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INFLUENCE OF pH ON PERMEABILITY OF CERAMIC MEMBRANES AND SELECTIVITY IN ULTRAFILTRATION OF MODEL BSA AND MYOGLOBIN SOLUTIONS

The results of ultrafiltration tests carried out with model BSA and myoglobin solutions using ceramic 50 and 150 kDa membranes have been presented. Membrane permeability and selectivity were investigated in function of pH, transmembrane pressure (TMP) as well as molecular modelling data for proteins such as size, geometrical parameters and pH of minimal free energy of folding. The study has shown that the permeate flux J_v depends on TMP, whereas the protein rejection is mainly influenced by pH. The results demonstrated that molecular modelling data are not sufficient to explain the membrane behaviour and the membrane–protein charge interactions and fouling effects must be also considered to explain the rejection mechanisms.

1. INTRODUCTION

Pressure-driven membrane techniques have been offered as an environmental and economical alternative for regeneration of used brines with the aim of closing water loops in the fish processing [1]. Several studies on application of microfiltration [2, 3], ultrafiltration [1], nanofiltration [4] and reverse osmosis [5] have been published. These processes are mainly used to concentration, fractionation and purification of the compounds present in the waste streams generated at various stages of production. The cross-flow filtration with use of ceramic membranes is demonstrated to be an advanced method for separating proteins from waste brines due to their excellent selectivity, permeability, as well as thermal and chemical stability.

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Casa et al. [6] present a review on the studies carried out to investigate the influence of pH and ionic strength on single protein filtration through ceramic membranes and the results of their own studies on BSA microfiltration. The authors investigated influence of pH and sodium chloride concentration on BSA rejection and permeate flux. Solutions with BSA concentration of 0.25 g/dm^3 and NaCl concentration of 0, 5 and 25 mM were used at pH range of 4.0–8.0. The highest BSA transmission was obtained at pH of 4.9 corresponding to the isoelectric point of BSA. An addition of 5 mM sodium chloride resulted in increase of permeability. The authors explain this effect with hindered aggregation of BSA molecules. The further increase of NaCl concentration up to 25 mM did not cause any improvement in protein transmission.

The main objective of this work was to reveal the mechanism of protein rejection from model solutions. For this purpose, both bovine serum albumin (BSA) and myoglobin (MG) molecular data as well as the experimental results of BSA and MG rejection by ceramic membrane of 150 kDa and 50 kDa have been collected and analysed. The effect of main parameters such as pH and transmembrane pressure (TMP) on protein rejection and permeate flux was discussed in the frame of molecular characteristics of BSA and MG molecules.

2. EXPERIMENTAL

Ceramic membranes with 50 and 150 kDa cut-offs were used and two kinds of membrane installation, laboratory and pilot one, respectively. The scheme of the laboratory installation is shown in Fig. 1, while the scheme of the pilot installation has been presented elsewhere [8, 9]. A flat membrane with the 50 kDa cut-off, diameter of 90 mm and membrane surface area of 0.00056 m^2 was used in experiments with model myoglobin solutions. The pilot installation used for BSA model solution ultrafiltration tests was equipped with 23-channeled module consisting of non-cylindrical membranes with the cut-off of 150 kDa, length of 1.178 m and membrane surface area of 0.35 m^2. The ultrafiltration tests with model feed were performed at constant temperature of 20 °C and at various transmembrane pressures (0.05–0.20 MPa) and pH (3–9).

Myoglobin from equine skeletal muscle (95–100%, essentially salt-free, lyophilized powder) and albumin from bovine serum (lyophilized powder, ≥96%) were used for preparing the model protein solutions with concentration of 0.005 and 0.1%, respectively. pH of the solution was adjusted with 1 M HCl or NaOH. The ultrafiltration tests were conducted with continuous permeate and retentate recycling. The membrane cleaning procedure, including acid and base washing, was carried out according to the following procedure: washing with water for 3–5 min at 30-60 °C, alkaline washing with 1.5% NaOH for 15–30 min at 75–85 °C, washing with water for 3–5 min at 40–60 °C, acid washing with 0.5% HNO_3 for 5–10 min at 50 °C and final washing with water for 3–5 min at 20–50 °C.

