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MEMBRANE-ASSISTED CHIRAL RESOLUTION OF PHARMACEUTICALS: IBUPROFEN SEPARATION BY ULTRAFILTRATION USING BOVINE SERUM ALBUMIN AS CHIRAL SELECTOR

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ABSTRACT

The chiral separation of ibuprofen has been performed by ultrafiltration in solutions containing the protein bovine serum albumin (BSA) as a chiral selector. The effectiveness of optical resolution was characterized in terms of enantiomeric enrichment and solute recovery in the permeate. Working conditions, including parameters of feed solution and hydrodynamic conditions, which can influence thermodynamic equilibrium and nonselective solute binding, have been investigated with regard to the separation effectiveness. Enantioselectivity is strongly pH-dependent and reaches a maximal value at pH 9.0–9.2. It was established that nonspecific interactions also played a significant role in total ibuprofen binding, decreasing both enantioselectivity and solute recovery in permeates. Nonspecific interactions are suppressed in

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the presence of organic solvents (acetonitrile, methanol) in the feed solution thereby increasing the solute recovery in the permeate. Increase in BSA content in the feed solution results in an increase of both enantioselectivity and solute binding. In ultrafiltration-based chiral separation, concentration polarization and gel formation worsen enantioselectivity and increase solute binding. A consequent enrichment of enantiomers was illustrated in a multi-stage chiral resolution. After six stages of separation, the permeate contains more than 95% of (*S*)-ibuprofen. Regeneration of both BSA and bound enantiomer may be fulfilled in the same ultrafiltration system by ultrafiltration of alkaline solutions.

Key Words: Chiral resolution; Bovine serum albumin; Ibuprofen; Ultrafiltration

INTRODUCTION

Chiral separations are important in a number of fields. Enantiomerically pure compounds are desirable, and in many cases vital, in the food, agrochemical, and pharmaceutical industries. Numerous examples of different biological activities of enantiomeric drug substances, herbicides, vitamins, and food components are well documented.[1,2] It is now generally recognized that the enantiomers of chiral bioactive substances are unlikely to be pharmacologically and toxicologically equivalent. So, production of chiral products in single-isomer form is an important task in drug development and manufacture.

Enantiomerically pure compounds can be produced by asymmetric synthesis, but this can become uneconomic if numerous steps and use of expensive enantiomeric reagents are needed.[3] In some cases, it is less expensive to separate the racemic mixture by physical methods. Membrane-based processes of separation are believed to have potential advantages for continuous or semi-batch large-scale chiral separation, such as easy and economical operation.[4] There are numerous studies on enantiomer separation by liquid membranes with an abundance of optically active substances tested as chiral selectors (CS) and carriers.[5–7] Successful separation was gained only at a low degree of solute recovery, which is determined by changes in thermodynamic equilibrium of enantiomer/CS interactions as a result of enrichment of feed solution by one optical isomer. The entire system will ultimately come to equilibrium with an essentially racemic mix of solute in each aqueous phase. It is worth noting that the problem can be solved in Cram's system[8] provided that there are two CSs, one for each enantiomer. However, the common drawbacks of liquid membranes are well known. So far, achievements in chiral resolution by solid membranes

with fixed optically active groups[11,12] or by membranes imprinted towards one of the enantiomers[9,10] in a continuous process are unconvincing.

However, it has been proven that membrane processes are useful methods in membrane-assisted chiral separation. Enantiomer resolution of several drug substances is successfully commercialized using membrane bioreactors.[13–15] Different enzymes, which are able to fulfill enantiospecific reactions are a key element of chiral separation in such devices. Membranes aid optimal functioning of enzymes and isolation of pure enantiomer as a product. Another promising approach in membrane-assisted chiral separation is based on the possibility of selective binding of one of the enantiomers by a soluble CS into large molecular aggregates. The later can be separated from unbound enantiomer by filtration through semi-permeable membranes.[16,17] For such membrane-assisted chiral separation, CSs in addition to high enantiospecificity have to satisfy a size criterion, namely the size of diastereomeric complex of substance/CS has to be sufficient to be retained by a membrane with free passage of unbound substance.

There are a few recent publications describing applications of serum albumin as CS,[16–21] for which the high affinity to L-amino acid is well known, for optical resolution of some amino acids. However, enantioselective binding to albumins is not restricted to amino acids. Protein binding is a significant factor in the transport and release of most drugs in human tissues and liquids. This suggests the possible use of albumins for chiral separation of a broad spectrum of optically active organic substances including pharmaceuticals. They are widely used for analytical separation of chiral substances and quantification of their enantiomeric composition by chiral HPLC and capillary electrophoresis.[22,23] In particular, albumins' usefulness in analytical chiral separation has been demonstrated on hundreds of enantiomeric substances including drugs. This suggests that the cheapest albumins could be used for preparative or even large-scale chiral separation based on ultrafiltration.

