Surface characterization by FTIR-ATR spectroscopy of polyethersulfone membranes—unmodified, modified and protein fouled

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Abstract

Polyethersulfone (PES) ultrafiltration membranes were modified by radical grafting with the aid of redox initiators to create new functional groups on the surface. Methacrylate-based monomers were used. The modified membrane surfaces were characterized by FTIR-ATR spectroscopy to detect chemical changes during modification. In addition to the common adsorption bands of carbonyl groups typical for all grafted polymers, the FTIR spectrum displayed new absorption bands in the region of aliphatic stretching vibration of the modified PES membrane (2921–2875 cm\(^{-1}\)), a region lacking absorption bands in the spectrum of the original membrane. It was also shown that the ATR technique enables recognition of the adsorption of albumin on the surface of the membranes. Preliminary results indicate, that spectral techniques such as subtracting and deconvolution could be applied in order to recognize the conformation of the adsorbed albumin. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Surface modification of UF membranes; Grafting of methacrylates; Redox initiation of grafting; Albumin adsorption; FTIR-ATR spectroscopy

1. Introduction

In this paper, we present the results of surface modification of commercial ultrafiltration (UF) polyethersulfone (PES) membranes. The membranes were modified by redox-initiated radical grafting of vinyl monomers. The study constitutes part of our ongoing research program on the surface modification of commercial membranes.

Surface modification is achieved by applying a very simple, inexpensive graft polymerization technique previously developed in our laboratory [1]. This method enables us to treat prefabricated membranes in aqueous solution at ambient temperatures without removal of oxygen. In addition to being simple and cheap, the method may be applied to ready-to-use devices such as reverse osmosis (RO) elements i.e. where such procedures as plasma or UV initiation are not feasible.

In our previous studies, commercial polyamide RO membranes were successfully grafted with a range of water-soluble monomers [1,2]. Unlike the polyamide composite asymmetric membranes, UF PES membranes are integrally skinned asymmetric membranes, i.e., the skin and the support are made of the same polymer material [3,4]. Such membranes are usually prepared by the phase inversion method. This method can be used to produce a wide range of membranes by varying the composition and properties of the initial polymer solution, as well as the temperature and...
composition of the precipitation solution. Ultrafiltration membranes also differ from RO membranes in that they have a more porous structure as a result of the method of preparation. Comprehensive reviews concerning the structure and mechanism of formation relationships of UF polysulfone and PES membranes may be found in the literature [3–5].

Redox reactions with peroxydisulfate and metabisulfite have been widely used to initiate free-radical polymerization grafting to cellulose and nylon [6,7]. However, to the best of our knowledge, chemical grafting with redox initiators has never been used for surface grafting of PES membranes. The most important aspect of grafting studies are the structural and functional changes of the membrane surfaces. The present study concentrated on the structure of grafted chains and how these new functionalities adsorb protein, a major class of foulants of UF membranes. Fourier transform infrared (FTIR) spectroscopy in combination with the attenuated total reflectance (ATR) technique, which was previously used by us to study grafting onto RO polyamide membranes [2], was applied in this study. This analytical method has been shown to be an extremely powerful tool for surface characterization of membranes, particularly when grafting is performed by different methods [8–11].

Because the spectrum of PES has no bands in the area below 3065 cm$^{-1}$, where the stretching vibrations of aliphatic hydrocarbons are located, we were able to detect the presence of new polymeric hydrocarbon \( -(\text{CH}_2 - \text{C} - \text{CH}_2 - \text{C} -)_n \) chains grafted onto the surface of the membrane. This area of aliphatic (CH) stretch is very sensitive to structural effects [12] and may, therefore, be diagnostic in studies on the macromolecular organization of grafts. This region may also find significance in studies of protein fouling of membranes because aliphatic segments of protein absorb in this region [13].

