Synthesis and characterisation of polymer containing dye-affinity ligand grafted to magnetic nanoparticles for enteric insulin delivery

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Published in Micro & Nano Letters; Received on 12th December 2017; Revised on 15th April 2018; Accepted on 1st June 2018

In the present work, a novel technique is reported for the surface grafting of polymer containing famous dye-affinity, Procion blue MX-R, onto modified iron oxide nanoparticles with 3-mercaptopropyltrimethoxysilane. The grafted nanocarrier was synthesised by free radical polymerisation followed by coupling with Procion blue and then characterised by Fourier transform infrared spectroscopy, thermogravimetric analysis, elemental analysis and also transmission electron microscopy. The grafted magnetic nanocarrier was used for enteric insulin delivery. The profile of the insulin uptake by synthesised nanocarrier indicated excellent accessibility of the polymer contains Procion blue. Scatchard analysis reflected that the capacity of the polymer containing dye-affinity ligand nanocarrier was about 12 μmol g^{-1} at pH 5. The balance sorption information of insulin by polymer contains Procion blue nanocarrier which were verified by Freundlich, Temkin, Langmuir and Redlich–Peterson models. The magnetic nanocarrier showed maximum of 95% drug release in acidic medium of pH 1.2, and about 80% drug release was recorded in intestine medium of pH 7.4.

1. Introduction: Magnetic nanoparticles (MNPs) are a significant class of nanoscale materials that has a great portion to revolutionise current clinical therapeutic methods. In order to their unique chemical and physical properties, recently MNPs considered as a carrier for targeted drug delivery [1–5]. MNPs can deliver the therapeutic agents to a disease site using a magnetic field. In other words, we can direct specifically the drugs to the target tissue by a magnetic field. Some scientist [6, 7] demonstrated the satisfactory toleration of colloidal iron oxide (Fe-O) particles by patients. In spite of non-toxicity, the capacity of magnetic nanocarrier for a therapeutic agent plays a significant role in choosing them. So, the modification of MNPs with ligands, which can interact with drugs, is a vital approach that scientist can take. Grafting techniques offer opportunities to have a good carrier with high capacity [8–10].

Insulin is an important hormone that effects on cell membrane for glucose absorption. In the absence or low concentration of insulin the body cannot take up the glucose from the blood and therefore begins to consume stored fat as an energy source [11]. In the best of our knowledge, disturbance in insulin metabolism occasions serious diabetic illness. Decreasing ability to consume or produce insulin in our body causes diabetes mellitus [12]. Regrettably, about 200 million people suffer from diabetes mellitus in the world, and the number of these people will go up increasingly [13]. So, fabrication of new type of nanocarrier can be interact with this hormone is vitally important [14]. In this research, a novel polymer grafted MNPs as a nanocarrier of insulin was introduced. MNPs were synthesised and grafted with polymer brushes containing of Procion blue, a famous dye in affinity chromatography. The purpose of the present Letter is to present the feasibility of using this grafted magnetic nanocarrier as a solid support for effective insulin delivery. The reason for selecting this functionalised polymer is its suitable retention and also longer controlled release for insulin delivery.

2. Materials and methods

2.1. Instruments: A Jasco Fourier transform infrared (FTIR) spectrometer, Jasco Inc., Easton, MD, USA FTIR-410 was used for FTIR spectra. Elemental (C, H and N) analysis was recorded on a Thermo-Finnigan analyser (Milan, Italy) model Flash EA. The transmission electron microscopy (TEM) micrographs were recorded on model of TEM-PHILIPS, CM 120, Netherlands. Thermogravimetric analysis (TGA) was obtained with a TGA-50H, Shimadzu (Kyoto, Japan).

2.2. Reagents and solutions: 3-mercaptopropyltrimethoxysilane was production of Steinheim, Germany. 2, 2′-Azobis (2-methylpropionitrile) was obtained from Acros Company (NJ, USA). Procion blue MX-R and allyl glycidyl ether were purchased from Fluka Chemica (Buchs Switzerland). Anhydrous 1, 4-dioxane, ethanol, methanol and all the other compounds were products of Merck (Darmstadt, Germany).

The stock solution of insulin in concentration of 500 mg l^{-1} was provided. An acetate or a phosphate buffer was used for pH adjustment.

2.3. Synthesis of polymer contains Procion blue-grafted MNPs: Procion blue-grafted MNP (PCPG-MN) was designed and synthesised in four steps: preparation of MNPs, modification of it with organosilane, polymer grafting and immobilisation of Procion blue. The synthesised methodology of PCPG-MN is summarised in Fig. 1.

