NOVEL SMART pH SENSITIVE CHITOSAN GRAFTED ALGINATE HYDROGEL MICROCAPSULES FOR ORAL PROTEIN DELIVERY: I. PREPARATION AND CHARACTERIZATION

MOHY EL DIN MS.1, OMER AM.2*, WASSEL MA.3, TAMER TM.2, ABD ELMONE M.4, IBRAHIM SA.4

1 Chemistry Department, Faculty of Science, University of Jeddah, Osfan, P. O. Box: 80203, Jeddah 21589, Saudi Arabia, 2Polymer Materials Research Department, Advanced Technology and New Materials Research Institute, City for Scientific Research and Technological Applications, Alexandria, Egypt, 3Department of Chemistry, Faculty of Science, AL-Azhar University, Cairo, Egypt, 4National Organizations for Drug Control and Research, Cairo, Egypt

Received: 30 Jun 2015 Revised and Accepted: 26 Aug 2015

ABSTRACT

Objectives: Preparation and characterization of a new pH sensitive chitosan (CS) grafted alginate (ALG) hydrogel microcapsules for the oral delivery of protein.

Methods: The pH sensitive hydrogel microcapsules were prepared for the first time using “grafting to” technique. Firstly, alginate was activated using p-Benzoquinone (PBQ) as a coupling agent to graft Chitosan chains later on. Both of activated and grafted alginate microcapsules were characterized by Fourier transform Infra-red spectroscopy (FT-IR), thermal gravimetric analysis (TGA) and the morphological structures were investigated using Scanning electron microscope (SEM) examination.

Results: It was found that the optimum conditions affecting the activation process and also the swelling degree of the prepared hydrogel microcapsules were 2% ALG, 0.04M PBQ pH10, 45 °C for 2h. In addition, the grafting process depends on the attached amount of PBQ and CS concentration. Maximum grafting efficiency (GE %) and chitosan add-on percentage were 98.6% and 14.8% respectively using 0.3% CSat 40 °C for 3h.

Conclusions: Novel pH sensitive hydrogel microcapsules were prepared via grafting of chitosan molecules on to activated alginate backbone. The formulated microcapsules can be applied as a new pH sensitive carrier for protein drugs.

Keywords: Alginate, Chitosan, Polyelectrolyte complex, Hydrogel, Grafting, Activation.

INTRODUCTION

Hydrogels are considered polymeric materials with three-dimensional cross-linked network structures [1-3]. These materials have the ability to absorb large amounts of water with maintaining their dimensional stability. A class of hydrogels which alter its shape, solubility, surface characteristics under the effect of different external conditions such as pH, temperature, ionic strength, solvent composition, light, or electric field, is considered to be a “smart hydrogels” [4]. All smart pH-sensitive hydrogels are polyelectrolytes contain acidic or basic groups that either accept or release protons in response to changes in environmental pH [5]. In addition, smart pH-sensitive hydrogels have great interest to polymer scientists in the field of pharmaceutical applications. Because of their excellent desirable biocompatible, biodegradable, hydrophilic and protective properties [6], these hydrogels have been most frequently used as delivery materials for drugs, especially to develop drug controlled release formulations for oral administration [7-10]. In recent years hydrogels made of polysaccharides, such as chitosan and alginate, have been suggested for many biomedical and pharmaceutical purposes [11].