Recently, it was reported that self-assembled monolayers of proteins could be constructed — among other methods — by spontaneous adsorption from solution onto a solid liquid interface [13,14]. The structural aspects of protein adsorption have become the objective of extensive studies mainly due to recent progress in the development of vibrational spectroscopy. FTIR-ATR is an attractive tool for studying protein adsorption, because it is a non-invasive surface-sensitive technique that provides a wealth of information [15–22]. A group of Finnish researchers investigated the density of protein adsorption on polysulfone membranes by depth profiling [16]. Giroux and Cooper [17] studied the absorption of fibronectin to polystyrene and plasma-treated polystyrene and interpreted spectral changes during adsorption in terms of conformational changes of the protein. Jakobsen et al. [18] showed that IR spectra could be used to compare the structure of proteins in solution with that of proteins adsorbed on surfaces. The systematic studies of Byler and Susi [19] established the presence of \( \alpha \)-helical, \( \beta \)-sheet and random coil structures of proteins by means of IR. Koehler et al. [20] explored spectral data for the kinetic study of albumin adsorption. Recently, the interpretation of IR spectra by subtraction of the spectrum of original substrate from the spectrum of the protein-adsorbed substrate has become a common technique, while the Fourier self-deconvolution technique has been employed to obtain better resolution of overlapping bands representing \( \alpha \)-helices, \( \beta \)-sheets, turns and other structures in the amide I band [21]. Although the dependence of the degree of modification on the nature of the monomer and on time is briefly discussed below, our main focus in this paper is on the novel features presented by the spectral aspects. As will be demonstrated in this report, grafted PES membranes provide a good model for demonstrating the value of the ATR-FTIR technique. The first objective of this work was to identify the grafting on the surface of the commercial UF membranes. The appearance of new functional groups as a result of grafting was confirmed by comparison of the IR spectra of modified and nonmodified membranes. The effect of modification was then studied by following adsorption of bovine serum albumin (BSA) by means of FTIR-ATR. We also extended the use of FTIR-ATR technique to obtain additional information on the conformational changes taking place in the albumin during adsorption.

2. Experimental

2.1. Materials

Three different PES membranes (Millipore, G.m.b., Austria) were used: PES PTG CO 6210, 10,0000
Table 1
Monomers used to modify the PES membranes

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacrylic acid (MA)</td>
<td>[ \text{OCH}_2\text{C-C-OH} ]</td>
</tr>
<tr>
<td>Polyethyleneglycol-methacrylate (PEG-MA)</td>
<td>[ \text{OCH}_2\text{C-O(CH}_2\text{CH}_2\text{O)}_n\text{OH} ]</td>
</tr>
<tr>
<td>Sulfopropylmethacrylate (SPM)</td>
<td>[ \text{OCH}_2\text{C-O(CH}_2\text{)}_2\text{SO}_3\text{-K} ]</td>
</tr>
</tbody>
</table>

MWCO; PES PTG CO 4020, 10,000 MWCO; and PTHK 0–6210, 100,000 MWCO (the first and the last having different cut-offs and the former two having the same cut-offs but different batch numbers). The PES membranes were modified as described below with the following monomers: methacrylic acid (MA), polyethyleneglycol methacrylate (PEG-MA, molecular weight 306) and sulfopropylmethacrylate (SPM). All monomers were supplied by Aldrich and used without purification. The model protein was bovine serum albumin (BSA; fraction V; Lot 105 H 1139, Sigma). The structural formulae of the monomers are given in Table 1.

2.2. Graft polymerization procedure

The graft polymerization procedure [1] was performed as follows: a mixture of monomer and initiators dissolved in water was poured into a glass container (Table 2). For instance to 64 ml of water with 5.9 g of MA, a mixture of 0.19 g K$_2$S$_2$O$_8$ and 0.15 g of K$_2$S$_2$O$_5$ was added and stirred till completed dissolving. PEG-MA has limited solubility in water, but in the range of concentrations used (5–10 and 12–20%) the macromer dissolved in pure water or in an isopropanol-water solution. By clasping the membrane between a glass container and its cover, only the active side of membrane (disk with external surface area of 12.7 cm$^{-2}$) came into contact with the reaction mixture. The container was kept at room temperature (25°C) without stirring. No deairation or purging with inert gas was employed. After an appropriate time interval, the membrane was taken out and washed thoroughly with water.

The washing of the membrane has special meaning because it is important to remove the unreacted monomer and homopolymer. Therefore, the membrane samples were placed in a large volume of washing water that was stirred continuously and periodically replaced until there was no detectable acid (pH) or organics (UV) in the washings. Usually, the membranes were used immediately after grafting in order to prevent the adsorption of undesired compounds.

