0273-2289/89/2202—0119$02.00

Removal of RNA During Protein Concentration by Means of Enzyme Membrane

MAGDALENA RUCKA* AND BOŻENA TURKIEWICZ

Institute of Organic and Physical Chemistry, Technical University of Wroclaw, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland

Received February 29, 1988; Accepted November 15, 1988

ABSTRACT

Immobilization of RNase in PVC ultrafiltration membranes was carried out. The obtained membranes were used for concentration of BSA solution, RNA being simultaneously removed. The yield of RNA hydrolysis was found to be controlled by the initial concentration of RNA in feed solution. The protein affected enzyme action as a result of its adsorption on the membrane surface at the beginning of ultrafiltration, whereas it did not inhibit RNase activity during the process.

Index Entries: Immobilized RNase; immobilization of RNase in ultrafiltration membranes; removal of RNA from RNA-BSA solution; immobilized enzyme; enzyme membrane; membrane reactor; enzymatic ultrafiltration membrane.

INTRODUCTION

Membrane and membrane separation processes have already established their important position in biotechnology. Membrane separation occurs as a result of the molecular weight differences of solutes present in feed solution. However, novel membrane-based bioseparation operations are increasingly being invented (*1,2*). In this paper, an attempt is made to apply ultrafiltration membrane with ribonuclease activity for concentration of protein with the simultaneous removal of RNA.

*Author to whom all correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

Reagents

Pancreatic ribonuclease, yeast ribonucleic acid (RNA), and bovine serum albumin (BSA) were supplied by POCh, Poland.

Preparation of the Ultrafiltration Membranes with Ribonuclease Activity

Enzyme was entrapped inside polymer matrix. A solution containing 10% w/w PVC and 0.02% w/w RNase in dimethylformamide was prepared. This solution was used for membrane casting. The membranes were cast manually on a glass plate kept at the temperature 50°C. Solvent evaporation time was 5 min and then the gelation process in water bath was carried out in the same temperature. RNase not entrapped in membrane structure was washed out during ultrafiltration of buffer solution.

Covalent attachment of the enzyme was accomplished. PVC membranes were cast using the same solution as described above but without enzyme. The obtained membranes were incubated for 17 h at 20°C in diethylenetriamine. After 5 h-lasting water washing under the pressure of 0.1 MPa, the membranes were immersed for 12 h in 0.05 *M* phosphate buffer, pH 6.5. RNase was coupled to the aminated membrane by cross-linking with glutaraldehyde. The membranes were left for 4 h in a solution of 8% glutaraldehyde in phosphate buffer in ambient temperature. Then they were washed for 2 h with buffer under the pressure of 0.1 MPa and immersed in a buffered solution of RNase for 72 h at 4°C. The concentration of RNase was 1.25 mg/dm^3.

Enzyme Loading

The amount of enzyme entrapped in PVC membrane was calculated in the same way as described previously (*3*).

The amount of enzyme bound to the aminated membrane was determined by measuring the enzyme coupling before and after the enzyme coupling stage by method of Lowry et al. (*4*).

Enzyme Activity

RNA was dialyzed for 48 h against buffer solution before all experiments. The amount of BSA and RNA in feed solution and in the permeate was calculated from the absorbances measured spectrophotometrically at 280 and 260 nm. The concentration factor was defined as the ratio of the concentration of BSA in the retentate to the concentration in the initial feed solution.

The yield of RNA removal was defined as the amount of RNA hydrolyzed during 1 h per 1 m^2 of membrane area.

