PCP nanoparticle, (b) insulin loaded in PCP nanoparticle, (c) PCP nanoparticles after drug release for 8h.

The swelling studies of PCP nanoparticles were studied as well. The swelling characteristics of PCP nanoparticles revealed that the degree of swelling of hydrogel nanoparticles was in the range of 35.6% and 21.7% after 480 min in the media with the pH value of 7.4 and 1.2, respectively (Fig 4). The High swelling degree of PCP nanoparticles in the neutral media compared to the acidic media can be explained by the ionization of carboxylic functional group of the polymer backbone in the neutral pH which subsequently could increase the swelling degree due to repulsion forces. It is clear that such characteristics make PCP nanoparticles as promising candidate for oral insulin delivery because of providing the possibility to protect insulin from harsh acidic gastric environment. As expected, it was also observed that the degree of swelling of nanoparticles increased as time increased.

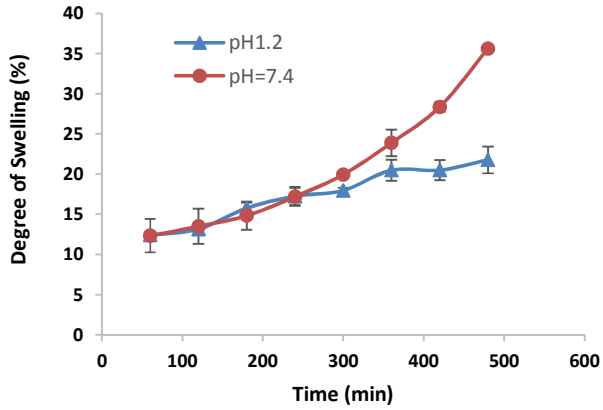

Fig. 4. Swelling experiments on PCP nanoparticles at pH=7.4, and 1.2 (n=3)

Fig. 5 shows the insulin release profile for PCP nanoparticles. It is clear that for both PBS buffer solution and gastric simulated media, the nanoparticles show pH responsive behavior where the extent of insulin release in the media with the pH value of 1.2 (gastric simulated media with and without pepsin) is considerably lower than that of the corresponding simulated intestinal media (about two fold). Such characteristics can be explained by the different swelling behavior of these nanoparticles at acidic medium compared to the neutral media. The finding also revealed that there is no considerable difference between the extent of drug released in the PBS buffer solution and gastric simulated media containing pepsin indicating the protecting ability of PCP nanoparticles from insulin degradation.

To evaluate the potential of PCP nanoparticles in protection of insulin from the harsh environment of the stomach, the biological activity of insulin and insulin payload PCP particles after treating with a simulated gastric solution containing pepsin were determined by HPLC and insulin ELISA kit. The biological activity is defined as the ratio of the extent of native insulin released in pH 7.0

buffer solutions for 2h after 1h treatment with the gastric simulated solution to the extent of native insulin released in the buffer solutions with the pH value of pH 7.0 for 3h. The findings showed that in case of free insulin almost all insulin was degraded immediately, while for insulin loaded PCP nanoparticles, the biological activity was determined to be 83.87±0.01.

The results exhibit that the maximum insulin attainable for 3h after 1h treatment with the gastric simulated solution was equivalent to 18.78 U/g $_{PCP}$ which could provide enough dose for diabete treatment.

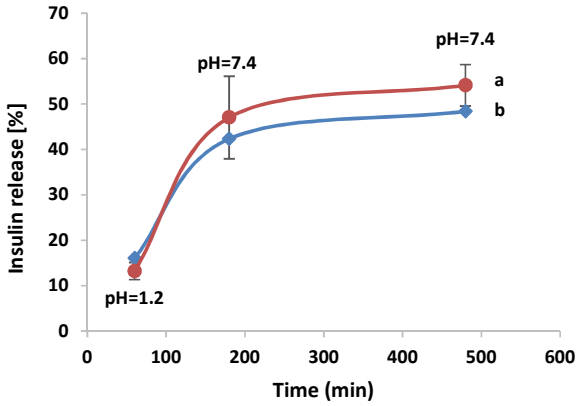

Fig. 5. In vitro release profiles of insulin loaded PCP nanoparticle in the simulated gastric media (PH=1.2 for 2h) and the simulated intestinal medium (PH=7.4 for 3h), a) PBS buffer solution, b) gastric simulated media containing pepsin

3.2 Experimental design and SVR model

The logic of this work was to connect the process variables with the characteristics PCP nanoparticles, and thereby allowing the identification of the aspects of the process factors that primarily dictate the PCP characteristics. The mathematical approach for modeling of PCP characteristics was based on SVR method. Determination of the optimum process parameters was done with the help of GA due to the conflicting nature of objective functions as well as multi-objective optimization process.

