Diffusion of Biological Molecules Through Hollow Chitosan Fibers

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ABSTRACT: Hollow chitosan fibers were tested for the diffusion of a series of biological macromolecules, including amino acids, vitamins, and antibiotics. The hollow fibers were immersed in the permeant solution, and water was circulated and recycled inside the lumen of the fiber. The concentration of the permeant in the hollow fiber loop was analyzed online by ultraviolet–visible spectrophotometry. The effect of process parameters such as the concentration, pH, and flow rate was tested. The permeability coefficient was calculated from the permeability. The limited influence of the flow rate indicated that resistance to film diffusion (at the surface of the fiber) was not the rate-limiting step: the limiting step remained diffusion through the membrane. The pH would be expected to influence the protonation of chitosan amine groups and the acid–base properties of the permeant; however, the diffusion profiles were little affected by this parameter. The impact of pH on the permeability coefficient decreased in the following order: tryptophan > chloramphenicol > vitamin B12. The change in the concentration of the permeant had a limited impact on the permeability coefficient. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3568–3578, 2008

Key words: biopolymers; diffusion; hydrogels; polysaccharides; separation techniques

INTRODUCTION

Chitosan is a widely studied amino–polysaccharide biopolymer used in a wide variety of applications, including wastewater treatment, textiles, papermaking, and biological and medical applications. The presence of amine groups gives chitosan interesting sorption properties for the binding of metal cations on nitrogen (through chelation at a nearly neutral pH) and anionic molecules on protonated amine groups (anionic dyes and metal anions by electrostatic attraction in acidic solutions). The acid–base properties of the chitosan biopolymer make it unique among natural polysaccharides. This property is also the key to a number of physical modifications: the protonation of amino groups causes the polymer to dissolve, and this a necessary step in the conditionings of new forms such as gel beads,[1–4] films (plane membranes),[5–9] fibers,[10,11] and hollow fibers.[12–16]

The contribution of diffusion mechanisms to the control of applications involving chitosan-based materials can be explained by the poor porosity of the raw material.[17–20] For this reason, a major challenge in recent decades has been the conditioning of chitosan in the form of gels to alleviate the impact of porosity limitations. Additionally, the specially tailored conditioning of chitosan in the form of plane membranes, fibers, hollow fibers, and so forth enables the critical length of the material to be decreased while an appropriate shape is maintained for practical applications (fine solid particles may cause removal problems or column clogging in wastewater treatment).

For example, hollow fibers of chitosan have been tested for the simultaneous sorption and desorption of chromate anions:[14,15] chromate anions in acidic solutions were adsorbed on the outer side of the fiber, whereas an extractant (Aliquat 336, a quaternary ammonium salt) was circulated in the lumen of the fiber for metal desorption and transfer. The fiber played the role of a reactive barrier between the aqueous and organic phases, confining the extractant and selectively sorbing the permeant because of its reactivity for the metal under the selected experimental conditions. Hollow chitosan fibers have also been tested for catalytic applications (hydrogenation of nitro-aromatics): Pd was immobilized in the fiber before being reduced *in situ*; hydrogen gas (or another hydrogen donor) was maintained in the outer compartment, whereas the solution was circulated in the lumen of the fiber.[13,21] In the field of biomedical applications, hollow chitosan fibers, either alone or in combination with other biopolymers or synthetic polymers, have been used as nerve conduits.[16,22,23] The hydrophilic character of chitosan, together with its biocompatibility, biodegradability,

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and structural similarity to glycosaminoglycans, explains the increasing number of studies focusing on the testing of chitin/chitosan tubes as nerve guidance channels for clinical applications.[16,24] Polysaccharides generally produce materials with poor mechanical properties that can be reinforced by (1) crosslinking or (2) combination with other polymers.[22] Chitin (the precursor of chitosan, which is its acetylated form) degrades faster than chitosan and forms much stronger materials[22] The conversion of chitosan into chitin, by means of selective reacetylation, is an alternative technique for reinforcing both mechanical and biodegradability properties. Another important parameter for evaluating the suitability of these fibers concerns their diffusion properties.

This work contributes to defining a practical method for their evaluation using a series of biological molecules. This methodology will be helpful for the evaluation of diffusion characteristics and the comparison of different materials derived from raw fibers (e.g., modification for controlling their porosity).

In this study, the diffusion properties were assayed for a series of biological molecules—an amino acid, a vitamin, and two antibiotics—under a number of experimental conditions. Tryptophan is an amino acid that is essential for human nutrition because it is not synthesized by the organism; it is characterized by an isoelectric point of 5.89 (pK_{a1}: 2.4, pK_{a2}: 9.34). Chloramphenicol is a bacteriostatic antibiotic (wide spectrum range). Amoxicillin is a β-lactam antibiotic (for Gram-positive bacteria) that inhibits the synthesis of bacterial cell walls. Vitamin B12 is one of the most complex vitamins; it participates in a number of enzyme-catalyzed reactions. B12 deficiency causes anemia. The effect of the concentration, pH, flow rate, and matrix (solvent or ionic strength) on the diffusion of the target molecules was investigated. The permeant was placed in the outside compartment, whereas the permeant-free solvent was circulated in a loop inside the lumen of the fiber. The transfer of the permeant through the wall of the fiber was quantified online by ultraviolet–visible (UV–vis) spectrophotometry. The mass balance equation was used to determine the permeant flux, from which the permeability and diffusion coefficients were deduced.

EXPERIMENTAL

Materials

The chitosan was provided by Mahtani Chitosan Pvt., Ltd. (Veraval, India). Other reagents (analytical-grade) were supplied by Sigma–Aldrich (Saint Quentin Fallavier, France; tryptophan), Acros Organics (Saint Quentin Fallavier, France; chloramphenicol), and Fluka (Saint Quentin Fallavier, France; amoxicillin and vitamin B12). Tryptophan [(*S*)-2-amino-3-(1*H*-indol-3-yl)-propionic acid; molecular weight: 204.23 g/mol] was supplied by Sigma–Aldrich). Chloramphenicol {chloromycetin or 2,2-dichloro-*N*-[1,3-dihydroxy-1-(4-nitro-phenyl)-propan-2-yl]-acetamide; molecular weight: 323.12 g/mol} was obtained from Acros Organics. Amoxicillin {7-[2-amino-2-(4-hydroxyphenyl)-acetyl] amino-3,3-dimethyl-6-ox-2-thia-5-azabicyclo[3.2.0]heptane-4-carboxylic acid; molecular weight: 365.4 g/mol} and vitamin B12 (cyanocobalamin; molecular weight: 1355.37 g/mol) were supplied by Fluka. Mother solutions were prepared in demineralized water, except for amoxicillin and chloramphenicol: their poor solubility in water made it necessary to add a small amount of ethanol to the mother solution (i.e., <0.5%).