The present paper deals with chiral resolution of ibuprofen, an anionic nonsteroidal anti-inflammatory drug substance, through enantioselective binding of enantiomers by bovine serum albumin (BSA) and separation of bound and unbound enantiomers by ultrafiltration. The effectiveness of optical resolution is characterized in terms of enantiomeric enrichment and solute recovery in the permeate. To optimize these parameters, their dependencies on the physico-chemical conditions of the feed solution as well as hydrodynamic conditions have been investigated.

THEORY

A large number of studies have been performed to examine the binding of ibuprofen to albumins. However, data on the strength of this binding and the

number of sites involved in this interaction are controversial. Most studies on human serum albumin (HSA) agree that ibuprofen has a primary binding site on this protein with an association constant in the range 10^5–$10^6\,M^{-1}$.[24,25] On the other hand, in several works it has been suggested that there are different numbers of sites involved in the interactions of HSA with *R*- and *S*-ibuprofen.[26,27] The BSA and HSA are closely related proteins and, consequently, the stereoselectivity binding characteristics of these proteins are similar.

By assuming that the protein binding corresponds to a 1:1 complex and the equilibrium is a reversible and kinetically rapid process, the separation principle is simply illustrated by equations:

$$R\text{-isomer} + \text{CS} \underset{k'_R}{\overset{k_R}{\rightleftarrows}} R\text{-isomer}-\text{CS} \tag{1}$$

$$K_R = \frac{k_R}{k'_R} = \frac{[R-\text{CS}]}{[R][\text{CS}]} \tag{2}$$

$$S\text{-isomer} + \text{CS} \underset{k'_S}{\overset{k_S}{\rightleftarrows}} S\text{-isomer}-\text{CS} \tag{3}$$

$$K_S = \frac{k_S}{k'_S} = \frac{[S-\text{CS}]}{[S][\text{CS}]} \tag{4}$$

where k_R and k_S are the second-order association rate constants for protein binding of *R*- and *S*-isomer, respectively; K_R and K_S are the association equilibrium constants for protein binding of *R*- and *S*-isomer, respectively.

The simplest method to determine the association constant is based on the study of solute/protein interactions using the individual enantiomers. At the same time, some methods for determining the binding constant in a mixture of two optical isomers have been developed in chromatographic or electrophoretic practice. One common way of using data for this purpose is to measure the bound and/or nonbound concentrations of the solute under various concentration conditions and to plot the results by using Scatchard analysis, for example according to:

$$\frac{C_\text{b}}{C_\text{u}} = -K_i C_\text{b} + nK_i C_\text{P} \tag{5}$$

where C_b and C_u are the concentrations of protein bound and unbound enantiomer, respectively; K_i is the association equilibrium constant for *R*- or *S*-isomer binding by protein; C_P is the total protein concentration; and n is the number of independent binding sites that predicts a dependence of the C_b/C_u ratio

on concentration of bound isomer as a straight line, thus providing the binding parameters for the solute–protein system.

The above reaction scheme (Eqs. (1) and (3)) assumes that the individual binding regions on the protein have constant and independent affinities for the solute. In the presence of both enantiomers, solute/protein interactions are competitive and more complex reaction models must be used. This is one of the reasons why results obtained in the systems with individual enantiomers and their mixture significantly differ. However, even this simplified model, Eqs. (1)–(4) can still provide a useful approximation of the net reaction, which takes place between a small solute and a protein, as well as of enantioselectivity of the reaction. The later depends on a ratio of binding constants of each enantiomer. Concentrations of free and bound enantiomers are determined by the law of mass action, i.e., by thermodynamic equilibrium.

Such enantiomeric binding strongly depends on solution parameters. The variety of functional groups that are available on proteins allows many different types of processes to play a role in stabilizing a solute–protein complex. Examples of these processes include nonpolar, dipole, or coulombic interactions, as well as hydrogen bonding and steric effects. These forces, combined with the well-defined three-dimensional structure of proteins, make it possible for proteins to discriminate between the different chiral forms of a solute. As a result, the ability of protein to recognize enantiomers depends on pH, ionic strength of solution, and the presence of organic substances, which strongly influence the actions of the above-mentioned forces, conformational state of protein, and ionization of ionogenic solutes. The combination of these various factors can lead to either the specific or general binding of solutes to a protein. So, parameters which can influence thermodynamic equilibrium and nonselective binding are important for effectiveness of chiral separation based on complexation/ultrafiltration.