In this study, characteristics of PCP nanoparticles for insulin oral delivery were optimized according to the formulations prepared according to the Table 3. The size, polydispersity index, zeta potential and insulin encapsulation and the extent of drug release were determined for each formulation and the results were used for SVR modeling and GA optimization (table 3).

Table 3. Formulation and processing conditions (training, and test data set) as well as dependent variables

	Independent variables			Dependent variables					
Batch	MAA / Cs ratio level (X1)	Cs /TPP ratio level (X2)	Insulin amount level (X3)	Particle size (nm) (mean + SD)	Polydispersity index (mean + SD)	Zeta Potential (mV)(mean + SD)	Entrapment Efficiency (%)(mean + SD)	Release simulated gastric fluid (% at 120 min) mean+SD	Release simulated intestinal fluid (% at 180 min) (mean +SD)
1	-1	-1	0	655.0±69.9	0.50±0.019	-27.85±0.07	98.5±0.979	23.95±4.06	58.92±8.04
2	1	-1	0	1338.1±107.6	0.62±0.39	-30.67±0.39	98.8±0.205	21.11±2.77	42.13±0.078
3	-1	1	0	395.8±51.55	0.63±0.178	-26.00±0.01	94.7±0.631	30.76±5.24	64.22±1.22
4	1	1	0	401.0±129.4	0.61±0.015	-23.40±0.01	96.4±0.057	23.58±2.24	52.22±1.22
5	-1	0	-1	474.1±12.16	0.53±0.07	-24.47±3.3	99.8±0.005	33.16±2.38	42.73±3.40
6	1	0	-1	861.6±72.5	0.65±0.107	-25.87±0.76	99.3±0.092	53.54±6.10	73.38±6.37
7	-1	0	1	475.7±107.2	0.50±0.018	-22.13±0.64	97.9±0.212	18.29±6.87	26.35±4.14
8	1	0	1	657.3±129	0.49±0.078	-27.17±0.06	97.9±0.090	29.61±2.62	33.49±1.58
9	0	-1	-1	577.4±32.27	0.36±0.300	-26.73±1.3	99.8±0.01	35.50±5.40	41.03±1.40
10	0	1	-1	697.2±67.1	0.33±0.385	-26.73±1.1	97.5±0.23	31.93±1.01	71.47±0.32
11	0	-1	1	577.4±32.26	0.36±0.300	-26.73±1.2	97.8±0.23	7.21±0.240	19.40±2.61

12	0	1	1	697.2±202	0.33±0.330	-26.73±1.1	99.0±0.750	7.12±0.073	16.84±0.132
13a	0	0	0	622.1±181.5	0.65±0.210	-27.17±0.85	98.5±0.115	12.78±0.065	74.03±6.51
14a	0	0	0	455.1±96.3	0.76±0.326	-24.80±1.7	98.7±0.057	15.66±6.28	51.85±0.94
15a	0	0	0	243.7±25.1	0.36±0.082	-27.33±2.5	99.37±1.45	24.65±1.70	66.86±10

SVR was selected to correlate relationship between various response (Y: including particle size, zeta potential, encapsulation efficiency and the extent of drug release at simulated gastric medium, and intestinal fluid) and the process variables (X: MAA/CS, CS/TPP, amount of insulin). The model was trained using the preparation variables parameters as the input and corresponding nanoparticles characteristics as the output. Data, obtained from conducting experiments using the data of fractional factorial design was analyzed using SVR. Based on the results, the best model was achieved for α, ω and b according to table 4.

