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Study the Different Preparation and Formation Conditions of Bovine Sarum Albumin (BSA) as a Model of Protein Drug.

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ABSTRACT

In case of the bovine sarum albumin (BSA) loaded grafted and mixed hydrogels, the drug loading content and loading efficiency have been obtained and the best obtained values for (BSA) loading parameters were:- (1) The percentage of loading content for grafted and mixed polyelectrolyte complex hydrogels were (17%, 16.6% respectively). (2) The percentage of loading efficiency of BSA for grafted and mixed polyelectrolyte complex hydrogels were (84.7%, 82.7% respectively). Also the study of the BSA release behavior from different grafted copolymers and mixed hydrogels have been studied. Factors affecting the BSA release behavior such as; CS (chitosan) concentration, (BSA) concentration, $CaCl_2$ concentration, cross-linking temperature and cross-linking time have been monitored, and the best obtained values for (BSA) release were (80%, 75%) for grafted and mixed hydrogels respectively. Also the (BSA) release behavior in gastrointestinal tract conditions has been studied. The bio-evaluation of the resultant loaded hydrogels with (BSA) from the point of biodegradability and antibacterial activity have been studied.

Key words: Chitosan, albumin, gastrointestinal, alginate, concentrations.

Introduction

Chitosan-based (PEC) networks have been produced by water-soluble anionic macromolecules like (DNA,) anionic polysaccharides (e.g. alginate, GAGs (chondroitin sulfate, hyaluronic acid, or heparin), carboxymethyl cellulose, pectin, dextran sulfate, xanthan, etc.), proteins (e.g. gelatin, albumin, fibroin, keratin, and collagen), and anionic synthetic polymers (e.g. polyacrylic acid). The stability of these compounds is dependent on charge density, solvent, ionic strength, pH, and temperature (Lee, *et al*, 2009). The choice of the anionic molecule for (PEC) formation is highly dependent upon its charge under physiological conditions because pH of the hydrogel environment modulates ionic interactions and, subsequently, (PEC) hydrogel properties. If the electrostatic interactions of the polymer are strong enough, the physical associations between the polymers at physiological pH can be maintained (Lee, *et al*, 2009).

There has been increasing interest in the study of alginate-chitosan microcapsules as carriers for controlled release of proteins and drugs. Chitosan-alginate complexes, being a pH sensitive hydrogel, have been studied for the development of oral delivery of peptide (Hari, *et al*, 2008).

Alginate-chitosan polyelectrolyte complexes are formed by ionic interactions as with ionically crosslinked networks. Alginate has the property of shrinking in low pH and getting dissolved in higher pH, whereas chitosan dissolves in low pH and is insoluble in higher pH ranges. In view of these limitations encountered in pure alginate and chitosan bead systems, the concept of alginate-chitosan polyelectrolyte complexes gained acceptance (Huguet, and Dellacherie, 2010). Upon mixing, the carboxyl residues of alginate and the amino groups of chitosan ionically interacts to form the polyelectrolyte complex (Huguet, and Dellacherie, 2010).

The polyelectrolyte complex between chitosan and alginate has been widely used in order to obtain microcapsules for cell encapsulation and devices for the controlled release of drugs or other substances (Meera, and Emitia, 2010). Complexation of chitosan with alinate reduces the porosity of alginate beads and decreases the leakage of the encapsulated drugs. Chitosan complex with alginate was studied as a coating on alginate beads., alginate-chitosan coacervates (Meera, and Emitia, 2010).

Experimental:

Bovine Serum Albumin (BSA) Loading step:

The (BSA), as a model protein drug, was dissolved with amount ranged from 10%-25% (w/w. to the total weight of grafted and mixed polyelectrolyte complexes) (Yongmei, *et al*, 2009) after the formation of homogeneous solution. The BSA loaded beads were rinsed with distilled water to remove the excess drug on surface. Finally, the rinsed beads were dried under vacuum at 30°C. (Yongmei, *et al*, 2009).

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(1) Determination of BSA Loading Content and Loading Efficiency:

After preparing the BSA loaded beads, the gelling medium solution was collected, and the drug content in the gelling medium (calcium chloride solution) was determined as the drug loss. The amount of (BSA) entrapped into the beads was determined by UV spectroscopy at 280 nm.

