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International Journal of Pharmaceutics 297 (2005) 22–29
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Cross-linked high amylose starch derivatives for drug release III. Diffusion properties

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Received 16 November 2004; received in revised form 7 February 2005; accepted 7 February 2005

Abstract

Acetate (Ac-), aminoethyl (AE-) and carboxymethyl (CM-) derivatives of cross-linked high amylose starch (HASCL-6)[1] were previously shown to control the release of drugs over 20 h from highly loaded (up to 60% drug) monolithic tablets. This report presents a diffusion analysis, aimed to facilitate a better understanding of the mechanisms involved in the control of the drug release from these hydrogels. The diffusion was found to depend on the molecular weight of the diffusant, whereas the partition coefficient depended on the affinities of the diffusant for the polymers and for the dissolution media via attractive or repulsive ionic interactions. The diffusion was also affected by the swelling of CM-HASCL-6, which, unexpectedly, increased with the decrease of the ionic strength. This diffusion analysis completes the swelling studies of HASCL-6 and of its derivatives, allowing the prediction of release kinetics of various active agents.

Keywords: Diffusion; Partition coefficients; Permeability; Hydrogels; High amylose starch derivatives; Controlled release mechanisms

1. Introduction

Cross-linked high amylose starch (HASCL) was introduced in the nineties as excipient (Contramid®) allowing drug controlled release over 18–24 h (Lenaerts et al., 1991; Mateescu et al., 1995). Partial substitution of the hydroxylic groups of HASCL-6 with ionic (carboxymethyl (CM-) and aminoethyl (AE-)) groups as well as with less polar (acetate (Ac-)) groups was shown to markedly increase the loading capacity of the tablet, from 20% for HASCL to 60% for HASCL derivatives (Mulhbacher et al., 2001). It was also shown that the carboxylic (CM-HASCL-6) and amino (AE-HASCL-6) derivatives were able to modulate the release of drug by ionic interactions whereas acetate groups can modulate the release by enhancing hydrophobic character of the matrix (Mulhbacher

Abbreviations: CM-HASCL-6, carboxymethyl high amylose starch cross-linked 6; AE-HASCL-6, aminoethyl high amylose starch cross-linked 6; Ac-HASCL-6, acetate high amylose starch cross-linked 6

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[1] Cross-linking degree (conventionally expressed in grams of bifunctional agent used to cross-link 100 g of polymer).

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doi:10.1016/j.ijpharm.2005.02.033

et al., 2001). Previous studies have showed the important role of hydroxylic groups and of hydrogen bonding (Dumoulin et al., 1998) in the organization of the high amylose starch matrices (Ispas-Szabo et al., 2000), contributing thus to the control of the drug release. It was now of interest to see the impact of various polar and non-polar functional groups introduced by starch derivatization on the network organization and on the release mechanisms.

The drug release from hydrophilic matrices can be controlled by three main mechanisms: (i) polymer swelling and drug solubility (which will determine the duration of drug dissolution), (ii) drug diffusion and (iii) tablet erosion. The swelling properties of HASCL-6 and its derivatives were recently investigated (Mulhbacher et al., 2004) being found that the swelling of HASCL-6, Ac-HASCL-6 and AE-HASCL-6 tablets was not affected by the ionic strength nor by the pH, whereas the CM-HASCL-6 swelling depended on both ionic strength and pH of the dissolution medium. The second mechanism, based on drug diffusion, will depend of the physicochemical properties of the drug and the matrix. The diffusibility will be influenced by chemical and frictional effects (Gehrke et al., 1997). The chemical effect consists in attractive or repulsive forces between the drug and the polymeric matrix, which can be reflected by the partition coefficient. The frictional effect results from physical size exclusion characteristics of the matrix, which is reflected by the diffusion coefficient. The drug will diffuse through the free-water present inside the matrix or through the gel (composed of water linked to polymer).

Rahmouni et al. (2003) showed that diffusibility of drug through HASCL (cross-linked high amylose starch) was not significantly affected by the compression force, by the particle size and by hydrophilic additives, whereas hydrophobic additives increased the diffusion coefficient.

