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Glycine Transport through a Charged Polysulfone Cation Exchange Membrane

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The transport of glycine through a charged polysulfone cation exchange membrane was studied as a function of pH at different initial conditions of less than pH 5.9 and the glycine permeability was explained under the experimental conditions as pH dependent interfacial transport. The glycine permeability increases with a decreasing pH in the receiver phase. The largest permeability was obtained if the initial pH on the source side was pH 5.9 and that on the opposite side was pH 2.0. The transport phenomena can be explained by the interfacial transport based on the interfacial chemical reaction (protonation, deprotonation or ion-exchange) between both phases in terms of the initial pH of the solution.

Key Words: amino acid transport; charged membranes; permeability; glycine.

Introduction

Amino acids are very important compounds, which can occur in cationic, anionic or neutral from depending on the solution pH[1−3]. Transport phenomena of amino acids through charged membranes were investigated[4−10] for an efficient purification process in biotechnology[11]. Recently, membrane mediated separations were found to be very attractive as alternative methods to most chemical methods of purification[12−20]. The main advantage of membrane based separation processes is their low-energy approach to bioseparations. It has been reported[21−24] that the membrane separation module could be conveniently coupled to a bioreactor for continuous product recovery without affecting the microbial cells, especially the recovery of organic acids such as citric acid or lactic acid from fermentation broth. Previous studies show that organic acid and amino acid permeation through an ion exchange membrane is pH dependent[4−9] and that interfacial transport can be the rate-limiting step[4] in the permeation process.

We are interested in obtaining information about the behavior of the transport process for recovering amino acids and the influence of the nature and pH gradient on the flux of amino acids through charged polysulfonated membranes, and in correlating these results systematically with the results in the literature. The present experiments are designed for the hydrogen ion a "pumping ion" for the process. Therefore, in this study, a series of experiments was especially designed to show the effects of pH on the interfacial transport of amino acids. Here glycine was especially chosen as the smallest one through a cation exchange

membrane. The experimental results are explained by considering that membrane interfacial transport is based on the interfacial chemical reaction.

Experimental

Glycine, HCl, citric acid, $Na_3HPO_4.12H_20$, ninhydrine from Merck and BDH Ltd., and the charged cation exchange membrane (SA_3S), obtained from Gelman Sciences (Pall Corp.), were incorporated into a strong polyelectrolyte with sulfonic acid groups as fixed charge groups. The ion exchange capacity of the charged membrane is 14 μeq. per disc 47 mm in diameter and δ 152.4 μm in thickness, supplied in the hydrogen ion form. Before the experiment, the membrane was pretreated with 1 M HCl, NaOH and deionized water; then the membrane piece was treated with 1 M HCl to insure conversion to the H^+form. It was then washed repeatedly with water before the transport experiments.

The amino acid solution was prepared by using a buffer solution (citric acid-phosphate) at different pH values. The pH values were determined with a pH apparatus (Orion ion meter EA 940), equipped with a pH (Orion 91-02) combined electrode. The ninhydrine solution was prepared by dissolving in ethanol.

Transport measurement

The experimental setup used in the glycine transport measurements is shown in Fig. 1. Three different experimental conditions were considered: in experiment (a) the pH in the source and receiver phases were both kept at pH 2, 4.0 and 5.9; in experiment (b), the pH in the source phase was approximately equal to 5.9, and the pH in the receiver phase was varied; and in experiment (c), the pH in the source phase was varied, and the pH in receiver phase was approximately equal to 5.9. Two compartment cells (made by Teflon) of volume 50 ml were separated by the charged membrane, the effective membrane area, A, was 7.8 cm^2. A constant temperature (25°C) was maintained during the experiments and both compartments were stirred by magnetic stirrers.

