Carbohydrate Polymers 99 (2014) 235–241

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Cationic amphiphilic dextran hydrogels with potential biomedical applications

Georgeta Mocanu*, Marieta Nichifor

"Petru Poni" Institute of Macromolecular Chemistry, 700487 Iasi, Romania

ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 3 July 2013
Accepted 26 July 2013
Available online 17 August 2013

Keywords:
Cationic dextran
Amphiphilic derivatives
Microspheres
Vaccine adjuvants
Tetanus anatoxin
BSA
Antioxidants

ABSTRACT

Dextran microparticles were chemically modified for the attachment of quaternary ammonium groups carrying substituents with different hydrophobicity, in order to obtain amphiphilic cationic hydrogels with different hydrophilic/lipophilic balance. These hydrogels retain various amounts of dyes: Rose Bengal, Brilliant Blue and Vitamin B12, used as models for hydrophobic, amphiphilic and hydrophilic drugs, as a function of their hydrophilic/hydrophobic properties. Bovine serum albumin (BSA) retention by hydrogels occurs in higher amounts at pH 6.9, and is influenced by electrostatic, hydrophobic forces and the swelling of the supports. Tetanus anatoxin is retained by the supports through electrostatic and/or hydrophobic forces, in amounts varying between 110 and 200 mg/g. Both proteins are gradually released, through increasing of the eluent ionic strengths. Alpha-tocopherol is retained by the hydrogels preponderantly through hydrophobic forces, in amounts varying between 130 and 300 mg/g. Measurement of the scavenging effect proved the antioxidant properties of the included drug. Based on the obtained results, one can appreciate the potential of the synthesized cationic hydrogels as supports for biomolecules or as vaccine adjuvants.

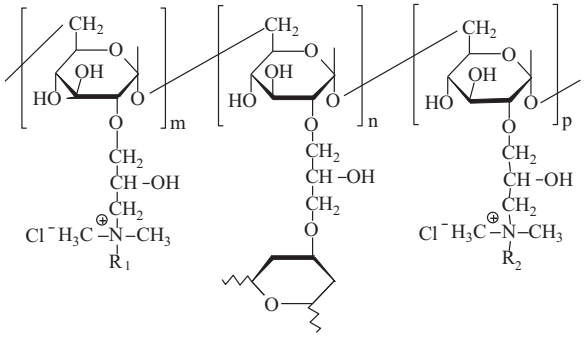
1. Introduction

Polysaccharides and their derivatives are considered valuable candidates for drug and vaccine carrier systems, due to their biocompatibility, biodegradability and absence of toxicity. Great attention has been paid in recent years to hydrophilic gels, which represent macromolecular networks, crosslinked by chemical or physical methods, able to absorb large amounts of water or biological fluids (Peppas, Bures, Leobandung, & Ichikawa, 2000; Peppas & Mikos, 1986). This interest is justified by their specific properties, which recommend them in biomedical, biotechnological and pharmaceutical applications. As a function of the nature of units linked on the polymeric chain, hydrogels may be: pH-sensitive (Dulong, LeCerf, Picton, & Muller, 2006), thermosensitive (Deguchi, Akiyoshi, & Sunamoto, 1994), associative (Akiyoshi, Yamaguchi, & Sunamoto, 1991; Henni-Silhadi et al., 2008) or multi-responsive (Rodriguez-Felix et al., 2011). Self-assembled amphiphilic polysaccharides (chitosan, dextran, pullulan, hyaluronic acid, amylase, heparin) can be used as drug delivery systems in pharmaceutical applications (Hassani, Hendra, & Bouchemal, 2012). Polysaccharide-based gels have been intensively studied as delivery systems for pharmaceutically-active peptides and protein drugs (Chen, Jo, & Park, 1995; Gao & Dubin, 1999). Self-assembled hydrogel nanoparticles of cholesterol-bearing pullulan were tested as carriers of protein drugs, such as insulin (Akiyoshi et al., 1998). Crosslinked DEAE-pullulan represents a matrix which could be a useful biomaterial for local gene transfer (San Juan, Hlawaty, Chaubet, Letourneur, & Feldman, 2007). Also, cationic hydrogels based on chitosan are well-known for their potential as delivery systems for protein therapeutics and antigens (Amidi, Mastrobattista, Jiskoot, & Hennink, 2010), including vaccines (Kang, Cho, & Yoo, 2009) (as Tetanus toxoid (Vila et al., 2004)) or enzymes (lipase (Nasratun et al., 2010; Palla, Pacheco, & Carrin, 2011)). The use of microparticles as vaccine adjuvants is based on their ability to bind the antigens through ionic and/or hydrophobic bonds. The use of microparticles as vaccine adjuvants for oral administration could reduce the inherent problems caused by the low pH of the stomach, enzymatic degradation, fast transit and low adsorption of large molecules. The microspheres of crosslinked dextran with a mean diameter between 20 and 340 μm were tested for tetanus toxoid encapsulation; a single immunization with a long-acting formulation elicited anti-tetanus toxoid response lasting for 1 year, comparable with the 12 weeks obtained when using conventional aluminum-adsorbed toxoid (Diwan, Khar, & Talwar, 2001).

Cationic dextran hydrogels were synthesized and their use as potential bile acid sequestrants was previously studied (Nichifor, Baille, Cristea, Zhu, & Carpov, 2001; Nichifor, Cristea, & Carpov, 2000; Nichifor, Cristea, Mocanu, & Carpov, 1998). In the present

* Corresponding author. Tel.: +40 232 217454; fax: +40 232 211299.
E-mail address: gmocanu@icmpp.ro (G. Mocanu).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.07.087



Scheme 1.

study, the investigations were devoted to the interaction of amphiphilic cationic dextran hydrogels with various hydrophilic, hydrophobic or amphiphilic molecules, as well as with antioxidants (alpha-tocopherol), proteins (BSA) and vaccines (tetanus toxoid), with the aim to appreciate their performances as supports for controlled release and/or as vaccine adjuvants.

