CHARACTERISTICS OF ASCORBIC ACID RELEASE FROM TPP-CROSSLINKED CHITOSAN/ALGINATE POLYELECTROLYTE COMPLEX MEMBRANES

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Abstract

Chitosan/alginate polyelectrolyte complex membranes (Ch/Alg) additionally cross-linked with tripolyphosphate (TPP) and containing ascorbic acid (AA) were prepared. The dynamic swelling behaviour of Ch/Alg/TPP and ascorbic acid release from the membrane were characterised in different buffer solutions. It has been found that the pH of the buffer solution affects the swelling and release behaviour of AA. Ascorbic acid release, observed over a period of 360 min, exhibited a biphasic pattern, characterised by a fast initial burst release, followed by a slow, sustained release. Different mathematical models were used to study the kinetics and transport mechanism of AA from Ch/Alg/TPP hydrogels. Drug release data were fitted to the zero order kinetic model and first order kinetic model. To characterise the drug mechanism, the release data were fitted to the Higuchi and Korsmeyer-Peppas equations. The initial burst AA release followed zero order kinetics and was quasi-Fickian in nature. The second step of AA release followed first order kinetics.

Keywords: *chitosan, alginate, polyelectrolyte complex, tripolyphosphate, hydrogel, drug release*

Received: 14.03.2018
Accepted: 04.06.2018

1. Introduction

Hydrogels are three-dimensional and hydrophilic polymer networks capable of swelling in water or biological fluids which retain a large amount of fluids in the swollen state (usually more than 20% of the total weight) [1]. These materials are generally divided into two classes depending on the nature of their network: chemical and physical hydrogels [2]. An interesting group of physical hydrogels is formed of polyelectrolyte complex hydrogels. Polyelectrolyte complexes (PECs) are defined as materials formed by combining oppositely charged polyelectrolytes together via ionic interactions [3]. Complexes composed of chitosan (cationic polyelectrolyte) and sodium alginate (anionic polyelectrolyte) are an example of such materials.

Chitosan (Ch) is a copolymer consisting of two residues: N-acetylglucosamine (2-acetamido-2-deoxy-β-D-glucopyranose) and glucosamine (2-amino-2-deoxy-β-glucopyranose). This cationic linear polysaccharide is usually obtained by the alkaline deacetylation of crustacean chitin from crab and shrimp shell wastes [4]. Alginates (Alg) are linear anionic polysaccharides, mainly extracted from three species of brown algae. They are composed of (1→4)-linked β-D-mannuronic acid (M) and α-L-gluluronic acid (G) residues arranged in a pattern of homopolymeric M blocks, homopolymeric G blocks and heteropolymeric sequentially alternating MG blocks along the chain [5].

Chitosan forms polyelectrolyte complexes with various synthetic and natural polyelectrolytes, including alginate [6-8]. Nowadays, polyelectrolyte complexes are growing in importance as materials in drug release technology, as materials for the encapsulation of various substances and as membranes in different industrial processes. PECs are materials of improved structural strength, mechanical stability and higher stability in swelling media of different pH. However, these properties are unsatisfactory; thus, PEC complexes, including chitosan/alginate (Ch/Alg) polyelectrolyte complexes, have certain limitations for use in different areas, including drug delivery [6, 9]. These limitations can be overcome by chemical or physical modification. For example, the crosslinking of PEC with glutaraldehyde, tripolyphosphate (TPP) or genipine has been proven to be a convenient and effective method for improving the chemical and physical properties of PECs [10-12].

In this paper, we prepared a TPP-cross-linked chitosan/alginate membrane (Ch/Alg/TPP), loaded with ascorbic acid (AA), as schematically shown in Figure 1. We characterised ascorbic acid release from that membrane into media of different pH. The main objective of the work was to study the kinetics and transport mechanism of ascorbic acid from Ch/Alg/TPP hydrogels.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan (Ch) from crab shells, sodium alginate (Alg) and pentasodium tripolyphosphate (TPP) of analytical grade were purchased from Sigma–Aldrich (Germany). Reagents for chitosan and alginate characterisation, the preparation of polymer films and buffer solutions (acetic acid, sodium acetate, hydrochloric acid, sodium hydroxide), Tris buffer, Hanks' balanced salt solution (HBSS) and model drugs (L-ascorbic acid, AA) were of analytical grade (POCh, Poland or Sigma-Aldrich, Germany). Deionised water was used as a solvent.