The drug loading content and loading efficiency are calculated as follows:

$$\text{Drug loading content (\%)} = \frac{\text{Mass of drug encapsulated in beads}}{\text{Mass of polymers in beads}} \times 100\%$$

$$\text{Drug loading content (\%)} = \frac{M_i - M_d}{M_i} \times 100\% .$$

Where **(M_i)** is the initial mass of drug dissolved in the homogeneous grafted and mixed solution and **(M_d)** is the mass of drug loss in the gelling medium right after the preparation of the drug-loaded beads (George and Nikolaos, 2011) and (Cui-Yun, *et al*, 2009).

(2) Determination of BSA Loading Content and Loading Efficiency:

After preparing the (BSA) loaded beads, the gelling medium solution was collected, and the drug content in the gelling medium (calcium chloride solution) was determined as the drug loss. The amount of BSA entrapped into the beads was determined by UV spectroscopy at 280 nm (George and Nikolaos, 2011) and (Cui-Yun, *et al*, 2009).

Results and Discussion

Based on the above swelling studies the release behavior of BSA (as a model of protein drug) from the grafted and mixed beads at different preparation and formulation conditions was studied, in which several factors affecting the release process have been studied in the following.

(1) Effect of Chitosan (CS) Concentration on the Percentage of Loading Content, Loading Efficiency and Release Profiles of BSA:

The effect of variation chitosan (CS) concentration on the percentage of loading content and loading efficiency of (BSA) in case of grafted and mixed beads was investigated. Table (1) show that increasing the (CS) concentration from (0.1-0.5%) clearly increased the percentage of loading content and loading efficiency of (BSA). In all cases the value of loading efficiency exceeded 80% regardless of the initial amount of (BSA) used at the loading process with increasing (CS) concentration from (0.3-0.5%) and reached the maximum values at 0.5% (CS) (88%, 86.1%) for grafted and mixed beads respectively.

On the other hand the release behavior of (BSA) was influenced by the amount of (CS) as shown in figures (1a,b), where with increasing of (CS) concentration up to 0.3% the release percentage slightly increased and reached the maximum values after 5h (80%, 75% for grafted and mixed beads respectively), and then tends to decreases with further increase of (CS) concentration. These results can be attributed to that with increasing (CS) concentration beyond 0.3% the swelling degree was decreased obviously as shown in swelling studies, where the poor solubility of chitosan at pH = 7.4 and the low binding with alginate under these conditions leads to decreasing the swelling degree of beads and thus the release amount of (BSA) decreased consequently.

Table 1: The effect of variation (CS) concentration on the percentage of loading content, loading efficiency of (BSA) for grafted and mixed beads. Activation conditions: (2%ALG, 0.04M PBQ, 45°C, 2h, pH10) at constant grafting and mixing conditions (CS, 40°C, 3h), formulation conditions (3%$CaCL_2$ for 1h at RT), using (BSA) 20% (w/w. to the total weight of polyelectrolyte complex) and wet beads 1g.

CS%	Drug Loading Content (%)		Drug Loading Efficiency (%)	
	Grafted Beads	Mixed Beads	Grafted Beads	Mixed Beads
0.1	11.5	11	57	54
0.2	13.4	12	64.9	58
0.3	17.0	16.6	84.7	82.7
0.4	17.1	17	83.33	82.8
0.5	18.7	18.3	88.0	86.1

Chitosan (CS) molecule has both amino and hydroxyl groups that can couple with proteins under mild conditions. Hydrophobic interactions between chitosan and (BSA) inside the network were favored. Strong charge to charge interactions haven been demonstrated between protein and chitosan with a less cumulative release amount of (BSA).