2. Experimental

2.1. Materials

Dextran (Dex-40) with molar mass 40,000 was purchased from Sicomed S.A. Drug Company (at present ZENTIVA S.A. Drug Company) (Bucharest, Romania). All tertiary amines and epichlorohydrin (ECH) were purchased from Aldrich and were used as received. Rose Bengal sodium salt (RB) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) – from Sigma Aldrich. Brilliant Blue (BB), Vitamin B12 (B12), alpha-tocopherol (α-TF) and bovine serum albumin (BSA) Mw ~ 60,000 were obtained from Fluka. Tetanus anatoxin (TA) was kindly supplied by the "Cantacuzino" National Institute for Microbiology and Immunology Research Development Iasi, Romania.

3. Methods

3.1. Synthesis of cationic dextran gels

The synthesis of cationic polymers, with the general chemical structure shown in Scheme 1 and characteristics included in Table 1, required several reaction steps: (a) synthesis of crosslinking dextran; (b) chemical modification of crosslinked dextran for the attachment of a first type of pendant quaternary ammonium groups (single-modified gels); (c) attachment of a second type of quaternary ammonium groups (double-modified gels).

(a) Preparation of crosslinked dextran microspheres, previously described in detail (Nichifor et al., 1998), was realized in the presence of ECH as a crosslinker and NaOH as a catalyst. Gels with different capacity for water retention (R_w) were obtained by variation of the ECH/glucopyranosidic unity (ECH/GU) ratio.
(b) The quaternization reaction of crosslinked dextran microspheres (0.10–0.20 mm in diameter) was performed according to a procedure described in detail elsewhere (Nichifor, Stanciu, & Simionescu, 2010). Generally, quaternization reactions were performed in water (polysaccharide/deionized water ratio = 1 g/10 ml), under constant stirring, with an equimolar mixture of epichlorohydrin and a tertiary amine as a reagent, for 6 h at 40–60 °C. The reaction mixture was filtered and the gel particles were sequentially rinsed on the filter with water, 0.1 N aqueous HCl, water (until the absence of chloride ions in the filtrate) and methanol. The gel particles were dried under vacuum. The content in amino groups of the sorbents (in chloride salt form) was determined by elemental analysis of nitrogen and by potentiometric determination of the chloride ions.
(c) In some cases, a second type of quaternary ammonium group was attached to dextran. This second chemical modification was realized on single-modified gels, after their purification and drying, by the same procedure applied in step (b). The most hydrophobic group (the tertiary amine used as a reagent, N,N-dimethyl-N-R_1, contained the most hydrophobic substituent R_1 = dodecyl, hexadecyl) was introduced in the first step, followed by the reaction with a more hydrophilic tertiary amine (usually N,N-dimethyl-N-ethylamine, where $R_2 = C_2$).

The degree of substitution of the first (DS_1) and second group (DS_2) was calculated with Eqs. (1) and (2), respectively.

$$DS_1 = \frac{162 \cdot Cl_1}{100 \times 35.5 - Cl_1 \cdot MS_1} 100\,(\text{mol}/100\,\text{GU}) \quad (1)$$

$$DS_2 = \left[\frac{X(MS_1 - MS_2 + (162 \times 100/DS_1))}{100 - X \cdot MS_2} - 1\right] DS_1\,(\text{mol}/100\,\text{GU}) \quad (2)$$

where $X = Cl_t/35.5$; Cl_1 and Cl_t are the chloride ions contents after first and second quaternization step, respectively; MS_1 and MS_2 are the molecular weights (in g) of the first and the second pendent ammonium group, respectively.

The codes of the hydrogels include the type of substituent R_1 and R_2 and the content of ammonium groups bearing

Table 1
Physico-chemical characteristics of the dextran cationic hydrogels.

Dextran gel sample	R_1	R_2	Content in amino groups			Water uptake of the dextran hydrogel (R_w, g/g)	
			DS_1 (mol %)[a]	DS_2 (mol %)[a]	Total (meq/g)	Neutral	Cationic
C2(50)	C_2H_5	–	50	–	1.96	2.5	3.97
C12(25)	$C_{12}H_{25}$	–	25	–	0.95	5.0	3.96
C12(25)–C2(35)	$C_{12}H_{25}$	C_2H_5	25	35	2.09	5.0	4.58
C12(10)–C2(47)	$C_{12}H_{25}$	C_2H_5	10	47	2.13	5.0	9.30
C16(10)–C2(47)	$C_{16}H_{33}$	C_2H_5	10	47	2.05	5.0	6.70
C12(10)–C2(48)	$C_{12}H_{25}$	C_2H_5	10	48	2.12	3.0	6.24
$(C2)_3$(58)	$(CH_2)_3$	–	58	–	2.12	6.0	13.0
C7(38)	Benzyl	–	38	–	1.54	5.0	–
C7(28)	Benzyl	–	28	–	1.23	2.5	3.07
C8(22)	C_8H_{17}	–	22	–	1.04	2.5	3.30
C8(20)	C_8H_{17}	–	20	–	0.91	7.5	60.00

[a] Moles/100 glucopyranosic units.

these substituents. For example C12(25)–C2(35) indicates the hydrogels containing both N-(2-hydroxypropyl)-N,N-dimethyl-N-dodecylammonium chloride groups with DS_1 = 25 mol% and N-(2-hydroxypropyl)-N,N-dimethyl-N-ethylammonium chloride groups with DS_2 = 35 mol% (Table 1).

Water retention at equilibrium R_w (g water/g dry microspheres) was determined according to a method described by Pepper, Reichenberg, and Hale (1952). R_w is an indirect measure of the swelling porosity of a hydrogel.

3.2. Retention and release experiments

Retention of dyes (RB, BB and B12) was determined by a procedure adapted according to previously described methods (Dulong et al., 2006; Gigimol & Mathew, 2003). The dry gel (50 mg) was equilibrated with an aqueous solution of dye (125×10^{-6} M) for 48 h, then the amount of dye bound by the polymer was determined by UV from the difference in the concentrations of the dye solution, before and after binding, using calibration curves built with known dye concentrations. The wavelengths for UV measurements were: 548 nm (RB), 586 nm (BB) and 550 nm (B12).