2.2. Chitosan and sodium alginate characterization

The viscosity average molecular weight (M_v) of Ch and Alg was equal to $(730\pm16)\times10^3$ g mol^{-1} and $(102\pm16)\times10^3$ g mol^{-1}, respectively. The degree of deacetylation (*DDA*) of Ch, determined by potentiometric titration method, was equal to (75.7±3.9)%. The details of M_v and *DDA* determinations were described elsewhere [13].

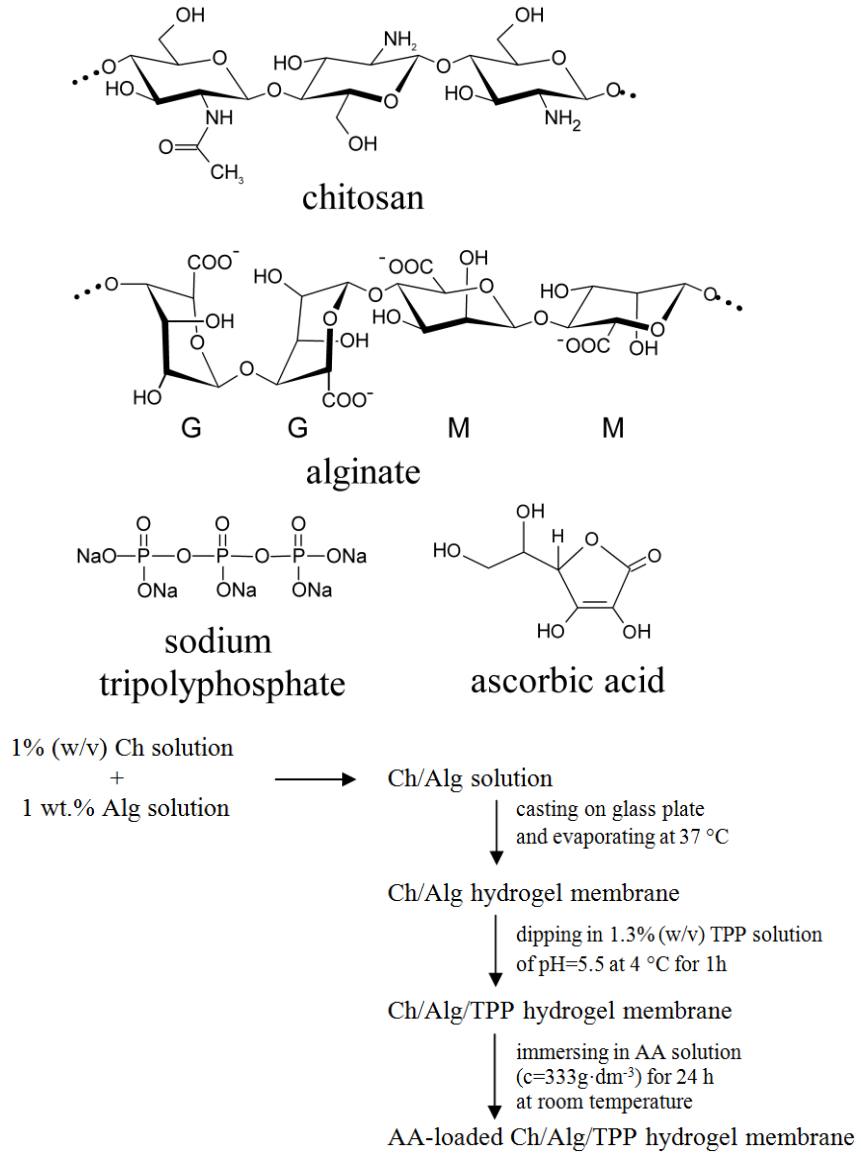

Figure 1. Chemical structure of components of drug-loaded hydrogel membranes and the schematic representation of the preparation of this membrane.