Preparation of the hollow chitosan fibers

The hollow chitosan fibers were prepared with a previously described procedure[11–13,15,25] derived from the work of Agboh and Qin,[11] Modrzejewska and Eckstein,[12] and Kaminski et al.[25] The chitosan was dissolved in an acetic acid solution (7% w/w) at the concentration of 7% (w/w). The solution was filtered to remove nondissolved material, and the viscous solution was debubbled *in vacuo*. The solution was then extruded into an alkaline coagulation bath, the external part of the fiber was first neutralized (with a 1*M* NaOH solution), and the inner part of the extruded material was not coagulated. The core of the fiber was removed with air flow first, and this was followed by an alkaline treatment (with a 1*M* NaOH solution). The fiber was stored in a 1*M* NaOH solution and abundantly rinsed before use. The standard length of the fibers used in the study was 0.5 m. The internal diameter of the wet hollow fibers was 400 ± 20 μm, and the thickness of the fiber walls was 60 ± 5 μm. The weight of the cut dry fibers (0.5 m) was 22.5 ±1 mg, whereas the weight was 125 ± 5 mg for the cut wet fibers. This means that the water volume in the wet membrane was close to 82%. Figure 1 shows a scanning electron micrograph of the hollow chitosan fibers after drying under supercritical CO_2 conditions.

When required, the fibers (length: 0.5 m; dry weight: 22.5 mg) were crosslinked with glutaraldehyde by the circulation (with recycling) of 50 mL of a glutaraldehyde solution (concentration: 0.25%) at a high velocity (3.5×10^3 m/h) through the lumen of the fiber. The high flow velocity was intended to facilitate the reaction of the crosslinking agent with the amine groups along the entire length of the fiber and thus ensure homogeneous crosslinking. The contact time was less than 1 s for a single flow-through, and the recirculation lasted for 1 h.

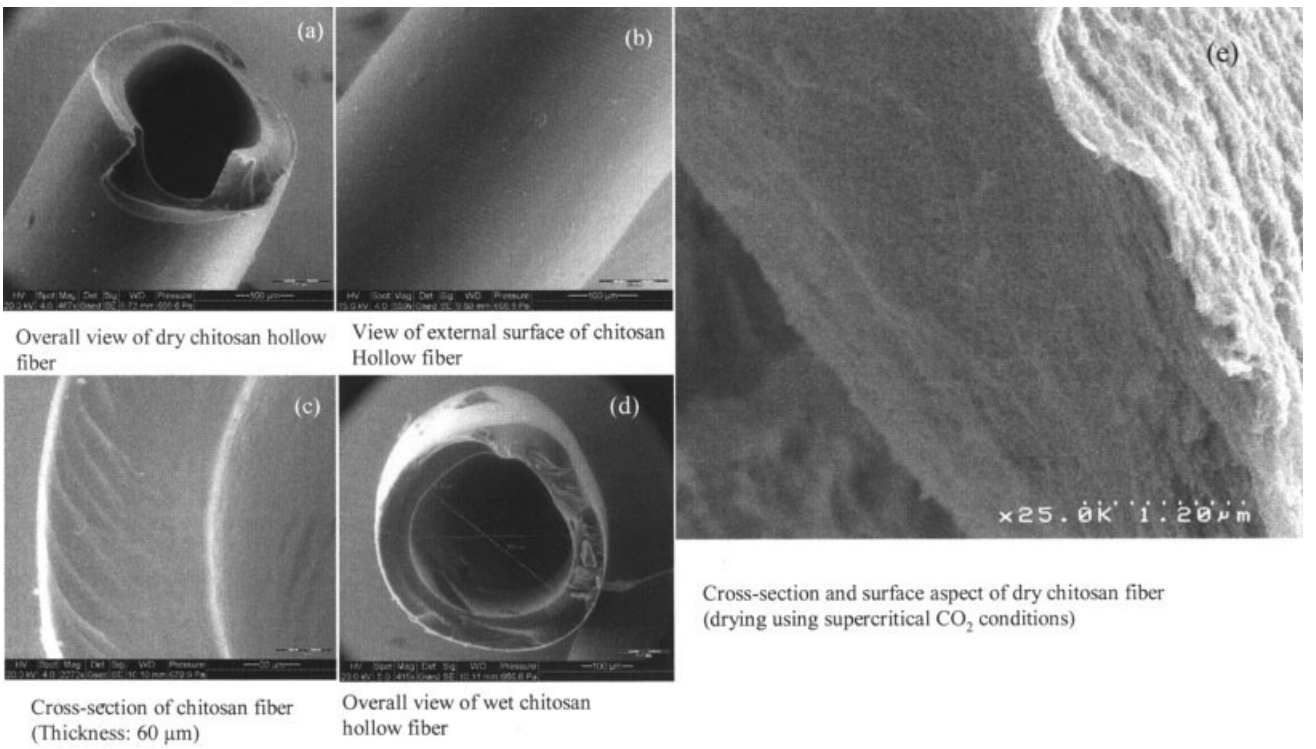

Figure 1 Scanning electron microscopy photographs of chitosan hollow fibers.

The drying of the fibers, when necessary, was performed through one of two procedures: (1) oven drying after preparation and (2) drying after the fiber had been saturated by immersion overnight in a saturated solution of sucrose (500 g/L). The sucrose-treated fibers were rinsed in demineralized water before use.

Investigation of the diffusion properties

The diffusion properties of the hollow chitosan fibers were investigated with the experimental setup shown in Figure 2. The tank reactor was filled with an aqueous solution of permeant. The volume of the tank reactor was fixed at 90–92 mL (depending on the experimental design). The hollow fiber was connected to the demineralized water reservoir (volume of water: 3–5 mL). For experiments carried out under acidic conditions, the pH was controlled with sulfuric acid to prevent the chitosan from dissolving. The water solution was circulated in a loop through the fiber immersed in the closed reactor, and the concentration of permeant was monitored online at the outlet of the fiber by UV–vis spectrophotometric analysis: the spectrum was collected at selected operating times, and the concentration was calculated from a calibration curve set at a fixed wavelength. Because the wavelength of maximum absorbance can differ slightly with changing experimental conditions (solvent and pH), the analytical wavelength was set for each experimental series. The standard wavelengths were set at 274 nm for tryptophan, 272 nm for chloramphenicol, 229 nm for amoxicillin, and 362 nm for vitamin B12.

The mass balance equation was used to determine the amount of permeant that migrated through the fiber to the outer compartment (tank reactor). The transmembrane permeant flux [F (μmol m^{-2} min^{-1})] was plotted versus the difference in permeant concentrations in the pores at the two sides of the membrane. Assuming that film diffusion could be neglected, this transmembrane flux was plotted versus the concentration gradient of the solution between the outer [C_R (μmol/L)] and inner compartments [C_{hf} (μmol/L)]. The permeability [Perm

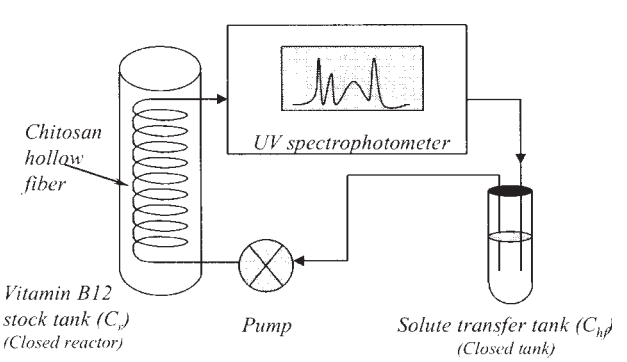

Figure 2 Experimental setup for the measurement of diffusion properties.