The retained dyes are not released from the supports during washing with water, as proved by UV measurements of the effluents.

Alpha-tocopherol retention was performed in a similar manner, in solutions containing 1 g drug/L (determination at 292 nm wavelength).

Retention of BSA and TA was performed in the presence of sodium azide as a preservative. Aliquots were withdrawn and protein concentration in the supernatant was determined according to the modified Folin method (Lowry, Rosenbrough, Lewis-Farr, & Randall, 1951). When the system reached equilibrium, the microparticles were filtered, washed with water to remove the physically-entrapped proteins, then with ethyl alcohol, and finally dried under vacuum.

Release of the proteins retained by supports was also performed under "batch" conditions, on a 50 mg support–protein complex, in a 50 mL NaCl solution, the ionic strength of which was gradually increased. After the release attained a plateau, the gel was removed and placed in a NaCl solution (50 mL) of higher ionic strength. The released protein amount was again determined by the Folin method. The desorption ratio of proteins was calculated with Eq. (3).

$$\text{Desorption ratio\%} = \frac{P_t}{P_T} \times 100 \quad (3)$$

Where P_T and P_t are the total amount of protein bound by support and the amount of protein released at time t, respectively.

3.3. Antioxidant activity

The antioxidant activity of the alpha-tocopherol retained by hydrogels was determined by the DPPH method (Ara & Nur, 2009) and compared with that of ascorbic acid and free alpha-tocopherol. Briefly, various amounts of samples containing the antioxidant product were immersed in a 2 mL NaCl aqueous solution (1 g/L) and a 1 mL DPPH solution (0.128 g/l methanol); after 30 min, absorbance of the supernatant at 517 nm was measured. The radical scavenging activity was calculated using the following Eq. (4):

$$\text{Scavenging effect\%} = \frac{A0 - A1}{A0} \times 100 \quad (4)$$

where A_0 is the absorbance of a standard solution prepared under the same conditions, but containing only DPPH and A_1 is the absorbance of the supernatant.

A decreased absorbance of DPPH indicates an increased antioxidant effect.

4. Results and discussion

4.1. General characteristics of cationic dextran gel microparticles

The procedure for the preparation of single modified polymers was elaborated for the quaternization of both linear and crosslinked polysaccharides. The reaction mechanism and the chemical structure (with formation of N-(2-hydroxypropyl)-N,N-dimethyl-N-ethylammonium chloride pendent groups) were ascertained by extensive ^{1}H NMR and ^{13}C NMR studies performed on linear (water soluble) quaternized dextran (described in detail by Nichifor et al., 2010). In the present work we used both single- and double modified cationic gels, in order to enlarge the spectrum of gel properties. The structure of the synthesized cationic dextran hydrogels is presented in Scheme 1 and their main characteristics are listed in Table 1.

The main goal of the present study was the design of new adsorbents for dyes, proteins and vaccines. Therefore, cationic gels properties, such as hydrophilic/lipophilic balance (HLB), content in amino groups and swelling were varied in order to establish the optimum properties for binding different compounds. The HLB of these gels, containing mainly N-(2-hydroxypropyl)-N,N-dimethyl-N-(R_1/R_2)-ammonium chloride groups, was varied in two ways: (i) by changing the hydrophobicity of the alkyl substituent R_1 in single-modified gels C2(50), C12(25), C8(22), C8(20) (R_1 = ethyl, dodecyl, octyl); (ii) by changing the ratio between DS_1 and DS_2 in double-modified gels: C12(25)–C2(35), C12(10)–C2(47), C16(10)–C2(47), C12(10)–C2(48) (R_1 = dodecyl or hexadecyl and R_2 = ethyl). The influence of the presence of other type of substituents, like benzyl [C7(38), C7(28)] or triethylammonium groups [$(C2)_3$(58)], was also studied. The swelling, which can be evaluated from the value of R_w, is influenced by the content in amino groups, hydrophobicity of the R_1/R_2 substituents and the R_w value of the unmodified (neutral) gel. When the amino groups contain $R_1 = C_2H_5$ or triethyl substituents and DS > 30 mol%, the cationic hydrogels become more hydrophilic than the parent dextran supports [samples: C2(50), $(C2)_3$(58)]. The cationic hydrogel C12(25) with $R_1 = C_{12}H_{25}$ (dodecyl) was more hydrophobic than the parent dextran support, and its hydrophobicity (water swelling decrease) could be exceeded only by the presence of a large amount of the second hydrophilic ammonium groups, as the comparison of R_w values for C12(25), C12(25)–C2(35) and C12(10)–C2(47) gel indicates. The increased swelling capacity of the parent neutral hydrogels determines a significant increase of the swelling of the cationic hydrogel [$(C2)_3$(58), C8(20)], even when DS is low and R_1 has a relatively high hydrophobicity [C8(20)].

The amphiphilic character of the hydrogels was evidenced by the retention of dyes with different hydrophobicities: hydrophobic Rose Bengal (RB), amphiphilic Brilliant Blue (BB) and hydrophilic vitamin B12. The results shown in Fig. 1 indicate a preponderance of the hydrophobic interaction between supports and dyes. Dye retention by all supports decreases in the order: RB > BB > B12. Retention of the hydrophobic RB is very high and reaches 60–95% of the initial amount of dye used for the retention experiment. It depends on the chemical structure of the support and increases with increasing the hydrophobicity of substituent R_1 of the single-substituted supports, having similar DS_1 and R_w [C7(28) < C8(22) < C12(25)], and also with increasing the hydrophobicity of R_1 and its DS_1 value for double-substituted supports [C2(50) ≈ C12(10)–C2(47) < C12(25)–C2(35) ≈ C16(10)–C2(47)]. Retention of the less hydrophobic BB is also influenced by the chemical composition of supports, but this influence is not very clear. The presence of oppositely charged groups on supports