Additionally, working with high molecular substances in membrane systems causes a necessary consideration of membrane phenomena such as concentration polarization and gel formation. These phenomena lead to an increase in concentration of solutes rejected by membranes in the boundary layer. In the present case, free BSA and BSA/ibuprofen complexes are rejected by membranes. During ultrafiltration, the concentration of these species will increase in the boundary layer. At the same time, the concentration of free enantiomers across the membrane should be very close to that in the bulk volume due to their low retention by a relatively large pore-size membrane. These changes in the boundary layer should influence the enantioselectivity of separation. At least two factors should be taken into consideration: continuous flow of solution enriched by one of the enantiomers through the boundary layer and increase in the density of protein functional groups in the boundary layer. The competitiveness of two enantiomers in protein binding from mixed solutions is very important in determining the effect of the first factor on enantioselectivity, while a possibility

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of an increase of nonspecific binding could be caused by the second factor. Thus, to improve enantioselectivity of chiral separation by the complexation/ultrafiltration method, it is important to study its dependence on hydrodynamic conditions determining levels of concentration polarization and gel formation.

MATERIALS AND METHODS

Materials

Racemic ibuprofen, (±)-4-isobutyl-α-methylphenylacetic acid (28,474-2), and *S*-ibuprofen (37,516-0) were purchased from Aldrich (Dorset, U.K.). *R*-Ibuprofen (I-107) and BSA obtained by cold alcohol precipitation (A-7638) were purchased from Sigma (Dorset, U.K.). Other chemicals were of reagent grade and were used without further purification.

Ultrafiltration

Filtration experiments were carried out using a dead-end stirred filtration cell (Amicon, model 8050 Fisher Scientific, Loughborough, U.K.) holding a flat sheet membrane having a diameter of 44.5 mm. The effective membrane area was 13.4 cm^2. The system was operated at 20°C with an applied pressure of 100 kPa and variation of a rotation speed from 200 to 400 rpm. All the feed solutions were prepared just before filtration experiments using demineralized water.

NADIR® C005F cellulose ultrafiltration membranes (NADIR Filtration, Wiesbaden, Germany) with molecular weight cut-off of 5000 Da was used in all ultrafiltration experiments. The membrane has a complete BSA rejection and ibuprofen retention does not exceed 10%.

Ibuprofen has low solubility in pure water. All the feed solutions contained about 80 mg/L (about 0.4 μmol/mL) that is close to the solubility limit. The molar ibuprofen/BSA ratio was varied from 3.6 to 10.2. Ibuprofen was dissolved in hot water. The BSA was dissolved after cooling of the ibuprofen solution. After preparation of ibuprofen/BSA solutions, pH adjustment was carried out in the range 3.5–12 by addition of 2 *M* sodium hydroxide or hydrochloric acid. Enantiomeric separation was studied in aqueous solution without and with background electrolyte, as well as with an organic modifier as co-solvent. A 0.1 *M* solution of sodium hydrogen phosphate was used as solvent in the case of separation in the presence of background electrolyte. Acetonitrile and methanol were used as organic modifiers and their content in the feed solution was varied from 0 to 15 vol%.

Ultrafiltration was continued up to degree of permeate recovery of 75% by volume with a discrete permeate sampling for analysis of the enantiomeric

composition. For multi-stage chiral resolution, the enantiomeric composition of the feed solution on each subsequent stage corresponded to the composition of permeate of a previous stage. However, the total ibuprofen concentration at each stage was about 80 mg/L. To prepare such solutions, calculated quantities of single ibuprofen enantiomers were mixed.

To check the possibility of protein regeneration, a concentrate was diluted by an equal volume of distilled water and the pH was adjusted to 11.8 by 2.0 *M* sodium hydroxide. Ultrafiltration was carried out at the same condition with permeate recovery of 50% by volume.

Analysis of Enantiomeric Composition by Chiral High Performance Liquid Chromatography

Enantiomeric composition of permeate was determined by chiral HPLC using a 4.0 mm × 100 mm Chiral-AGP column (ChromTech International AB, Hägersten, Sweden) and 100 m*M* sodium phosphate buffer at pH 7.0 as mobile phase at flow rate of 0.9 mL/min. The chromatographic system included a Pro Star model 210 solvent delivery module (Varian Ltd. Walton-on-Thames, U.K.) and Pro Star model 320 UV–VIS detector. Ibuprofen detection was performed at 225 nm. The retention times of *R*- and *S*-ibuprofen were 4.1 ± 0.2 and 6.5 ± 0.2 min, respectively.