Aliquots (1 ml) of the aqueous solutions of both phases were withdrawn at appropriate intervals and the volume taken was not replaced. Transport rate J was the number of moles of glycine transported and calculated at the beginning of the linear concentration curve vs. time. The concentration of glycine on both sides of the membrane was measured by UV-visible spectrophotometer (Shimadzu UV-160A) at 570 nm. Before measurement, the glycine solution was pretreated with ninhydrine solution for 24 h. The determination procedure of amino acid with ninhydrine has been given in the literature[25]. Each experiment was repeated at least twice and the results were consistent within ±10%.

Results and Discussion

The series of experiments depicted in Fig. 1 was especially designed to show the effects of pH on the interfacial transport of glycine in aqueous solution through the cation exchange membrane. For these reasons, three different experimental conditions were considered for the transport process: in experiment (a), the pH in the source and the receiver phases were the same; in experiment (b), the pH in the source phase was approximately equal to 5.9, and the pH in the receiver phase was varied; and in experiment (c), the pH in the source phase was varied, and the pH in the receiver phase was approximately equal to 5.9. The

concentration of glycine was 0.05 mol dm^{-3} in the source phase, and was initially set to zero in the receiver phase for all experiments.

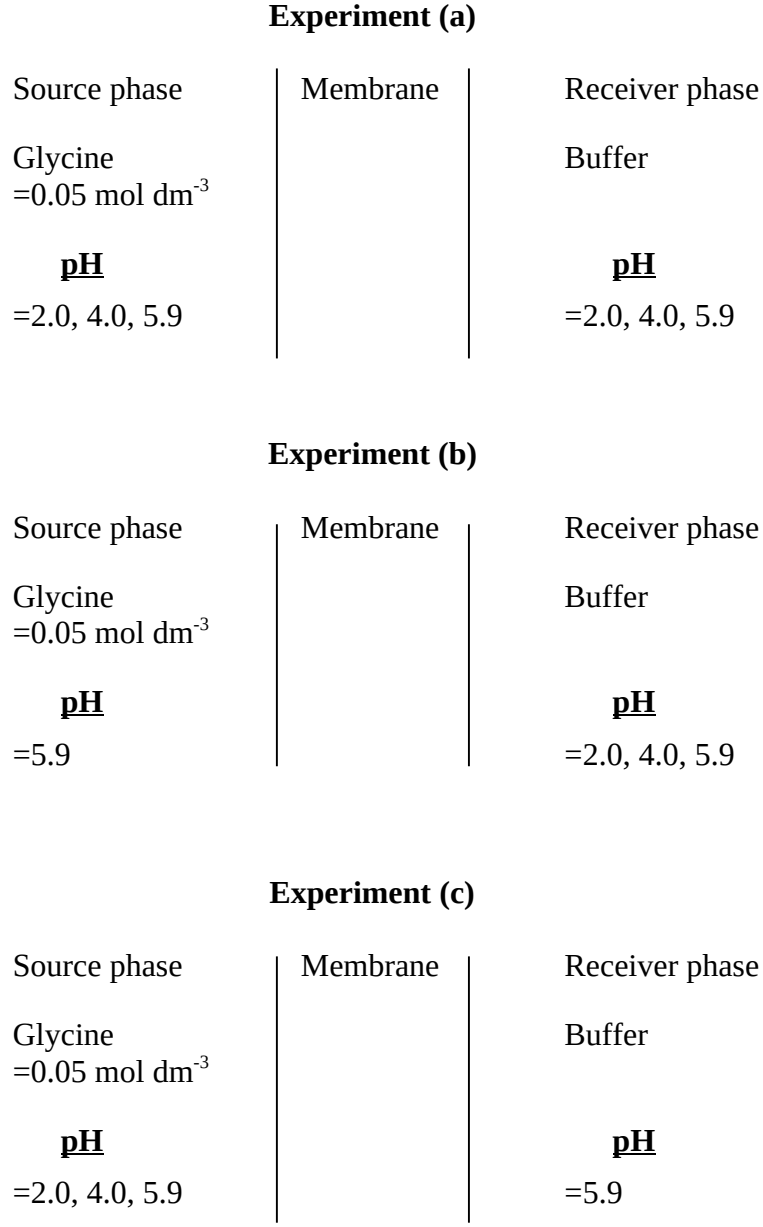

Figure 1. Schematic diagram for experiment (a-c) for the different initial interfacial conditions on the charged polysulfone (SA3S) membrane.