(m/min)] and the permeability coefficient [P (m^2/min)] were obtained as follows:[26–28]

$$F = 1000 \text{ Perm } (C_R - C_{hf}) \quad (1)$$

$$F = 1000 \frac{P}{l} (C_R - C_{hf}) \quad (2)$$

where l is the thickness of the fiber (m).

Matsuyama et al.[29] defined the relation between the permeability coefficient and the effective diffusion coefficient (m^2/min) as a function of the partition coefficient of the permeant (K), which is also defined as the permeant solubility coefficient:

$$D_{gel} = \frac{P}{K} = \frac{P}{HK'} \quad (3)$$

where D_{gel} is the diffusion coefficient in the gel (m^2 s^{-1}), K is defined as the ratio of the permeant concentration in the gel membrane standardized by the total membrane volume to that in the bulk solution, K' is defined as the ratio of the permeant concentration in the gel membrane standardized by the water volume in the membrane to that in the bulk solution, and H is the water volume fraction of the membrane.

RESULTS AND DISCUSSION

Preliminary remarks

A series of experiments was performed to check systematically that the permeant was not significantly adsorbed onto the fiber. Figure 3 shows the time course of the permeant concentration (in this case vitamin B12) in both the external and internal compartments. At a long contact time (i.e., 8 h), the concentrations in the tank and in the hollow fiber circuit tended to the same value, suggesting that equilibrium had been reached. Additionally, the asymptotic value was consistent with the theoretical equilibrium value (because the permeant was indifferently transferred between the compartments, i.e., the transfer was homogeneous and not selectively oriented), with the permeant distributed in the whole volume (i.e., the reactor volume plus the volume of the hollow fiber circuit). The amount of permeant possibly adsorbed was negligible.

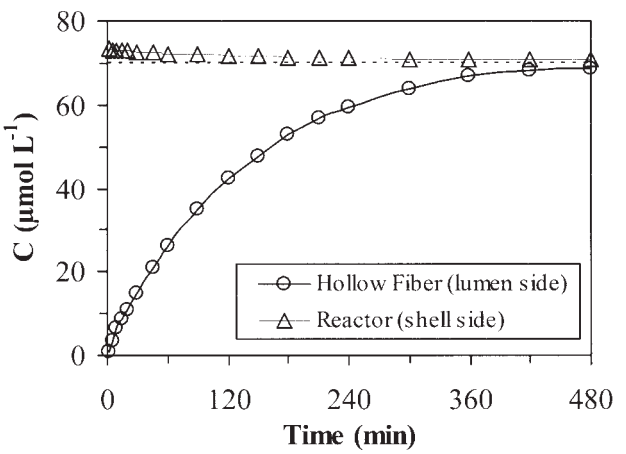

Figure 3 Comparison of the time course of the solute concentration (vitamin B12) in both the outer [shell side (reactor)] and inner [lumen side (hollow fiber)] compartments (the dashed line shows the theoretical concentration at equilibrium).

To be able to approach the diffusivity with accuracy, it is necessary to decrease as much as possible the contribution of external diffusion to the control of mass transfer. Mass transfer results from various diffusion mechanisms: (1) bulk diffusion in the tank reactor, (2) film diffusion at the external surface of the fiber, (3) diffusion in the membrane, (4) film diffusion at the internal surface of the fiber, and (5) bulk diffusion in the hollow fiber loop. The experimental conditions were selected to minimize the resistance to mass transfer in the bulk and film compartments. For example, the volume of the tank compartment was so much larger than that of the hollow fiber loop that the amount of permeant to be transferred remained negligible and the concentration in the tank reactor could be considered constant. This means that the gradients in the bulk of the tank reactor and in the film on that side of the fiber could be neglected. On the other hand, the velocity of the circulation in the loop (flow rates ranging from 0.5 to 3.5 mL/min) corresponded to values in the range of 0.13–1 m/s for superficial velocity. The Reynolds number (Re) was calculated with the following equation:[30]

$$Re = \frac{D\nu\rho}{\mu} \quad (4)$$

where D is the internal diameter of the tube (m), ν is the average fluid velocity (m/s), ρ is the fluid density, and μ is the kinematic viscosity (m^2/s). Because of the low concentrations of the permeant, the values of the fluid density and kinematic viscosity were considered equal to those of pure water. Re ranged from 38 to 265. This means that the hydrodynamic conditions corresponded to laminar flow; it is commonly accepted that turbulent flow appears when Re exceeds 2000. It was thus necessary to check whether resistance to bulk and film mass transfer could control the overall mass-transfer rate.

Figure 4 shows the influence of the flow rate on the diffusion of chloramphenicol. The concentration in the hollow fiber loop was not significantly affected by the velocity of the solution in the fiber (not shown). This is confirmed by the plot of the permeant flux versus the gradient concentration

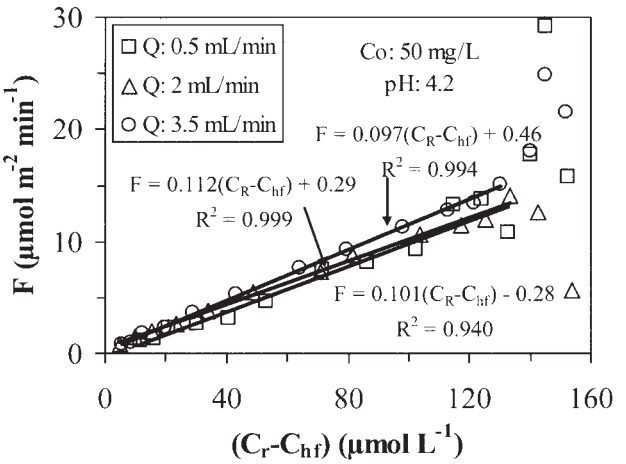

Figure 4 Influence of the flow rate on the diffusion of chloramphenicol through chitosan hollow fibers.

between the two compartments: the permeability coefficient remained in the range of 5.82–6.06–6.72 $\times$ 10^{-9} m^2/min. At the initial stage of the transfer procedure (corresponding to the high concentration gradient between the two compartments; right part of the figure), the flux did not follow a linear trend: this step corresponds to the unsteady stage of the process.