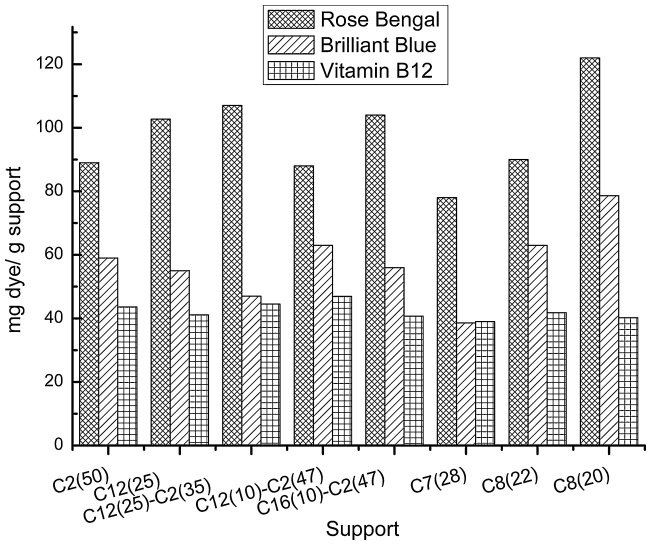


Fig. 1. Retention of Rose Bengal, Brilliant Blue, Vitamin B12 by cationic amphiphilic hydrogels; the values are the mean of three independent measurements that deviated: 2–4%.

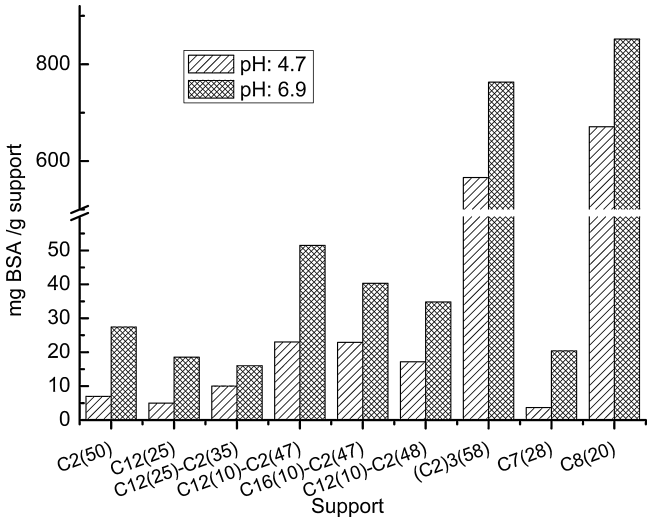


Fig. 2. Retention of BSA by cationic amphiphilic hydrogels; the values are the mean of three independent measurements that deviated: 2–3%.

(cationic) and dyes (carboxylic groups for RB and sulfonic groups for BB) should have enhanced dye binding through electrostatic interactions. However, this effect is very weak, as the increase of the total degree of substitution from 1 to 2 meq/g led to a very small increase in binding capacity, for both RB and BB [see C12(25) and C12(25)–C2(35)]. Besides hydrophobicity, the swelling degree of cationic supports has a decisive role on dye binding capacity. This aspect is highlighted by the highest amounts of both RB and BB retained by the support C8(20) having the highest swelling, which allows a non-hindered access of the dye into the macromolecular network. Hence, dyes retention is the resultant of the hydrophobic forces and diffusion of molecules into the polysaccharide network. Vitamin B12 is retained by the hydrogels in lower amounts than the previously discussed dyes, and the influence of support's chemical structure is negligible. Vitamin B12 can form interpolymer complexes only with a weak acid cation exchange resin (Mahore, Wadher, Umekar, & Bhoyar, 2010) or with poly(methacrylic acid-g-ethylene glycol) hydrogels (Fogueri & Singh, 2009); its retention by the cationic gels can be due to other kind of interactions (hydrophobic, hydrogen bonds) between the support and the solute (Mocanu, Souguir, Picton, & LeCerf, 2012).

Interaction with bovin serum albumin. BSA is a protein with M_w = 60,000 and isoelectric point of 4.7; its retention was studied by both anionic hydrogels (PNIPAM-carboxymethyl interpenetrating cellulose polymeric network (Ekici, 2011)) and cationic hydrogels (chitosan micro- and nanoparticles crosslinked with glutaraldehyde (Wei et al., 2008) or tri-polyphosphate (Kafshgari, Khorram, Khodadoost, & Khavari, 2011; Zhang, Wu, Tao, Zhang, & Su, 2010), DEAE dextran (Can & Güner, 2006; Yoshida, Fujita, & Kudo, 1999)). BSA absorption by the supports can be the result of many cumulative effects, for example, hydrophobic interactions, hydrogen bonds, electrostatic forces (Hou, Liu, Deng, Zhang, & Yan, 2007).

The pH of the medium can influence BSA retention, as the protein becomes positively or negatively charged in acidic or basic environment, respectively. By anionic hydrogels, BSA is retained in higher amounts at a pH around its isoelectric point (4.7) (Ekici, 2011). The optimum pH values for maximum BSA adsorption were reported to be 4.7 (pI; BSA has no net charge) or 6.9–7.2 (the dissociation of anionic groups of the BSA molecule is completed and BSA has an anionic character) (Can & Güner, 2006). For this reason, BSA retention by the dextran hydrogels was studied at both pH = 4.7 and 6.9 values. The results are presented in Fig. 2. BSA retention is clearly influenced by medium's pH. Higher amounts of protein are retained at pH: 6.9, indicating that dissociation of the anionic groups favors binding through electrostatic forces by the cationic dextran support. The swelling of the support also influences BSA retention. Samples $(C2)_3(58)$ and C8(20), with a higher water uptake (R_w > 10), retain large amounts of BSA, probably due to a more free access of the large protein molecule into the hydrogel network. The uptake by the other supports occurs primarily by surface adsorption, but some diffusive penetration into the matrix can be also possible.