Some examples of typical curves for the glycine concentration in the receiver phase vs. time are shown in Figs. 2-4 for three different experimental conditions (a), (b) and (c), respectively. The maximum time is 15 hours. For all values on the receiver side, the concentration of glycine increased to a maximum value and then leveled off. The transport of glycine through the membrane from the source phase to the receiver phase occurred by pH changes on both sides. Because of the lower pH, the isoelectric points of glycine are positive (ionic forms) and the positively charged form in the source phase can permeate the membrane and diffuse

by its concentration gradient to increase the total concentration of glycine in the receiver phase. However, these observed concentration changes are important enough to be experimentally monitored. The flux and permeability of glycine are determined from the slope using eq.(1). For the experiments, the concentration of glycine was C_a in the source phase and was initially set to zero in the receiver phase for all experiments. The flux of glycine J_a and the permeability coefficient, P, defined as $J_a l/C_a$, were determined from the concentration changes with time using the following equation[9]:

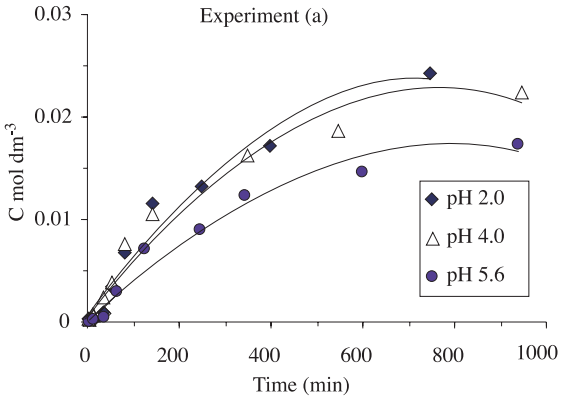

Figure 2. Typical curve of the glycine concentration in the receiver phase versus time for experiment (a), the initial glycine concentration 0.05 M.

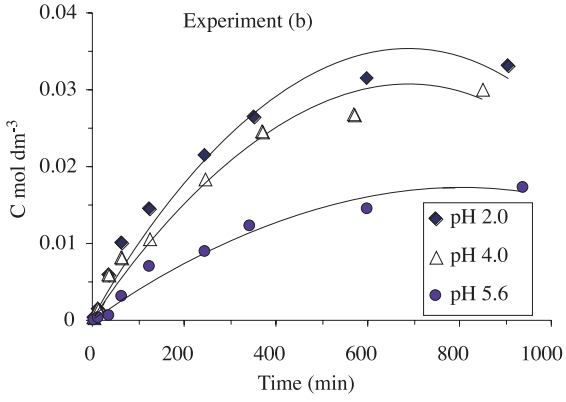

Figure 3. Typical curve of the glycine concentration in the receiver phase versus time for experiment (b), the initial glycine concentration 0.05 M.

$$P = \frac{l}{A}\,\frac{1}{1/V_s + 1/V_r}\,\frac{1}{\Delta t}\,\ln\frac{\Delta C_a(t)}{\Delta C_a\,(t\,+\,\Delta t)} \tag{1}$$

where l is membrane thickness; A is the active area of the membrane; V_s (V_r) is the volume of source (receiver) solution, and $\Delta C_{a(t)}$ and $\Delta C_{a(t+}\Delta_{t)}$ are the concentration differences between the receiver solution, $C_{a,r}$, and the source solution, $C_{a,s}$, measured at times t and t+Δt, respectively.

In Fig. 2, the amount of glycine transported through charged polysulfone cation exchange membranes over time is shown for experiment (a), when the initial pH for both phases was the same. The highest transport was observed when the initial pH in both phases was 2; the lowest transport was observed when the initial pH was 5.9; and intermediate transport was observed when the initial pH was 4 (Fig. 2).