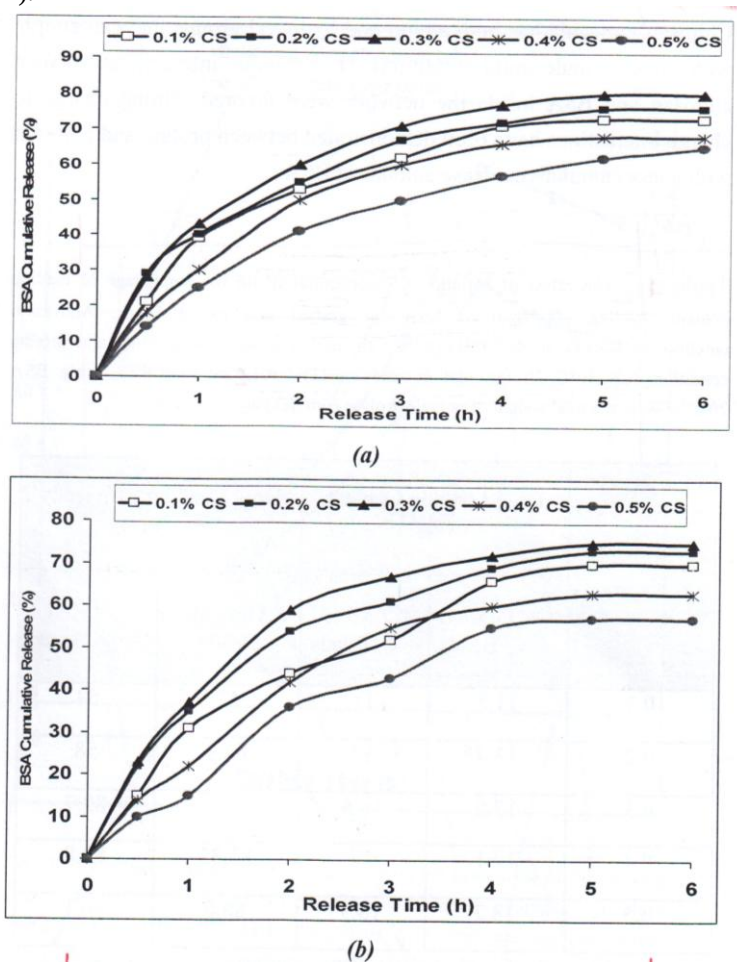


Fig. 1: Effect of variation the (CS) concentration on the release profiles of (BSA) of (a) CS-g-ALG copolymers and (b) ALG-CS mixed hydrogels. Activation conditions: (2%ALG, 0.4M PBQ, 45°C, 2h, pH10) at constant grafting and mixing conditions (CS, 40°C, 3h), formulation conditions (3%$CaCL_2$ for 1h at RT) and release conditions (at 37°C, SPB pH 7.4) using BSA 20% (w/w, to the total weight of polyelectrolyte complex) and wet beads 1 gm.

(2) Effect of Calcium Chloride ($CaCl_2$) Concentration on the Percentage of Loading Content, Loading Efficiency and Release Profiles of (BSA):

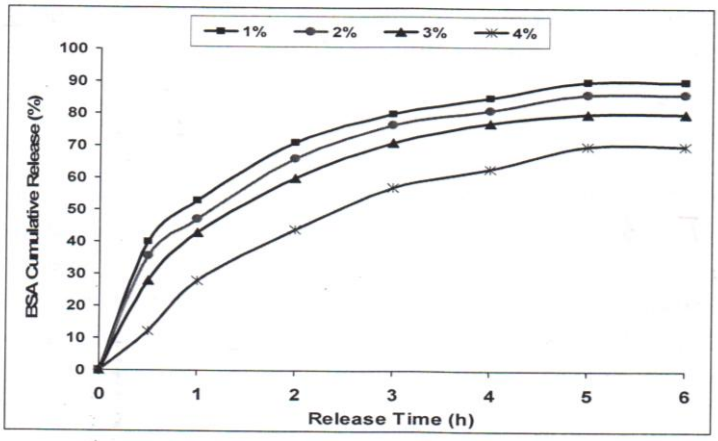
In the present study the effect of variation $CaCl_2$ concentration on the percentage of loading content and loading efficiency of (BSA) in case of grafted and mixed beads was studied with different concentrations (1-4%) as shown in Table(2). The results shows that increase in $CaCl_2$ concentration for the concentration of (1-4%) increases the loading content and loading efficiency, in which the maximum loading efficiency of (BSA) for grafted and mixed beads (89.1,84.6%) were obtained at 4% $CaCl_2$.

These results can be attributed to that with increasing $CaCl_2$ concentration the wall beads thickness increases, and the pores size of beads also decreased and consequently this leads to hindering the passage of soluble (BSA) molecules from the pores of beads and this cause a lack of release rate of (BSA). Thus the amount of loading and loading efficiency increased.