Alginate (ALG) is a natural occurring biopolymer derived from brown algae, is a linear polysaccharide composed of α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues. Sodium alginate is a water soluble sodium salt which has the ability to form gel in the presence of multivalent cations like calcium ions in aqueous medium [12, 13]. Hydrogels made from alginate have excellent properties such as good biocompatibility, biodegradable, high hydrophilic, mucoadhesive and non-toxic which makes it widely used in different biomedical and pharmaceutical applications such as wound dressing [14] and microencapsulation researches as a carrier for controlled release of drugs [15]. It was reported that the physicochemical modification of alginate may enable control of properties generally not possible with the native polymer such as improved mechanical properties, pH sensitivity, and control of biodegradability, swelling and diffusion/release characteristics [16-18]. Additionally, alginate exhibits a pH-dependent anionic nature and has the ability to interact with other cationic polymers like chitosan to form polyelectrolyte complexes [19, 20]. Chitosan (CS) is a natural cationic biopolymer composed of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucopyranosyl and 2-amino-2-deoxy-D-glucopyranose [21]. Chitosan is considered the second most abundant polymer in nature after cellulose, obtained by alkaline deacetylation of chitin which previously isolated from crustacean exoskeletons such as shrimps [22, 23]. Much attention has been focused on chitosan hydrogels and their use in controlled release of drugs, wound dressing, tissue engineering scaffolds, and implants [24-27]. This is because of their outstanding characteristics and properties such as favorable biological properties, such as biodegradability non-toxicity, mucosadhesive properties and availability [28, 29]. To improve the drug delivery properties of chitosan, several physical and chemical modifications have also been studied to be suitable for the peroral drug delivery purpose [21, 30]. Upon mixing alginate and chitosan, the COOH residues of alginate ionically interacts with NH2 groups of chitosan to form polyelectrolyte complexes (PECs) as shown in fig. 1 [31]. These PEC shave been produced for drug delivery applications in micro or nano scale forms [32-34]. In addition, there are numerous factors affecting the previous properties of the alginate/chitosan microcapsules, among these factors are the chemical composition of the alginate used and the molecular weight and degree of deacetylation of chitosan [21]. Furthermore, alginate/chitosan PEC can be used in oral peptide delivery systems. It was reported that complexation of alginate with chitosan can decreases the leakage of the encapsulated drugs and reduces the porosity of the alginate microcapsules [35].

This study focused on preparation and characterization of a new smart pH sensitive polyelectrolyte complex hydrogel composed from alginate and chitosan using “grafting to” technique. This goal has been achieved through activation of OH of alginate using p-Benzoquinone (PBQ) as the coupling agent followed by click grafting of chitosan chains via NH2 groups. Factors affecting both the activation step of alginate and the grafting step of CS have been investigated. The swelling degree of the prepared beads have been
measured and the impact of variation pH on the swelling behavior of
grafted beads was explored. Finally, the physico-chemical characters of
the graft copolymer microcapsules were correlated to their composition.

Fig. 1: Schematic representation of alginate and chitosan
polyelectrolyte complex

MATERIALS AND METHODS

Materials
Sodium alginate (Low viscosity) obtained from Sigma-Aldrich Chemicals
Ltd. (Germany). Chitosan (M. wt. 10000-300000) was obtained from
Across Organics. (New Jersey, USA). P-Benzooquinone (PBQ) (Purity 99 %)
was obtained from Sigma-Aldrich Chemicals Ltd. (Germany). Calcium
chloride (anhydrous Fine GRG 90%) was purchased from Fisher
Scientific (Fairlawn, NJ, USA). Methyl alcohol (99%) was obtained from
El-Nasr Pharmaceutical Co. for Chemicals. (Egypt). Thiourea was
obtained from Sigma-Aldrich Chemicals Ltd. (Germany).

Methods

Preparation of PBQ-activated alginate

The activated beads were prepared according to the method
described previously by Mohy Eldin et al. [36]. Firstly; alginate was
dissolved in distilled water with continuous stirring and heating, p-
benzooquinone (PBQ) solution was added to the alginate solution
with the final concentration (0.01-0.05M) and 2% (w/v) for alginate.
The mixture was stirred for a definite time interval at temperature
ranged from 30°C to 45°C. The mixture was precipitated and washed
many times using methyl alcohol to remove the excess of PBQ. The
activated alginate was left to dry at room temperature.

Determination of PBQ concentration

The attached amount of PBQ was determined according to the published procedure by De Oliveira et al. [37]. Different
concentrations of PBQ were used to obtain the calibration curve. In
brief, 1 ml of PBQ-activated alginate solution was taken in a test tube
and the volume was adjusted to 2 ml with 0.2M acetic acid/acetate
sodium pH3.200 μl of 0.1M thiourea was added to each tube,
shaken and incubated at 37 °C for 10 min and cooled to room
temperature. After 20 min at room temperature, the absorbance was
read against the blank at 410 nm.