In Fig. 3, the amount of glycine transported for experiment (c) is given, when the initial pH in the source phase was constant and that on the opposite phase was varied. The highest transport was observed when the initial pH in the receiver phase was 2, the lowest transport at pH 5.9 and intermediate transport at pH 4. It is known that the lower pH of amino acid solution from the isoelectric point forms positive charges sufficient to cause electrostatic interactions with the negative charges of the sulfonyl groups of the membrane. In this case, H^+ ions are the driving force in the transport of the glycine, because protons are diffused from the receiver phase to the source phase in an equal ratio. Fig. 4 was drawn for the data of experiment (c), in which the transport increased when the pH on the source side was 2. In the case of pH values of 4 and 5.9, the transport of glycine is similar. Each experimental value in Figs 2-4 corresponds to the average value of 2 or 3 independent measurements. The maximum deviation between measurements was less than 10% in all cases.

The interest here was in the transport of amino acids against its pH difference. The pH gradient of glycine on the source phase of membrane - the driving force of the transport of the glycine - is generated by

a pH difference between both phases. This is in good agreement with a simple mechanism for the transport, in which the driving force of the process is the pH, as well as the concentration gradient.

Many studies concerning ion transport phenomena in membrane-electrolyte solution systems have been reported[26−32]. In the case of the amino acids, the transport phenomenon is still under investigation. There may be several factors controlling the membrane phenomena such as the solution pH (the charge of amino acid either positive, negative or neutral form), ion flux through the membrane, partition coefficients at the interface between the membrane and solution, and polymer swelling. Of these, the charges of ions either in the positive or the negative state within the membrane may play an important role in the transport phenomena. The ions may exist in two different states due to ion exchange with sites and Donnan adsorption. It would be interesting to separate the two states experimentally.

It is generally known that, in acidic meda, amino acids are protonated according to the following reaction

$$H_3N^+ - R - COO^- + H^+ \longleftrightarrow H_3N^+ - R - COOH$$

In a basic medium the following reaction occurs;

$$H_3H^+ - R - COO^- + OH^- \longleftrightarrow H_2N - R - COO^- + H_2O$$

Consequently, the permeation of glycine from the source phase into the membrane phase occurs. On the other hand, on the source side, positively charged amino acid molecules can penetrate the membrane, interact with sulfonyl anions in a boundary region in which the membrane swells, and be exchanged by the H^+ ion. Thus, the pH gradient between the two aqueous phases promotes the transport of amino acid against its pH or concentration gradient. Consequently, the acid medium of the source phase allows the protonation of amino acids, which are attached to the sulfonyl groups in the bulk membrane phase. The receiver phase facilitates the ion exchange mechanism of amino acids with the proton, and thus the amino acids pass from the membrane interface into the receiver phase. It can be seen in Figs. 2-4 that the glycine flux does not linearly increase with the concentration difference imposed at pH 5.9, which suggests some tendency towards saturation in the glycine transport through the membrane. This is in agreement with Sikdar's result [4], where the diffusion of the protonated glycine was assumed to occur along the negatively charged sulfonic groups of the cation exchange membrane.

Transport of glycine through a charged membrane has been qualitatively explained by Minegowa and Tanioka[9], who suggested a theoretical model. In the model, their assumptions were the following: i) the equilibrium dissociation equations for the fraction of amino acids forming at different pH values; ii) a rate of equation for the interfacial transport based on the interfacial chemical reaction between amino acid and hydrogen ion or alternatively, Donnan equilibrium; and iii) the Nernst-Planck flux equations for the amino acid and hydrogen transport through the cation exchange membrane. They considered that the fixed charge concentration of the cation exchange membrane is higher than the HCl concentration in the pH range 1-6. The fluxes of co-ions were neglected according to Donnan co-ion exclusion, since the membrane fixed groups contain negatively charged sulfonyl groups.