Diffusion of tryptophan

The diffusion of tryptophan through the hollow chitosan fibers was investigated, with the pH and the concentration of the permeant being varied (Figs. 5 and 6, respectively). The figures show both the time course of the tryptophan concentration in the hollow fiber loop and the flux variation versus the concentration gradient between the two compartments. Figure 5 shows that, at a long contact time (i.e., 4 h), the concentration of tryptophan in the hollow fiber loop was comparable for all the pHs tested. The tryptophan concentration was close to 174 mmol/m^3, which was much less than the level expected (i.e., 232 μmol/L) from the mass balance for the distribution of the permeant between the two compartments (based on the volumes of each compartment). A longer contact time was required for full equilibrium; this is confirmed by the slightly increasing trend followed by the tryptophan concentration in the time range of 3–4 h. The most representative differences were observed for operating times lower than 2 h; with respect to kinetic diffusion profiles, the pH can be ranked according to the following sequence: pH 6 > pH 9 > pH 2. This means that tryptophan transfer was facilitated when the pH was close to the isoelectric point. In the case of plane membranes, Matsuyama et al.[27,29] investigated the diffusion of three permeants with different acid–base properties (anionic, cationic, and neutral behavior for benzenesulfonic acid, theophylline, and styrene glycol, respectively, in the pH range investigated). The change in the pH caused a change in the charge density of the chitosan membrane: decreasing the pH enhanced the protonation of amine groups and improved membrane swelling because of electrostatic repulsion. This increased the water volume fraction of the membrane. When the pH increased, the permeability of benzenesulfonic acid (anionic permeant) increased, whereas the permeability of theophylline (cationic permeant) decreased; the permeability of neutral species (styrene glycol) was not affected by changes in the pH. The variations of both the partition coefficient and diffusion coefficient (gel) followed the same increasing trend as permeability for increasing pH.

The second panel of the figure shows the flux of tryptophan versus the concentration gradient. This figure confirms that tryptophan permeability was improved by a nearly neutral pH: alkaline media

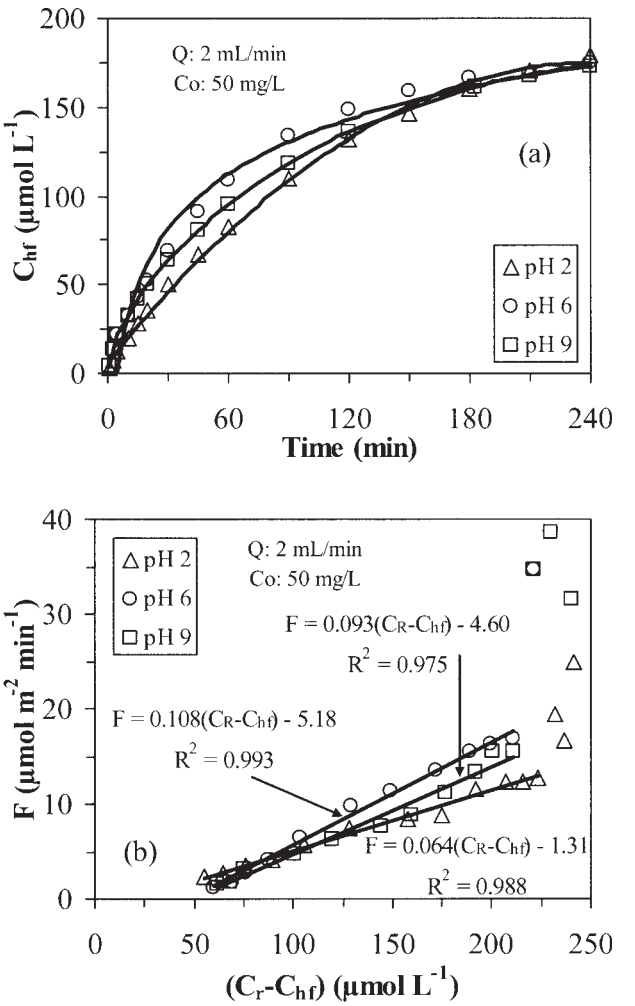

Figure 5 Influence of pH on tryptophan diffusion through chitosan hollow fibers: (a) concentration versus time and (b) flux versus gradient concentration.

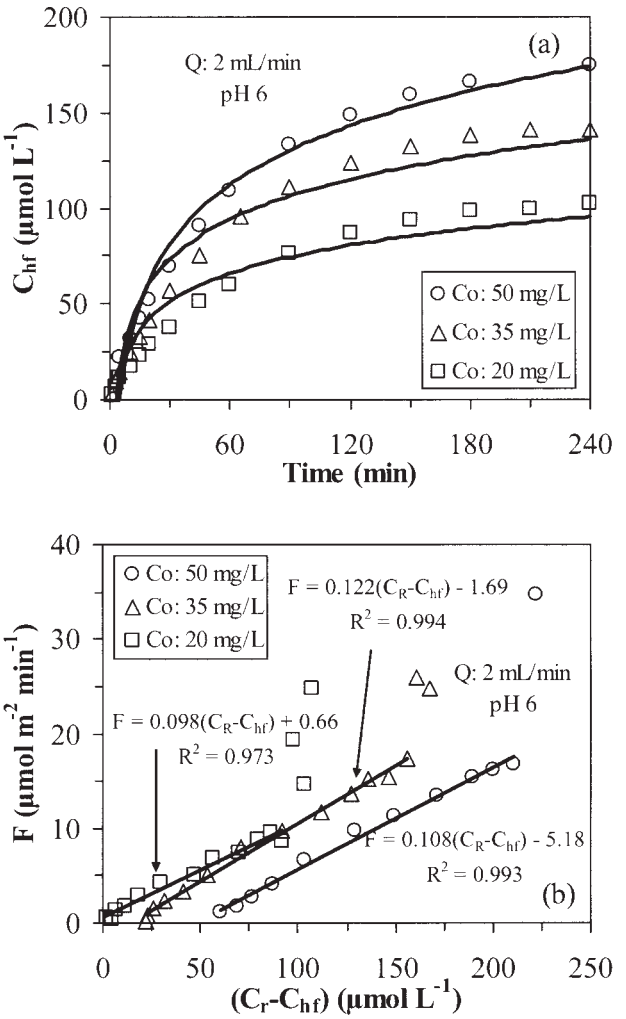

Figure 6 Influence of the initial concentration on tryptophan diffusion through chitosan hollow fibers: (a) concentration versus time and (b) flux versus gradient concentration.

induced a much lower decrease of the permeability coefficient than pH 2. The permeability at pH 2 was about 40% less than that reached at a nearly neutral pH. The free molecular diffusivity of tryptophan in water was 1.31×10^{-8} m^2/min.[31] The permeability coefficient was found to be close to 6.5×10^{-9} m^2/min. This is much less than the decrease in diffusivity observed by Clogston and Caffrey[31] for the diffusion of tryptophan from lipid cubic gels prepared from monoacylglycerol (MAG). Indeed, tryptophan diffusivity in water decreased by 10–20 times when it was enclosed in a series of MAG cubes: the decrease in diffusion properties depended on the hydration level and the type of MAG.

As expected, decreasing the initial concentration reduced the concentration of tryptophan in the hollow fiber loop, which remained below the theoretical equilibrium concentration (i.e., 231, 162, and 105 µmol/L for initial concentrations of 50, 35, and 20 mg/L, respectively), as shown in Figure 6. The permeability coefficient was little affected by the initial concentration; it ranged between 5.9×10^{-9} and 7.3×10^{-9} m^2/min. For ion-exchange processes, Helfferich[32] observed that when the sorption process is controlled by intraparticle diffusion, the kinetic rate does not depend on the permeant concentration. This is consistent with the results obtained with this diffusion system.