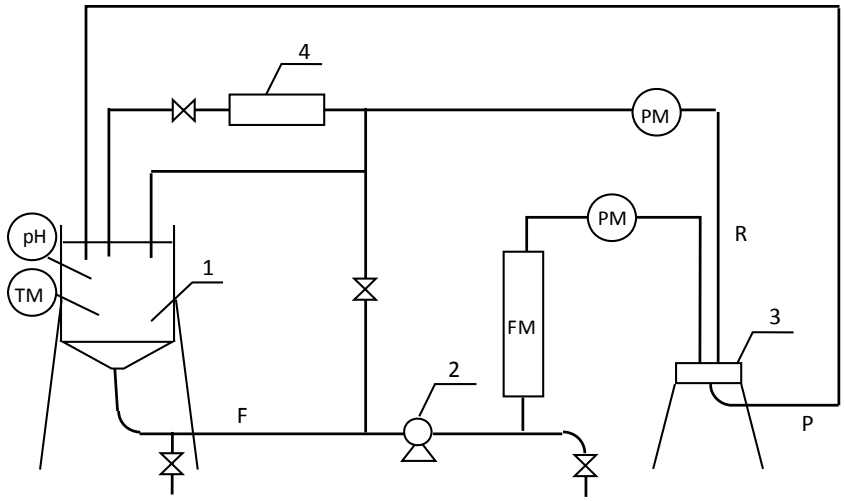

Fig. 1. Laboratory experimental unit: 1 – feed tank, 2 – pump, 3 – membrane module, 4 – heat exchanger, F – feed, P – permeate, R – retentate, PM – pressure meter, TM – temperature meter, FM – flow meter, pH – pH meter

The concentration of proteins in the feed and permeate samples was determined with application of UV-Vis absorbance measurements performed at 260 and 280 nm using quartz cuvettes of 10 mm path length from the following equation [10]:

$$C = 1.55A_{280} - 0.76A_{260} \tag{1}$$

where A_{280} and A_{260} are the absorbances at 280 and 260 nm, respectively.

The rejection of proteins was calculated from the formula:

$$R = 1 - \frac{C_P}{C_F} \tag{2}$$

where C_P is protein content in permeate, C_F – protein content in feed.

The molecular modelling calculations on optimal protein structures were carried out using the HyperChem software (release 8.0.9 for Windows). To estimate additional geometrical parameters Vega ZZ software was used [11]. The dependence of protein stability on pH was determined by pK_a calculations performed for the ionisable residues with use of the PROPKA 3.1 Web Interface [12].

The starting structure of MG was obtained from XRD based 4DC8 pdb file [13] and the BSA structure from 4F5S pdb file [14] deposited at RCSB Protein Data Bank.

The ligands were removed using the Chimera package developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311) [15]. In the next step, both struc-

tures were geometrically optimised (GO) in vacuo using molecular mechanics (MM) methods with the AMBER force field and applying Polak-Ribiere minimization algorithm. The myoglobin structure was further geometrically optimised in aqueous solution with explicit solvent and optimised with use of the molecular dynamics (MD).

Applying the Vega ZZ software, approximate dimensions, molecular weight, radius of gyration and ovality of the optimised structures were determined.

3. RESULTS AND DISCUSSION

The results of the calculations on size, geometrical parameters and pH corresponding to minimal free energy of folding are presented in Table 1. The results of the calculations on protein stability versus pH along with the protein rejection in function of pH are also presented in Fig. 2.

Table 1

Results of molecular modelling

Calculated parameter	Myoglobin (in solution after MD and GO)	BSA (in vacuo after MM and GO)
Minimum energy, kcal/mol	–77 278.69	–33 195.17
Molecular weight, Daltons	16 905.29	123 617.27
Approximate dimensions, Å	48.976, 31.656, 49.646	147.302, 67.037, 93.500
Radius of gyration, Å	15.159	38.54
Ovality	5.895	11.42
pH of minimal free energy of folding	6.4	6.1