Protonation and deprotonation interfacial chemical reactions at the surfaces between membrane-solution and the ion exchange play a role between protonated amino acid and hydrogen ion. In this case, a steady-state equation may be used to calculate the transport parameter. The other effect is that the neutral form of amino acids can be transported due to the scarcity of hydrogen ions. For these circumstances, a rapid

ion exchange interfacial equilibrium cannot be assumed if interfacial kinetic control is the rate-determining step. The entrance of amino acid from the source phase to the bulk membrane phase is controlled by the protonation mechanism; and the exit from the membrane to the receiver phase occurs by deprotonation or is controlled by the ion-exchange mechanism.

Glycine transfer rates observed for the different experimental conditions were found to be nearly two times higher when the initial pH on the receiver phase was 2. On the other hand, it can be supposed that the H^+ flux drives the transport of the protonated glycine ion through the membrane. The transport rate is related to proton activity. The flux of H^+ ions from the receiver to the source solution governs glycine transport. This means that a larger pH difference between the feed and receiver solutions is necessary to obtain a higher amino acid transport rate. This fact is due to the higher mobility of H^+ ions. It can be concluded that glycine transport rates were affected mainly by the pH difference between the source and the receiver solutions. The explanation of this may be simply that the pH gradient increases the partitioning of the glycine at the membrane/feed phase interface.

For our goal, it is interesting to note that the largest flux of glycine was measured with the pH around 2 on the receiver phase and that on the opposite side the pH was 5.9. A much higher flux of glycine could, however, have been achieved by applying an electrical field by inserting electrodes into chambers and enforcing the migration of protonated glycine through polysulfonated charged membranes. Comparisons show that the data obtained by the charged polysulfone cation exchange membrane seem to be in good agreement with the K 101 membrane.

In Fig. 5, the permeability coefficients obtained for experimental conditions are given as a function of the initial pH. For experiment (a) the permeabilities were very close to each other between the initial pH 4 and 5-6; for experiment (b) permeability increases when the amino acid phase pH was 5.9 and the other sides pH was 2.0. The permeability coefficients for glycine were in the range of 10^{-8}-10^{-9} cm^2 s^{-1}, and for alkali metal cations in cation exchange membranes, these permeability coefficients were in the range of 10^{-7} cm^2 s^{-1} [33].

The permeability coefficients for all experiments, (a), (b) and (c), were found to be lowest at pH 5.9. This can be explained by the low concentration of hydrogen ions in the receiver solution for glycine. In this case, the scarcity of hydrogen ions in the bathing solutions has two effects on the amino acid transport through the membrane. First, the concentration of protonated glycine in the solution is on the order of, or even higher than, that of the hydrogen ion, which is the pumping ion, and thus the membrane is not in the H^+-form throughout. Second, this scarcity of hydrogen ions forces the glycine to enter and to exit the membrane in neutral form.

As seen in Fig. 5, the permeability coefficients of experiment (b) decrease while the permeability coefficients of experiment (a) remain nearly constant with increasing of pH. The experimental results of the glycine P increase slightly for experiment (c). Such differences among the three experiments may be caused by the pH changes in both cells. From a theoretical point of view, the glycine permeability should remain constant as in experiment (a), as reported[9].

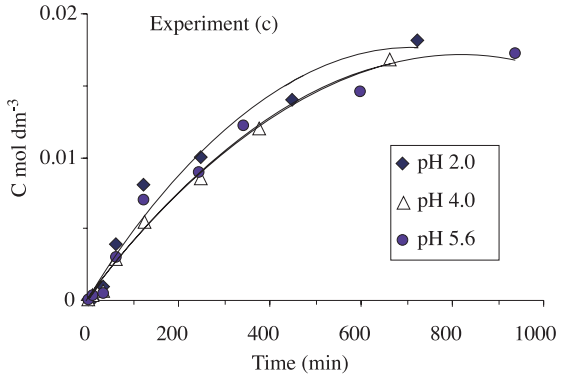

Figure 4. Typical curve of the glycine concentration in the receiver phase versus time for experiment (c), the initial glycine concentration 0.05 M.