2.3.1. Preparation of MNPs: Details of the synthesis and characterisation of MNPs were mentioned in another work [15]. Ferric and ferrochloride were co-precipitated using ammonia solution at 85°C in vigorous mechanical stirring and under nitrogen atmosphere. The resulting magnetic nanocompounds were separated magnetically, washed with distilled water and ethanol and dried in vacuum oven at 40°C.

2.3.2. Modification of MNPs with organosilane: The MNPs (3 g) from previous step were reacted with a boiling solution of 3-mercaptopropyltrimethoxysilane (5%) in 1, 4-dioxane (anhydrous) for 48 h. Then, the modified MNPs were washed with dioxane and dried in ambient condition.

doi: 10.1049/mnl.2017.0384

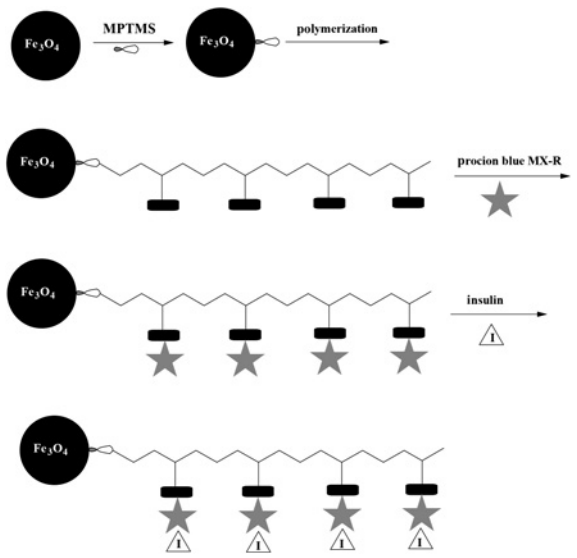

Fig. 1 *Synthesis process of PCPG-MN*

2.3.3. Polymer grafting: Allyl glycidyl ether was grafted onto modified MNPs by free radical polymerisation. The modified MNPs (3 g), 20 ml ethanol as solvent, 10 ml allyl glycidyl ether as functional monomer and 0.1 g 2, 2′-azobis (2-methylpropionitrile) as initiator were degassed and heated for 7 h at 65–70°C under nitrogen bubbling. The resulting allyl glycidyl ether grafted onto MNPs was separated magnetically from the reactor, washed with ethanol to remove adsorbed homopolymer and then dried under vacuum.

2.3.4. Immobilisation of Procion blue onto polymer grafted onto MNPs: The reason for designing this polymer containing Procion blue as an interacting ligand is increasing of insulin sorption capacity. The immobilisation process was adapted with other methods, which consist of opening the epoxy groups in the presence of a sodium chloride (NaCl) (aq)/N, N-dimethylformamide mixture [16–19]. Allyl glycidyl ether grafted onto MNPs (2 g), from the previous step, was added to a solution of NaCl 0.5 M and *N, N*-dimethylformamide (40:10 v/v) containing 0.5 g Procion blue MX-R. The mixture was shaken for 30 h at 40°C. The resulting PCPG-MN was separated magnetically, whished with distilled water and then dried under vacuum. PCPG-MN was characterised by FTIR, TGA, elemental analysis and TEM.

2.4. Batch method: A set of solutions (50 ml) containing insulin (20 μg ml^{-1}) was taken in an Erlenmeyer flask and their pH levels were adjusted to 5. The PCPG-MN (0.05 g) was enhanced to each solution and the mixture was shaken for 15 min. The sorbed insulin was estimated by ultraviolet–visible (UV/vis) spectrophotometry method at 257 nm.

2.5. Isotherm studies: Isotherm studies were performed by adding 0.05 g of PCPG-MN to a series of Erlenmeyer flasks filled with 50 ml diluted solutions of insulin (10–80 μg ml^{-1}) at pH 5. The sealed Erlenmeyer flasks were shaken at 20°C for 30 min. The amount of insulin on PCPG-MN (q_e, mg g^{-1}) was calculated using

$$q_e = (C_0 - C_e)V/W \quad (1)$$

where C_0 and C_e (mg l^{-1}) are concentrations of the insulin before and after sorption, respectively, $V(L)$ is the initial solution volume and W (g) is the bulk of the PCPG-MN.