Diffusion of chloramphenicol

The influence of pH was tested on the diffusion of chloramphenicol (Fig. 7). The concentration profile for acidic pH solutions (i.e., pHs 2.4 and 4.2) were superimposed, whereas for alkaline solutions (pH 8.8), a slight difference was observed: mass transfer was slightly improved by alkaline media. The concentration of chloramphenicol remained lower than the theoretical equilibrium value (i.e., 143 µmol/L).

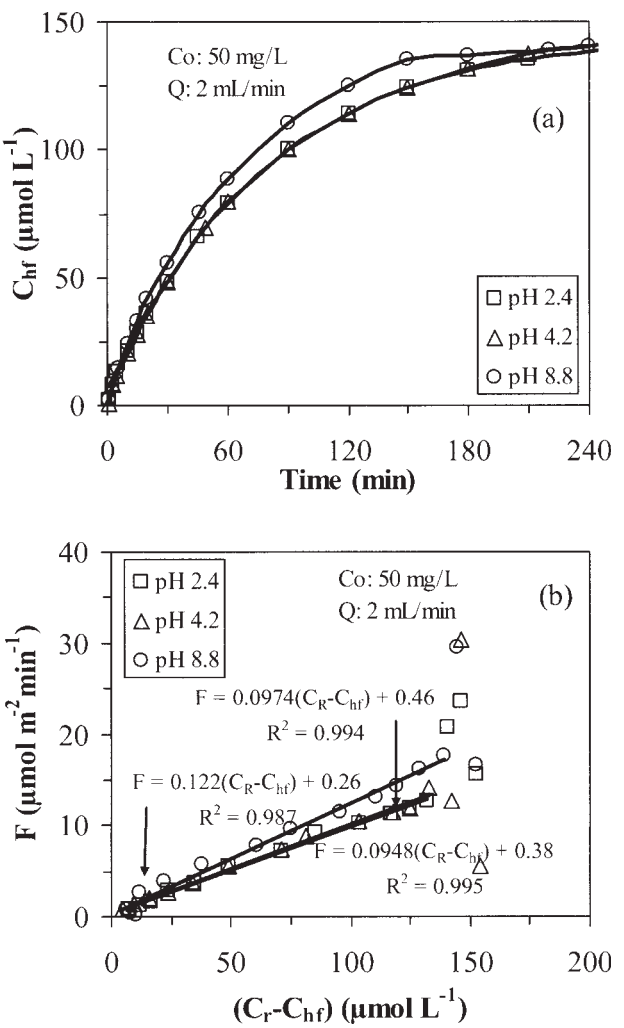

Figure 7 Influence of pH on chloramphenicol diffusion through chitosan hollow fibers: (a) concentration versus time and (b) flux versus gradient concentration.

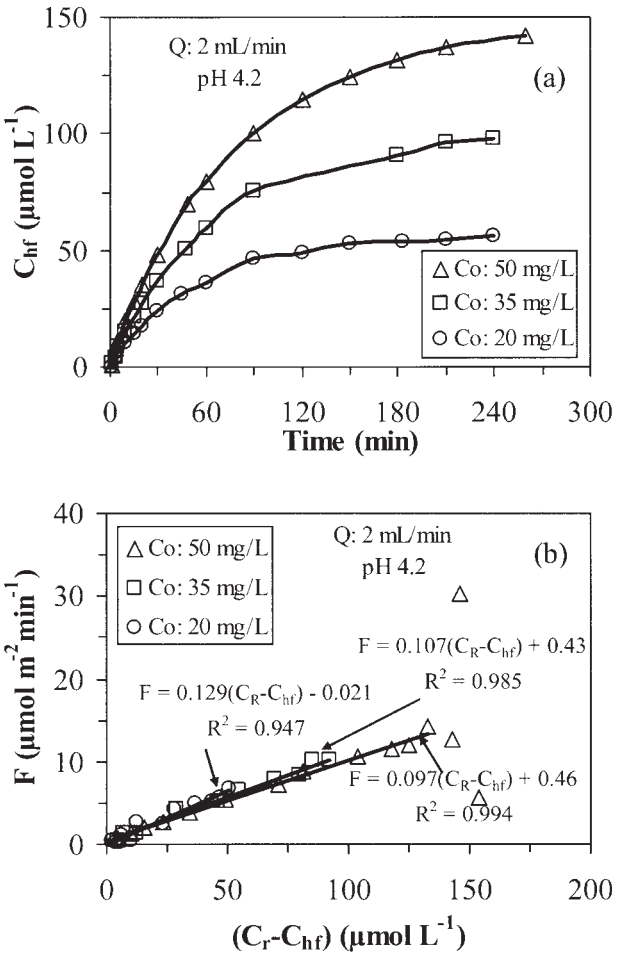

Figure 8 Influence of the initial concentration on chloramphenicol diffusion through chitosan hollow fibers: (a) concentration versus time and (b) flux versus gradient concentration.

This means that the diffusion is not selectively oriented. These conclusions are confirmed by the plot of the chloramphenicol flux versus the concentration gradient. The permeability coefficient was comparable for solutions at pH 2.4 and pH 4.2 (5.7×10^{-9} and 5.82×10^{-9} m^2/min, respectively); increasing the pH to the alkaline region (i.e., pH 8.8) increased the permeability coefficient to 7.32×10^{-9} m^2/min.

This is close to the values obtained for tryptophan.

The diffusion of chloramphenicol was not significantly influenced by the initial concentration in the selected experimental range (Fig. 8). Permeability coefficients varied between 5.82×10^{-9} and 7.7×10^{-9} m^2/min. Hada et al.[33] investigated the diffusion of two antibiotics (including chloramphenicol) through artificial reconstituted skin. They found a diffusion coefficient close to 5×10^{-10} m^2/min.

Diffusion of amoxicillin

Figure 9 shows the diffusion of amoxicillin through the hollow chitosan fibers. This figure demonstrates the good reproducibility of the experiments: the test was performed in triplicate, and the curves overlapped. The concentration after an operating time of 6 h was close to 120 μmol/L, which is less than the theoretical equilibrium concentration (i.e., 140 μmol/L). This means that even after 7 h of contact, the equilibrium was not reached; the approach to equilibrium lasted longer than that for tryptophan and chloramphenicol. Compared to the theoretical equilibrium concentration, after 7 h of contact, amoxicillin transfer was around 83%, whereas for other substrates, the time required to reach such a transfer yield was around 3–4 h. This is probably due to low sorption on the fiber, which (1) contributed to limiting amoxicillin diffusion and (2) did not allow the complete balance of the substrate between the two compartments to be maintained.