2.3. Preparation of chitosan, chitosan/alginate and chitosan/alginate/tripolyphosphate membranes

The chitosan/alginate/tripolyphosphate (Ch/Alg/TPP) membrane was prepared by the method schematically presented in Figure 1. The chitosan/alginate (Ch/Alg) membrane was obtained according to a previously described procedure [13]. Briefly, to obtain this membrane, 1% (w/v) Ch solution in 2% (v/w) acetic acid was mixed with 1 wt.% Alg solution in water in the weight ratio 3:1. The resulting solution was cast on a clean glass plate and evaporated to dryness in an oven at 37ºC. The obtained Ch/Alg membrane was additionally washed with distilled water. The Ch/Alg/TPP membrane was prepared by dipping the Ch/Alg membrane (ca. 100 mg) into 100 mL of 1.3% (w/v) aqueous TPP

DOI: 10.15259/PCACD.23.07

solution for 1 h. The applied cross-linking conditions were as follows: temperature T = 4°C, and pH of TPP solution 5.5 (initial TPP solution acidified with hydrochloric acid). The obtained Ch/Alg/TPP membrane was thoroughly washed with distilled water, air-dried at 37°C and then under vacuum at 60°C for 24 h, and stored in a desiccator over P_2O_5 at ambient temperature.

2.4. FTIR measurements

FTIR-ATR spectra of Ch/Alg/TPP and AA-loaded Ch/Alg/TPP membranes and AA powder were recorded using a Genesis II FTIR spectrometer (Mattson, USA) equipped in an ATR device (MiracleTM PIKE Technologies) with zinc-selenide crystals. All spectra were recorded in an absorption mode from 400–4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans.

2.5. Preparation of ascorbic acid-loaded membranes

The Ch/Alg/TPP membrane was loaded with L-ascorbic acid (AA) by immersing in an AA-saturated aqueous solution (333 $g \cdot dm^{-3}$) at room temperature for 24 h, until equilibrium was reached. Then, the membrane was washed with water and dried as described above.

2.6. Swelling studies in buffer solutions

In order to study the swelling behaviour, dry hydrogel membrane samples were immersed in swelling medium (buffer solutions) at 37.0±0.1°C. At certain time intervals, the samples were removed from the solution, blotted with filter paper to eliminate excess solvent from the surface and weighed. The measurements were continued until a constant weight was reached for each sample. The following buffered solutions were used: 10 mM acetic acid/sodium acetate solution (pH 3.5, I=0.145 M), 10 mM Tris buffer solution (pH 7.4, I=0.145 M), and Hanks′ balanced salt solution (HBSS, pH 7.4, I=0.17 M). The ionic strength of acetate and Tris buffer solutions was carefully adjusted to 0.145 M by adding an appropriate amount of sodium chloride. The ionic strength of HBSS was calculated from its composition and was equal to 0.17 M.

The swelling ratio (*S*) at different times was expressed as gram of solvent sorbed per gram of dry membrane and was calculated using Eq. (1):

$$S = \frac{M_{wet} - M_{dry}}{M_{dry}} \quad (1)$$

where M_{wet} and M_{dry} are the masses of the membrane sample swollen in solution and in the completely dry state, respectively. All experiments were performed at least three times for each membrane and the mean value of *S* at any time *t* was evaluated. Standard deviations were less than 5%.

2.7. *In vitro* drug release studies

To investigate the release behaviour of ascorbic acid from the Ch/Alg/TPP membrane, dried AA-loaded membrane specimens (ca. 150 mg) were immersed in 70 cm^3 of release medium (acetate buffer (pH 3.5), Tris buffer (pH 7.4) and HBSS (pH 7.4)) at 37±0.1°C under continuous stirring. Media with a pH=7.4 were chosen to mimic the environment of the human body (blood plasma and small intestine fluid). HBSS buffer resembles the pH and ion concentration of the small intestinal fluid. At the predetermined time interval, 0.5 cm^3 release medium was taken out and an analogous volume of the fresh medium was added to the flask to maintain the unchanged volume. The amount of AA released from the membrane was determined at 245 nm (acetate buffer) and 266 nm (Tris buffer and HBSS) using a Shimadzu UV-2101PC

Spectrophotometer. The cumulative release (Q%) of ascorbic acid from the membrane was calculated using the following equation:

$$Q(\%) = \frac{M_t}{M_0} \cdot 100 \quad (2)$$

where M_t is the cumulative amount of drug released at time t and M_0 is the total amount of drug in the membrane.