Release of the BSA retained by the supports was tested in NaCl solutions with various ionic strengths (Fig. 3). Desorption of proteins in saline solutions is the result of the shrinkage of the macromolecular network, due to either an increased ionic strength, which reduces porosity and the availability of the binding sites, or to the competition between the salt counterions and protein ions for the support binding sites. BSA release from all supports did not exceed 25% in a 0.1 M NaCl solution; when the ionic strength of the NaCl solution was increased at 1 M, a slight rise of BSA release occurs. Partial BSA desorption was previously

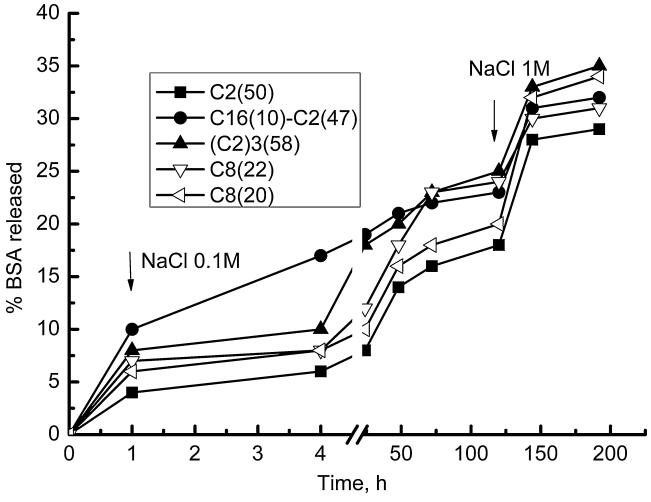


Fig. 3. Release of BSA retained by cationic amphiphilic hydrogels; the values are the mean of three independent measurements that deviated: 3–4%. Arrows indicate the moment when the ionic strength of the release medium was changed.

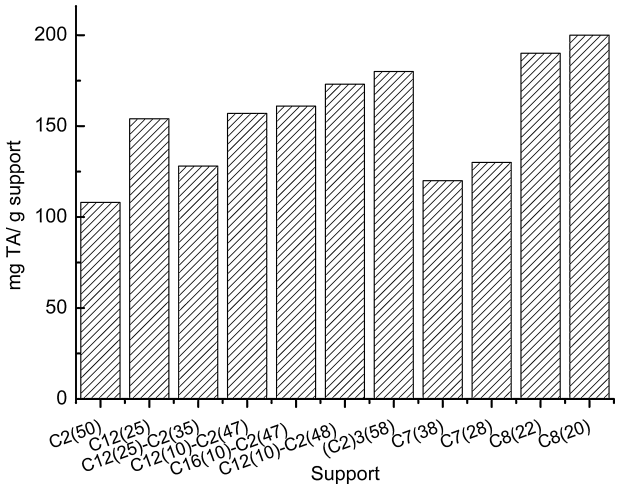


Fig. 4. Retention of TA by cationic amphiphilic hydrogels; the values are the mean of three independent measurements that deviated: 2–3%.

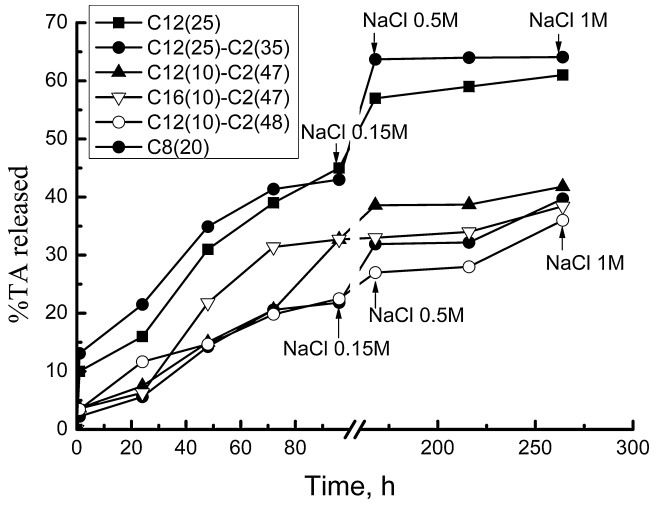


Fig. 5. Release of TA retained by cationic amphiphilic hydrogels; the values are the mean of three independent measurements that deviated: 3–5%. Arrows indicate the moment when the ionic strength of the release medium was changed.

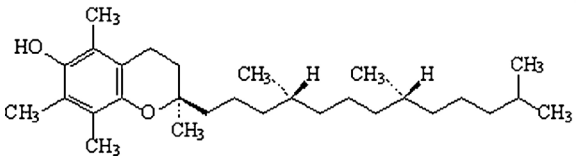
reported for protein retention by DEAE dextran (Can & Güner, 2006). A possible explanation can be the significant contribution of the hydrophobic forces to BSA binding by the amphiphilic cationic dextran hydrogels, which are enhanced and not weakened by ionic strength rise. (Tanford, 1980). Even if the BSA adsorption capacity decreases when the ionic strength of the adsorption medium increases (Yoshida et al., 1999), once retained by the supports, BSA is difficult to be released, probably due to the hydrophobic forces established between the support and the hydrophobic units of the protein.

Hence, one can appreciate that the synthesized supports can be useful for immobilization/separation of proteins.

Interaction with tetanus anatoxin (TA). Tetanus is a potentially life threatening disease affecting 1 million people worldwide every year (Larroche, 2004). Immunization through vaccination is highly effective and is the key of prevention. Researches in the vaccines domain were equally focused on attempts to diversify their variety and to improve the existing ones, by using efficient adjuvants. The common adjuvants employed until now for human use are: aluminum hydroxide, aluminum phosphate, calcium phosphate and oleic emulsions; other adjuvants, such as: derivatives of muramyl dipeptide, monophosphoryl lipid A, liposomes, immunostimulating complexes can be also mentioned. An important aspect in the use of adjuvants for human vaccines is the toxicity and adverse side-effects of most adjuvant formulations (Gupta & Siber, 1995). Besides the biodegradable polymers used as vaccine adjuvants [poly(lactic acid), poly(glycolic acid) or their derivatives] (Jung, Noneberg, Hungerer, & Kissel, 2002), micro- or nano-particles of chitosan were studied for preparation of both therapeutic peptides/protein and antigen formulations to be applied in protein and antigen delivery through parenteral and mucosal (particularly nasal and pulmonary) paths (Amidi, Mastrobattista, Jiskoot, & Hennink, 2010). Low molecular chitosan nanoparticles loaded with tetanus toxoid could be considered as promising new nasal vaccine delivery systems able to provide high and long-lasting mucosal and humoral immune responses (Vila et al., 2004). Previous studies revealed that curdlan microspheres retain important amounts of TA, appearing as promising candidates as vaccine adjuvants; in this case, the reciprocally enhanced immunological activity of the curdlan–protein complexes should be also considered (Mocanu, Mihai, Moscovici, Picton, & Lecerf, 2009).