From the results of chromatographic analysis, enantiomeric excess in the permeate, ee%, and solute binding, SB_i were calculated using the following equations:

$$\text{ee\%} = \frac{C_{\text{predominant}} - C_{\text{minor}}}{C_{\text{predominant}} + C_{\text{minor}}} \tag{6}$$

$$\text{SB}_i = \left(1 - \frac{C_{\text{perm}}}{C_{\text{feed}}}\right) \times 100 \tag{7}$$

where $C_{\text{predominant}}$ and C_{minor} are the concentrations of predominant and minor enantiomers in permeate, respectively; C_{feed} and C_{perm} are total enantiomer concentrations in feed solution and permeate, respectively.

RESULTS AND DISCUSSION

It is known[26,27] that *R*-ibuprofen is preferentially bound by albumins compared to its counterpart. As expected, the permeate during ultrafiltration is enriched by *S*-ibuprofen due to lower binding of this isomer by BSA. As discussed, feed solution pH should play an important role in enantiospecificity of ibuprofen

binding by BSA. As shown in Fig. 1, ee%, of (*S*)-ibuprofen in the permeate increases with an increase in the pH of the feed solution up to 9.05–9.10. At this pH, enantiomeric excess reaches about 24% starting from a racemic mixture. At low pH (below the isoelectric point of BSA) solute binding exceeds 90% for both enantiomers (Fig. 1), showing a high nonspecific binding of carboxylic-containing ibuprofen. Increase in the degree of dissociation of ibuprofen and suppression of the ionization of basic groups of BSA with increasing pH reduces nonspecific binding. As a result, at pH ~9.0 the solute binding drops to 75 and ~60% for (*R*)- and (*S*)-isomers, respectively. However, it can be seen from Table 1 that nonspecific binding is significant even at pH = 9.1. At this condition the total solute binding was 2.7 mol per mole of protein. That is, notably higher than the stoichiometric ratio, which is assumed in the model described by Eqs. (1) and (3). Thus, nonspecific binding plays a significant role in association of ibuprofen with BSA.

Decrease in solute binding occurs simultaneously with increasing enantioselectivity, suggesting that the three-dimensional structure of BSA's binding site for ibuprofen is stable in the pH range 7–9. At pH values above 9.1 the enantiomeric enrichment drastically diminishes. The main causes are likely to be conformational changes of protein structure destroying the well-organized structure of the binding site. On the other hand, alkaline solution can be used to regenerate BSA and release bound solutes for repeated utilization.

It should be noted that the degree of solute binding by protein is a very important factor for applicability of separation methods. The common drawback

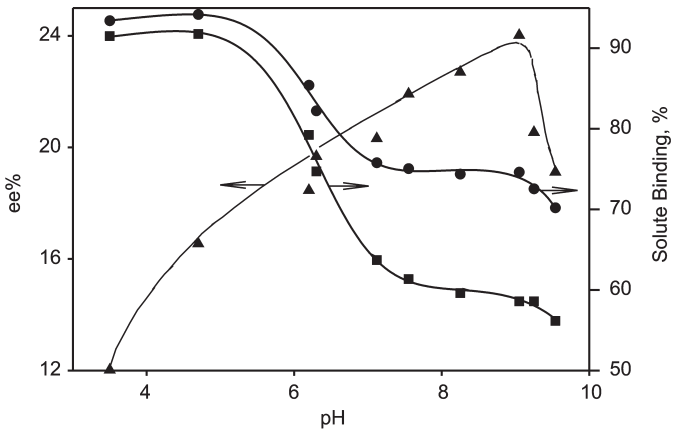

Figure 1. Enantiomeric excess (▲) in permeate and binding of (*R*)-ibuprofen (●) and (*S*)-ibuprofen (■) by BSA vs. pH of feed solution. Ibuprofen/BSA ratio, 4 mol/mol; stirring rate, 400 rpm; operating pressure, 100 kPa; degree of permeate recovery by volume, 10%.