2.8. Mathematical models of drug release

Different mathematical models of drug release have been used to fit the *in vitro* release of drugs from drug delivery systems [14]. Some of the most important models are: the diffusion model, the zero order kinetic model, the first order kinetic model, the Higuchi model and the Korsmeyer-Peppas model (the power law). In order to study the kinetics and transport mechanism of ascorbic acid release from Ch/Alg/TPP hydrogels in buffer solutions, four mathematical models were used.

Model I is based on the zero order kinetic equation. It describes the system where the release rate of the drug is independent of its concentration. The drug release is expressed by the following equation:

$$W_0 - W_t = k_0 \cdot t \quad (3)$$

where W_0 is the initial amount of drug in the drug formulation, W_t is the amount of drug in the drug formulation at time t, and k_0 is the proportionality constant. After dividing Equation 3 by W_0, a simplified equation can be written as:

$$f_t = k_0 \cdot t \quad (4)$$

where $f_t = 1 - (W_t/W_0)$; f_t represents the fraction of drug released at time t and k_0 is the apparent dissolution rate constant or zero order release constant.
The zero order model, in a simple way, can be also described by Equation 5:

$$M_t = M_0 + k_0 \cdot t \quad (5)$$

where M_t is the cumulative amount of drug released at time t, M_0 is the initial amount of drug in the solution (most often $M_0 = 0$) and k_0 is the zero order release constant.

Model II is based on the first order kinetic equation. The drug release which follows the first order kinetics can be expressed by Equation 6 or 7.

$$M_t = M_0 \cdot e^{-k_1 \cdot t} \quad (6)$$

$$\ln M_t = \ln M_0 - k_1 \cdot t \quad (7)$$

where M_t is the amount of drug released at time t and M_0 is the initial amount of drug in the solution and k_1 is the first order release constant.

Model III is based on the Higuchi equation:

$$\frac{M_t}{M_\infty} = k_H \cdot t^{\frac{1}{2}} \quad (8)$$

where M_t is the cumulative amount of drug released at time t, M_∞ is the amount of drug released after infinite time, and k_H is the Higuchi rate constant.

Model IV was described by the Korsmeyer-Peppas equation:

$$\frac{M_t}{M_\infty} = k_{KP} \cdot t^n \quad (9)$$

where M_t is the cumulative amount of drug released at time *t*, M_∞ is the amount of drug released after infinite time, k_{KP} is the constant incorporating structural and geometric characteristics of the device, and *n* is the release exponent that can be related to the drug transport mechanism (Table 1). This equation is applicable for small values of *t* or short times. The portion of release curve, where M_t/M_∞<0.6, should be used to determine the exponent *n*. Moreover, Equation 9 can only be used when release occurs in a one-dimensional way (the system length to thickness ratio should be at least 10). If *n*=0.5, then the Korsmeyer-Peppas model reduces to the Higuchi equation.

Table 1. Release exponent *n* of the Korsmeyer-Peppas equation and drug release mechanism from polymeric films [14, 15]

Release exponent *n*	Drug transport mechanism	Rate as a function of time
$n < 0.5$	Quasi-Fickian diffusion	
$n = 0.5$	Fickian diffusion	$t^{-0.5}$
$0.5 < n < 1.0$	Non-Fickian (anomalous) transport	t^{n-1}
$n = 1.0$	Case II transport	Zero order release
$n > 1.0$	Super case II transport	t^{n-1}

3. Results and Discussion

3.1. FTIR studies of AA-loaded membranes

The FTIR spectra of Ch/Alg/TPP (Figure 2A), AA (Figure 2B) and AA-loaded Ch/Alg/TPP hydrogel (Figure 2C) have been used to verify the loading of ascorbic acid into TPP-cross-linked chitosan/alginate polyelectrolyte complex membrane.