Dextran cationic hydrogels retain important amounts of TA (Fig. 4); taking into account that the isoelectric point of TA is around 6.2–6.5 (Laferriere et al., 2011) one may assume that it can be adsorbed through electrostatic forces by the strongly basic functions of the supports. In the case of TA, the differences in adsorption between supports are lower than in the case of BSA. The obtained data for AT retention cannot be correlated with the amount of ionic groups and with the amount or length of the alkyl radical R_1 at the amino group. TA has a high molecular weight (~150,000 g/mol); one may assume that its access into the macromolecular network will be hindered, hence, it will be retained both through electrostatic and/or hydrophobic forces preponderantly on the support surface. Samples C2(50), C7(38) and C7(28), which have less hydrophobic R_1 substituents, retain smaller amounts of TA. Sample C8(20), with moderate hydrophobicity, but with very high swelling presents the highest TA adsorption, perhaps due to the TA retention both inside the polysaccharide network and on the support surface. Hence, the retention capacity by the cationic hydrogels depends on the length and amount of radicals at the amino groups, hydrophilic/hydrophobic balance of the support, and also on its swelling, which determines the access of the protein to the binding sites; however, at least for the time being, a clear structure–adsorption capacity relationship can not be established. This aspect needs more attention in the future.

The TA release was determined first in isotonic NaCl solutions (that mimic the osmolality of human blood, approximately 0.15 M NaCl). As shown in Fig. 5, TA is released gradually, in time, from its complexes with all supports. To verify if TA can be entirely released, at certain time periods the concentration of eluents was increased at 0.5 M and then at 1 M; it was observed that desorption attained 100% for no support, similarly as for BSA release. The salts counterions are in competition with the proteins ions for binding sites, thus decreasing the attraction between the cationic dextran amino group and protein molecules, but hydrophobic interactions still exist, influencing on the protein release. Samples C12(25) and C8(20), having the smallest amount of ionic groups and moderate hydrophobicity, release faster AT than samples C12(25)–C2(35), C12(10)–C2(47), C16(10)–C2(47), C12(10)–C2(48) which have a higher content of amino groups and higher hidrophobicity. Hence, the release of TA is governed by a co-operative effect of both electrostatic and hydrophobic forces. This behavior permits the conclusion that the amphiphilic cationic dextran hydrogels may be tested as vaccine adjuvants for long lasting single dose application. Further *in vivo* studies will reveal the performances of these supports for this purpose.



Scheme 2. Alpha tocopherol.

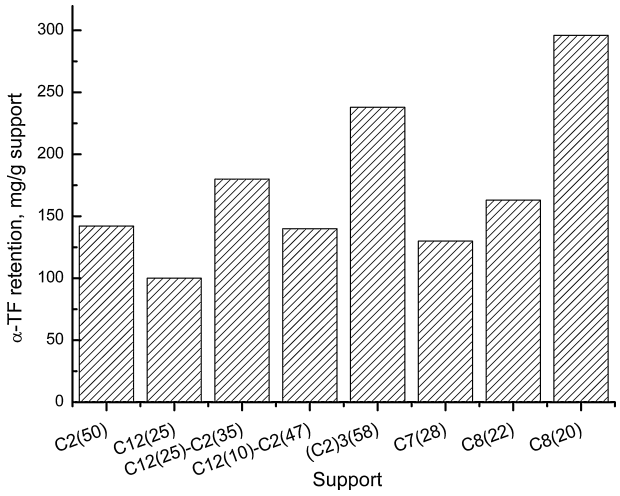


Fig. 6. Retention of α-TF by cationic amphiphilic hydrogels; the values are the mean of three independent measurements that deviated: 2–3%.

4.2. Interaction with antioxidants (α-TF)

Tocopherols (Scheme 2) are methylated phenols which act as fat-soluble antioxidants and have vitamin E activity. α-TF is the most biologically-active form of vitamin E, that is preferentially absorbed and accumulated in humans. Being fat-soluble, it is incorporated into cell membranes, protecting them against oxidative damage. Other important functions of α-TF are the regulatory effect on enzymatic activity or gene expression. It also plays a role in neurological functions.

Retention of α-TF by the studied supports is presented in Fig. 6; due to their hydrophobic character, the assumption may be that it will be retained preponderantly through hydrophobic forces. α-TF is retained by amphiphilic cationic dextran gels in various amounts, depending on their hydrophobicity and accesibility of the solute inside the macromolecular network. Thus, C8(20) presents the highest capacity of α-TF retention, followed by $(C2)_3(58)$, due to their higher swelling. The other samples, more hydrophobic, but with lower swelling, retain lower α-TF amounts.

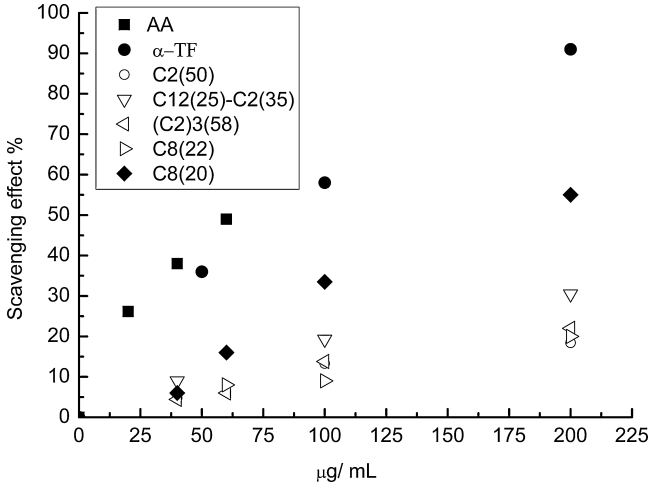


Fig. 7. Scavenging effect of α-TF retained by cationic amphiphilic hydrogels, comparatively with that of ascorbic acid and α-TF substance; the values are the mean of three independent measurements that deviated: 1–3%.