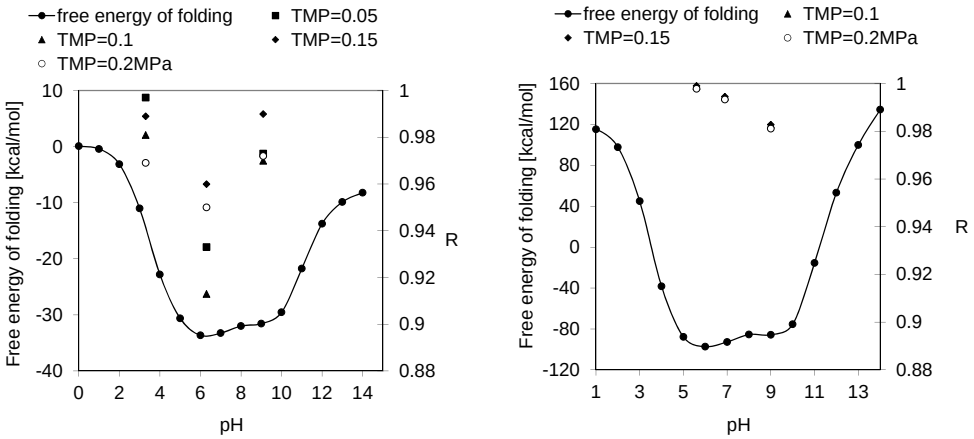

Fig. 2. Free energy of folding and protein rejection in function of pH: a) MG 0.005% solution, flat membrane, cut-off – 50 kDa, b) BSA 0.1% solution, 23-channelled membrane cut-off – 150 kDa

The results obtained for ultrafiltration tests of model BSA and MG solutions indicated that the membrane permeability J_v is mainly influenced by TMP. On the contrary, membrane selectivity is mainly dependent on pH. The effects of these two mentioned operands on BSA and MG rejection and permeate flux for the UF tests of model BSA and MG solutions are presented in Figs. 3 and 4, respectively.

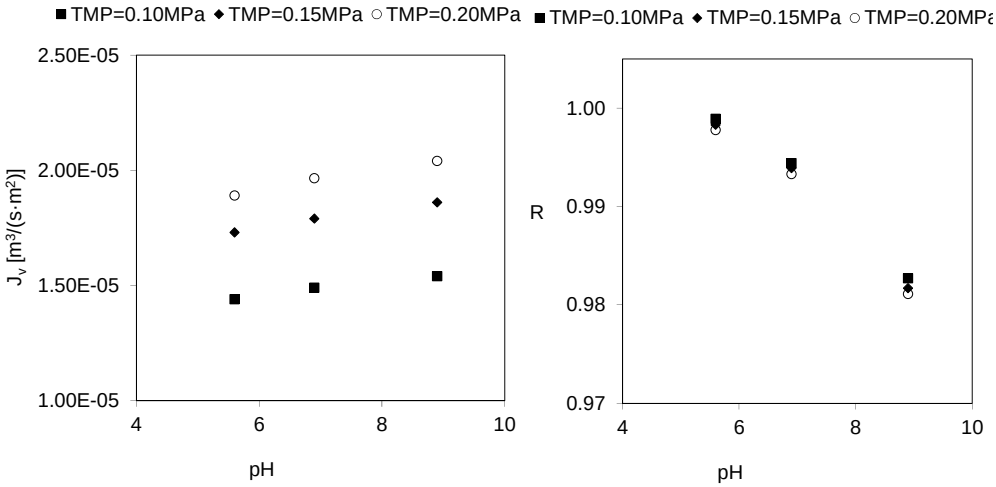

Fig. 3. Dependence of: a) membrane permeability, b) BSA rejection on pH and TMP; pilot installation, membrane surface area – 0.35 m^2, cut-off – 150 kDa

