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MECHANISMS OF PROTEIN FOULING IN MICROFILTRATION. II. ADSORPTION AND DEPOSITION OF PROTEINS ON MICROFILTRATION MEMBRANES

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ABSTRACT

Adsorption and deposition of proteins in/on membranes were two main mechanisms of protein fouling in microfiltration. To distinguish the two mechanisms, adsorption and deposition were studied under both static and filtration conditions. The results demonstrated that under static conditions, adsorption had an equilibrium and the equilibrium amount of adsorption was almost constant even in solutions with different concentrations. The maximum adsorption occurred at isoelectric point pH value, and the amount of protein absorbed was similar to that of monolayer type adsorption. In actual microfiltration, adsorption and deposition of proteins occur simultaneously and the adsorption has an equilibrium with adsorption values similar to that of static adsorption. The amount of protein that deposited onto the membranes was simply proportional to filtration

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volume of the solution. The result can be described by a mathematical model.

Key Words: Microfiltration; Protein fouling; Adsorption; Deposition

INTRODUCTION

Microfiltration is widely used in various separation processes, e.g., for the recovery of extracellular proteins produced via fermentation, for sterilization of pharmaceutical products, and for clarification of fruit juice, wine, or beer. Most of these applications are treated with protein-containing solutions. One of the severe problems in microfiltration of protein-containing solutions is fouling. Membrane fouling causes filtration flux decline and eventually complete blockage of the filters.

It is generally considered that membrane fouling occurs due to the deposition and accumulation of submicron particles on the membrane surface and within the pores of the membrane itself.[1] On basis of the location of foulants, protein fouling can be classified into two types. One is external fouling that occurs at the front face of the membrane, and the other is internal fouling occurring on the inner surfaces of the membrane pores. In actual microfiltration, the two types of fouling are expected to occur simultaneously. Studies of protein fouling in microfiltration have been reviewed by Nilsson,[2] Marshall et al.,[3] and Belfort et al.[4]

Adsorption of macromolecular solutes is one of the causes for such a fouling phenomenon. Adsorption of proteins on microfiltration membranes is generally studied in terms of "static adsorption," in which no pressure is applied. The adsorption procedure is governed only by the diffusion of protein molecules. The effect of adsorption was estimated by comparing flux of pure water or solution between a clean membrane and a protein pre-adsorbed one.[5] Adsorption was also studied using surface force apparatus;[6,7] it was concluded that the amount of protein adsorbed onto the membrane was linearly related to the adhesion force. However, in the actual filtration, in which the solute is forced to flow through the pore, the adsorption behavior is expected to be different from that in the immersing method. Bowen and Gan[8] studied dynamic adsorption by pumping bovine serum albumin (BSA) solutions through membranes, and multilayer adsorption was observed in their experiments. They concluded that adsorption was introduced by shear while other researchers considered that deposition of protein aggregates in or on membranes was the main factor for flux decline. Kim et al.[9] observed fouling layers of protein, using field emission scanning electron microscopy on various microfiltration and ultrafiltration

membranes fouled with BSA. They observed a heavy cake of aggregates and sheets on the surface after 40 min of filtration, with some large holes still evident in the cake layer. They concluded that fouling appeared to be a surface phenomenon, even for membranes in which substantial amount of protein was passed through. The BSA aggregates on the surfaces of track-etched and asymmetric microfiltration membranes have also been observed by Hlavacek and Bouchet.[10] Using light scattering, Chandavarkar[11] found that aggregates formed by peristaltic pumping of BSA solutions and that the initial fouling behavior may be attributed to the deposition of these aggregates on the membrane surface. Kelly et al.[12] also concluded that protein aggregates were the cause of membrane fouling. They observed that the fouling increased with an increase in the number of denatured protein monomers in solution, and that fouling could be substantially reduced or delayed by prefiltration to remove the aggregates.

In practical microfiltration, adsorption and deposition exist simultaneously. It is difficult to distinguish proteins adsorbed or deposited on membranes. In this article, proteins on membrane were determined by amido black 10 B method, and the adsorption and deposition of protein was described by a mathematical model.