Table 1. Content of Bound Enantiomer in the Presence of BSA

	Solute Binding (mol/mol of BSA)		
Condition of Feed Solution	Total	(*R*)	(*S*)
Aqueous solution, pH 6.0, ibuprofen/BSA ratio = 4.0	3.5	1.8	1.7
Aqueous solution, pH 9.1, ibuprofen/BSA ratio = 4.0	2.7	1.5	1.2
15 vol% of acetonitrile, pH 9.1, ibuprofen/BSA ratio = 3.4	2.0	1.2	0.8

of the methods of chiral resolution is the loss of at least half of the total substrate as one another enantiomer as by-product. If selective binding is the basis of chiral resolution, nonselective binding worsens enantiomer recovery. Nonselective binding may be partially suppressed by variation of the properties of the feed solution.

As can be seen from Fig. 2, the presence of acetonitrile in the feed solution decreases both enantiomeric excess and solute binding. It is important for solute recovery that at acetonitrile content higher than 10 vol%, solute binding decreases substantially. Similar results were obtained using methanol as organic modifier. Organic solvents were not used at concentrations higher than 15 vol% because of the danger of BSA denaturation.

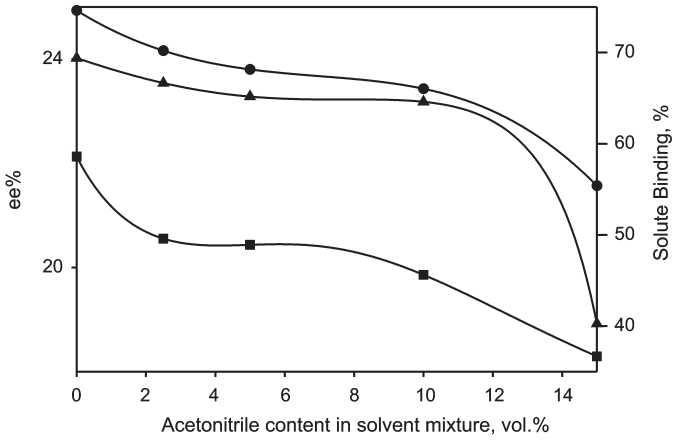

Figure 2. Influence of organic modifier on enantiomeric excess in permeate (▲) and binding of (*R*)-ibuprofen (●) and (*S*)-ibuprofen (■) by BSA. Ibuprofen/BSA ratio, 4 mol/mol; pH = 9.1; stirring rate, 400 rpm; operating pressure, 100 kPa; degree of permeate recovery by volume, 10%.

According to Table 1 the total solute binding in the presence of acetonitrile can be decreased to 2.0 molecules per protein molecule. Nonspecific binding is significantly suppressed, but it cannot be avoided. Even so enantiomeric excess in the permeate slightly decreases in the presence of acetonitrile in the feed solution. Use of organic additives to feed solutions may be useful for maximizing recovery of solute in the permeate. At an acetonitrile content of 15 vol%, solute binding did not exceed 56 and 40% for (*R*)- and (*S*)-isomers, respectively. Thus, more than half of (*S*)-ibuprofen can be transported through the membrane during ultrafiltration.

The variation of ibuprofen/BSA ratio should influence both enantioselectivity and solute binding. This may allow choice of a BSA content to improve enantioselectivity or solute recovery. At high ionic strength of background electrolyte, binding of ibuprofen was found to be relatively high, ~80% for an ibuprofen/BSA ratio of 4 (Fig. 3a). At the same time, for similar conditions but in the presence of acetonitrile it does not exceed 60%. Thus, a high ionic strength induces greater nonspecific ibuprofen/BSA binding. In such a case, increase in BSA content could eventually result in almost complete solute binding. As can be seen from Fig. 3a, increase in ibuprofen content gives decreasing solute binding, though enantioselectivity also decreased significantly. The levels of enantioselectivity are lower in the case of aqueous solutions with high ionic strength compared to acetonitrile-containing systems. Figure 3b shows that low levels of solute binding in the presence of acetonitrile allow increase in BSA content to compensate the enantioselectivity decrease owing to acetonitrile action.

To sum up the influence of feed solution conditions on chiral resolution based on diastereomeric complex formation and ultrafiltration, it should be noted that the highest enantiospecificity gains are obtained in aqueous solutions. However, taking into consideration that this method of separation needs several stages to achieve a high enantiopurity (enantiomeric excess higher than 95%), it is clear that reduction in nonspecific solute binding is an important factor in minimizing the loss of substances with the by-product. From this point of view, it is better to carry out the separation from solution containing organic additives. Moreover, at acetonitrile content up to 15 vol%, enantioselectivity is maintained at a reasonable level. For further studies we used feed solutions containing 15 vol% of acetonitrile with pH about 9.1 and ibuprofen/BSA ratio about 3.4. These conditions during the separation of racemic ibuprofen provide enantiomeric excess in permeate of ~23% with approximately 67 and 47% of binding for (*R*)- and (*S*)-ibuprofen, respectively.