In order to know if α-TF retained by the supports still presents antioxidant activity, the antioxidant activity (expressed as Scavenging effect %) of the supports containing α-TF was studied, comparatively with that of ascorbic acid (AA) (as standard) and of the drug itself. As can be seen from Fig. 7, the scavenging effect of AA is higher than that of α-TF; the α-TF retained by the studied supports presents antioxidant activity. The scavenging effects of the released α-TF are lower than those of the parent α-TF, due to the gradual release of the drug retained on supports. Sample C8(20), with the highest swelling and α-TF content, releases faster the drug *in vitro*. This behavior can be important in the potential use of supports for controlled delivery of antioxidant substances.

5. Conclusions

Cationic amphiphilic hydrogels with various content of amino groups and lengths of alkyl substituents were synthesized and characterized. Their amphiphilic behavior was evidenced through retention of hydrophobic/amphiphilic/hydrophilic substances. The hydrogels interaction with proteins (BSA) and vaccines (tetanus anatoxin) occurs through electrostatic and/or hydrophobic forces, being also influenced by the swelling of the support; BSA retention was higher when using an initial solution having pH: 6.9. Release of proteins takes place in saline solutions; when increasing the ionic strength of the eluent, protein desorption increases but is not complete, due to the hydrophobic interaction forces, as already mentioned in literature. The obtained hydrogels also retain important amounts of antioxidants (α-TF) through hydrophobic forces; the drug is released gradually, its antioxidant activity being preserved.

All these results recommend the cationic amphiphilic hydrogels based on dextran as potential supports for drug release or as vaccine adjuvants. Further *in vitro* and *in vivo* studies are required for confirmation the biological potential of these new hydrogels.

Acknowledgements

This work is supported by a grant of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project number PN-II-ID-PCE-2011-3-0622.

References

Akiyoshi, K., Kabayashi, S., Shichibe, S., Mix, D., Baudys, M., Wan Kim, S., et al. (1998). Self-assembled hydrogel nanoparticles of cholesterol-bearing pullulan as carrier of protein drugs: Complexation and stabilization of insulin. *Journal of Controlled Release*, *54*(3), 313–320.

Akiyoshi, K., Yamaguchi, S., & Sunamoto, J. (1991). Self-aggregates of hydrophobic polysaccharide derivatives. *Chemistry Letters*, *20*, 1263–1266.

Amidi, M., Mastrobattista, E., Jiskoot, W., & Hennink, W. E. (2010). Chitosan-based delivery systems for protein therapeutics and antigens. *Advanced Drug Delivery Reviews*, *62*, 59–82.

Ara, N., & Nur, H. (2009). In vitro antioxidant activity of methanolic leaves and flowers extracts of *Lippia alba*. *Research Journal of Medicine and Medical Sciences*, *4*(1), 107–110.

Can, H. K., & Güner, A. (2006). Investigation of adsorption–desorption dynamism of bovine serum albumin on crosslinked *NN*-diethylaminoethyl dextran microbeads: Solution phase. *Journal of Applied Polymer Sciences*, *99*, 2288–2299.

Chen, J., Jo, S., & Park, K. (1995). Polysaccharide gels for protein drug delivery. *Carbohydrate Polymers*, *28*, 69–76.

Deguchi, S., Akiyoshi, K., & Sunamoto, J. (1994). Solution property of hydrophobized pullulan conjugated with poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer. Formation of nanoparticles and their thermosensitivity. *Makromolecular Rapid Communications*, *15*(9), 705–711.

Diwan, M., Khar, R. K., & Talwar, G. P. (2001). Tetanus toxoid loaded preformed microspheres of crosslinked dextran. *Vaccine*, *19*(28–29), 3853–3859.

Dulong, V., LeCerf, D., Picton, L., & Muller, G. (2006). Carboxymethylpullulan hydrogels with an ionic and/or amphiphilic behavior: Swelling properties and entrapment of cationic and/or hydrophobic molecules. *Colloid and Surfaces A: Physicochemical and Engineering Aspects*, *274*, 163–169.

Ekici, S. (2011). Intelligent poly(N-isopropylacrylamide)-carboxymethyl cellulose full interpenetrating polymeric networks for protein adsorption studies. *Journal of Material Sciences*, *46*, 2843–2850.

Fogueri, L. R., & Singh, S. (2009). Smart polymers for controlled delivery of proteins and peptides: A review of patents. *Recent Patents on Drug Delivery & Formulation*, *3*, 40–48.

Gao, J. Y., & Dubin, P. L. (1999). Binding of proteins to copolymers of varying hydrophobicity. *Biopolymers*, *49*, 185–193.

Gigimol, M. G., & Mathew, B. (2003). Effect of the nature and degree of crosslinking on Rose Bengal binding by DVB-, NNMBA-,HDODA- and TTEGDA-crosslinked poly(*N*-vinylpyrrolidone)s. *Polymer International*, *52*, 973–980.

Gupta, R. K., & Siber, G. R. (1995). Adjuvants for hyman vaccines. Current status, problems and future prospects. *Vaccine*, *13*(14), 1263–1276.

Hassani, L. N., Hendra, F., & Bouchemal, K. (2012). Auto-associative amphiphilic polysaccharides as drug delivery systems. *Drug Discovery Today*, *17*(11/12), 608–614.

Henni-Silhadi, W., Deyme, M., Ruiz de Hoyos, M., LeCerf, D., Picton, L., & Rosilio, V. (2008). Influence of alkyl chains length on the conformation and solubilization properties of amphiphilic carboxymethylpullulans. *Colloid and Polymer Science*, *286*, 1299–1305.

Hou, X., Liu, B., Deng, X., Zhang, B., & Yan, J. (2007). Monodisperse polystyrene microspheres by dispersion copolymerization of styrene and other vinyl comonomers: Characterization and protein adsorption properties. *Journal of Biomedical Materials Research A*, *83*(2), 280–289.