In the case of amoxicillin diffusion in human jejunum, Lennernäs et al.[34] obtained a permeability close to 2×10^{-5} m/min. The permeability coefficient

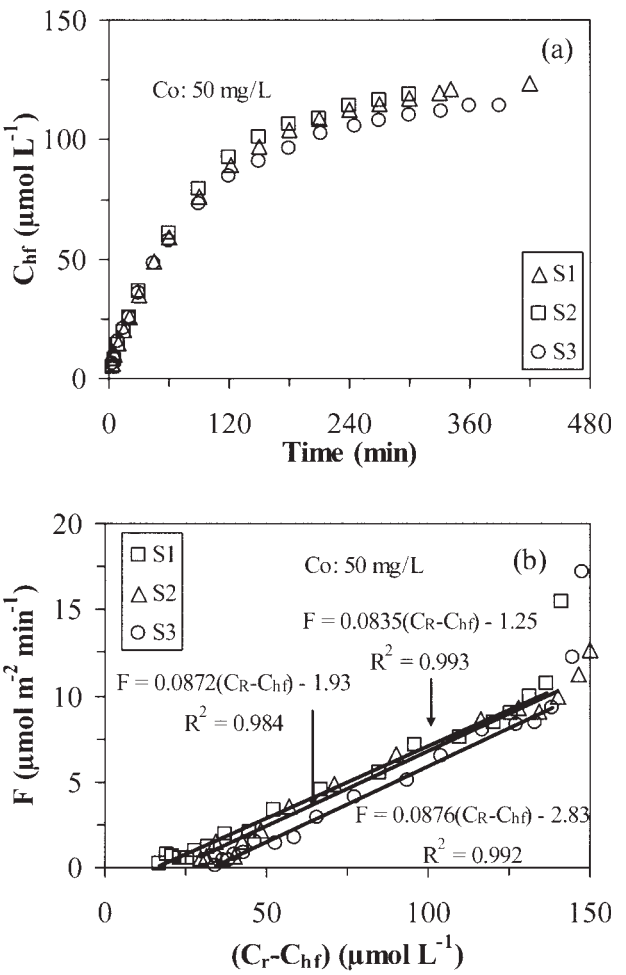

Figure 9 Amoxicillin diffusion through chitosan hollow fibers—an example of experimental reproducibility: (a) concentration versus time and (b) flux versus gradient concentration.

through the hollow chitosan fibers was close to 5.2 $\times$ 10^{-9} m^2/min. Although slightly lower than the values reached with other substrates, this was of the same order of magnitude. This is several orders of magnitude lower than the molecular diffusivity cited by Adriano et al.,[17] who used the Wilke–Chang method to determine amoxicillin diffusion in water (close to 4.02 $\times$ 10^{-6} m^2/min). Adriano et al. investigated the sorption of amoxicillin onto chitosan beads; they found that sorption at pH 6.5 did not exceed 8.7 mg of amoxicillin per gram (wet adsorbent) and that the diffusion coefficient of the antibiotic in the gel beads was close to 6.5 $\times$ 10^{-11} m^2/min.

Diffusion of vitamin B12

With the conditioning of the fibers varied, the diffusion of vitamin B12 was investigated with dried materials (prepared by oven drying or the so-called sucrose-drying procedure, i.e., drying after saturation with a sucrose solution and rehydration; Fig. 10) and crosslinked material (Fig. 11). Uncontrolled drying of chitosan hydrogels leads to a significant decrease in diffusion properties.[35] Indeed, oven drying causes an irreversible collapse of the porous structure. Impregnation of the fiber with a sucrose-saturated solution before the drying step limits the collapse of the structure and restores the diffusion properties of the hydrogel after re-hydration. Figure 10 compares the transport properties of vitamin B12 for dry and wet hollow fibers (for fibers that were used as produced, which are called raw, and those that were impregnated with sucrose before the drying step). After drying, the hollow fibers partially lost their porous structure, and the time required to reach equilibrium significantly exceeded the time for wet fibers (close to 8 h). The permeability coefficient was significantly affected by the drying treatment regardless of the pretreatment of the beads (with or

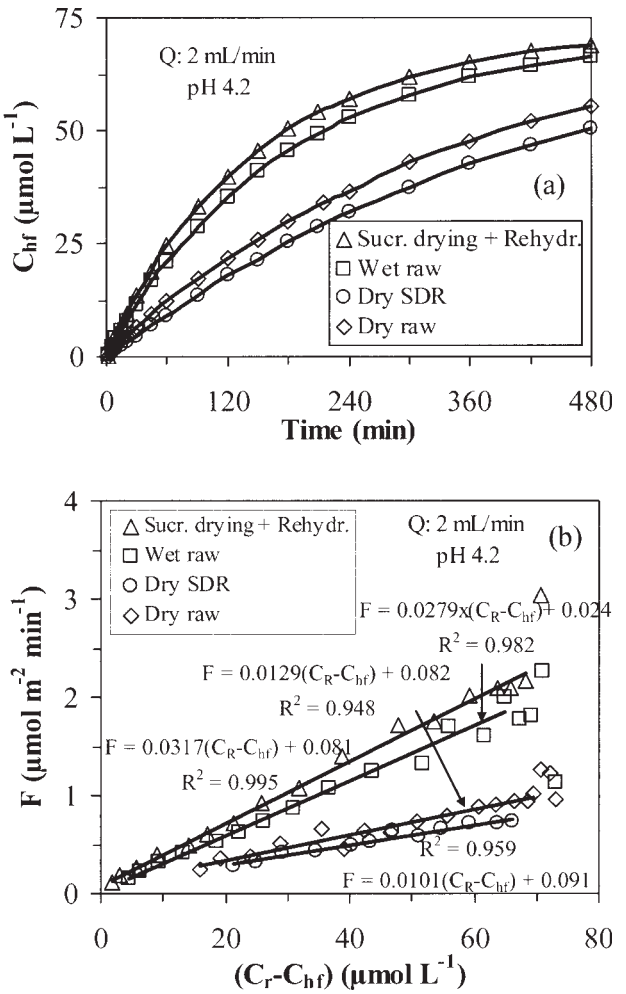

Figure 10 Influence of fiber drying on the diffusion of vitamin B12 through chitosan hollow fibers (sucrose-drying treatment): (a) concentration versus time and (b) flux versus gradient concentration.

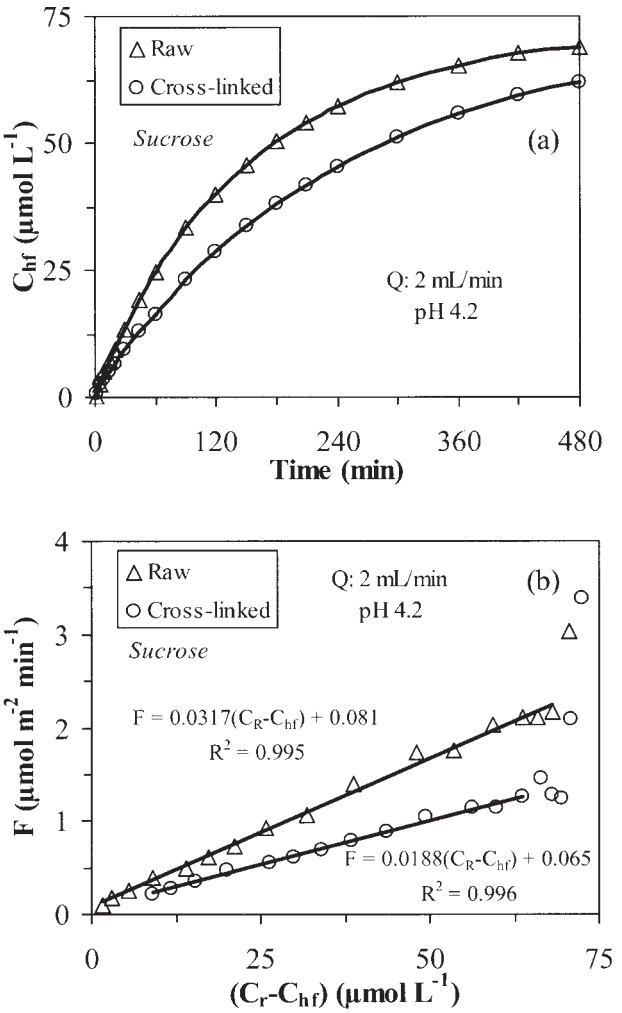

Figure 11 Influence of the crosslinking treatment on the diffusion of vitamin B12 through chitosan hollow fibers (sucrose-drying treatment): (a) concentration versus time and (b) flux versus gradient concentration.