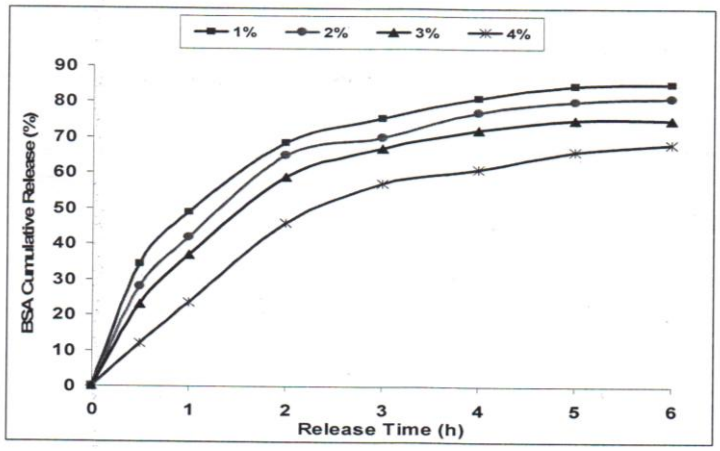
On the contrary, the release percentage of (BSA) was decreased with increasing $CaCl_2$ concentration as shown in Figures (2a,b), in which maximum release percentage form grafted and mixed beads (90.1, 85% respectively) were obtained at 1% $CaCl_2$ and this due to that at low concentrations of $CaCl_2$ the wall beads be thinner and the pores size of beads also increasing in the release percentage.

Table 2: The effect of variation $CaCl_2$ concentration on the percentage of loading content, loading efficiency of (BSA) for grafted and mixed beads. Activation conditions: (2%ALG, 0.04M PBQ, 45°C, 2h, pH10) at constant grafting and mixing conditions (0.3CS 40°C, 3h), formulation conditions (3%$CaCL_2$ for 1h at RT), using (BSA) 20% (w/w. to the total weight of polyelectrolyte complex) and wet beads 1g.

$CaCl_2$ %	Drug Loading Content (%)		Drug Loading Efficiency (%)	
	Grafted Beads	Mixed Beads	Grafted Beads	Mixed Beads
1	8.7	9.0	43.4	44.8
2	13.9	12.8	69.5	64.0
3	17.0	16.6	84.7	82.7
4	17.9	17.0	89.1	84.6



(a)



(b)

Fig. 2: Effect of variation $CaCl_2$ concentration on the release profiles of (BSA) of (a) CS-g-ALG copolymers and (b) ALG-CS mixed hydrogels. Activation conditions: (2%ALG, 0.4M PBQ, 45°C, 2h, pH10) at constant grafting and mixing conditions (0.3CS, 40°C, 3h), formulation conditions ($CaCL_2$ for 1h at RT) and release conditions (at 37°C, SPB pH 7.4) using (BSA) 20% (w/w, to the total weight of polyelectrolyte complex) and wet beads 1 gm.

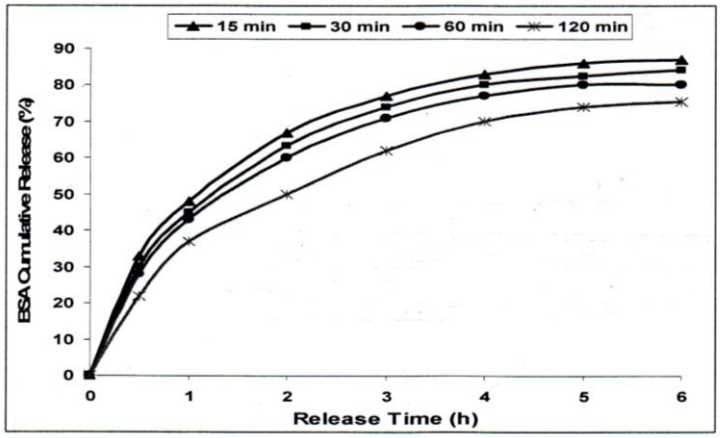
(3) Effect of Cross-linking time (Aging time) on the Percentage of Loading Content,Loading Efficiency and Release Profiles of (BSA):

The effect of variation the cross-linking time in $CaCl_2$ solution on the percentage of loading content and loading efficiency of BSA in case of grafted and mixed beads was studied in the range (15.120 min) as shown in Table 3). It was clear from results that the loading content and loading efficiency of (BSA) were decreased gradually with increasing time of cross-linking up to 120 min. These results may be attributed to increase the amount of (BSA) lost in the solution of $CaCl_2$ with increasing time, and this obviously leads to decrease the loading content and loading efficiency.