Jung, T., Noneberg, R., Hungerer, K. D., & Kissel, T. (2002). Tetanus toxoid microspheres consisting of biodegradable poly(lactide-co-glycolide) and ABA-triblock-copolymers: Immune response in mice. *International Journal of Pharmaceutics*, *234*, 75–90.

Kafshgari, M. H., Khorram, M., Khodadoost, M., & Khavari, S. (2011). Reinforcement of chitosan nanoparticles obtained by an ionic cross-linking process. *Iranian Polymer Journal*, *20*(5), 445–456.

Kang, M. L., Cho, C. S., & Yoo, H. S. (2009). Application of chitosan microspheres for nasal delivery of vaccines. *Biotechnology Advances*, *27*, 857–865.

Laferriere, C., Ravenscroft, N., Wilson, S., Combrink, J., Gordon, L., & Petre, J. (2011). Experimental design to optimize an Haemophilus influenzae type b conjugate vaccine made with hydrazide-derivatized tetanus toxoid. *Glycoconjugate Journal*, *28*, 463–472.

Larroche, C. (2004). Tetanus: A review on always severe infection disease. *Antibiotiques*, *6*(1), 23–28.

Lowry, H. H., Rosebrough, N. J., Lewis Farr, A., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Mahore, J. G., Wadher, K. J., Umekar, M. J., & Bhoyar, P. K. (2010). Ion exchange resins: pharmaceutical applications and recent advancement. *International Journal of Pharmaceutical Sciences Review and Research*, *1*(2), 8–13.

Mocanu, G., Mihai, D., Moscovici, M., Picton, L., & Lecerf, D. (2009). Curdlan microspheres. Synthesis, characterization and interaction with proteins (enzymes, vaccines). *International Journal of Biological Macromolecules*, *44*, 215–221.

Mocanu, G., Souguir, Z., Picton, L., & Lecerf, D. (2012). Multi-responsive carboxymethyl polysaccharide crosslinked hydrogels containing Jeffamine side-chains. *Carbohydrate Polymers*, *89*, 578–585.

Nasratun, M., Hasrul, A. S., Sureena, A., Narul Aini, M. A., Ruwaida, A. R., Shalida, M. S., et al. (2010). Immobilization of lipase from *Candida rugosa* on chitosan beads for transesterification reaction. *Journal of Applied Sciences*, *10*(21), 2701–2704.

Nichifor, M., Baille, W. E., Cristea, D., Zhu, X. X., & Carpov, A. (2001). Bile acid sequestrants based on cationic dextran hydrogel microspheres. 2. Influence of the length of alkyl substituents at the amino groups of the sorbents on the sorption of bile salts. *Journal of Pharmaceutical Sciences*, *90*, 681–689.

Nichifor, M., Cristea, D., & Carpov, A. (2000). Bile acid sequestrants based on cationic dextran hydrogel microspheres. 1. Influence of the chemical structure of functional groups on the sodium cholate sorption. *International Journal of Biological Macromolecules*, *28*, 15–21.

Nichifor, M., Cristea, D., Mocanu, G., & Carpov, A. (1998). Aminated polysaccharides as bile acid sorbents: In vitro study. *Journal of Biomaterials Sciences Polymer Edition*, *9*, 519–534.

Nichifor, M., Stanciu, M. C., & Simionescu, B. C. (2010). New cationic hydrophilic and amphiphilic polysaccharides synthesized by one pot procedure. *Carbohydrate Polymers*, *82*, 965–975.

Peppas, N. A., & Mikos, A. G. (1986). Preparation methods and structure of hydrogels. In N. A. Peppas (Ed.), *Hydrogels in medicine and pharmacy* (Vol. 1) (pp. 1–27). Boca Raton, FL: CRC Press.

Palla, C. A., Pacheco, C., & Carrín, M. E. (2011). Preparation and modification of chitosan particles for Rhizomucor miehei lipase immobilization. *Biochemical Engineering Journal*, *55*, 199–207.

Peppas, N. A., Bures, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics*, *50*, 27–46.

Pepper, K., Reichenberg, D., & Hale, D. K. (1952). Properties of ion-exchange in relation to their structure. Part IV. Swelling and shrinkage of sulphonated polystyrenes of different cross-linking. *Journal of Chemical Society*, 3129–3136.

Rodriguez-Felix, D. E., Castillo-Ortega, M. M., Real-Felix, D., Romero-Garcia, J., Ledezma-Perez, A. S., & Rodriguez-Felix, F. (2011). Synthesis and swelling properties of pH and temperature-sensitive interpenetrating polymer network composed of polyacrylamide and poly(γ-glutamic acid). *Journal of Applied Polymer Sciences*, *119*, 3531–3537.

San Juan, A., Hlawaty, H., Chaubet, F., Letourneur, D., & Feldman, L. J. (2007). Cationized pullulan 3D matrices as new materials for gene transfer. *Journal of Biomedical Materials Research*, *82A*(2), 354–362.

Tanford, C. (1980). *The hydrophobic effect. Formation of micelles and biological membranes* (2nd ed.). New York: Wiley.

Vila, A., Sánchez, A., Janes, K., Behrens, I., Kissel, T., Vila Jato, J. L., et al. (2004). Low molecular weight chitosan nanoparticles as new carriers for nasal vaccine delivery in mice. *European Journal of Pharmaceutics and Biopharmaceutics*, *57*, 123–131.

Wei, W., Yuan, L., Hu, G., Wang, L. Y., Wu, J., Hu, X., et al. (2008). Monodisperse chitosan microspheres with interesting structures for protein drug delivery. *Advanced Materials*, *20*, 2292–2296.

Yoshida, H., Fujita, K., & Kudo, K. (1999). Fabrication of hard dextran DEAE: Adsorption equilibria of BSA. *Adsorption*, *5*, 63–71.

Zhang, H. L., Wu, S. H., Tao, Y., Zang, L. Q., & Su, Z. Q. (2010). Preparation and characterization of water-soluble chitosan nanoparticles as protein delivery system. *Journal of Nanomaterials*, *2010*. Article ID 898910, 5 pages doi:10.1155/2010/898910.