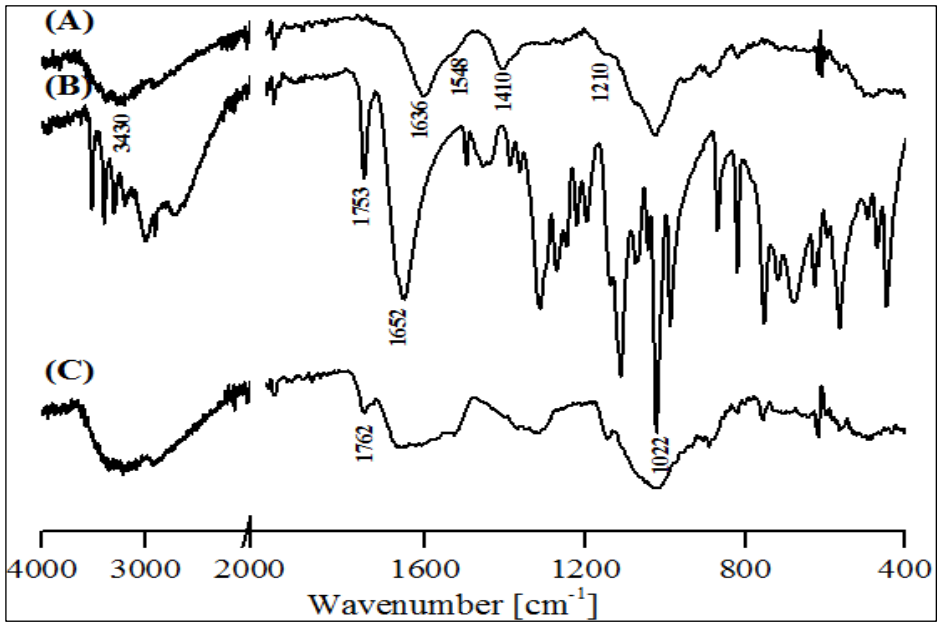

Figure 2. FTIR spectra of Ch/Alg/TPP (A), AA (B), AA-loaded Ch/Alg/TPP (C).

The FTIR spectrum of Ch/Alg/TPP shows the band at 3430 cm^{-1} with a shoulder at 3350 cm^{-1} (O-H and N-H stretching vibrations), complex band with two maxima at 1636 cm^{-1} and 1548 cm^{-1} (asymmetric and symmetric N-H deformation vibrations in $–NH_3^+$, symmetric COO stretching vibrations, amide I and amide II bands), the band at 1210 cm^{-1} (asymmetric POO stretching vibrations) and the band at 1410 cm^{-1} (asymmetric COO stretching vibrations [13, 16]. The FTIR data indicate that in the Ch/Alg/TPP membrane, carboxylate ions of alginate chains and phosphate ions of cross-linking agents interact with $–NH_3^+$ ions of chitosan chains and a three-dimensional structure is formed.

The main absorption bands of AA are seen at 1753 cm^{-1} (C=O stretching vibrations), 1652 cm^{-1} (C=C stretching vibrations) and 1022 cm^{-1} (C-O-C stretching vibrations) [16]. The TPP-cross-linked Ch/Alg hydrogel, after loading with ascorbic acid, shows an additional characteristic absorption band at 1762 cm^{-1} (C=O stretching vibrations). The appearance of a characteristic peak in the AA-loaded membrane has confirmed the loading of ascorbic acid into TPP-cross-linked PEC.

3.2. Swelling studies

The rate and degree of swelling are very important parameters that affect the release patterns of solvents and drugs from polymeric hydrogel networks. Therefore, we studied the kinetics of swelling of Ch/Alg/TPP membranes in three different release media.

Figure 3 presents the dynamic swelling data for the membrane in buffered aqueous solutions at 37°C. Curves A and C in Figure 3 are similar; at the beginning, the swelling is very fast and then slower, finally reaching the equilibrium maximum swelling ratio. Swelling curve B, corresponding to Ch/Alg/TPP in Tris buffer (pH 7.4, I=0.145M), exhibits a remarkable "overshooting effect". The membrane swells to a maximum S value and then gradually de-swells until S_{eq} is reached.