Fig. 2: Freshly prepared chitosan grafted alginate (CS-g-ALG)
PEC beads

Schematic diagram describes the proposed mechanistic pathway for
synthesis of chitosan grafted alginate (CS-g-ALG) hydrogel PEC is
presented; fig. 3.

Two parameters have been monitored for evaluation the grafting
process namely: grafting efficiency (GE %) and polymer add-on (%) and can calculated as follow [38-40]

\[
GE \% = \left( \frac{W_2 - W_1}{W_1} \right) \times 100
\]

\[
% Add-on = \left( \frac{W_3 - W_2}{W_2} \right) \times 100
\]

Where; \( W_1, W_2 \) and \( W_3 \) are the weights of alginate, grafted
copolymer (CS-g-ALG), and chitosan respectively.

Determination of the swelling degree

The swelling studies were conducted using wet beads. Pre weighed
grafted hydrogel beads (1g) were immersed in the swelling medium
solution at 37°C using HCL-KCL, Citrate-Phosphate and saline
phosphate buffer solutions with different pHs in the range (1.0-10)
and allowed to swell for 6h. Finally; the swollen beads were taken
out and pressed gently in between two filter papers to remove liquid
adhering to the surface, immediately followed by weighing on an
electronic balance. All swelling experiments were performed in
triplelicate and the mean values are reported.

The percentage of swelling degree can be determined as a function
of time as following:

\[
Swelling \text{ degree} \% = \left( \frac{M_t - M_0}{M_0} \right) \times 100
\]

Where; \( M_t, M_0 \) weight of the swollen hydrogel sample after 6h of
swelling, and \( M_0 \) is the initial weight of the sample.

Physicochemical characterization

FT-IR spectroscopic analysis

The chemical structure of alginate, chitosan, PBQ-activated alginate and
chitosan grafted alginate PEC was investigated via FT-IR spectroscopy
(Shimadzu FTIR-8400 S, Japan) using the absorbance mode.

Fig. 3: Schematic representation for synthesis of chitosan
grafted alginate hydrogel

Swelling degree (%) = \( \left( \frac{M_t - M_0}{M_0} \right) \times 100 \)
Thermal gravimetric analysis

The thermal degradation behaviors of alginate, chitosan, PBQ-activated alginate and chitosan grafted alginate PEC were studied using thermal gravimetric analyzer (Shimadzu (TGA)-50, Japan).

Morphological characterization

The changes in the surface morphology of alginate, chitosan, PBQ-activated alginate and chitosan grafted alginate PEC were observed using scanning electron microscopy (SEM; Joel Jsm 6360LA, Japan).

RESULTS AND DISCUSSION

Activation step

The reason for the activation process is to create active sites on the alginate backbone for further covalent binding of chitosan chains via "grafting to" technique. This goal has been achieved through reaction of PBQ molecules with OH- groups of alginate backbone. The effects of variation conditions of activation process on the attached amount of PBQ molecules were explored. In addition, these conditions affect the amount of covalently grafted chitosan chains and consequently affecting the swelling degree latter was discussed in the following.

Effect of PBQ concentration

The attached amount of PBQ and the swelling degree of the grafted PEC hydrogel beads were affected by variation of PBQ concentration as shown in Fig 4. It was clear that increasing of PBQ concentration has the positive effect on the attached amount of PBQ and the swelling degree until definite value (0.04M). Where, the attached amount of PBQ increased up to 10 mg/g [36]. Increasing the swelling degree of the grafted beads with increasing PBQ concentration can be explained in the light of increasing the number of activated hydroxyl groups and so the amount of covalently grafted chitosan. In fact, the COO-groups of alginate and NH₂ groups of chitosan are hydrophilic ones which contribute the swelling process of hydrogel. Almost all of OH-groups of alginate have been activated using 0.04M concentration and the left numbers of un-activated OH-groups are very few. This could be the explanation of the un-affected swelling degree with increasing the PBQ up to 0.05M.