Table 4. The learning parameters for individual dependant variables

Particle size (nm)	α	1.32	-1.09	-0.18	-0.18	-1.09	1.32	1.32	-1.09	1.32	-1.09	-0.18	-0.18
		-1.12	-0.14	-1.12	1.30	-1.12	-0.14	1.30	-0.14	-0.14	1.30	1.30	-1.12
		-0.14	1.30	1.30	1.30	-0.14	1.30	-0.14	-1.12	-1.12	-0.14	-1.12	-1.12
	w	-2496	429	1068	-1215	-1045	1008	1654	310	484	1507	-1464	1914
	b	-1255.61015											
Polydispersity index	α	1.32	1.32	-0.18	-1.09	-0.18	-1.09	-1.09	1.32	-1.09	1.32	-0.18	-0.18
		-1.12	-0.14	1.30	-0.14	-1.12	-0.14	-1.12	1.30	1.30	-0.14	-1.12	1.30
		-0.14	-1.12	-1.12	-1.12	1.30	1.30	-0.14	-0.14	-0.14	1.30	-1.12	1.30
	w	-0.33	-0.44	1.50	-0.44	0.58	-0.30	-0.21	-0.50	-1.15	1.02	0.06	0.96
	b	-0.1794											
Zeta Potential (mV)	α	-0.18	1.32	-1.09	-1.09	-0.18	-0.18	-1.09	1.32	1.32	1.32	-0.18	-1.09
		-1.12	-0.14	1.30	-0.14	1.30	-1.12	-0.14	-1.12	1.30	-0.14	1.30	-1.12
		-1.12	1.30	-0.14	-1.12	1.30	1.30	1.30	-0.14	-0.14	-1.12	-1.12	-0.14
	w	-6.73	-0.87	1.62	-11.13	13.76	2.35	-21	14.93	-12.5	-4.87	12.60	18.15
	b	-31.7605											
Entrapment Efficiency (%)	α	-1.09	-1.09	-1.09	-0.18	-0.18	-0.18	-0.18	1.32	-1.09	1.32	1.32	1.32
		1.30	-1.12	-0.14	-1.12	-1.12	1.30	1.30	-0.14	-0.14	-1.12	1.30	-0.14
		-0.14	-0.14	1.30	1.30	-1.12	-1.12	1.30	1.30	-1.12	-0.14	-0.14	-1.12
	w	17.55	1.96	-1.27	1.72	1.51	-6.08	-13.17	0.7	-10.6	-0.93	6.61	-3.62
	b	103.0787											
Release simulated gastric fluid (% at 120 min)	α	-0.18	1.32	1.32	-1.09	1.32	-0.18	-0.18	1.32	-0.18	-1.09	-1.09	-1.09
		1.30	-0.14	-1.12	-0.14	1.30	-1.12	-1.12	-0.14	1.30	1.30	-1.12	-0.14
		1.30	-1.12	-0.14	-1.12	-0.14	1.30	-1.12	1.30	-1.12	-0.14	-0.14	1.30
	w	63.65	-110	98	28.26	49.7	56.8	-15.76	-84.6	27.6	-52.55	-8.05	-35.96
	b	10.4758											
Release simulated intestinal fluid (% at 180 min)	α	-1.09	-0.18	-0.18	-1.09	-0.18	1.32	-1.09	1.32	-0.18	1.32	-1.09	1.32
		-0.14	1.30	-1.12	-0.14	-1.12	-0.14	-1.12	-0.14	1.30	1.30	1.30	-1.12
		-1.12	-1.12	-1.12	1.30	1.30	1.30	-0.14	-1.12	1.30	-0.14	-0.14	-0.14
	w	122.82	-46.3	66.27	8.13	56.01	-66.3	-178	-115	68.89	28.3	-96.7	41.6
	b	141.976											

The performance of SVR model was evaluated by calculation of the corresponding MAE. As shown in table 5, the small error corresponding to the test set of all dependent variables indicates that SVR was successful to build a good model that addresses the nonlinearity between the input and the output data.

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Table 5. The SVR prediction error corresponding to various indepandant variables for test data.

Indepandant variable	Particle size (nm)	Polydispersity index	Zeta Potential (mV)	Entrapment Efficiency (%)	Release simulated gastric fluid (% at 120 min)	Release simulated intestinal fluid (% at 180 min)
MAE×10^7	5.1824	9.9987	5.4485	9.1519	9.1773	6.7958

The genetic algorithm was integrated with the SVR model to find the optimum condition where the target was to minimize size of nanoparticle, polydispersity index, and the extent of drug release at simulated gastric media, and simultaneously maximize the zeta potential, entrapment efficiency, and the amount of drug release at simulated intestinal fluid. After the modeling of process by SVR, the output was passed to GA in order to optimize the trained model giving rise to the best process parameters and their corresponding response.

Table 6 shows the weight coefficient for main responses. The positive and negative values indicate that the goal is to reach maximum, and minimum, respectively. Since, the most concern for oral insulin delivery is instability of insulin in gastric media, the highest weight coefficient was dedicated to the extent of drug release at acidic media.