MATERIALS AND METHODS

All membranes used in the experiments were mixed esters of cellulose nitrate and acetate membrane (cellulose membrane) with a nominal pore size of 0.22 μm. The membranes were purchased from Shanghai Xingya Corporation (Shanghai, China). Bovine serum albumin (BSA, Fraction V) with isoelectric point of about pH 4.7 was obtained from Boehringer Mannheim (Germany). Amido black 10 B was purchased from Beijing Chemical Reagent Corporation (Beijing, China).

The BSA solutions were prepared by dissolving BSA powder in desired pH buffers or pure water. Buffers of pH 3.0 and 4.7 were 10 mmol/L citrate buffer. Buffer of pH 7.2 was phosphate buffer. The pH of BSA–pure water solution was pH 7.1. All buffers were pre-filtered with 0.22 μm cellulose membranes.

In static adsorption experiments, cellulose membrane disks with a diameter of 25 mm were used. Before the experiments, the membranes were soaked in pure water for an hour to remove glycerin, which was used as a protectant in membranes. The static adsorption was carried out by simply immersing membranes into protein solutions for a specific time. The concentrations of protein ranged from 0.1 to 5.0 mg/mL. Then, the membranes were soaked in pure water for 2 hr to remove the proteins that did not bind to membranes. Finally, the amount of proteins on the membranes was measured. For measurement of protein coverage, the membranes were soaked in protein solution for 24 hr, which is enough for adsorption to reach the equilibrium state.

Microfiltration was performed at 10 kPa. Membrane disks with a diameter of 13 mm were used in these experiments. In each microfiltration, 20 mL of pure water was filtered through membranes to remove glycerin from the membrane and get an equilibrium flux. Then, the water was replaced with BSA solution. After desired volumes of the BSA solutions were filtered, the BSA solution was replaced with pure water, and 10 mL pure water was filtered subsequently to remove any protein that did not bind to the membrane. The BSA solutions that were pre-filtered with 0.22 μm cellulose membrane were also used in experiments. The amount of proteins fouled on membranes was measured by amido black 10 B.[13]

RESULTS AND DISCUSSION

Static Adsorption

The time profile for the adsorption of BSA is shown in Fig. 1. There is an initial period of rapid adsorption followed by a slower approach to a limiting value. The overall profile was similar at all the studied protein concentrations. The limiting value was 170 μg per membrane with the BSA concentration

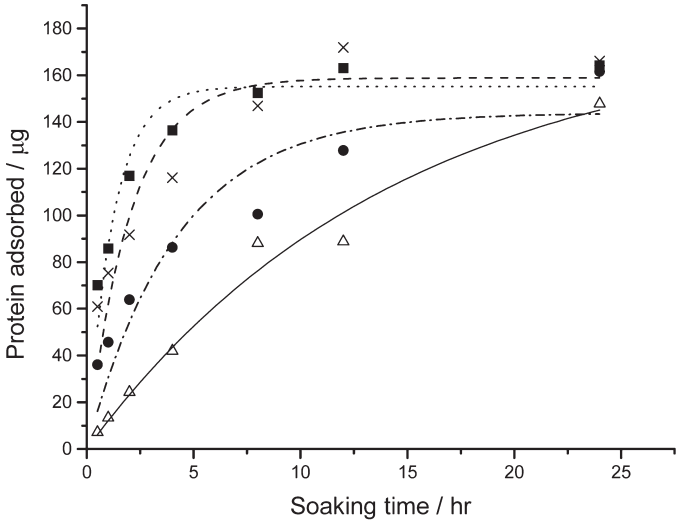

Figure 1. Adsorption profile of BSA on cellulose membrane. The concentration of BSA is ■: 5.0 mg/mL; ×: 1.0 mg/mL; ●: 0.5 mg/mL; △: 0.1 mg/mL. The lines are predicted data. dot line: 5.0 mg/mL; dash line:1.0 mg/mL; dash dot line: 0.5 mg/mL; solid line: 0.1 mg/mL.

ranging from 0.1 to 5 mg/mL. This indicated that there was an equilibrium between the solution and the adsorbed protein.