without the sucrose impregnation): for wet materials, the permeability coefficients were in the range of 1.7–1.9 $\times 10^{-9}$ m^2/min (for raw and sucrose-treated fibers, respectively), and the values decreased to 2.6 $\times 10^{-10}$ and 2.0 $\times 10^{-10}$ m^2/min, respectively. Freier et al.[16] also observed that vitamin B12 diffusion coefficients for both chitin and chitosan tubes decreased when the fibers were dried. The control of porosity and diffusion properties is a key parameter in the design of chitosan-based materials whatever the application is (sorption processes, catalytic applications, drug release, supported cell culture, etc.), especially when drying steps are involved in the synthesis procedure. This explains the growing interest in designing alternative drying processes (drying in the presence of sucrose and drying under supercritical CO_2 conditions) or processes for pore size control (compounds are introduced during the conditioning step before being dissolved and removed from the material at the end of the process).

The crosslinking of chitosan with glutaraldehyde through a Schiff base reaction (the dialdehyde involves the formation of ceto–imine linkages between amine functions of chitosan on different chains) is commonly used to reinforce the resistance of chitosan to dissolving in acidic solutions. These new linkages may contribute to decreasing the number of reactive groups (e.g., when amine groups are involved in chelation reactions for the binding of metal cations) and may affect diffusion properties. In the case of gel beads, Kulkarni et al.[36] showed that the diffusion of diclofenac (an anti-inflammatory drug) decreased when the gel beads were crosslinked with chitosan. Figure 11 shows the influence of a crosslinking treatment for fibers conditioned by the sucrose-drying procedure. The transfer of vitamin B12 decreased after crosslinking, as shown both by the time course of the permeant concentration in the hollow fiber loop and by the permeability coefficients. The permeability coefficient decreased from 1.9 $\times 10^{-9}$ to 1.1 $\times 10^{-9}$ m^2/min.

The influence of the permeant concentration on the diffusion of vitamin B12 is shown in Figure 12. The concentration of the substrate in the hollow fiber loop remained slightly lower than the theoretical equilibrium concentration (i.e., 71, 36, and 18 μmol/L for initial concentrations of 100, 50, and 25 mg/L, respectively). The size of vitamin B12 may cause diffusion limitations, and an 8-h contact time was not sufficient to reach theoretical equilibrium values.

The permeability was not significantly affected by concentration changes: the values ranged between 2.84 $\times 10^{-5}$ and 3.17 $\times 10^{-5}$ m/min. In the case of poly(methacrylic acid-*g*-ethylene glycol) hydrogels, Bell and Pepas[37] observed that permeability varied between 3 $\times 10^{-6}$ and 6 $\times 10^{-6}$ m/min, depending on the state of the hydrogel, which was complexed

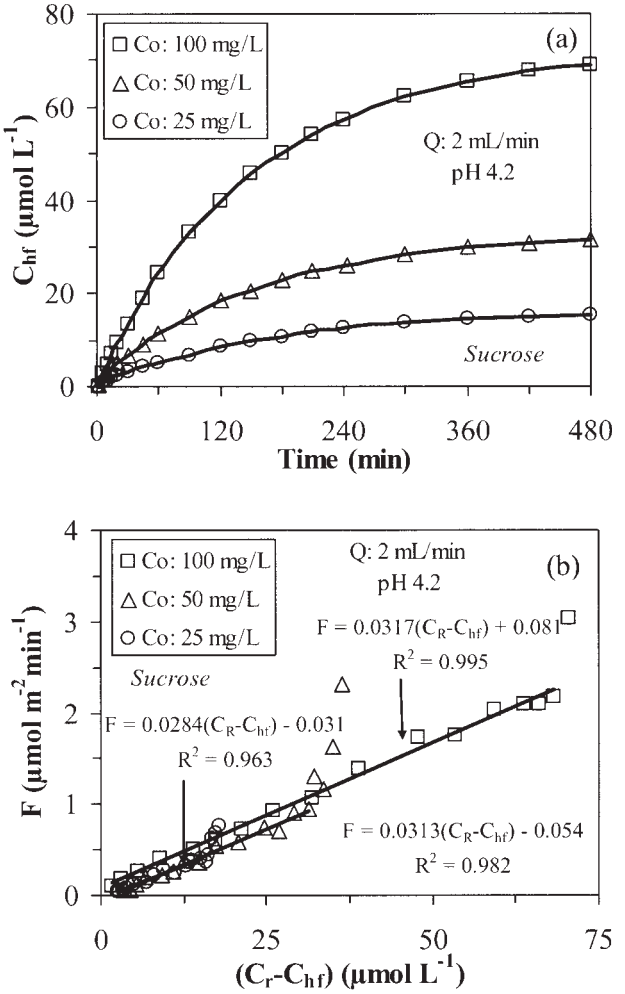

Figure 12 Influence of the initial concentration on vitamin B12 diffusion through chitosan hollow fibers: (a) concentration versus time and (b) flux versus gradient concentration.

or uncomplexed (because of intrachain interactions between copolymers). The permeability coefficient was 1.88 ± 0.08 $\times 10^{-9}$ m^2/min; this was about 10 times lower than the value of the molecular diffusivity (2.27 $\times 10^{-8}$ m^2/min). Sokolnicki et al.[38] determined the diffusivity of vitamin B12 through bacterial cellulose membranes: the measured diffusion coefficient was 4.32 $\times 10^{-9}$ m^2/min. This was of the same order of magnitude as the value obtained with hollow chitosan fibers (without taking into account the partition coefficient).

The diffusion profiles for vitamin B12 were tested in two solvents: water and methanol. The time course of the concentration in the hollow fiber loop was not significantly changed (not shown), and this was confirmed by the very similar values obtained for permeability coefficients close to 1.9 $\times 10^{-9}$ m^2/min. The change in the flux values for water and methanol solutions (Fig. 13) can be attributed to both (1) the small change in the volume of the hollow

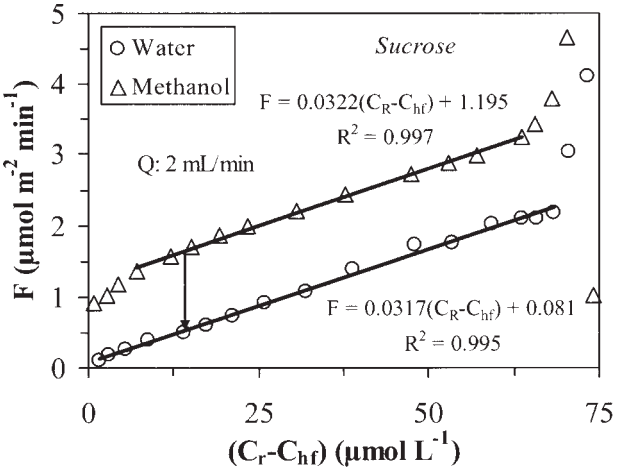

Figure 13 Influence of the solvent on the diffusion of vitamin B12 through chitosan hollow fibers (sucrose-drying treatment).

fiber loop (i.e., 4 mL for methanol as the solvent versus 3 mL for water as the solvent) and (2) the reduction of the solvating effect of water.