All of the above results were obtained at the same hydrodynamic conditions, an operating pressure of 100 kPa and a stirring rate of 400 rpm. At these conditions the initial trans-membrane flux (~18.5 L/m^2 hr) (Table 2) during ultrafiltration of a solution with an ibuprofen/BSA ratio of 3.4 (BSA concentration was ~7.5 g/L) practically coincides with the membrane pure water flux (18.6 L/m^2 hr). Thus, at these conditions concentration polarization

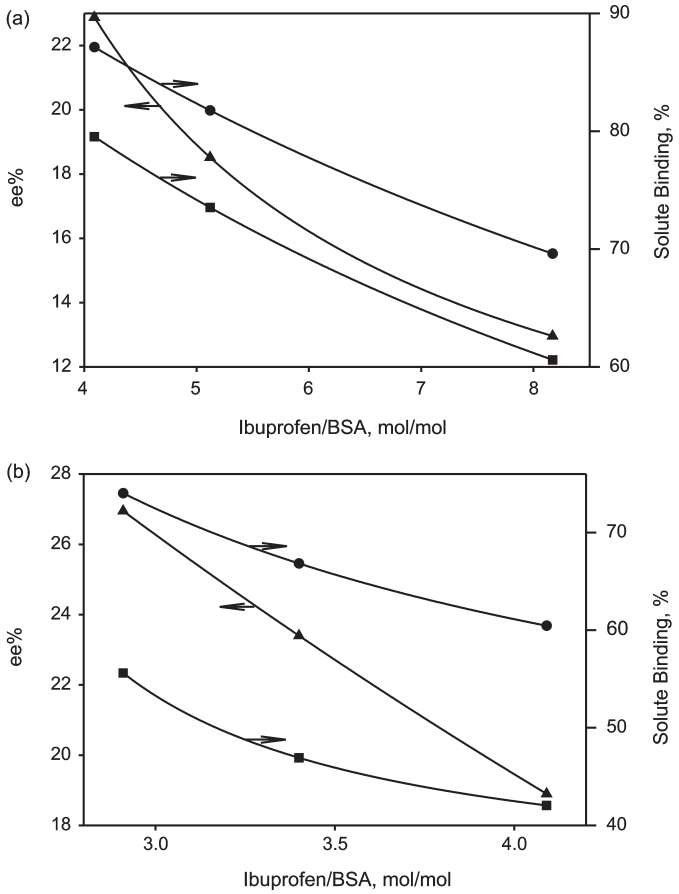

Figure 3. Influence of ibuprofen/BSA molar ratio on enantiomeric excess in permeate (▲) and binding of (*R*)-ibuprofen (●) and (*S*)-ibuprofen (■) by BSA for separation in (a) 0.1 *M* Na_2HPO_4 buffer solution and (b) in 85:15 water/acetonitrile mixture. pH = 9.1; stirring rate, 400 rpm; operating pressure, 100 kPa; degree of permeate recovery by volume, 10%.

and gel formation were practically eliminated. As is shown in Table 2, there is a slight decrease in trans-membrane flux at high degrees of permeate recovery. This shows slight concentration polarization as a result of increasing BSA concentration. Enantiomeric excess slightly decreases with increase in permeate recovery, which can be attributed to the effect of concentration polarization. Separation was carried out at different stirring rates to quantify this contribution to enantioselectivity. Figure 4 shows that low stirring rate, which gives an

Table 2. Volume Flux and Enantiomeric Excess in Permeate at Different Degrees of Permeate Recovery

Permeate Recovery (%)	Volume Flux (L/m^2 hr)	ee%
12.1	18.5	23.40
44.4	18.5	22.62
60.5	18.3	22.58
68.5	18.2	22.08
76.6	18.0	20.94

Solvent: 85:15 water/acetonitrile mixture; pH = 9.1; Ibuprofen/BSA ratio—3.4 mol/mol.

increase in concentration polarization, worsens enantioselectivity. Minimization of concentration polarization can improve not only enantioselectivity but also recovery of enantiomers. Increase in CS concentration and, consequently, density of functional groups in the boundary layer at higher levels of concentration leads to an increase in solute binding. This mainly causes an increase in nonselective solute binding.