Effect of activation temperature

The effect of variation activation temperature on the attached amount of PBQ and the swelling degree of the grafted PEC hydrogel beads was shown in Fig 5. It was observed that with increasing the activation temperature from 30 °C to 45 °C the attached amount of PBQ and the swelling degree increased gradually. This behavior could be explained by an increase of the activation rate of the reaction due to decreasing the reaction medium viscosity, so the number of activated OH groups increased and in turn the amount of covalently grafted NH₂ of chitosan increased consequently. Furthermore, with using fixed amount of chitosan (0.2%), the probability of formation multi-attached covalent bonds with chitosan molecules leads to improvement of the three network structure of the formulated polyelectrolyte and enhance the swelling degree.

Effect of activation time

The effect of activation time was studied in the range 15 to 240 min as shown in Fig 6. It was observed that the attached amount of PBQ and the swelling degree of the grafted PEC beads were increased with increasing activation time up to 120 min. However, there is no effect on the attached amount of PBQ with increasing activation time beyond 120 min. These results can be attributed to that almost all of the available OH groups in of alginate backbone were activated after 120 min, and so the amount of covalently grafted NH₂ groups of chitosan chains also increases, thus, the swelling degree increased consequently.

Effect of activation pH

Fig. 7 show the effect of variation pH value of PBQ solution on the attached amount of PBQ and the swelling degree of the grafted PEC hydrogel beads. Activation conditions: (2%ALG, 0.01M PBQ, 1h,pH10) at constant grafting conditions (0.2% CS for 3h at 40 °C)
and PBQ molecules increased, and consequently the amount of covalently grafted NH2 of chitosan.

Effect of activation pH

Fig. 7: Effect of variation activation pH on the attached amount of PBQ and the swelling degree of the grafted PEC hydrogel beads. Activation conditions: (2%ALG, 0.01M PBQ, 30 °C, 1h) at constant grafting conditions (0.2% CS for 3h at 40 °C)

Effect of alginate concentration

The effect of alginate concentration on the attached amount of PBQ and the swelling degree of the grafted PEC hydrogel beads was studied as shown in fig. 8. A positive effect on the attached amount of PBQ and the swelling degree was observed with increasing alginate concentration up to 2%. This behavior may simply be attributed to that with increasing alginate concentration from 1 to 2%, the number of activated OH groups increased. In addition, the number of COO- groups increased which responsible for the hydrophilic nature of sodium alginate and impart the swellable behavior of the network structure. However, this increment was not observed with increasing alginate concentration beyond 2%, since, the used amount of PBQ is the limiting factor in this study.

Effect of chitosan concentration

Fig 9 shows that increasing CS concentration clearly increased the percentage of grafting efficiency and % add-on. Maximum grafting efficiency (96.6%) and % add-on (14.5%) were obtained at 0.3% CS, and then the grafting efficiency tends to decrease with further increase of CS concentration beyond 0.3%. Also % add-on values were observed to be increased with small values (nearly stable). These observations may be attributed to that beyond 0.3% CS the viscosity of the mixture increased, so, the movement of chitosan molecules decrease and the rate of chitosan diffusion into the formed polyelectrolyte complex also decreased, and then the grafting efficiency decreased consequently. On the other hand, % add-on values were nearly stable with increasing of CS above 0.3%; this may be attributed to the left numbers of activated OH groups of alginate were limited. Further increase of CS was not effective. Higher concentrations of chitosan beyond 0.5% leads to more increasing in viscosity of the mixture, thereby hindering the formation of beads.

Effect of grafting temperature

A small increase in the grafting efficiency and % add-on was noticed as shown in fig. 10 with increasing grafting temperature up to 40 °C, and then tends to slightly decrease with further increase of temperature. The enhancement in grafting of CS onto activated alginate chains with increasing the grafting temperature from 30 to 40 °C might be attributed to decreasing the viscosity of the activated alginate solution,
which facilitate reaching of CS chains to the active sites of ALG-PBQ phase. On the other hand, the grafting efficiency and % add-on decreases as temperature increased beyond 40 °C. The rate of grafting chitosan chains onto PBQ activated alginate is logically big at elevated temperature. This leads to increase the viscosity of the formed grafted polyelectrolyte complex in the early stage of reaction time and may be lead to formation of higher density of covalent bonds between PBQ activated alginate and chitosan chains which consuming the active site to bind less number of chitosan chains.