Figure 14 reports the diffusion profiles for vitamin B12, with the flow rate and pH varied. The curves overlap well, indicating that these experimental parameters did not influence mass transfer. The low effect of the flow rate confirms the results obtained with chloramphenicol (Fig. 4) and that the film diffusion in the inner compartment can be neglected: the mass transfer was only slightly improved at the highest flow rate (i.e., 3.5 mL/h). The protonation of amine groups resulting from an acidic pH is expected to promote the electrostatic repulsion of polymer chains and thus the swelling of the membrane with a possible effect on diffusion properties, as pointed out by Matsuyama et al.;[29] however, this figure shows that this effect can be neglected in this case.

The Renkin equation can be used to measure the size of the pores of membranes [R_p (nm)] under critical experimental conditions and model hypotheses. The Renkin equation is based on the molecular diffusivity of vitamin B12 in water (D_{mol}; i.e., 2.274×10^{-8} m^2/min) and the hydrodynamic radius of vitamin B12 (R_s; i.e., 0.85 nm):[39]

$$\frac{D}{D_{mol}} = \left(1 - \frac{R_s}{R_p}\right)\left[1 - 2.104\left(\frac{R_s}{R_p}\right) + 2.09\left(\frac{R_s}{R_p}\right)^3 - 0.95\left(\frac{R_s}{R_p}\right)^5\right] \quad (5)$$

The Renkin equation should be taken to be indicative of the order of magnitude of the pore size because the relation requires R_s/R_p to be greater than 0.5 (i.e., $R_p > 1.7$ nm). The interactions of the permeant with the fiber may also affect the diffusion properties and consequently the evaluation of the pore size. The diffusion coefficient in the hollow fiber is correlated to the permeability coefficient through the equation $P = KD$. The partition coefficient or permeant solubility of the membrane is based on the amount of permeant in a unit volume of the membrane divided by the amount of permeant in a unit volume of the solution. At equilibrium, assuming (1) that adsorption of the permeant on the fiber is negligible and (2) that the concentration in the pore volume is at equilibrium with (and equal to) the concentration in the bulk solution (Fig. 3), partition coefficient K can be approximated by the water volume fraction in the fiber (i.e., H: 0.82). This means that the diffusivity of vitamin B12 can be approximated by the value 1.56×10^{-9} m^2/min. With the Renkin equation, the value of the pore radius was found to be close to 1.9 nm. The surface properties of hollow fibers were investigated by Peirano et al.[40] using Brunauer–Emmett–Teller surface analysis after the samples had been dried under supercritical CO_2 conditions; this technique is appro-

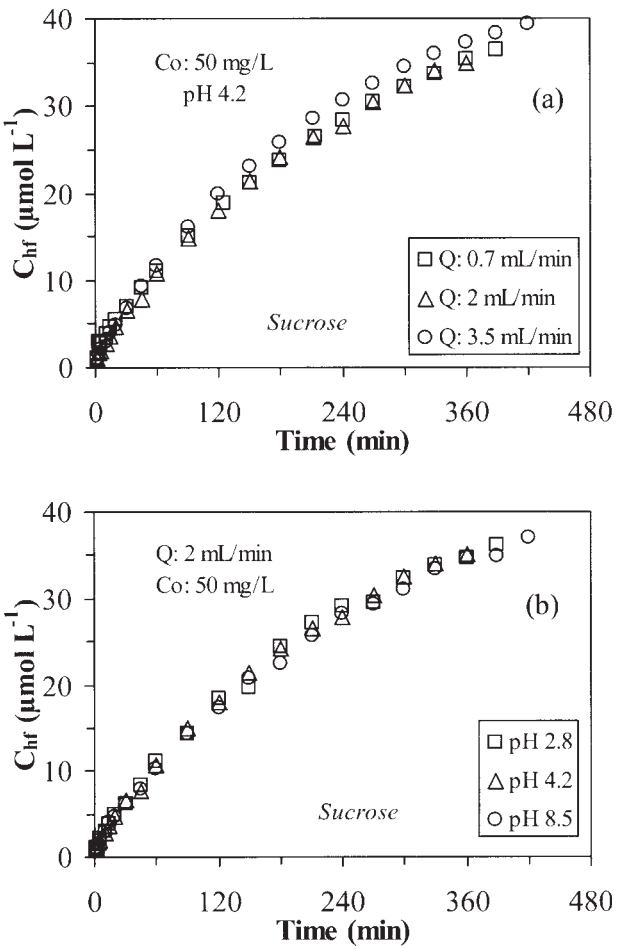

Figure 14 Influence of (a) the flow rate [Q (mL/min)] and (b) pH on the diffusion of vitamin B12 through chitosan hollow fibers (sucrose-drying treatment).

priate only for measuring the mesoporosity of the material. The pore size was found to be close to 10 nm.

Influence of the permeant molecular weight on the permeability coefficient

Diffusion profiles clearly showed, as expected, that the rate of diffusion decreased with increasing molecular weight. The permeability coefficient was plotted versus the molecular weight of the permeants (not shown). Although the variations in the permeability coefficient as a function of experimental conditions were relatively slight, they were significant enough to make a clear comparison of the relationship between the permeability coefficient and the molecular weight. The logarithmic equation gives a first approximation of the decreasing trend that is consistent with the literature:[29,41]

$$P \times 10^9 = -2.66 \text{ Ln MW} + 21.2 \quad (6)$$

where MW is the molecular weight. The permeability and diffusion coefficients are generally plotted versus the permeant radius, also giving a decreasing trend.

CONCLUSIONS

The diffusion of a series of biomolecules through hollow chitosan fibers was investigated under different experimental conditions (pH, permeant concentration, flow rate, etc.). The permeant concentration and flow rate did not significantly affect the diffusion properties. The pH had a more significant impact, although less than expected: the influence of the pH depended on the permeant. Tryptophan diffusion was much less influenced than chloramphenicol diffusion, whereas the diffusion of vitamin B12 was almost unaffected by pH. The permeability coefficient decreased with the molecular weight of the permeant. Drying the hollow fiber induced a significant decrease in diffusion properties. The irreversible collapse of the porous structure limited the permeability coefficient. Such hollow fibers could be envisaged in the field of supported catalysis[13,21,42] or as supports for biological molecules or enzymes as an alternative to gel beads for drug release[35] or enzyme immobilization[43,44] or for biomedical applications (including nerve reparation).[16,45,46]

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