The aim of the present study is to evaluate the impact of the polymer derivatization with polar (carboxymethyl, aminoethyl) or non-polar (acetate) groups on diffusibility of drug. The effects of drug ionic charges and molecular weight on the diffusibility through the mentioned polymeric derivatives were also studied.

2. Materials and methods

High amylose starch (Hylon VII) was from National Starch (USA) and Kollidon was a gift from Mr. R. Salagan CerCan Corp./BASF (Canada). The tracers (benzylamine chlorohydrate, phenylacetic acid and acetaminophen), the derivatization agents (monochloroacetic acid, chloroethylamine hydrochloride, and acetic anhydride) and the other chemicals were reagent grade and used without further purification.

2.1. Synthesis of HAS derivatives

The syntheses were realized in conditions previously described by Mulhbacher et al. (2001).

2.1.1. Synthesis of CM-HASCL-6

An amount of 70 g of HAS was first suspended in 170 mL of water, completed with 235 mL of NaOH 1.5 M and then cross-linked by 3.5 mL of epichlorohydrin for 40 min at 50 °C (Mateescu et al., 1997). The reaction medium was then treated with 5 g of monochloroacetic acid at the same temperature for 1 h (Schell et al., 1978). When the reaction time was ended, the CM-HASCL-6 suspension slurry was neutralized with acetic acid at room temperature, then washed with an equal volume of acetone/water 85/15 (v/v), kept 20 min and filtered. The washing procedure (Mulhbacher et al., 2001) was repeated by resuspending and filtration two more times with 1/2 equivalent volume of acetone/water 70/30 (v/v) and then with 85/15 (v/v). The remaining wet gel was dried with pure acetone (1/2 equivalent of final reaction volume three times).

2.1.2. Synthesis of AE-HASCL-6

A similar procedure as for CM-HASCL-6 was followed. The same amount of HAS was first cross-linked and then treated with 86 g chloroethylamine hydrochloride (rapidly solubilized, just before the synthesis, in a minimal volume of water) for 2 h at 70 °C (Mateescu et al., 1988). The pH was maintained between 9 and 10 during the synthesis (by adding small amounts of 5 M NaOH).

2.1.3. Synthesis of Ac-HASCL-6

A similar procedure to that used for CM-HASCL-6 was followed for synthesis of acetate derivatives. A

batch of 70 g of HAS was treated with epichlorohydrin (Mateescu et al., 1997) and then with 15 mL of acetic anhydride (Jarowenko, 1986) at room temperature for 1 h.

2.2. Evaluation of substitution degree of derivatives

The carboxylic groups of CM-HASCL-6, were potentiometrically titrated (Corning pH-meter) with 0.1 M NaOH. The amine groups of AE-HASCL-6 were determined with trinitrobenzene sulfonic acid (TNBS) (Habeeb, 1966). The acetate groups of Ac-HASCL-6 were assayed by ^{1}H NMR, as previously described (Mulhbacher et al., 2001). The obtained substitution degrees were in the same order of magnitude: 0.092 mmol/g for CM-HASCL-6, 0.049 mmol/g for AE-HASCL-6 and 0.029 mmol/g for Ac-HASCL-6.

2.3. Diffusion test

Thin slabs (100 mg) were obtained by dry compression at 29.4 kN (2.3 T/cm^2) in a Carver hydraulic press using a punch of 13 mm diameter. Irrespective to the polymeric composition or drug loading, dry tablets (slabs) dimensions were of 12.72 ± 0.22 mm diameter and 1.23 ± 0.08 mm thickness. Tablets were first hydrated in the appropriate buffer for 24 h and then cut to fit in the hole (11.72 mm diameter) of the tablet support which was placed between the two cells of the diffusion device, as described by Peppas and Wright (1998). The donor cell was filled with the appropriate buffer containing the diffusant whereas acceptor cell was filled with the same buffer without diffusant. Each cell was magnetically stirred. The stirring was adjusted to ensure that the hydrated slab was the only parameter limiting the diffusion. The accumulation of the diffusant transferred through the swollen slab in the acceptor cell was measured at every 15 min, using a spectrophotometer Beckman DU 65 with an auto-sampling system. An appropriate absorbency wavelength was set for the detection of diffusant used.