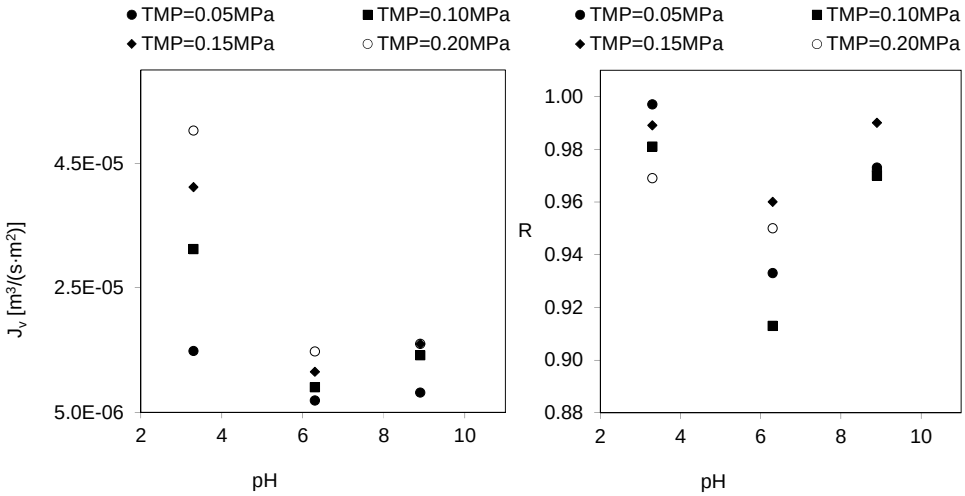

Fig. 4. Dependence of: a) membrane permeability, b) BSA rejection on pH and TMP; laboratory installation, membrane surface area – 0.00056 m^2, cut-off – 50 kDa)

According to the PropKa calculations, the pH of optimum stability is 6.4 for the MG structure and 6.1 for the BSA structure with the corresponding free energies of –33.9 kcal/mol and –97.4 kcal/mol at 298 K for MG and BSA, respectively (Table 1). In the case of myoglobin, for similar pH, minimal values of protein rejection are also observed. This can be attributed to the protein unfolding, occurring beyond the ranges of maximum stability, causing increase in protein rejection.

Experimental results showed that BSA rejection was high (0.982–0.998) and slightly dependent on TMP and pH, in the ranges of 0.1–0.2 MPa and 5.6–9.0, respectively (Fig. 3b). BSA rejection increased upon decreasing pH and reached maximal value for pH of 5.6. For analysed BSA structure, the pH of optimum stability which corresponds to the most compact structure and minimal calculated free energy of folding, is 6.1 (Table 1, Fig. 2b). In this case, the minimum of BSA rejection is shifted into higher pH in relation to the pH of maximal compactness of the protein. As presented by Casa et al. [6], the point of zero charge for $Al_2O_3/TiO_2/ZrO_2$ membrane is 6.9 and the isoelectric point for BSA is 4.9. The occurrence of maximal value of protein rejection at pH around 5.6 can be explained by the presence of attraction forces, occurring between the positively charged membrane and the negatively charged BSA, causing protein adsorption to the membrane.

The permeate flux in BSA model system is dependent on TMP and pH and increases with the increase of both operands (Fig. 3a). The highest value of J_v was obtained for TMP 0.2 MPa and pH 9.0. This could be explained by the fact that for pH 9.0 the extent of fouling in the ultrafiltration of model BSA solution is the lowest and increases upon decreasing pH [8]. The permeate flux in MG ultrafiltration tests showed dependence on pH and TMP (Fig. 4a). The highest value of J_v was obtained for TMP 0.2 MPa and pH 3.3.

The MG rejection was in the range of 0.913–0.990, showing dependence on pH and slightly on TMP (Fig. 4b). The minimal rejection was obtained for TMP 0.1 MPa and pH 6.3 and increased for pH of 3.3 and 8.9. This behaviour is in accordance with the results of molecular modelling (Table 1, Fig. 2a). The pH of maximal stability of MG structure is 6.4 for which the free energy takes the minimal value. Along with the increase of free energy of folding, the increase in MG rejection was observed. When taking into consideration the electrostatic interactions between the myoglobin and the membrane the minimal value of rejection should be obtained at pH around 7 as the isoelectric point of myoglobin is 7.1 and point of zero charge for the membrane is 6.9. For pH below 6.9 and above 7.1, both membrane and protein have charge of the same sign and repulsion effects will occur resulting in increased myoglobin rejection (Fig. 4b).

4. CONCLUSION

The results of ultrafiltration tests showed that the permeate flux took highest values for pH 3.3 for MG and 9 in the case of BSA and increased upon increasing TMP.

The lowest values of rejection were observed for pH 6.3 in the case of MG and 9 in case of BSA. In the case of MG it overlaps with the minimum of free energy of folding corresponding to maximum stability of protein (more compact structure). The calculated molecular weights show that the tested protein molecules are smaller than the cut-off of investigated membranes. Thus the membrane–protein charge interactions and fouling effects must be also considered to explain the rejection mechanisms.

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