Effect of grafting time

The effect of variation time of grafting on the studied grafting parameters was investigated in fig. 11. It was clear from results that the grafting efficiency and % add-on largely increased with increasing the grafting time from 0.5h to 1h. Further increase of the grafting time up to 3h, slightly increased the grafting parameters and finally stays approximately constant at 4h. The large increase of the grafting efficiency and % add-on below 1h is due to an increase in the diffusion rate of chitosan chains into the activated alginate phase, and the constancy of grafting parameters at about 3h is a clear remark on the almost complete consumption of chitosan in the grafting to activated alginate chains.

Fig. 11: Effect of variation grafting time on the grafting efficiency (GE %) and % polymer add-on. Activation conditions: (2%ALG, 0.04M PBQ, 45 °C, 2h, pH10) at constant grafting conditions (0.3%CS at 40 °C)

Effect of chitosan molecular weight

In the present study the effect of chitosan molecular weight on the grafting efficiency and % add-on was studied with different molecular weights (low, medium and high) as shown in fig. 12. From results it was clear that the grafting efficiency and % add-on largely increased with increasing chitosan molecular weight from low to medium, then it's slightly decreased with small value (nearly stable) at high molecular weight, where, maximum grafting efficiency and % add-on were obtained using medium molecular weight (96.6%, 14.8% respectively). These observations may be attributed to that increasing molecular weight increase the length of chitosan chains, which enhance the grafting probability of chitosan on the PBQ activated alginate backbone, explain the increasing of grafting efficiency and % add-on. On the other hand, using low molecular weight of chitosan may produce short chains of chitosan, which reflects consequently on the drop of both grafting efficiency and % add-on. The slightly decreasing value of the grafting parameters at high molecular weight may be attributed to that the viscosity of PEC mixture increased and the rate of chitosan diffusion into the PBQ activated alginate matrix slightly decreased and so on the grafting parameters.

Fig. 12: Effect of variation molecular weight of chitosan on the grafting efficiency (GE %) and % polymer add-on. Activation conditions: (2%ALG, 0.04M PBQ, 45 °C, 2h, pH10) at constant grafting conditions (0.3%CS, 3h, 40 °C)

pH Sensitivity of the grafted beads

In the present work, the effect of the swelling medium pH on the sensitivity and swelling behavior of the grafted PEC hydrogel beads was studied in the range (1–10) as shown in fig. 13. As known, both chitosan and sodium alginate are polyelectrolytes, which can undergo protonation/deprotonation processes. In deed, the sensitivity of the studied grafted PEC beads for pH involves deprotonation of COO of the activated alginate at high pHs and protonation of NH2 groups of chitosan under low pHs. Also, it has been reported that alginate has a pKa around 3.5 depending on the content of M blocks and G blocks and chitosan has a pKa of 6.1–6.5 depending on the degree of deacetylation and its molecular weight [41]. It can be noted that the swelling degree of grafted PEC beads increased gradually with increasing pH up to 6.8, and then tends to decrease further increase of pH. As the pH increases from 3 to 7.4, the concentration of the negatively charged COO groups in the grafted beads increases, and can undergo ionization process producing chain repulsion, thus the swelling degree increased consequently. As well known the interaction between chitosan and alginate is known to be pH-dependent and strong complexes are obtained at pH around 4–6. When pH increases from 6 to 7.4, the ionization process of NH2 groups decreases greatly, where, most NH2 groups of chitosan are deprotonated. So, the electrostatic interaction between alginate and chitosan become weak, and the electrostatic repulsion between the ionized COO groups in alginate causes the further swelling of the grafted PEC beads. As a result the grafted PEC beads at pH 6.8 have the highest swelling degree. However, when pH increases above 7.4 all beads were not stable and that may be attributed to the disruption of calcium-alginate gel beads which occurred in the phosphate buffer solution, hence, the affinity of phosphate for calcium is higher than that of alginate, thus largely shrinking occurred for beads.