2.3.1. The swelling effect on permeability

Citric acid/dibasic phosphate buffers having constant pH (pH 7), and different ionic strengths (0.1, 0.2 and 0.4 M) were used to swell the tablets (consisting in the polymer excipient only). Each tablet was first incubated alone at 37 °C, in 50 mL of the desired buffer for 24 h. Then the tablet was fixed in the tablet holder and submitted to the diffusion test using 30 mM acetaminophen as tracer (detection at 280 nm).

2.3.2. The ionic charge effect on permeability

The influence of ionic charge of tracer was evaluated on tablets incubated at 37 °C, in 50 mL of citric acid/dibasic phosphate buffers pH 7, 0.2 M ionic strength, for 24 h and then submitted to the diffusion test using benzylamine or phenylacetic acid (30 mM) as tracer (in both cases detection was at 258 nm).

2.3.3. The effect of diffusant molecular weight on the permeability

Each tablet was first incubated at 37 °C, in 50 mL of citric acid/dibasic phosphate buffers pH 7, 0.2 M ionic strength, for 24 h. Then, each of them was submitted to the diffusion test using as diffusant tracer various Kollidon (4%) of different molecular weight: 12 PF (2500 g/mol), 17 PF (9000 g/mol), 25 (31,000 g/mol), 30 (49,000 g/mol) and 90 F (1,250,000 g/mol) with detection at 250 nm.

2.4. Determination of partition coefficients

Tablets were weighed and incubated in 50 mL of citric acid/dibasic phosphate buffers pH 7, of appropriated ionic strength, for 24 h. Each tablet was then incubated in the buffer (50 mL) containing either: acetaminophen (30 mM), benzylamine (30 mM), phenylacetic acid (30 mM) or one of the Kollidon variants (4%), for 4 h. Then, in each case, tablet was taken, weighed again and incubated in tracer free buffer (50 mL). After 4 h, for each tablet the concentration of the tracer molecule (acetaminophen, benzylamine, phenylacetic acid or Kollidon) in the buffer was determined spectrophotometrically at the appropriate wavelength. The concentration of tracer molecule inside the tablet was calculated by reporting the measured concentration of liberated tracer in 50 mL of buffer to the volume of the swollen tablet. All incubations were at 37 °C, under stirring (50 rpm).

2.5. Data analysis and quantification

The permeability coefficient (P) was determined following the Flynn et al. (1974) equation:

$$\frac{dM}{dt} = \frac{P \times C_0}{h}$$

where M is the cumulative mass of diffusant per unit area of tablet, t is the time, P is the permeability coefficient, C_0 is the initial concentration of diffusant in the donor cell and h is the swollen tablet thickness.

The partition coefficient (K) of tracer molecule was evaluated following Gehrke et al. (1997) as:

$$K = \frac{C_g}{C_s}$$

where C_g is tracer concentration in tablet gel and C_s is tracer concentration in buffer solution.

The diffusion coefficient (D) was calculated from (Gehrke et al., 1997):

$$P = K \times D$$

2.6. Scanning electron microscopy

The CM-HASCL-6 tablet were allowed to swell in 0.2 or 0.4 M ionic strength buffer pH 7 for 24 h. The swollen tablet were frozen in liquid nitrogen and then lyophilized for 5 days. Lyophilized tablets were than covered with a thin gold layer and observed by a scanning electron microscopy (Hitachi S-2300 coupled with a Kevex image analyzer system).