2.6. *In vitro* drug release: The kinetic release of insulin from PCPG-MN were obtained at pH level of 1.2 (simulated gastric fluid) and pH level of 7.4 (simulated intestinal fluid). The insulin -loaded PCPG-MN was put into Erlenmeyer flasks with shaking (35 rpm) at 37°C. The samples were collected at specific times and the insulin content was measured by UV/vis spectrophotometric method at 257 nm.

3. Results and discussion

3.1. Characterisation: The peaks in FTIR spectrum at 561, 3389, 1619, 1296, 1023, 1560 and 1112 cm^{-1} were related to Fe-O, OH, C = O, CH, C-O, N-H and Si-O stretching bands, respectively, that confirmed the modification was accomplished. The elemental analysis information is presented in Table 1. The increasing of carbon and hydrogen percentages in each step approved the correct chemical modifications. On the other hand, the increasing nitrogen percentages in two last steps endorse that the polymer grafting and subsequently coupling with Procion blue was done successfully. The thermal behaviour of PCPG-MN was considered by TGA. Fig. 2 indicated a 2.1% weight loss is due to the removal of water content of MNPs, whereas PCPG-MN indicated an 18% weight loss, so 15.9% of weight loss is due to the decomposition of the organic section that was grafted on the MNPs. The morphology and particle size of PCPG-MN were verified using TEM. The spherical agglomerated PCPG-MN with particle diameter of <100 nm can be observed in Fig. 3.

3.2. Optimisation of parameters: The pH investigation exhibited in Fig. 4 indicate the best sorption was carried out at pH 5. An alkaline pH was not examined because the MNPs were dissolved in higher pH. The effect of temperature on insulin sorption at pH 5 was shown in Fig. 5. The insulin sorption was increased with temperature up to 40°C. The lower sorption of insulin at upper 40°C may be due to enhanced mobility of the hormone in the solution and subsequence less interaction with Procion blue on the PCPG-MN. The kinetic sorption of insulin at pH 5 is presented in Fig. 6. The sorption rate of insulin was quite rapid, such that the half time of saturation sorption is <1 min and about 15 min shaking was required for 100% saturation sorption. The fast rate of sorption of the insulin can be due to the accessibility of grafted polymer containing Procion blue on the PCPG-MN. To

Table 1 Elemental analysis information

	C, %	N, %	H, %
MNPs	0.69	0.22	0.35
3-mercaptopropyltrimethoxy silane-MNPs	1.28	0.23	0.52
polymer grafted modified MNPs	2.36	0.34	0.65
PCPG-MN	3.43	0.41	0.88

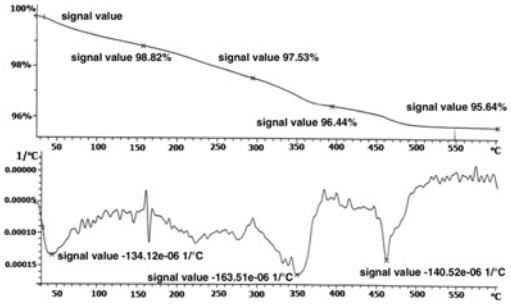

Fig. 2 *TGA images of PCPG-MN*

evaluate sorption capacity of PCPG-MN, Scatchard analysis was performed as follows:

$$Q/C = (Q_{max} - Q)/Kd \quad (2)$$

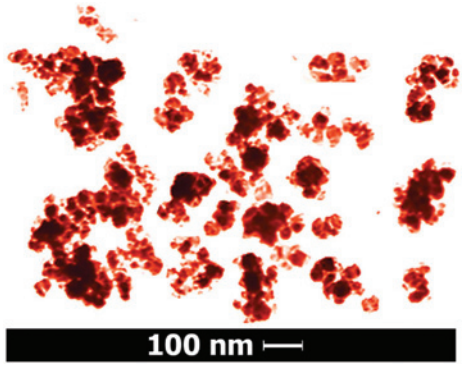

Fig. 3 *TEM images of PCPG-MN*

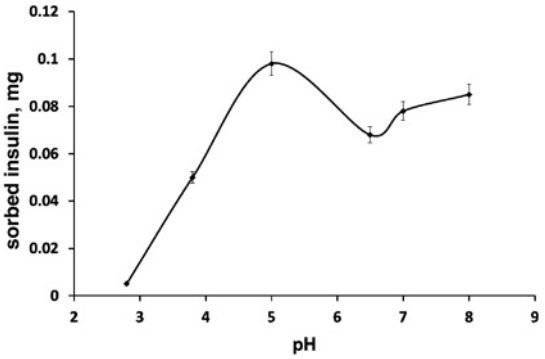

Fig. 4 *Effect of pH on sorption of insulin onto PCPG-MN*

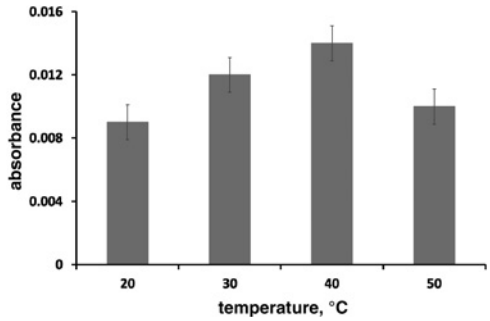

Fig. 5 *Effect of temperature on sorption of insulin onto PCPG-MN*

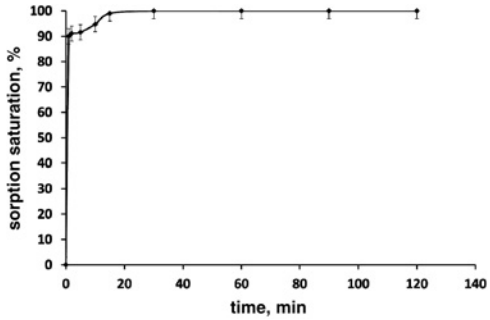

Fig. 6 *Kinetics of insulin sorption on PCPG-MN*

where K_d (μmol ml^{-1}) is the equilibrium dissociation constant, Q (μmol g^{-1}) is the equilibrium insulin sorption; C (μmol ml^{-1}) is the insulin concentration and Q_{max} (μmol g^{-1}) is the sorption capacity of PCPG-MN for adsorption of insulin. From Fig. 7, the K_d and Q_{max} were obtained as 0.0016 μmol ml^{-1} and 11.98 μmol g^{-1}, respectively.

3.3. Adsorption isotherms: Adsorption isotherms, at 20°C, were verified to optimise the insulin interaction (Fig. 8). The most important isotherm model is Langmuir which is valid for monolayer sorption on PCPG-MN surface with number of identical sites that can be interacted with insulin. In this model, we assume uniform energies of sorption on the PCPG-MN surface and no transmigration of insulin in the plane of the surface [20]. The Langmuir model with its calculated parameters is presented in Table 2. In this table, q_{max} and K_L are the maximum insulin sorption capacity corresponding to complete monolayer coverage on the PCPG-MN surface and the Langmuir constant, respectively. The essential characteristic of the

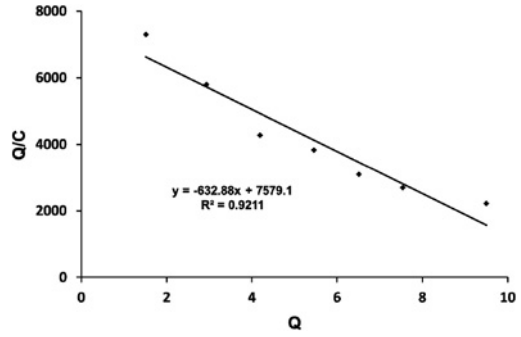

Fig. 7 *Scatchard analysis of insulin sorption onto PCPG-MN*

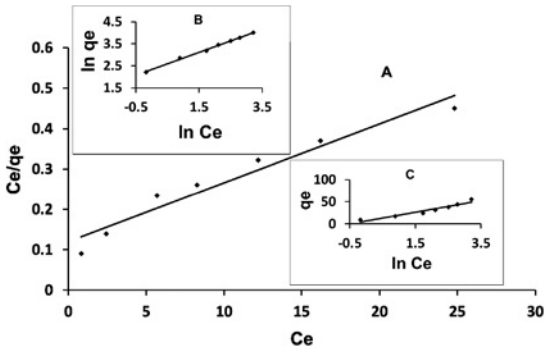

Fig. 8 *Langmuir (A), Freundlich (B) and Temkin (C) models for insulin sorption onto PCPG-MN at 20°C*