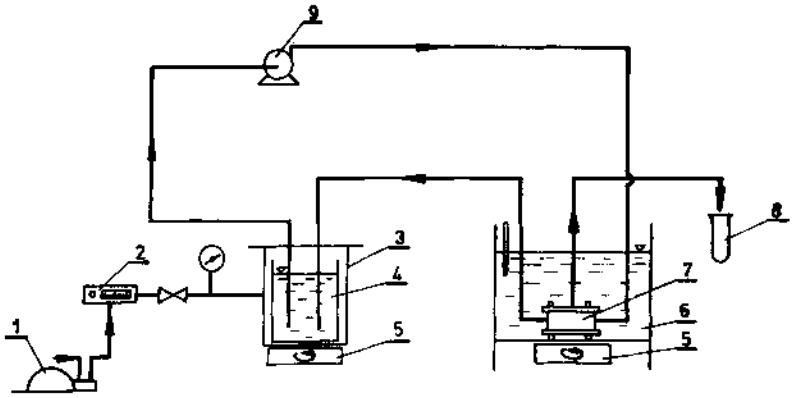

Fig. 1. Schematic diagram of apparatus 1-air compressor, 2-pressure control, 3-pressure vessel, 4-feed reservoir, 5-magnetic stirrer, 6-thermostat, 7-ultrafiltration cell with enzyme membrane, 8-fraction collector.

RNase effectiveness was determined as the amount of the removed RNA caused by 1 mg of enzyme per 1 h.

Membrane Reactor

A schematic diagram of the apparatus is shown in Fig. 1. It consists of a magnetically stirred UF cell (id 5 cm, 15 mL total capacity) with a plane membrane. The effective membrane area was 15.9 cm^2, cutoff 700 D. The experiments were carried out in a continuous recirculating ultrafiltration system with a pressure drop of 0.1 MPa across the membrane. The retentate was returned to the feed reservoir and permeate was collected by means of an automatic fraction collector. The retentate was circulated at 10 cm^3/min by a peristaltic pump. The initial volume of feed solution was 250 cm^3.

RESULTS

The PVC ultrafiltration membranes with ribonuclease activity retained RNA and BSA, whereas nucleotides, which were the product of RNA hydrolysis, passed through. To examine the pH value at which the process can be carried out, the influence of pH on enzyme activity was studied. The results are shown in Fig. 2. The enzyme immobilized by both chemical and physical means have shown the same pH optimum at the value of 5.0. Thus, all the experiments with RNA-BSA solutions were carried out at this pH.

The obtained results, when different concentrations of RNA and BSA in feed solution were employed, are given in Table 1. All the experiments

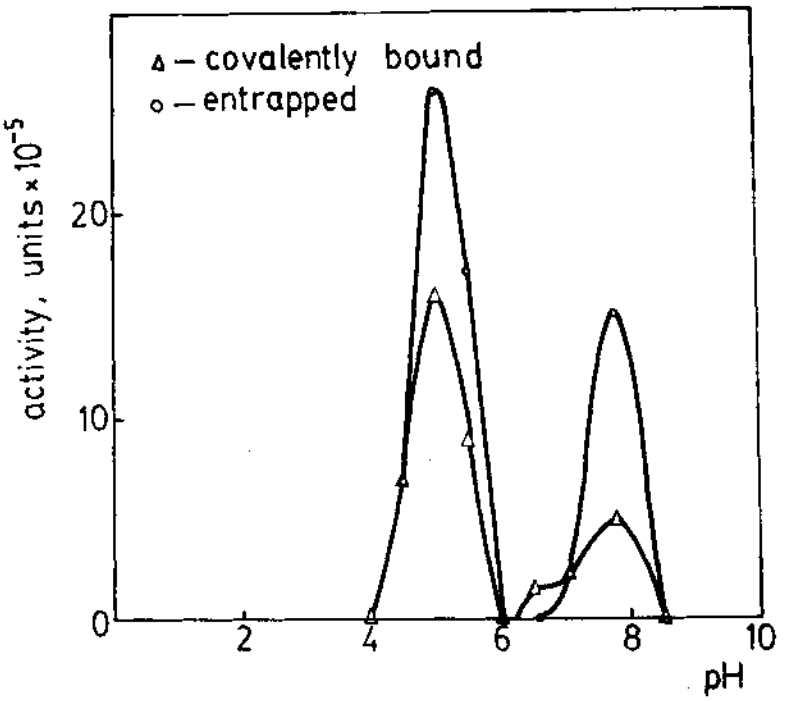

Fig. 2. Dependence of immobilized RNase activity on pH: △—covalently bound; ○—entrapped.