3. Results and discussion

The ionic strength of the medium was previously shown (Mulhbacher et al., 2004) to modulate the swelling volume of CM-HASCL-6 matrix but not that of HASCL-6, AE-HASCL-6 and Ac-HASCL-6. In agreement with the previous data, for the last three matrices, the increase of the ionic strength exerted no significant effect on the permeability of the acetaminophen (Fig. 1a). A modification of the swelling volume is expected to influence the diffusion properties of drugs through the tablet. Thus, it was supposed that an increase of the swelling volume of the matrix would enhance the drug permeability due to a larger space between the matrix chains whereas decreasing swelling will reduce it. Unexpectedly, in the particular case of the CM-HASCL-6 a higher permeability of acetaminophen at increasing ionic strength (lower swelling) was found (Fig. 1a). Similar results (not shown) were obtained with acetylsalicylic acid as diffusant. A possible explanation of this behavior can be that the increase of the swelling volume could enhance the water regain (associated with the gel) and thus the local viscosity within the tablet, which will induce a decrease of permeability to acetaminophen. However, no viscosity differences were found among the swollen gel granules (not tablets) with the increase of ionic strength for all derivatives. Scanning electron microscopy evaluation (Fig. 2) showed a more compact structure and a decrease of pores size of tablets previously swollen at higher ionic strength. For instance, the size of the pores appears two times greater for the CM-

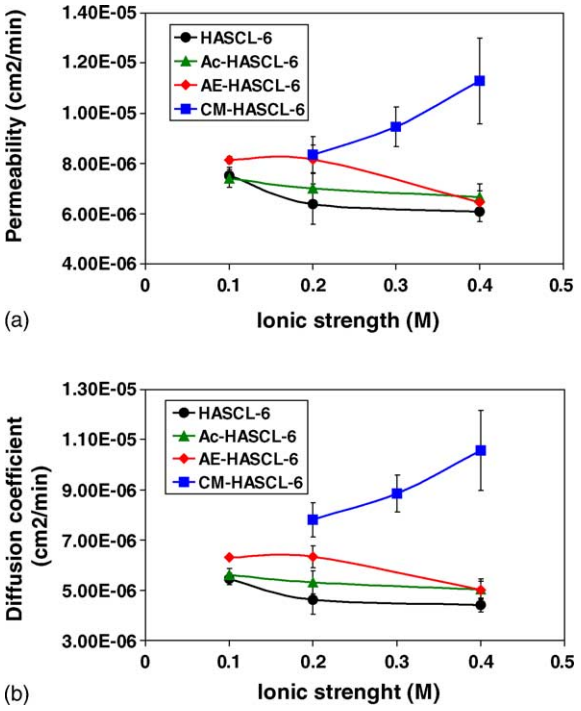

Fig. 1. Coefficients of permeability (a) and diffusion (b) of acetaminophen through swollen tablets of HASCL-6 and its derivatives in function of ionic strengths. Tablets (200 mg) were obtained by dry compression, and acetaminophen permeability was followed, in appropriate media at 37 °C, with a diffusion apparatus. Diffusion was calculated from the permeability and the partition coefficients.

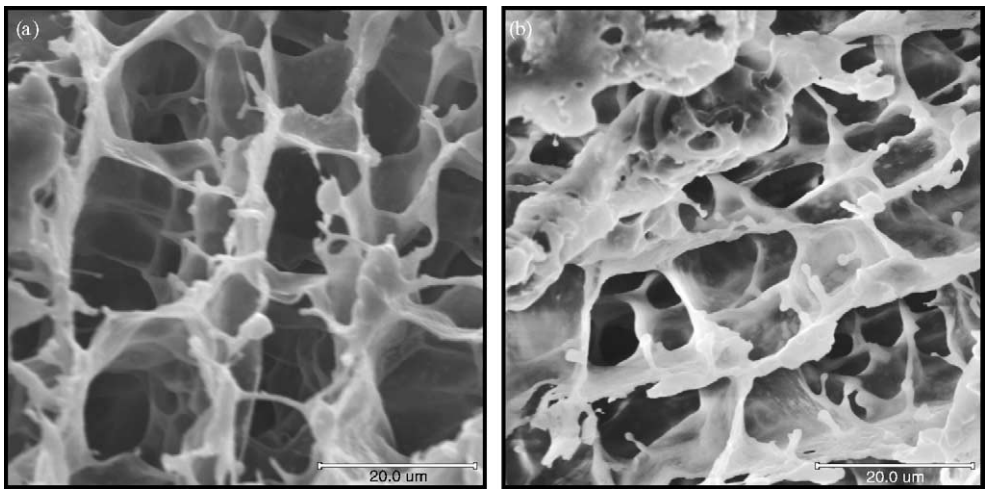

Fig. 2. Scanning electron microscopy (magnitude ×1500) of lyophilized CM-HASCL-6 previously swollen in 0.2 M (a) and 0.4 M (b) ionic strength buffer. The CM-HASCL-6 tablets were swollen each in the proper buffer, then frozen in nitrogen and lyophilized.