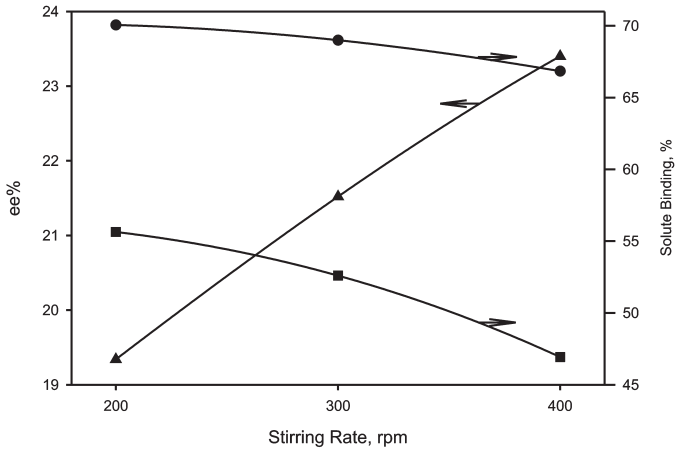

Figure 4. Influence of stirring rate on enantiomeric excess in permeate (▲) and binding of (*R*)-ibuprofen (●) and (*S*)-ibuprofen (■) by BSA. Solvent: 85:15 water/acetonitrile mixture; pH = 9.1; ibuprofen/BSA ratio, 3.4 mol/mol; operating pressure, 100 kPa; degree of permeate recovery by volume, 10%.

As can be seen from Table 2, enantiomeric excess remains at a high level even at degrees of permeate recovery higher than 75%. Starting from a racemic mixture, the enantiomeric excess of total permeate is approximately 22.5% at about 77% of volume recovery. Further, during the diafiltration of the final concentrate with an equal volume of acetonitrile/water mixture, the enantiomeric excess in the permeate was 20.7%. This shows the high stability of the ibuprofen/BSA binding. It allows an increase in solute recovery by using diafiltration.

After ultrafiltration of a feed solution containing a racemic mixture, the total permeate contains about 61% of (*S*)-ibuprofen. In light of the stringent requirements for enantiomeric purity in the pharmaceutical industry, additional stages of resolution are necessary to increase the purity of product. Figure 5 shows the results of an experimental multi-stage chiral resolution of ibuprofen in six consequent stages of ultrafiltration. Each consecutive stage in ultrafiltration gives a rise of enantiomer enrichment in the permeate. However, the relative increase in enantiomeric excess in the permeate gradually declines with the number of consecutive stages. This is a result of competitive nature of the interaction. After the sixth stage of ultrafiltration, the content of (*S*)-ibuprofen in the permeate is ~94%. Further increase in the number of stages will increase the enantiomeric purity. However, each stage of ultrafiltration is accompanied by a loss of substrate in retentate as a bound fraction and unbound isomers in the remaining solution. As can be seen from Table 3, total recovery of both isomers

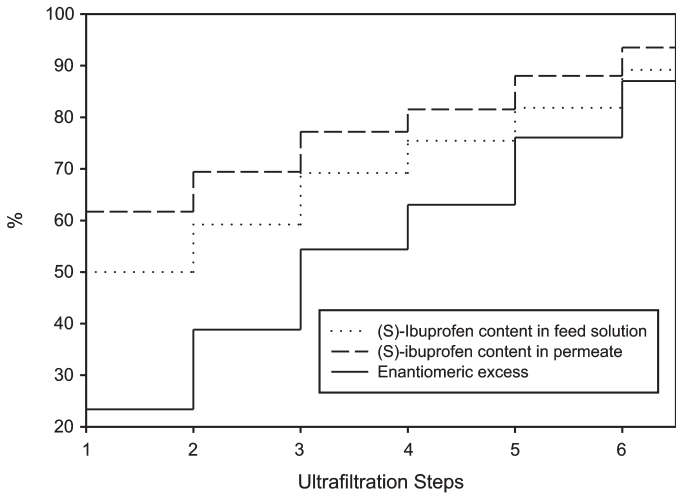

Figure 5. Experimental chiral resolution of ibuprofen in multi-stage ultrafiltration process. Degree of permeate recovery by volume on each ultrafiltration stage—80%.