Table 1
Concentration of Protein and Removal of RNA

Feed concentrations			Protein concentration factor	Yield of RNA removal	RNase effectiveness
RNA	BSA	Ratio			
mg/dm^3	mg/dm^3	$\frac{BSA}{RNA}$		g/m^2h	g/mgh
RNase entrapped					
148	375	2.5	1.3	13.0	0.36
90	375	4.2	1.3	6.3	0.18
80	350	4.3	1.3	5.8	0.16
54	320	5.9	2.1	3.1	0.09
190	510	2.7	2.7	0.0	—
RNase covalently bound					
190	360	1.9	1.4	12.1	0.0038
150	320	2.1	1.4	6.3	0.0020
90	360	4.1	1.2	5.9	0.0019
80	320	4.0	1.3	4.6	0.0015
60	300	5.0	1.2	3.8	0.0012
200	600	4.0	1.3	0.0	—

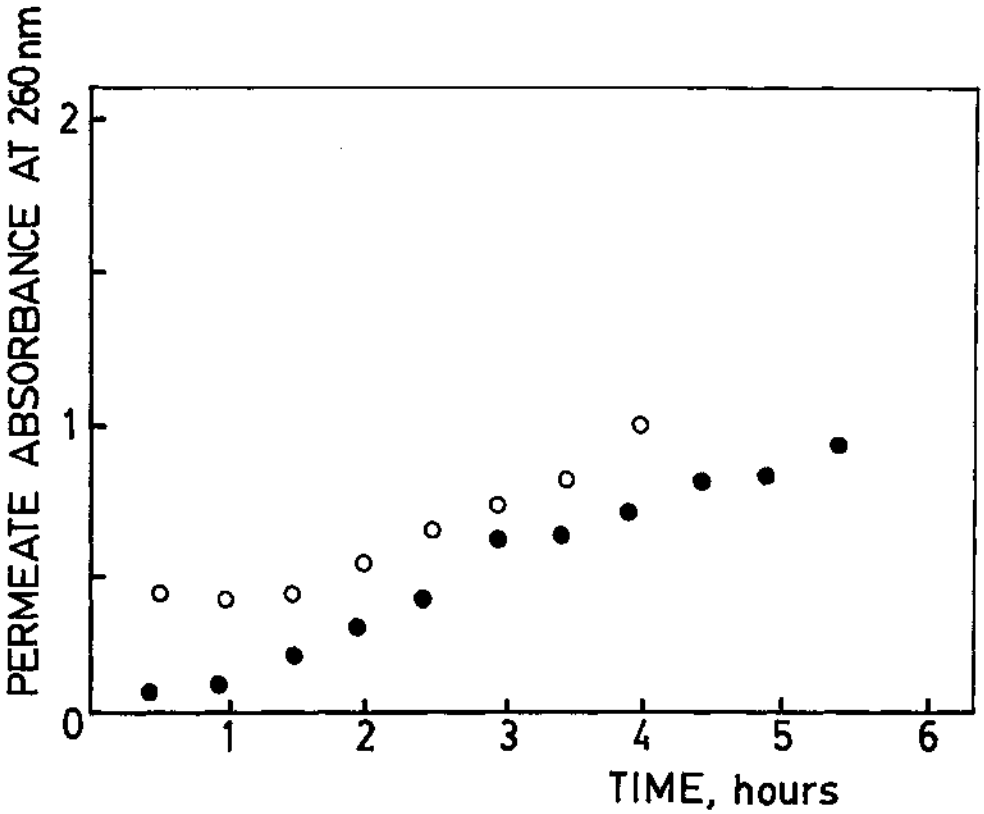

Fig. 3. Time course of RNase covalently bound. Initial concentration of RNA: 120 mg/dm^3. ○—without BSA; ●—initial concentration of BSA: 375 mg/dm^3.

presented here were held in the temperature of 37°C. The yield of the removed RNA was established as the difference between the RNA mass before and after the ultrafiltration. During the process the nucleotides can pass through the membrane but some of them may be washed away back to the retentate. It was examined by the absorption measurement before and after adding the RNA precipitation reagent (25% perchloric acid solution containing 0.75% uranyl acetate). It turned out that there were no nucleotides in the retentate. The absorbances at 260 nm were the same before and after the precipitation.