HASCL-6 previously swollen in 0.2 M than in 0.4 M ionic strength buffer. It is worth to note that, prior to lyophilization, the CM-HASCL-6 tablets swollen in 0.2 M ionic strength buffer were larger than that swollen in 0.4 M (Mulhbacher et al., 2004) whereas once lyophilized they have the same size irrespective to the buffer in which they were swollen. At low ionic strength, there is more water associated to the highly swollen hydrogel. Increase of ionic strength signifies more hydrated ions, higher fluidity and an easier diffusion of molecules (Fig. 3).

The diffusion coefficients were influenced in the same way as the permeability by the modification of swelling volume. Similarly, the diffusion coefficients of acetaminophen through HASCL-6, AE-HASCL-6 and Ac-HASCL-6 were not affected by ionic strength

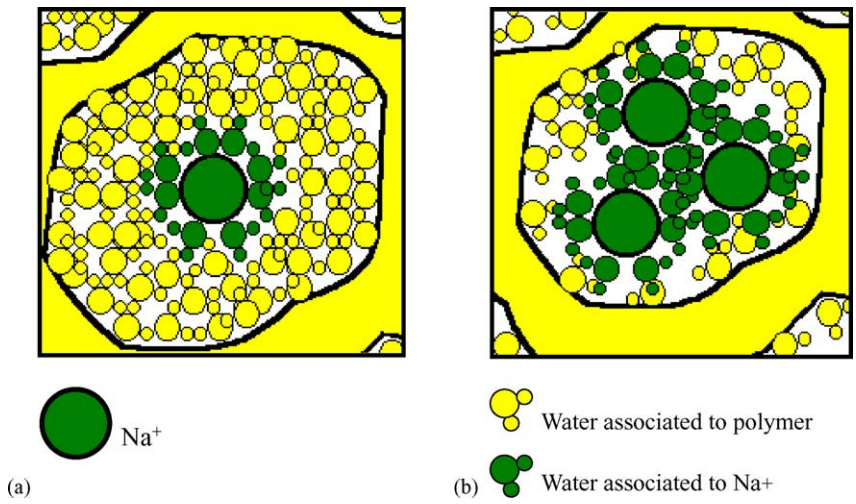

Fig. 3. Schematic representation of CM-HASCL-6 swollen in 0.2 M (a) and 0.4 M (b) ionic strength buffer. The increase of ionic strength (lesser amount of water available for the polymer hydration) decreases the swelling. It is supposed, as depicted, that an increase of ionic strength generates more ions within the pores retaining water by hydration and creating more fluidity than in case of lower ionic strength, allowing thus the molecule to diffuse more easily.

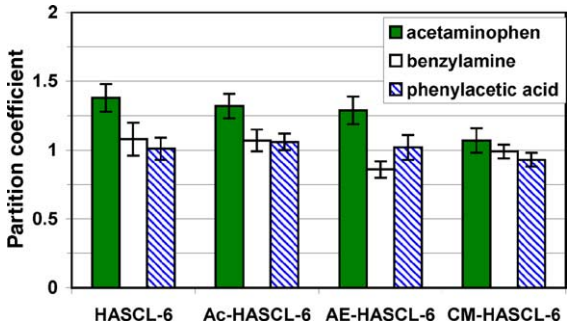

Fig. 4. Partition coefficients of acetaminophen, benzylamine and phenylacetic acid in HASCL-6 and derivatives matrices. Tablets (200 mg) were obtained by dry compression, and partition coefficients were determined after incubation in appropriate media (Section 2) at 37 °C, 50 rpm.