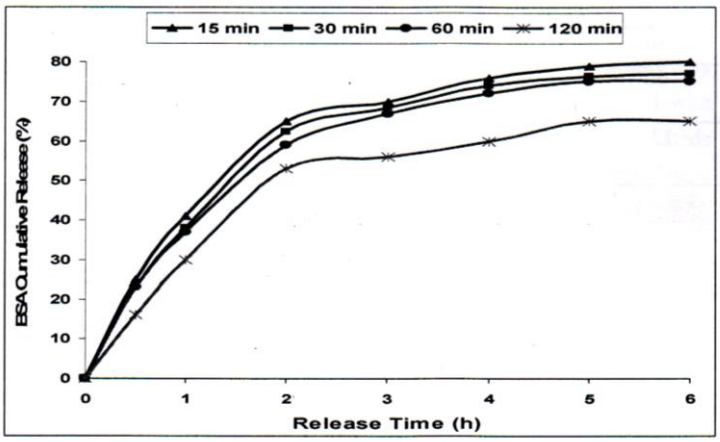
On the other hand the cumulative release of (BSA) decreased with increasing time of cross-linking up to 120 min. these observations may be attributed to that the stiffness and hardness of beads increase with increasing time of cross-linking up to 120 min, and this obviously leads to obstruct the passage of (BSA) molecules from the beads resulting a decreasing in the cumulative release of (BSA) as shown in figures (3a,b).

Table 3: The effect of cross-linking time on the percentage of loading content, loading efficiency of BSA for grafted and mixed beads. Activation conditions: (2%ALG, 0.04M PBQ, 45°C, 2h, pH10) at constant grafting and mixing conditions (0.3CS 40°C, 3h), formulation conditions (3%$CaCL_2$ at RT), using BSA 20% (w/w. to the total weight of polyelectrolyte complex) and wet beads 1gm.

$CaCl_2$ %	Drug Loading Content (%)		Drug Loading Efficiency (%)	
	Grafted Beads	Mixed Beads	Grafted Beads	Mixed Beads
15	18.0	18.5	89.6	99.0
30	17.6	17.2	87.9	88.0
60	17.0	16.6	84.7	82.7
120	15.9	14.5	79.2	72.2



(a)



(b)

Fig. 3: Effect of variation the cross-linking time on the release profiles of (BSA) of (a) CS-g-ALG copolymers and (b) ALG-CS mixed hydrogels. Activation conditions: (2%ALG, 0.04M PBQ, 45°C, 2h, ph pH10) at constant grafting and mixing conditions (0.3%CS, 40°C, 3h), formulation conditions (3% $CaCl_2$ at RT) and release conditions (at 37°C, SPB pH 7.4), using (BSA) 20% (w/w, to the total weight of polyelectrolyte complex and wet beads 1 gm.

(4) Effect of Cross-linking Temperature (Aging temperature) on the Percentage of Loading Content, Loading Efficiency and Release Profiles of (BSA):

The effect of variation the cross-linking temperature in $CaCl_2$ solution on the percentage of loading content and loading efficiency of (BSA) in case of grafted and mixed beads was studied in the range (25-55°C) as shown in Table 4). It was clear from results that the loading content and loading efficiency were decreased with increasing temperature, this may be attributed to increase the loss of (BSA) during the cross-linking process with elevating temperature. This leads consequently to decrease the loading content and loading efficiency.

Of the other hand the release of (BSA) increased gradually at the first hour of releasing with increasing temperature of cross-linking solution. Beyond 1 h it was observed that there is no difference of release values tat all different cross-linking temperatures as shown in figures (4a, b)

These observations may be attributed to that increasing temperature of cross-linking accelerate the release of (BSA) molecules to network of beads at the initial release time (1h), After this time, all beads which prepared at different cross-linking temperatures took the same temperature of the swelling medium (37°C), and this explains the constancy values of the BSA cumulative release at different cross-linking temperature.