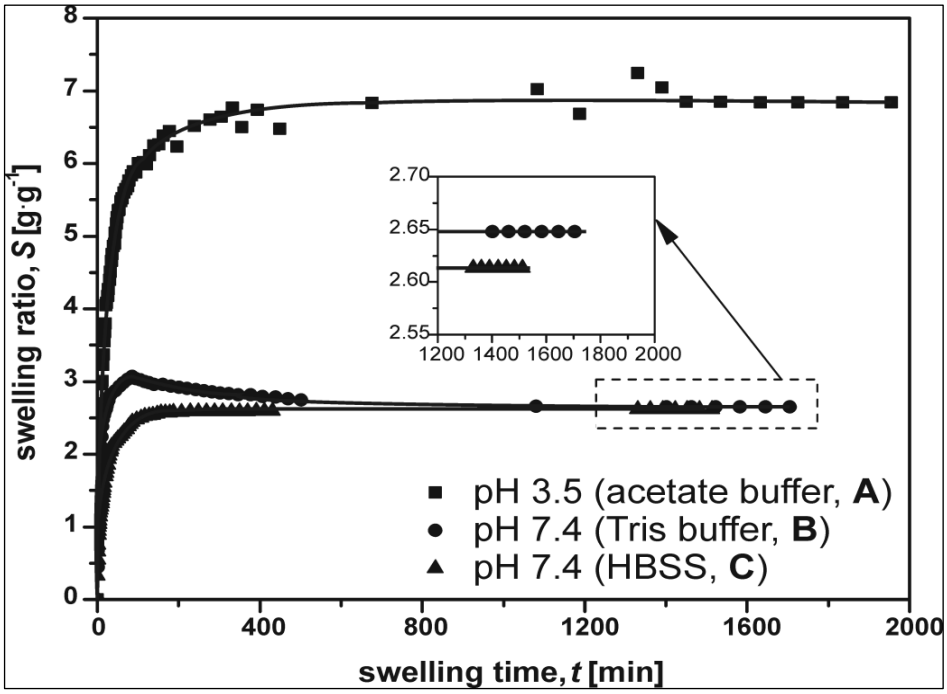

Figure 3. Swelling curves of Ch/Alg/TPP membrane in buffer solutions at 37 ºC.

The results for buffers of different pH (3.5 and 7.4) and the same ionic strength indicate that the hydrogel membrane shows a pH responsive swelling. S_{eq} decreases with increasing solution pH. The results for buffers of different ionic strength and the same pH indicate that swelling depends on the ionic strength of the swelling medium. Ionic interactions between ionised amine groups of chitosan and carboxyl or phosphate groups of alginate and tripolyphosphate are responsible for pH-dependent and/or pI-dependent swelling or de-swelling of the hydrogel membrane.

3.3. *In vitro* AA release studies

3.3.1. Characteristics of AA release profiles in buffer solutions

To investigate the pH-responsive release behaviour of the Ch/Alg/TPP hydrogel membrane, the ascorbic acid-loaded membrane specimens were placed in buffers of pH=3.5 and pH=7.4. The release profiles, characterised by the cumulative amount of ascorbic acid released from Ch/Alg/TPP membrane as a function of time, are presented in Figure 4. It can be seen that AA release exhibited a biphasic pattern characterised by a fast initial burst during the first 6–8 min, followed by a slow, sustained release. The cumulative amount of ascorbic acid released from the membrane over a period of 360 min was 98% in acetate buffer (pH=3.5), 65% in HBSS buffer (pH=7.4) and 56% in Tris buffer (pH=7.4). Burst release has been associated with different physical, chemical, and processing parameters [17].