Figure 2 shows a comparison between adsorption in BSA–pure water solution and that in BSA–phosphate buffered solution. The concentration of both the solutions is 1 mg/mL. The adsorption in BSA–phosphate buffered solution was more rapid than that in BSA–pure water solution. The maximum amount of protein adsorbed on the membrane in the case of BSA–phosphate buffered solution was larger than that in the BSA–pure water solution. The static adsorption was governed by adsorption rate constant and diffusion of molecules from bulk solution to membrane surface. On comparing curves (a) and (b) of Fig. 2, it is clear that adsorption under conditions of curve (a) was so fast that the amount of protein adsorbed on the membrane rapidly reached the maximum value while the adsorption under conditions of curve (b) was slower. The diffusion coefficient of BSA under conditions of curves (a) and (b) should be the same. Thus, the adsorption under conditions of curve (b) was mainly controlled by the adsorption rate constant.

The rate of protein adsorption on membranes was proportional to the uncovered membrane surface area and protein concentration, so

$$\frac{dq_a}{dt} = k_{ad} S_{Uncover} C = k_{ad}(S_0 - S_{Adsorb})C \quad (1)$$

where q_a is the amount of protein adsorbed, t the time the membrane contacts the protein solution, k_{ad} the adsorption rate constant, C the concentration of protein

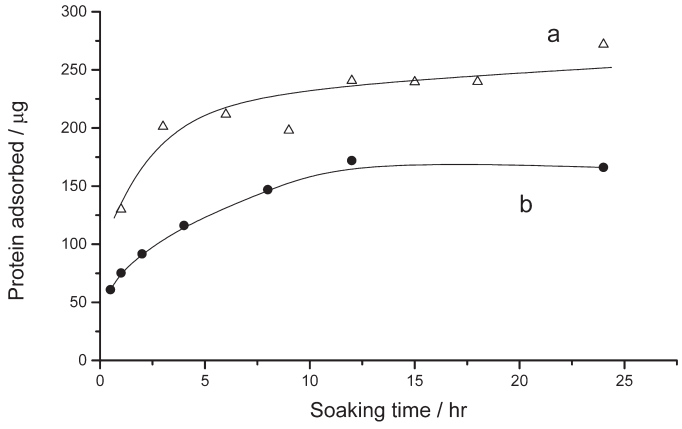

Figure 2. Adsorption profile of BSA on cellulose membranes in different solutions. The BSA concentration was 1.0 mg/mL. a: phosphate buffed BSA solution, b: BSA–pure water solution.

solution, S_0 the total internal membrane surface area, S_{Uncover} the internal membrane surface area that has not been covered by adsorbed protein, and S_{Adsorb} the internal membrane surface area that was covered by adsorbed protein.

Assuming that saturation amount of protein adsorbed per unit membrane surface area is q', the following expression was obtained.

$$S_{\text{Adsorb}} = \frac{q_a}{q'} \tag{2}$$

Substituting Eq. (2) into Eq. (1)

$$\frac{dq_a}{dt} = k_{ad}\left(S_0 - \frac{q_a}{q'}\right)C = \frac{k_{ad}}{q'}(S_0 q' - q_a)C = \frac{k_{ad}}{q'}(q_m - q_a)C \tag{3}$$

let $k_1 = k_{ad}/q'$ then

$$\frac{dq_a}{dt} = k_1(q_m - q_a)C \tag{4}$$

Integrating Eq. (4)

$$q_a = q_m(1 - e^{-k_1 Ct}) \tag{5}$$

where $q_m = S_0 q'$ is the maximum amount of protein adsorbed on the membrane.

Equation (5) is a theoretical model under ideal conditions with k_1 as a constant in theory while in practice, the concentration of protein in membrane was not uniform. This would introduce deviations in k_1 from the ideal value, but it was still able to show the trends of the adsorption of protein on membranes. The parameters that fit the data in Fig. 1 by Eq. (5) with Levenberg–Marquardt (LM) algorithm are shown in Table 1. The maximum amount of adsorbed protein on membranes at different concentrations was in the same order while the coefficient k_1 was different. In practice, diffusion of proteins into membrane also influences adsorption procedure, so the appearance of k_1 was also affected by diffusion of proteins from bulk solution to internal surface through membrane pores. Because transmission coefficient was directly proportional to protein concentration, Eq. (5) could fit the experimental data very well with different values of k_1 at various concentrations. Later in experiments, the BSA solution was filtered through

Table 1. Parameters of Adsorption Profile in Fig. 1 Fitted by Eq. (5)

	5.0 mg/mL	1.0 mg/mL	0.5 mg/mL	0.1 mg/mL
q_m	155	159	144	178
k_1	0.00276	0.00823	0.00786	0.0116

membranes. Under those conditions, the concentration of protein in pores was nearly the same as that of bulk solutions and the effect of diffusion could be neglected. The nearly ideal condition of adsorption was obtained.