Table 6. The weight coefficient for different cost function of GA

Independent variables	Particle size (nm)	Polydispersity index	Zeta Potential (mV)	Entrapment Efficiency (%)	Release simulated gastric fluid (% at 120 min)	Release simulated intestinal fluid (% at 180 min)
Weight coefficient (μi)	18	9	3	-1	150	-86

Our objective was to search the best process variables in their admissible limits to achieve the optimization process parameter for PCP nanoparticles preparation. This goal was equivalent to getting a minimum cost value in the searching process. The optimum settings obtained through GA were as follows: MAA / Cs ratio (%): 297.35, Cs/TPP ratio (%): 51.4, and initial insulin amount (mg): 50.3 and the corresponding cost value was -1.9×10^5.

3.3 Verification of the optimization condition

To confirm the model adequacy for predicting the optimum condition, the model was validated by carrying out some experiments under the optimum condition. The corresponding experimental value of the PCP nanoparticles under the optimum condition of the variables is shown in table 7, which was well consistent with the theoretically optimized value. This result confirms the validity of the optimal point.

Table 7. The observed and predicted dependant variables for test data.

Indepandant variable	Particle size (nm)	Polydispersity index	Zeta Potential (mV)	Entrapment Efficiency (%)	Release simulated gastric fluid (% at 120 min)	Release simulated intestinal fluid (% at 180 min)
Predicted response	261.22	0.35	-26.88	98.25	17.27	57.73

Observed response	253.67	0.32	-26.60	98.81	13.20	50.52
error	7.55	0.03	-0.28	-0.56	4.07	7.21

The findings demonstrate that SVR/GA as hybrid modeling approach was successful to determine an optimised preparation conditions for the preparation process of insulin loaded PCP nanoparticles for oral insulin delivery in order to achieve the best performance in terms of lower particle size, lower PDI, higher zeta potential, as well as lower release at pH=1.2 and higher release at pH=7.4.

3.4 Relative importance of input variable factors

The resulting model introduced not only the idea of simultaneous optimization of multi objective process by SVR, but also a valuable method to investigate the impact of various parameters. Apparently, insights into the important factors related to the characteristics of PCP nanoparticles provide the opportunity for further designing novel drug delivery systems with optimum activity. Hence, the extent of process parameters influence on nanoparticles properties, it was investigated as well to identify which aspects of the preparation process had the most impact on the PCP properties. The results introduce a new approach for understanding which factors control drug release and has important implications for the rational design of new polymeric carriers for oral insulin delivery.

Global sensitivity analysis (SA) was conducted on the obtained model to characterize the impact of the independent variables on dependant variables. SAs are formalised procedures to identify the impact of changes in model inputs and components on a model's output (table 8). Surprisingly it was found the MAA / Cs ratio (%) exerts a significant influence on the particle size, polydispersity index and zeta potential of PCP nanoparticles, while the extent of drug encapsulation was identified to be mostly affected by MAA/Cs ratio (%) and Cs/TPP ratio (%). These results can be explained by the impact of insulin amount on the swelling behavior of PCP nanoparticle through formation of hydrogen bond with carboxylic groups of MAA which consequently dictates the extent of nanoparticles swelling.

Table 8. Relative contribution of input data in output data

Independent variables / Dependant variables	MAA / Cs mole ratio (%)	Cs /TPP mole ratio (%)	insulin amount (mg)
Particle size (nm)	0.638	0.406	0.081
Polydispersity index	0.850	0.007	0.007
Zeta Potential (mV)	0.731	0.373	0.098
Entrapment Efficiency (%)	0.610	0.503	0.108
Release simulated gastric fluid (% at 120 min)	0.090	0.064	0.787
Release simulated intestinal fluid (% at 180 min	0.080	0.106	0.779

4. Conclusions

This study presents the successful implication of a hybrid modeling approach based on SVR and GA to correlate between preparation process parameters and characteristics of PCP nanoparticles for oral insulin delivery. The results showed that in order to achieve the best performance of nanoparticles in terms of lower particle size, lower PDI, higher zeta potential, as well as lower release at pH=1.2 and higher release at pH=7.4, the optimum process parameters are as follows: MAA / Cs ratio (%): 297.35, Cs/TPP ratio (%): 51.4, and initial insulin amount (mg): 50.3. Global sensitivity analysis also revealed that the MAA / Cs ratio (%) exerts a significant influence on the particle size, polydispersity index and zeta potential of PCP nanoparticles, while the extent of drug encapsulation was identified to be mostly affected by MAA / Cs ratio (%) and Cs /TPP. The most important finding was that the amount of initial insulin exhibits determinant effect on the extent of drug release at different media. The potential of SVR/GA method in modeling of PCP nanoparticles as a complex process with multi objective parameters can be considered as a promising approach for modeling of various drug delivery systems in order to rational design of various carriers to achieve the properties of interest.

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