Table 3. Total Recovery of Ibuprofen Isomers with Respect to Quantities Introduced at the First Stage for Different Stages of Ultrafiltration

	Total Solute Recovery (%)	
Stage	(*R*)-Ibuprofen	(*S*) -Ibuprofen
1	27.0	42.0
2	6.70	16.8
3	1.95	7.32
4	0.662	3.58
5	0.174	1.54
6	0.044	0.67

Degree of permeate recovery by volume at each ultrafiltration stage 80%.

with respect to their quantities introduced on the first stage of the separation calculated from the results of six ultrafiltration stages drastically decreases with the number of stages. Recovery of the unbound portion of isomers can be increased by performing a diafiltration of each retentate. However, it should be noted that enantiomeric purity of permeate decreases during diafiltration. Although the degree of total recovery can be slightly improved by diafiltration, the best results can be achieved by minimizing or avoiding nonspecific binding. For such complex molecules as BSA with the versatility and plurality of functional groups, this is not possible. The future prospects in membrane-assisted chiral resolution of pharmaceuticals may be connected with application of simpler substances providing stoichiometric substrate/CS interactions.

The practicality of such a separation will also depend on the possibility of recovering both CS for recurrent utilization and substrate for racemization and subsequent resolution. Membrane processes may also be used for such recovery of solutes. Alkaline medium can be used for regeneration of CS and substrate. Thus, ultrafiltration of concentrates at pH 11.8 gives permeates enriched by (*R*)-ibuprofen with enantiomeric excess of $\sim 12\%$ and very low solute binding (less than 5%). This shows the possibility of recovery of BSA and bound ibuprofen for sequential use.

In multi-stage separation, systematic changes in quantities of bound and unbound enantiomers occur. This allows application of Scatchard analysis using Eq. (5) to determine association constants for both enantiomers. Based on this analysis, association constants with BSA in acetonitrile/water mixture were found to be 5.8×10^3 and $1.4 \times 10^3\, M^{-1}$ for (*R*)- and (*S*)-ibuprofen, respectively. These values are much lower than those for aqueous solutions,

showing that the organic solvent weakens ibuprofen/BSA interactions. However, binding of (*R*)-ibuprofen is more than four times stronger than its counterpart.

CONCLUSIONS

The chiral separation of ibuprofen has been performed by ultrafiltration in solutions containing the protein BSA as a CS. The effectiveness of optical resolution was characterized in terms of enantiomeric enrichment in the permeate and solute recovery in the permeate. Working conditions, including parameters of feed solution and hydrodynamic conditions, which can influence thermodynamic equilibrium and nonselective solute binding, have been investigated with regard to the separation effectiveness.

The higher binding constant of (*R*)-ibuprofen compared with the (*S*)-isomer allows recovery of permeates enriched by (*S*)-ibuprofen. Enantioselectivity is strongly pH-dependent and reaches a maximal value at pH of 9.0–9.2. It was established that nonspecific interactions also played a significant role in total ibuprofen binding, decreasing both enantioselectivity and solute recovery in permeates. Nonspecific interactions are suppressed in the presence of organic solvents (acetonitrile, methanol) in the feed solution that allows an increase in solute recovery in the permeate.

Increase in BSA content in the feed solution results in increase in both enantioselectivity and solute binding. To increase solute recovery in the permeate, it is preferred to restrict BSA content at levels allowing solute binding lower than 60%. In ultrafiltration-based chiral separation concentration polarization and gel formation worsen enantioselectivity and increase solute binding. For improved effectiveness of chiral separation, the choice of operating parameters should minimize these phenomena.

A sequential enrichment of enantiomers was obtained in a multi-stage chiral resolution. After six stages of separation the permeate contained more than 95% of (*S*)-ibuprofen. Regeneration of both BSA and bound enantiomer may be achieved in the same ultrafiltration system by ultrafiltration of alkaline solutions.

Successful separation of optical isomers of ibuprofen based on enantioselective binding of enantiomers by BSA and separation of bound and unbound enantiomers by ultrafiltration demonstrates that such a method of separation can be used for chiral resolution of different pharmaceuticals. As enantiospecific binding of many pharmaceuticals by BSA is well known, results obtained in the present work on membrane-assisted chiral separation have a general character.

The main problem of using protein as CS is the versatility and plurality of functional groups and binding sites in such large macromolecules. For this type of CS, nonspecific binding is inevitable. The future prospects in membrane-assisted

chiral resolution of pharmaceuticals may be connected with application of simpler substances such as cyclodextrines, maltodextrines, and macrocyclic glycopeptide antibiotics. For such substances, a stoichiometric interaction is more probable. As a result, nonspecific interactions can be avoided. However, use of such substances as CS tightens the demands of semi-permeable membrane selectivity. In this case, the membrane should provide a reliable separation of substances with molecular weight of a few thousands from substances of molecular weight of several hundred Daltons.

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