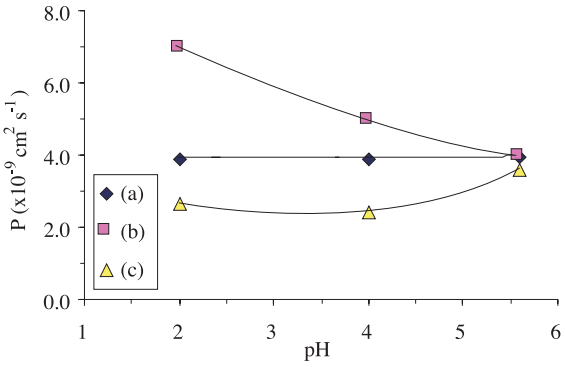

Figure 5. Permeability coefficients obtained for glycine (0.05 M) in experiment (a-c) as a function of the pH.

In Fig. 6, the glycine flux for three different conditions is shown as a function of pH for all experiments. Glugla and Dindi[5] have pointed out that protonation is faster than ion exchange and ion exchange is faster than deprotonation. Taking into account their rule, glycine transport in experiment (a) is intermediate, in experiment (b) it is highest and in experiment (c) it is lowest, as shown in Figs. 2-4. In Fig, 5, at initial pH 2, the permeability coefficients of experiment (b) are at leas twofold greater than those of experiments (a) and (b). All of the permeability coefficient values were quite similar to each other when the initial pH was 5.9. In the case of the glycine transport phenomena at pH 1, they correspond with the transport behavior of aniline, nitroaniline, chloraniline and urea at pH 1 through perfluorosulfonated ionomer membranes based on the work of Glugla and Dindi[5]. They pointed out that the highest permeabilities were seen (I) when their source side had no supporting electrolyte and the sink side was at pH 1, which corresponds to experiment (b). The lowest permeability was seen (II) when both sides have no supporting electrolyte, which corresponds to experiment (a) at pH 5.9, and (III) with pH 1 on the source side and no supporting electrolyte on the sink side, which corresponds to experiment (c).

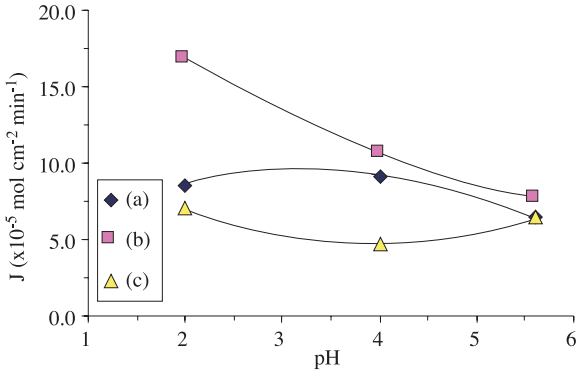

Figure 6. Flux obtained for glycine (0.05 M) in experiment (a-c) as a function of the pH.

Conclusions

In experiment (a), the entrance of glycine from the source phase to the membrane is largely controlled by protonation, or the ion-exchange mechanism, in the pH ranges 2.5-4, and partly by ion-exchange mechanism

or protonation at pH 5.9, and the exit from the membrane to the receiver phase is controlled by the ion exchange mechanism and partly by deprotonation.

In experiment (b), the entrance of glycine from the source phase to the membrane is controlled by protonation or partly ion exchange mechanism and the exit from the membrane to the receiver phase by ion exchange mechanism.

In experiment (c), the entrance of glycine from the source phase to the membrane is largely controlled by the ion exchange mechanism and the exit from the membrane to the receiver phase mainly by deprotonation and partly by the ion-exchange mechanism.

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List of Symbols

J_a flux of glycine (mol/cm^{2}s)
l membrane thickness (mm) P permeability coefficient (cm^2/s)
$C_{a,rors}$source or receiver solution concentration.
A active area of the membrane (cm^2)
$V_{s,r}$, volume of source or receiver solution
$C_{a,r\ or\ s}$source or receiver solution concentration

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