The hydrolysis products in the permeate during the experiments, for both types of membranes, are shown in Figs. 3 and 4. To investigate if the RNA removal is affected by the concentration polarization, the experiments were done at various agitation speeds. The results shown in Fig. 5 indicate that no measurable differences were observed. The decrease of permeate flux for both membrane types during the experiments with the agitation speed of 660 rpm are shown in Fig. 6.

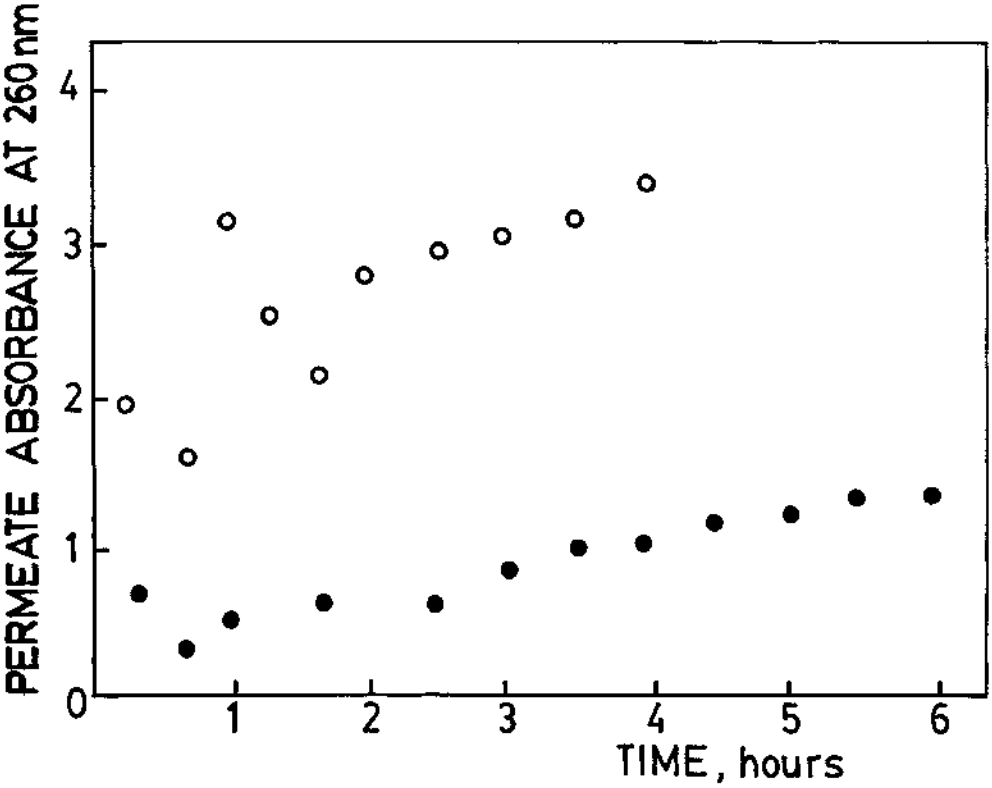

Fig. 4. Time course of RNase entrapped. Initial concentration of RNA: 120 mg/dm^3. ○—without BSA; ●—initial concentration of BSA: 375 mg/dm^3.

We have observed that if the temperature was lowered up to 20°C there was no hydrolysis of RNA and concentration factors achieved for BSA and RNA are of the same value. This was valid for both types of membranes. These experiments were done when the initial concentration of BSA was 350 mg/dm^3 and RNA 90 mg/dm^3.

The enzymatic membranes were used up to ten times, each of them. They were in operation for 6 h a day on the average, and after each experiment they were washed with water and stored refrigerated. We have not observed any decrease of activity during this time of operation, and this was valid for both types of membrane.