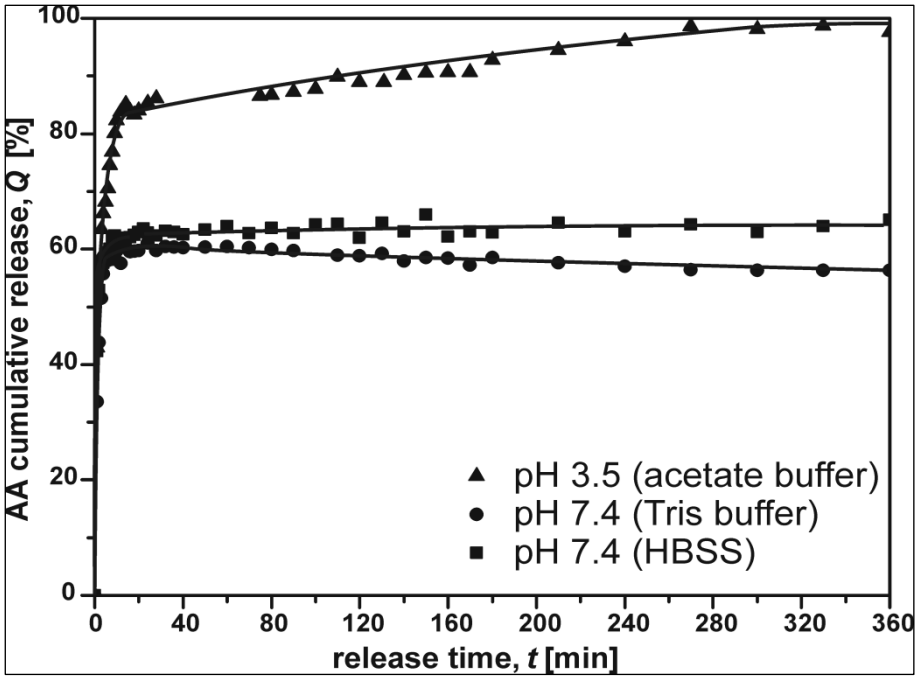

Figure 4. Release profiles of AA from Ch/Alg/TPP membrane in buffer solutions at 37ºC.

The burst or rapid release step on the release curve was observed earlier in many of the controlled release formulations. Because burst release happens in a very short period

of time compared to the entire release process, it has been ignored in most mathematical models. There are only a few papers where burst release was characterised in detail [18-20].

The results presented in Figure 4 indicate that the pH of the release medium affected the release of ascorbic acid from modified chitosan membranes. The Ch/Alg/TPP hydrogel membrane showed a pH-responsive release behaviour that was due to the change in the mesh size of the hydrogel network. The data presented above in Figure 3 indicate that the swelling of the Ch/Alg/TPP membrane depends on pH and is therefore an example of a pH-responsive hydrogel. Hydrogels are swelling-controlled drug delivery systems. Such systems consist of polymeric materials with a three-dimensionally cross-linked network; the mesh size plays a central role in controlling the drug release behaviour. Small changes in pH can result in a significant change in the mesh size of the polymeric networks [21]. When the pH of the release medium decreased over the pK_a of chitosan, electrostatic repulsion forces between protonated amine groups brought an increase in the mesh size of the hydrogel and an abrupt release of ascorbic acid from the hydrogel membrane.

pH-responsive drug release was observed earlier for AA release from microparticles of alginate coated with chitosan [22], vitamin C release from TTP-cross-linked nanoparticles of chitosan [23] and for another drug release from ionically cross-linked chitosan films and beads [24, 25]. Some differences in cumulative drug release found for two buffers of pH=7.4 most probably result from their differences in ionic strength and chemical composition. The ionic strength of the HBSS buffer used, calculated from its composition, was equal to 0.17 M. It has been found that pH, ionic strength and media composition can influence the drug release properties of hydrogels. In general, drug release increases with decreasing pH and increasing ionic strength [21, 24, 25].

The burst effect is often regarded as a negative effect when considering the long-term drug delivery system. However, the burst release may be the optimal mechanism of delivery in several cases. Many drugs need to be administered at varying rates, for example those used at the beginning of wound treatment. Food companies also have an interest in the development of burst release systems. Coatings are used to protect flavours and aromas during processing and storage, but they must allow the rapid release of these substances when the products are consumed. Thus, it can be concluded that the pH-sensitive release behaviour of the Ch/Alg/TPP membrane can be used as an on-off switch for special ingredients, triggered by an external pH change.