Table 4: The effect of variation cross-linking temperature on the percentage of loading content, loading efficiency of BSA for grafted and mixed beads. Activation conditions: (2%ALG, 0.04M PBQ, 45°, 2h, pH10) at constant grafting and mixing conditions (0.3%CS, 40° C,40°C,3h), formulation conditions (3%$CaCL_2$ for 1h), using (BSA) 20% (w/w. to the total weight of polyelectrolyte complex) and wet beads 1 gm.

Cross-linking temperature (°C)	Drug Loading Content (%)		Drug Loading Efficiency (%)	
	Grafted Beads	Mixed Beads	Grafted Beads	Mixed Beads
25	17.0	16.40	84.7	82.0
35	16.3	16.00	81.2	80.0
45	14.0	14.00	69.7	70.0
55	11.2	10.38	55.8	54.0

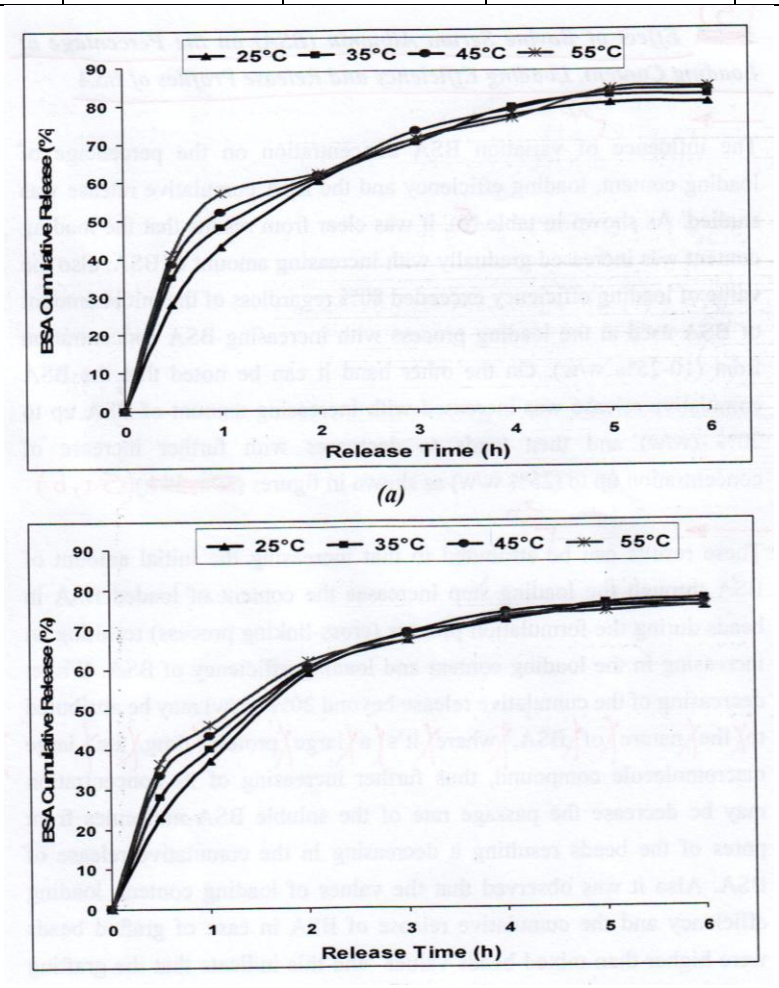


Fig. 4: Effect of variation the cross-linking temperature on the release profiles of (BSA) of (a) CS-g-ALG copolymers and (b) ALG-CS mixed hydrogels. Activation conditions: (2%ALG, 0.04M PBQ, 45°C, 2h, pH10) at constant grafting and mixing conditions (0.3% CS, 40°C, 3h), formulation conditions (3%$CaCl_2$, for 1h) and release conditions (at 37°C, SPB pH 7.4), using (BSA) 20% (w/w, to the total weight of polyelectrolyte complex) and wet beads 1 gm.

(5) Effect of Bovine Serum Albumin (BSA) on the Percentage of Loading Content, Loading Efficiency and Release Profiles of (BSA):

The influence of variation (BSA) concentration on the percentage of loading content, loading efficiency and the (BSA) cumulative release was studied. As shown in Table (5), it was clear from results that the loading content was increased gradually with increasing amount of (BSA) also the value of loading efficiency exceeded 80% regardless of the initial amount of (BSA) used at the loading process with increasing (BSA) concentration from (10-25%w/w). On the other hand it can be noted that the (BSA) cumulative release was increased with

increasing amount of (BSA) up to 20% (w/w) and then tends to decreases with further increase of concentration up to (25%) w/w) as shown in figures (5a, b).