Protein Coverage of Adsorption

In order to evaluate the protein surface coverage, it is first necessary to estimate the surface area of membranes. The structure of porous membranes is extremely complicated. The pores are strongly interconnected, and the path of a stream through the membrane is tortuous. Therefore, the Kozeny–Carman relationship, which has been applied to the laminar flow in a granular bed, was used to describe the porous membranes' behavior, and the pore surface area was estimated from measurements of the hydraulic permeability using the Kozeny–Carman equation

$$Q = \frac{p}{\mu R_{\mathrm{m}}} = \frac{\varepsilon^3}{k S_{\mathrm{SSA}}^2 (1 - \varepsilon^2)} \frac{p}{\mu L} \tag{6}$$

where Q is the permeate flux, p the permeation pressure, μ the viscosity of the permeate solutions, R_{m} the initial membrane resistance to flow, ε the porosity of the membrane, S_{SSA} the effective specific surface area of the membrane, L the thickness of the membrane, and k generally known as Kozeny's constant. A commonly accepted value for k is 5.0.

Protein coverage at equilibrium state in different solutions is shown in Fig. 3. The BSA concentrations of solutions were 1 mg/mL. The maximum adsorption was obtained at pH 4.7, which is the isoelectric point of BSA. At the isoelectric point, the net charge of protein molecules was zero, and there was minimum repulsion between molecules. So, protein molecules were easily adsorbed onto membranes at the isoelectric point. This was consistent with most of the previous investigations.[5] The protein coverage at pH 4.7 was 3.9 μg/cm^2. This value is nearly monolayer coverage.

Adsorption and Deposition in Microfiltration

Adsorption of protein onto membrane was mostly studied at static condition, in which there was no shear present while in practice, solutions were filtered through the membranes. Fouling of membrane consists of two procedures. One is adsorption of protein molecules onto membranes and another is deposition of trace aggregates of protein on membrane surface or in pores. The two procedures exist simultaneously. It is difficult to isolate the two procedures.

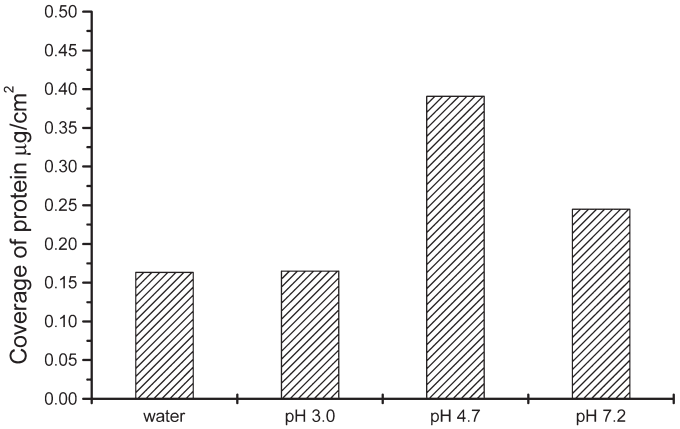

Figure 3. Protein coverage at equilibrium state in different solutions. The pH value of BSA–water solution is pH 7.1.

The adsorption procedure could be described by Eq. (5) while the deposition was directly proportional to the volume microfiltered.

$$q_d = k_2 CV \tag{7}$$

where q_d is the amount of protein deposited on the membrane, k_2 the fraction of aggregates in total protein, C the concentration of protein, and V the volume filtered.