Table 2 Isotherm data at 20°C

Langmuir isotherm model			
q_{max}(mg g^{-1})	K_L (l mg^{-1})	R_L	R^2
68.49	0.12	0.09	0.9459
K_F (mg g^{-1}) (l mg^{-1})$^{1/n}$	n	R^2	
10.36	1.92	0.9964	
Temkin isotherm model			
A (l g^{-1})	B	b (J mol^{-1})	R^2
1.74	12.97	187.87	0.9321
Redlich–Peterson isotherm model			
g	B (l mg^{-1})^g	A (l g^{-1})	R^2
0.96	0.29	13	0.9822

doi: 10.1049/mnl.2017.0384

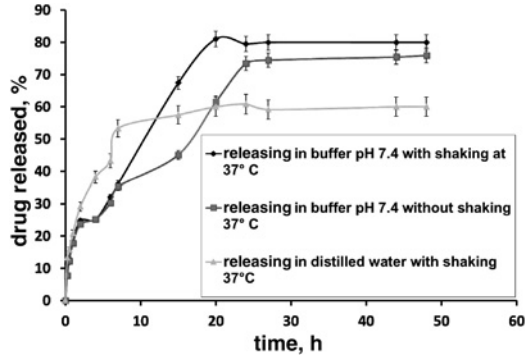

Fig. 9 *Insulin release profile in simulated intestinal fluid (pH 7.4)*

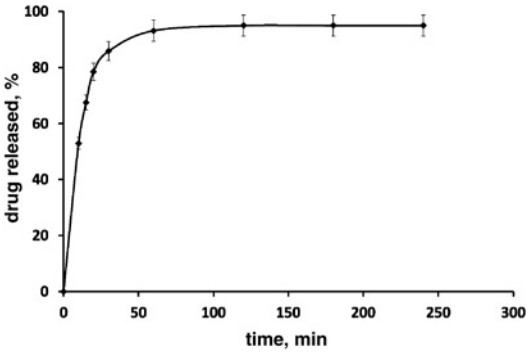

Fig. 10 *Insulin release profile in simulated gastric fluid (pH 1.2)*

Langmuir model (R_L) indicates the adsorption nature can be irreversible (with $R_L = 0$), favourable (with $0 < R_L < 1$), linear (with $R_L = 1$) or unfavourable (with $R_L > 1$) [21]. The R_L is expressed with

$$R_L = 1/(1 + K_L C_0) \tag{3}$$

The R_L was calculated as 0.09 that is in the range of 0–1 that confirms the favourable sorption of insulin.

Another isotherm model, Freundlich (see Table 2), is an empirical isotherm employed to explain heterogeneous systems [22] in which it is determined by the heterogeneity factor $1/n$ with the empirical equation. In Table 2, the K_F was representative of Freundlich constant.

Under discussion, model assumes adsorption on the heterogeneous surface and also a logarithmic decrease in the enthalpy of sorption with the increase in the fraction of Procion blue on the PCPG-MN. Freundlich model can predict that the insulin concentration on the PCPG-MN will increase when the insulin concentration goes up in the solution.

The next model is Temkin isotherm (Table 2) that offers a linear decrease of insulin sorption energy as the degree of completion of the sorptional centres of PCPG-MN is increased [23]. In Temkin model, $B = RT/b$, where R (J mol^{-1} K^{-1}) is the gas constant, T is the absolute temperature (K), b (J mol^{-1}) is the constant related to the heat of insulin sorption and the A (l g^{-1}) the isotherm constant.

The last model is Redlich–Peterson isotherm with three constants A (l g^{-1}), B (L mg^{-1})^g and g ($0 < g < 1$). This model incorporates the features of the Langmuir and the Freundlich isotherm models. The Redlich–Peterson parameters for the sorption of insulin onto PCPG-MN using the linear method are listed in Table 2. The isotherm constants presented in Table 2 was estimated from the linear plot with a trial and error procedure with application of Microsoft Excel [24]. The g value of 0.96 (approach to 1) means that the isotherms are close to the Langmuir form.

3.4. Insulin release: Figs. 9 and 10 show the emancipation of insulin from PCPG-MN in simulated intestinal fluid (pH 7.4), distilled water and simulated gastric fluid (pH 1.2). Approximately, 76–80% of the insulin were released at pH 7.4 (intestinal media) over a period of 48 h at 37°C. In other words, in pH 1.2 (gastric fluid) due to the high acetic media, insulin can be released in about 2 h at 37°C. More investigation indicates that just 60% of insulin was released in distilled water after 48 h at 37°C.

4. Conclusion: Novel polymer grafted MNPs beads on Procion blue were introduced by free radical polymerisation for the intestinal delivery of insulin. The FTIR spectra and elemental analysis confirmed the grafting of allyl glycidyl ether and then coupling with Procion blue. The drug sorption and release depends on the pH in the solution. The grafted nanosorbent has high chemical stability and sorption capacity. The rate of insulin sorption on the PCPG-MN was excellent. The sorption mechanism was also investigated by sorption isotherm models.

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