There results can be attributed to that increasing the initial amount of (BSA) through the loading step increases the content of loaded (BSA) in beads during the formulation process (cross-linking process) resulting an increasing in the loading content and loading efficiency of (BSA). While; decreasing of the cumulative release beyond 20% (w/w) may be attributed to the nature of (BSA), where it's a large protein drug, i.e large macromolecule compound, thus further increasing of its concentration may be decrease the passage rate of the soluble (BSA) molecules from pores of the beads resulting a decreasing in the cumulative release of (BSA). Also it was observed that the values of loading content, loading efficiency and the cumulative release of (BSA) in case of grafted beads were higher than mixed beads values and this indicate that the grafting process was enhanced the release process.

Table 5: The effect of variation (BSA) concentration on the percentage of loading content, loading efficiency of (BSA) for grafted and mixed beads. Activation conditions (2%ALG, 0.04M PBQ, 45°, 2h, pH10) at constant grafting and mixing conditions (0.3%CS, 40°C, 3h), formulation conditions (3%$CaCL_2$ for 1h, at R.T.), using wet beads 1 gm.

BSA (w/w. to the total weight of polyelectrolyte complex)	Drug Loading Content (%)		Drug Loading Efficiency (%)	
	Grafted Beads	Mixed Beads	Grafted Beads	Mixed Beads
10	6.5	7	65.2	70
15	12.2	12	81.1	79.7
20	17	16.6	84.7	82.7
25	20.9	20.5	83.4	81.8

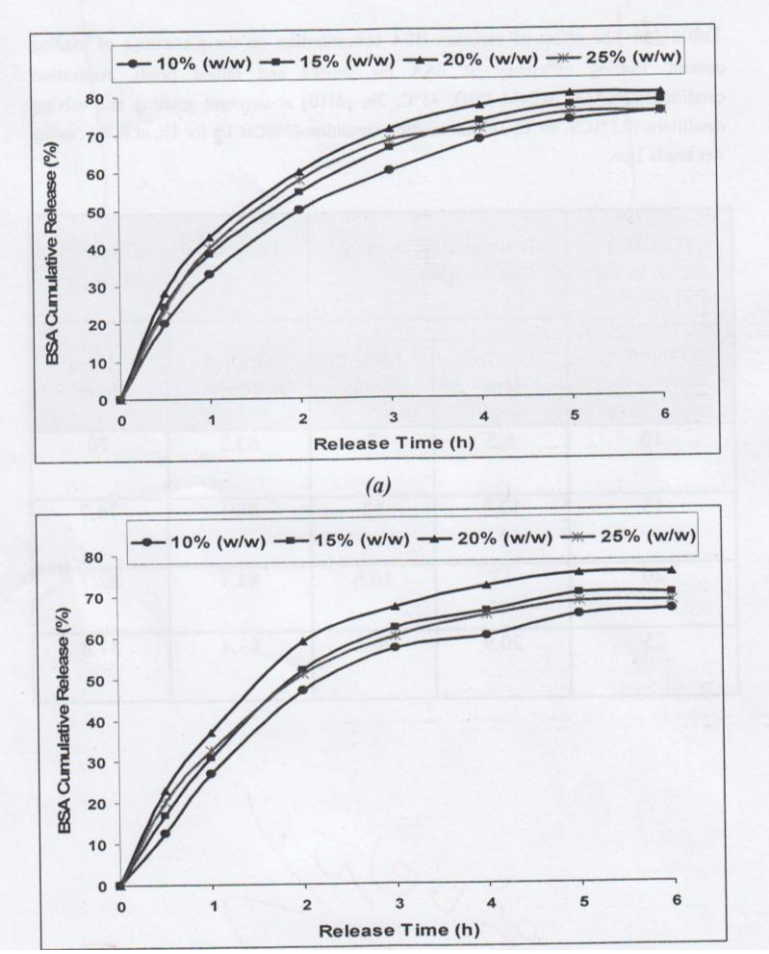


Fig. 5: Effect of variation (BSA) concentration on the release profiles of BSA of (a) CS-G-ALG copolymers and (b) ALG-CS mixed hydrogels. Activation conditions (2%ALG, 0.04M PBQ, 45°, 2h, pH10) at constant grafting and mixing conditions (0.3%CS, 40°C, 3h), formulation conditions (3%$CaCL_2$ for 1h, at R.T.) and release conditions (at 37°C, SPB pH 7.4), using wet beads 1 gm.