Combining Eqs. (5) and (7), one obtains

$$q = q_m(1 - e^{-k_1 Ct}) + k_2 CV \tag{8}$$

Figures 4 and 5 show the relations between the amount of protein fouled on membranes and the filtration volume. In Fig. 5, the protein solutions were prefiltered with 0.22 μm cellulose membranes. It can be seen that the amount of protein on the membrane increased rapidly at the beginning of filtration, and then increased slowly with the volume filtered. The amount of protein on the membrane was linearly proportional to the volume filtered after 20 mL solution was filtered. The rapid increase in the beginning was due to adsorption of protein molecules onto the internal surface of the membrane. This procedure was caused by interaction between the membrane and protein molecules when membranes came into contact with solutions. After several minutes, adsorption reached an equilibrium state, and the increase in the amount of protein on the membrane was completely due to deposition of protein aggregates that were brought to the

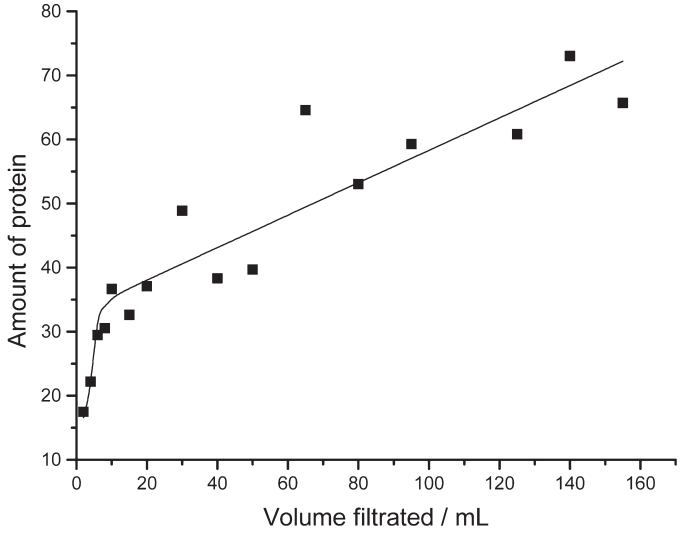

Figure 4. Protein amount fouled on membrane as a function of filtration volume. The BSA concentration was 1.0 mg/mL, and the solution was not pre-filtered.

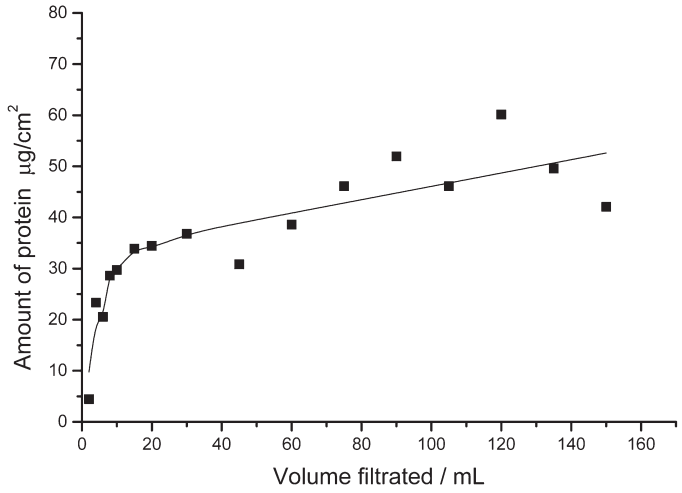

Figure 5. Protein amount fouled on membrane as a function of filtration volume. The BSA concentration was 1.0 mg/mL, and the solution was pre-filtered.

membrane surface convectively from bulk solution. Parameters derived from data in Figs. 4 and 5 by fitting with Eq. (8) are shown in Table 2.

In Table 2, it was shown that the maximum adsorption under the two conditions was similar. This indicated that the adsorption procedure was independent of pre-filtration. The protein coverage caused by q_m was 0.38 μg/cm^2, which was more than two times higher than that of the equilibrium static adsorption in BSA–pure water solution at the same concentration while it is interesting to note that the value is similar to that of the maximum static adsorption in pH 4.7 solution, and the rate of adsorption was more rapid than that in static adsorption. This indicated that protein molecules, which were driven by shear, could overcome electric repulsion to access membrane surface. So the coverage of adsorption in microfiltration was more like that of static adsorption at the isoelectric point at which the electric repulsion was nearly zero. The coefficients that derived from nonpre-filtered BSA solution and pre-filtered BSA solution were different, this was also seen in Figs. 4 and 5. k_2 is the fraction of aggregates in BSA solution. Pre-filtration could eliminate aggregates in solution, so a lower k_2 was obtained with microfiltrate pre-filtered solution. But the value of the k_2 indicated that there were also aggregates in pre-filtered solutions, the aggregates may be newly generated in operation or remained from pre-filtration procedure. The exact reasons for the existence of aggregates need to be investigated. Using fitted parameters with Fig. 4 and Eq (8), it could be calculated that contributions of adsorption and deposition to fouling were 32 and 5 μg, respectively, per membrane within the first 20 mL solution filtered. This indicated that the rapid increase in the beginning was due to adsorption of protein molecules onto membrane's internal surface.