Fig. 13: Effect of variation pH on the percentage of swelling degree of CS-g-ALG. Activation conditions: (2%ALG, 0.04M PBQ, 45 °C, 2h, pH10) at constant grafting conditions (0.3%CS, 40 °C, 3h), formulation conditions (3%CaCl2 for 1h at RT) and swelling conditions (6h, at 37 °C)
Materials characterization

Fourier transform infra red (FT-IR) analysis

The FT-IR spectra of ALG, ALG-PBQ, CS and CS-g-ALG beads are shown in Fig.14(a-d) respectively. It was evident that it shows a broad absorption band at 3446.5 and 3443 cm⁻¹ [28], due to the stretching frequency of the OH group in case of ALG and ALG-PBQ, also characteristic functional groups (COO⁻ stretching) were present, with a broad asymmetrical band at 1630 and 1627 cm⁻¹ and a narrower symmetrical band at 1410 and 1435 cm⁻¹. An even broader absorption was observed near 1026 and 1081 cm⁻¹, which can be attributed to COH stretching. The IR spectra of CS confirm the presence of N-H stretching vibration at 3425 cm⁻¹. Stretching vibration band for CH aliphatic at 2920-2950 cm⁻¹, which can be attributed to COH stretching. The IR spectra of CS-g-ALG Polyelectrolyte complex confirm the presence of OH and N-H stretching vibration at 3440 cm⁻¹, in which the OH stretching vibration are overlapped by N-H stretching which is due to the presence of chitosan. The appearance of peak at 1060, 1040 cm⁻¹, possibly as a result of ionic interaction between the COO⁻ and NH⁺ groups of the grafted PEC beads.

Thermal gravimetric analysis (TGA)

Thermal analysis for ALG, ALG-PBQ, CS and CS-g-ALG beads was evaluated out using thermo gravimetric analyzer in nitrogen atmosphere at the heating rate 10 °C/min. The data was summarized in table 1. It was clear that the formation of grafted PEC beads improved the thermal stability relative to the parent polymers; alginate and chitosan. On the other hand, ALG-PBQ show different behavior probably due to the gained thermal stability of the beads as compared with alginate case. The weight loss at 230 °C has been reduced as compared with in the case of the ALG. This behavior could be attributed to the attaching aromatic of PBQ molecules onto the alginate backbone which enhances the thermal stability of ALG.

The noticeable reduction of weight loss observed at temperature ranged from room temperature to 150 °C confirmed these explanations. Finally, it can be concluded that the beads demonstrate fair stability in the vicinity of the body temperature (i.e. 37 °C). It can also be anticipated that chitosan grafted alginate PEC, should also be thermally stable at the physiological temperature of the human body.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T_{25}°C</th>
<th>T_{300}°C</th>
<th>Weight loss (%) at Ambient-150°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALG</td>
<td>230</td>
<td>315</td>
<td>25.67</td>
</tr>
<tr>
<td>ALG-PBQ</td>
<td>265</td>
<td>343</td>
<td>10.9</td>
</tr>
<tr>
<td>Chitosan</td>
<td>290.53</td>
<td>341.8</td>
<td>11.545</td>
</tr>
<tr>
<td>CS-g-ALG</td>
<td>211.44</td>
<td>439</td>
<td>3.981</td>
</tr>
</tbody>
</table>

CONCLUSION

In this study, Chitosan grafted alginate (CS-g-ALG) PEC hydrogel microcapsules have been prepared for the first time. It was found that the optimum conditions affecting the activation step and also the swelling degree were obtained using 2% ALG and 0.04M PBQ at pH10 and 45 °C for 2h. In addition to the activation of alginate, covalent binding of chitosan chains, via grafting process was done. The optimum conditions for the grafting process were 40 °C for 3h using 0.3%CS, in which the best obtained values for the grafting efficiency, were 96.6% and % Add-on 14.5%. FT-IR, TGA, and SEM analysis of the modified beads show changes in the physicochemical characters which prove the occurrence of the activation and grafting processes. In conclusion, the prepared grafted PEC hydrogel microcapsules have different hydrophilic groups which can be applied as a new carrier for different biomedical and pharmaceutical applications such as oral protein delivery system.

CONFLICT OF INTERESTS

Declared None

REFERENCES