(6) The BAS Release in Gastrointestinal Tract conditions:

When a dosage form is taken orally, first of all it goes to the stomach and resides there for a certain period. Then it passes on the small intestine and finally reaches the colon. The total gastrointestinal transit time for an oral dosage form may vary depending on the physiology of the patient (Bajpai and Rasika; 2008), the most important site for drug absorption is the small intestine, it is known that the small intestine transit time ranges from 3 to 4 h for most healthy subjects.

In this study along gastrointestinal tract the formulation has to get exposed to a sharp pH change in the range 1-2 to 6-8 along the whole tract based on the previous obtained results from the swelling behavior in the gastrointestinal tract. Therefore behavior of beads as oral dosage form can be best visualized by exposing the beads to the environment of changing pH as shown in figure (6).

It was observed from results that when beads were incubated for 2h in SGF (pH1.2) the cumulative release rate of (BSA) in case of grafted and mixed beads was increased very slowly (6, 13% respectively). Transferring into SIF (pH 6.8) for 3h, the BSA release rate was accelerated and increased rapidly (78.5, 71.5% respectively), and lastly, when beads transferred into SCF (pH 7.4) for another 3h, the BSA release rate also increased (93.8, 90% respectively).

These results can be attributed to that at pH 1.2; both amino groups in chitosan and carboxyl groups in alginate are protonated. Due to the dominate effect of the protonated carboxyl groups in alginate, the hydrogel network deswells, and the beads have the lowest swelling ratio. As a result, the (BSA) release from the hydrogel network is strongly retarded, leading to the slow release rate.

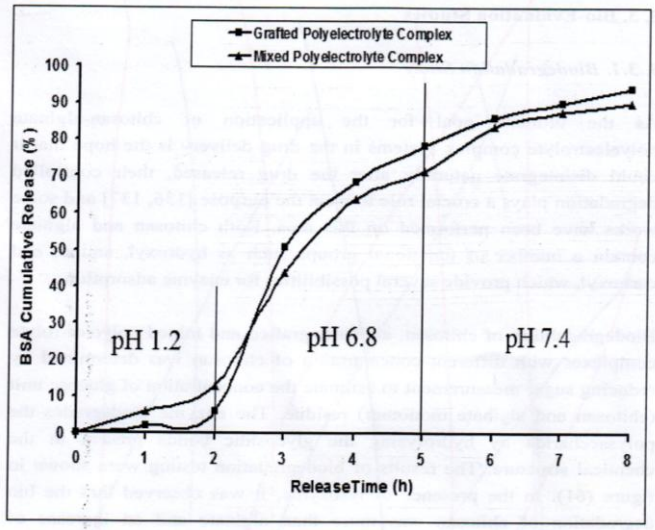


Fig. 6: The BSA release behavior in gastrointestinal tract conditions of grafted and mixed polyelectrolyte complex hydrogels. Activation conditions: (2%ALG, 0.04M PBQ, 45°, 2h, pH10) at constant grafting and mixing conditions (0.3%CS, 40°C, 3h), formulation conditions (3%$CaCaCL_2$ for 1h, at R.T.) and release conditions (at 37°C, SPB PH 7.4), using BSA 20% (w/w, to the total weight of polyelectrolyte complex).

If pH further increases to 6.8 then to 7.4, the carboxyl groups on the alginate become progressively ionized. As a result, the hydrogel swells more significantly due to a large swelling force created by the electrostatic repulsion between the ionized groups. For chitosan, the opposite is the case as the ionization of amine groups decreases greatly when the pH increases above 6.0 (around the pKa of chitosan), and at pH 7.4 most amino groups in chitosan are deprotonated.

As a result the electrostatic interaction between chitosan and alginate becomes weak, and the electrostatic repulsion between the ionized carboxyl groups in alginate causes the further swelling of the hydrogel network. So the beads at pH 7.4 have the highest swelling ratio, which leads to the fast release of BSA.

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