DISCUSSION

We have applied 2 methods of immobilization. The most effective, referring to the enzyme loading, appeared to be covalent binding, the amount of RNase in the membrane running up to the level of 170 mg per 1 g of dry membrane, whereas the entrapment in polymer matrix gave 1.9 mg of enzyme per 1 g of obtained membrane. Nevertheless, the effective-

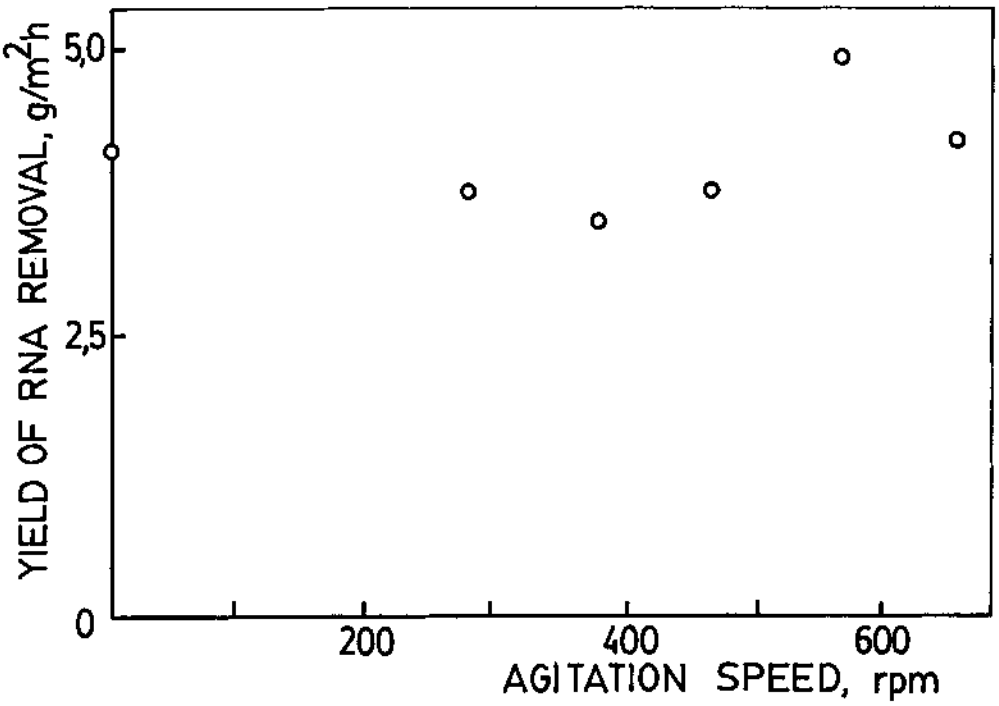

Fig. 5. The dependence of RNA removal on agitation speed. Initial BSA concentration: 300 mg/dm^3, RNA 50 mg/dm^3.