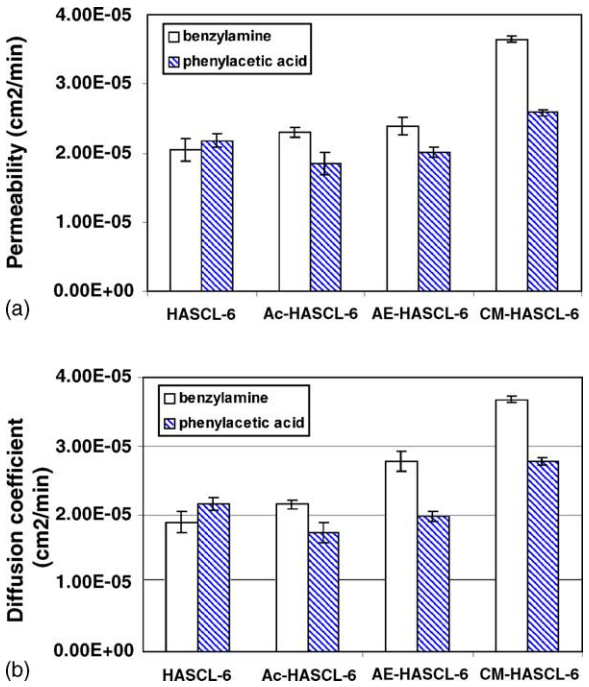

Fig. 5. Permeability (a) and diffusion (b) coefficients of benzylamine and phenylacetic acid through HASCL-6 and its derivatives. Tablets (200 mg) were obtained by dry compression, and benzylamine and phenylacetic permeability was followed with the diffusion apparatus in appropriate media at 37 °C. Diffusion was calculated from the permeability and the partition coefficients.

(no modifications of swelling volume), whereas in the case of CM-HASCL-6 tablets (Fig. 1b), they increase (similarly to the permeability coefficient) at higher ionic strength (which generates the decrease of matrix swelling volume).

The partition coefficient (*K*) of acetaminophen was about 1.33 for HASCL-6 and its amino and acetate derivatives, and slightly lower (1.07) for CM-HASCL-6 (Fig. 4). These data suggest some interaction between starch and acetaminophen. In the case of the highly swollen CM-HASCL-6, the lower value (1.07) could be due to the fact that interactions between acetaminophen and starch were, at least in part, dissipated by the higher water content into the tablet. If two tablets contain the same amount of starch but differing in the swelling volume, the tablet with higher swelling will present a lower partition coefficient (same number of interaction sites in a higher volume). This explanation also applies to HASCL-6, Ac-HASCL-6 and AE-HASCL-6, with the mention that only slightly decreasing values of K 1.38, 1.32 and 1.29 for moderately increasing swelling of 4.5, 4.8 and 5.6 g of water/g of polymer, respectively were found (Mulhbacher et al., 2004).

The effect of the ionic charge of the drug on permeability, partition and diffusion coefficients was evaluated with benzylamine and phenylacetic acid as tracers. The benzylamine and phenylacetic acid permeability coefficients through HASCL-6 were similar. For the CM-HASCL-6, Ac-HASCL-6 and AE-HASCL-6 matrices the permeability coefficients of the benzylamine were higher than those of phenylacetic acid tracer (Fig. 5a). A possible explanation of these differences can reside on the fact that, even though the two molecules have similar molecular weight (benzylamine: 105 g/mol, phenylacetic acid: 134 g/mol), the phenylacetic acid would have a higher hydration volume than benzylamine due to its carboxylic group. It is however difficult to understand why the difference was not also noticed for HASCL-6.