Relationship Between Permeate Flux and Fouling Style of Protein on Membrane

In Fig. 6, it can be seen that protein fouled on membrane increased rapidly within the first 5 mL BSA solution filtered at initial stage while the initial permeate flux has no significant decline. This phenomenon indicated that permeate flux decline was not caused by the rapid increase of protein onto

Table 2. Parameters Derived from Data in Figs. 4 and 5 by Fitting with Eq. (8)

	q_m	k_1	k_2
Nonpre-filtered BSA solution	32.9	0.66	0.25
Pre-filtered BSA solution	33.0	0.30	0.13

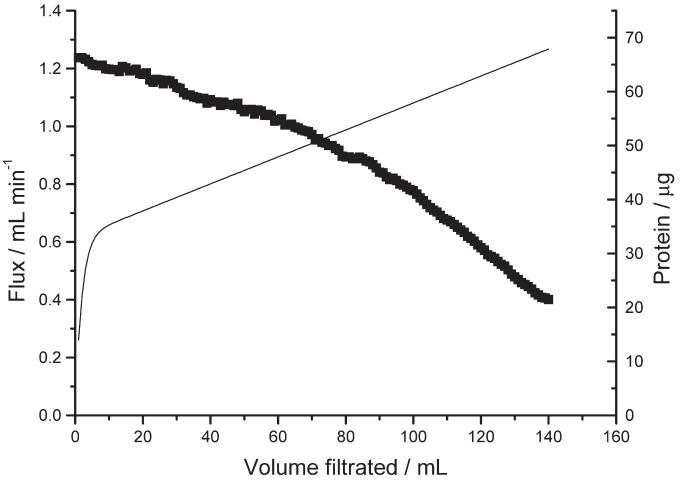

Figure 6. Permeate flux and protein amount on membrane as a function of volume filtered through membrane.

membrane, due to adsorption of protein on membrane, but was caused mainly by the deposition of protein on membrane. A comparison between the flux of protein pre-adsorbed membranes and clean membranes also showed the same result. This result was also supported by previous works; Belfort et al.[4] had calculated the effect of protein adsorption on flux—they concluded that the flux reduction caused by monolayer adsorption of BSA was only 2–12% in microfiltration system. In recent years, some researches focused on the microfiltration of protein solutions, which contain large particles (yeast cells).[14–16] They all concluded that the presence of yeast cells could enhance long-term flux of microfiltration and protein transmission. The mechanisms were proposed that large particles could form secondary dynamic membrane, which decreases fouling of the primary membrane by small particles while protein molecules could permeate the membrane easily. This also indicated that deposition of protein aggregates was the main cause of fouling.

CONCLUSION

Adsorption and deposition of protein on membranes was studied by determining protein on membranes with amido black 10B. The amount of protein absorbed on membranes at static state has apparently an equilibrium, which is almost constant within a concentration range from 0.1 to 5.0 mg/mL.

The maximum equilibrium was obtained at pH 4.7 solution, which is the isoelectric point of BSA. The maximum protein coverage of adsorption at pH 4.7 is 0.38 μg/cm^2, which is similar to that of monolayer adsorption. In microfiltration, the adsorption of proteins also had an equilibrium while protein coverage at the equilibrium was similar to that of static adsorption at isoelectric point but not similar to that of static adsorption at the same pH conditions. This phenomenon clearly indicated that adsorption in microfiltration was different from that at static conditions and the shear force could overcome the electrical repulsion between molecules, and the molecules could have more access to the membrane surface. The amount of protein fouled onto membrane in microfiltration was described by a model, which was a combination of adsorption and deposition mechanisms. The results also showed that flux decline was mainly caused by deposition of proteins.

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