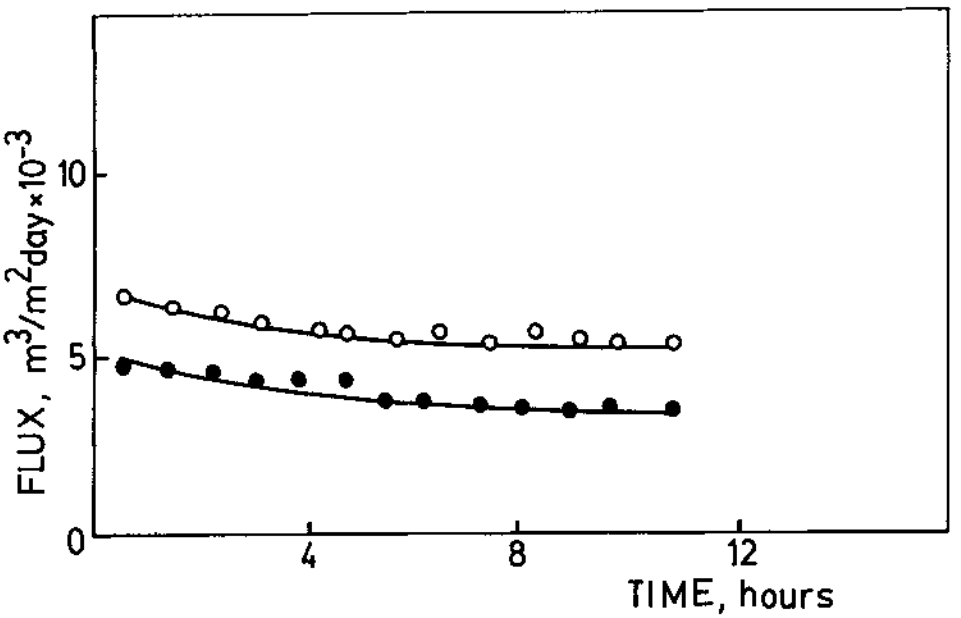

Fig. 6. The permeate flux vs time. Initial concentration of BSA: 350 mg/dm^3, RNA: 80 mg/dm^3. ○—membrane with entrapped RNase; ●—RNase covalently bound.

ness of the entrapped RNase has been found to be much better compared with the covalently bound one (Table 1). It may have resulted from the method of immobilization and/or from the adsorption of protein (BSA) on the membrane surface. We have found that the enzymatic activity of RNase did not decrease after a 1 h exposition to the temperature of 50°C, and after a 24-h exposition to dimethylformamide. This phenomenon was discussed elsewhere (3).

Additionally, we have found that the permeate flux during ultrafiltration of BSA solution using aminated membrane decreased to a great extent, compared with that for the untreated PVC membrane (unpublished data). It could be suggested that the amination encouraged the adsorption of BSA on membrane surface, which results in obstruction of the enzyme. The smaller permeate flux shown in Fig. 6 (aminated membrane) confirms this suggestion and makes this immobilization method less helpful.

Characteristic changes in the concentration of hydrolysis products in permeate shown in Figs. 3 and 4 were commonly observed for all the experiments. The absorbance of permeate decreased and then increased. This was confirmed by the computer simulation of the enzymatic hydrolysis provided in UF membranes (*5*). At the beginning of the run, the concentration at the membrane surface changed considerably. Then, the enzyme activity increases because of the concentration polarization phenomenon. Despite stirring, for macromolecules like ribonucleic acid, the membrane rejection is very strong and the back diffusion is extremely slow. Therefore, the concentration of substrate at the membrane surface is higher than that in the bulk solution. The initial RNA concentration appeared to have a substantial influence on the rate of its removal (Table 1). Nevertheless, the initial BSA concentration of 500 mg/dm^3 completely stopped the reaction, even if the high RNA concentration was employed. The suggestion is that when the adsorption of protein, taking place just at the beginning of ultrafiltration (*6*), was strong with the high initial concentration of BSA, the substrate did not reach an enzyme. The increase of BSA concentration even above the load of 500 mg/dm^3 during the concentration process has been found not to inhibit the hydrolysis of RNA. Although the process of RNA removal during the concentration of protein is effective, the rate of RNA degradation seems to be controlled by the rate of protein adsorption on the membrane surface. Later on, the development of a polarization layer does not affect the hydrolysis of RNA, because the phenomenon of concentration of protein goes together with concentration of RNA.

ACKNOWLEDGMENTS

This work has been supported by the Research Program CPBP 02.11 "Membranes." We also thank A. Mika for membrane preparation.

REFERENCES

1. Michaels, A. S. and Matson, S. L. (1985), *Desalination* **53,** 231.
2. Tone, S. and Nakamura, H. (1987), *The International Congress on Membranes and Membrane Processes,* June, Tokyo, Japan.
3. Rucka, M. (1987), *Biotechnol. Tech.* **1,** 189.
4. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem.* **193,** 265.
5. Zuk, J., Poster presented at the 8th International Biotechnol Symposium Paris (1988), July 17–22.
6. Mathiasson, E. and Sivik, B. (1980), *Desalination* **35,** 59.