The partition coefficients of benzylamine and phenylacetic acid were similar for HASCL-6 and Ac-HASCL-6 matrices (slightly higher than 1). The partition coefficient of benzylamine (0.86) was lower than that of the phenylacetic acid (1.02) for AE-HASCL-6, whereas for CM-HASCL-6, it was slightly higher (0.99 but the difference being not significant) than that of the phenylacetic acid (0.93) (Fig. 4). Partition coefficients lower than 1 can be explained by the fact that when the matrix and drug have the same charge they will repel each other. For CM-HASCL-6, the fact that the difference was not statistically significant could be due to its

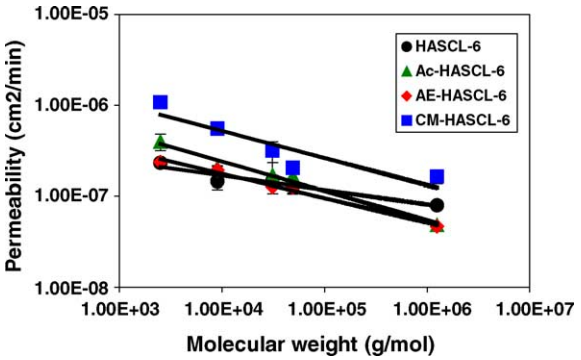

Fig. 6. Permeability coefficients of various Kollidon types with different molecular weight, through HASCL-6 and its derivatives. Tablets (200 mg) were obtained by dry compression at 29.4 kN, and Kollidon permeability was followed with the diffusion device in appropriate media at 37 °C.

higher swelling which decreases the number of charges per volume units leading thus to an under-evaluation.

The diffusion coefficients of benzylamine and phenylacetic acid through HASCL-6 and its derivatives were similar to the partition coefficient because the coefficients of partition of the two molecules were close to 1 (Fig. 5b).

The permeability coefficients for each Kollidon of different molecular weight in case of CM-HASCL-6 were higher than for HASCL-6, Ac-HASCL-6 and AE-HASCL-6 (Fig. 6), such as observed for other tracers tested. To obtain a linear relation between the permeability and the molecular weight, the data were plotted on a log–log scale. The experimental values of permeability coefficient for the five Kollidon variants of different molecular weight, through each starch derivative fit well with a power trendline (Table 1). These results are in agreement with those of Ju et al. (1997), on the diffusion coefficients of polymers in the layer adjacent to a swollen matrix. The partition coefficient of Kollidon 12 PF (2500 g/mol) and 30 PF (49,000 g/mol) were found close to 1.01 when tested with HASCL-6 and its derivatives. The increase of the Kollidon molecular weight did not affect the partition coefficients. These results can be useful for the pharmaceutical formulation of drugs of high molecular weight.

Table 1
Equations of the trendline obtained from permeability coefficients of Kollidon series through the starch derivatives tablets

Matrices	Equations	R^2
HASCL-6	$y = 7 \times 10^{-7} x^{-0.1577}$	0.9332
Ac-HASCL-6	$y = 5 \times 10^{-6} x^{-0.322}$	0.9729
AE-HASCL-6	$y = 2 \times 10^{-6} x^{-0.2665}$	0.9874
CM-HASCL-6	$y = 8 \times 10^{-6} x^{-0.298}$	0.8202

In conclusion, this study allows a better understanding of the diffusion mechanism of molecules through HASCL-6 and its derivatives. The diffusion was shown to depend on the molecular weight of the diffusant, whereas the partition coefficients were affected by the affinity of the diffusant for the polymeric matrices and for the dissolution medium modulated by attractive or repulsive ionic interactions. Unexpectedly, in the case of CM-HASCL-6, the diffusion was lowered at higher swelling volumes. Knowledge of the swelling and diffusion characteristics of polymeric excipients are useful to predict the drug release kinetics. The fact that for the HASCL-6, Ac-HASCL-6 and AE-HASCL-6 matrices there are no major differences in terms of diffusion and permeability at various ionic strength is important, showing the possibility to use one or another of these matrices in function of the desired formulation characteristics. This also suggests a certain stability of release profile, irrespective to the site of delivery in the intestinal tract. Furthermore, diffusion and partition studies can represent a useful tool to evaluate eventual drug–excipient interactions, important for the formulation approach of various active agents.

Acknowledgements

J. Mulhbacher is the recipient of a University-Industry graduate studentship from FQRNT (formerly FCAR, Québec, Canada) with participation of Labopharm Inc. Both contributions are gratefully acknowledged.

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