3.3.2. Kinetics and mechanism of AA release in buffer solutions

In order to study the kinetics and transport mechanism of ascorbic acid from Ch/Alg/TPP hydrogels in buffer solutions, four mathematical models were used. Drug release data were fitted to the zero order kinetic model and first order kinetic model. To characterise the drug mechanism, the release data were fitted to the Higuchi and Korsmeyer-Peppas equations.

The kinetic rate constants k_0, k_1, k_H, k_{KP}, release exponent n and values of R^2 for each model were calculated by linear least-squares regression analysis and are presented in Table 2. The most appropriate model was selected based on correlation coefficient values. The higher the value of R^2, the better the model adjusts the data and the better the model describes the drug kinetics and transport mechanism. For fitting the experimental data to the Higuchi and Korsmeyer-Peppas models, only the points within 10–60% of cumulative drug amounts were used. The Korsmeyer-Peppas model is valid up to 60% cumulative drug released.

Table 2. Kinetic parameters of ascorbic acid release from Ch/Alg/TPP membranes

Buffer	Steps on curve release	zero-order		first-order		Higuchi		Korsmeyer-Peppas		
		k_0 (% min^{-1})	R^2	k_1 (min^{-1})	R^2	k_H (%$min^{-1/2}$)	R^2	k_{KP} (%min^{-n})	n	R^2
Acetate buffer pH 3.5	1st step *)	3.653	0.896	0.025	0.823	0.292	0.904	0.442	0.319	0.955
	2nd step*)	0.025	0.823	0.044	0.956	-	-	-	-	-
Tris buffer pH 7.4	1st step*)	7.431	0.968	0.073	0.942	0.224	0.979	0.552	0.373	0.997
	2nd step*)	0.0163	0.884	0.002	0.887	-	-	-	-	
HBSS pH 7.4	1st step*)	3.835	0.816	0.033	0.785	0.494	0.868	0.625	0.205	0.935
	2nd step*)	**)	**)	**)	**)	-	-	-	-	-

*) 1st step – burst release, 2nd step – slow release
**) results encumbered with high errors

Analysis of the data presented in Table 2 indicates that the initial burst release of AA from the Ch/Alg/TPP membrane in all buffer solutions followed zero order kinetics. Therefore, the AA release from that membrane was independent of the ascorbic acid concentration in the hydrogel network.

R^2 values calculated for the Higuchi and Korsmeyer-Peppas mathematical models suggest that the Korsmeyer-Peppas model is more suitable for the release of AA from the Ch/Alg/TPP membrane in buffer solutions. The Korsmeyer-Peppas model describes an initial 60% AA release very well, with high correlation coefficients ($R^2 \geq 0.93$). Parameter n was equal to 0.319 for AA release from the membrane in acetate buffer solution, 0.373 for the same sample in Tris buffer solution and 0.205 for the sample in HBSS. All n values were smaller than 0.5. A value of $n < 0.5$ indicates a quasi-Fickian mechanism of AA release from the hydrogel matrix (Table 1). The diffusion exponent $n < 0.5$ was observed earlier for release of different drugs from various hydrogels. Recently, a value of $n = 0.30$ was obtained for the release of vancomycin from chitosan/alginate polyelectrolyte microparticles [26].

4. Conclusions

Ascorbic acid release from a TPP-cross-linked Ch/Alg hydrogel membrane was studied in three buffer solutions: acetate buffer (pH 3.5, I=0.145 M), Tris buffer (pH 7.4, I=0.145 M), and HBSS (pH 7.4, I=0.17 M). It has been found that pH, ionic strength and/or the composition of buffers affect the swelling and release behaviour of AA. Ascorbic acid was released from the hydrogel membrane in two different steps, i.e. as a burst release and as a sustained release. The initial burst release followed zero order kinetics, but the second step followed first order kinetics. Parameter n of the Korsmeyer-Peppas equation was dependent of the pH of release medium and was smaller than 0.5. Thus, the release mechanism of AA from the Ch/Alg/TPP membrane in the studied buffer solutions was found to be quasi-Fickian.

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