2.3. Spectra

ATR-FTIR spectra were recorded on a Nicolet spectrometer. The ATR accessory contained a ZnSe crystal (25 mm × 5 mm × 2 mm) at a nominal incident angle of 45°, yielding about 12 internal reflections at the sample surface. All spectra (100 scans at 4.0 cm$^{-1}$ resolution and ratioed to the appropriate background spectra) were recorded at 25°C. A special
dry system was constructed to prevent interference of atmospheric moisture with the spectra.

The incident beam was polarized in the p-direction (perpendicular to the plane of incidence) or the s-parallel direction to determine the orientation of adsorbed albumin. For this purpose, the Brewster rotating Ge polarizer was placed in the beam path in front of the entrance face of ATR crystal.

2.4. Albumin adsorption

Two procedures were used for static adsorption [23,24], as described below.

2.4.1. Procedure A

This procedure was employed to obtain densely packed, tightly bound to the surface protein. For this purpose, low protein concentrations were taken, as was recommended in the literature [20,22]. The membrane sample was mounted in the 150 ml batch cell of the UF (Model 8200, Amicon) unit and stabilized in water by allowing water to permeate through it for at least 5 min. The water flux was measured. Water was then replaced with a 0.08 g/l solution of BSA at pH 4.8. BSA solution, 10 ml, was placed on top of the membrane. Neither pressure nor stirring was applied. In this way protein could adsorb only on the surface accessible to diffusion. After 2 h — while still mounted in the cell — the membrane was rinsed gently three times with water. The water flux was measured again. The significant decline in flux indicated that the membrane had adsorbed albumin. The membrane was washed three times with methanol and kept overnight in ethanol to reproduce the conditions of ‘cleaning’ used for the original PES (see below). Then, the membrane was dried in vacuum and subjected to IR spectrometry.

2.4.2. Procedure B

For a higher albumin concentration, the whole procedure was simplified. Membrane samples (5 cm² external surface area) were brought into contact with buffered BSA solution (10 mg/ml) for 2 h. A control sample was placed in buffer solution without albumin. The membrane samples were then rinsed with distilled water three times for 10 min each time to remove un-bound albumin and finally dried under vacuum at room temperature for 24 h.

3. Results and discussion

3.1. Characterization and spectral analysis of the initial membranes

A number of problems were encountered in the characterization of PES membranes. These membranes are produced by a phase-inversion method involving the precipitation of the polymer solution into an aqueous phase. [25]. The structure of phase inversion membranes stems from a phase change of initially stable solutions that have been brought to an unstable state. The resulting asymmetric membrane structure generally consists of a thin, selective skin layer supported by porous substructure.

A difficulty most commonly encountered in the evaluation of supposedly identical membranes is the batch-to-batch variation in their porous structure, which results from the inherently variable conditions of preparation. Small changes in the composition of the casting solution, the coagulation bath or the additives influence the structure of the membrane active layer. It was found, for example, that the addition of a high-molecular-weight component to the polymer solution could drastically change the porous structure of the membrane. On the other hand, it was established [27] that adding polyvinyl pyrrolidone (PVP) to a solution of poly(ether-p-phenylsulfone) PES in N-methyl-pyrrolidone caused the permanent entrapment of PVP molecules in the PES matrix which could contribute to the spectral bands of membrane. Another difficulty is simply terminological and lies in the ambiguous use of the term ‘polysulfone’ for membranes actually having different chemical compositions. For instance, our spectral analysis shows noticeable differences between polyethersulfone I (usually known as Victrex) and polysulfones known as Udel membranes, which have a bisphenol A unit [II] in their structure. Such differences, first described by Oldani and Schock [8] and later by Fontyn [22] et al., find spectroscopic expression as follows: aromatic bands at 1578 and 1486 cm⁻¹ are characteristic for PES, while 1586 and 1488 cm⁻¹ are characteristic for polysulfone. Two weak bands at 1385 and 1365 cm⁻¹
are due to methyl groups and are present exclusively in the spectrum of polysulfone.

According to the spectra obtained, our Millipore membranes are composed solely of PES. Spectral inspection of the ‘as-received’ PES membrane revealed the presence of preservatives (a very strong band at 3400 cm\(^{-1}\) and three bands at 1650, 1040, and 920 cm\(^{-1}\)) (Fig. 1a). The preservatives were washed out with water (complete disappearance of the above-mentioned bands) (Fig. 1b). After washing, the membranes underwent a drying procedure, but water removal was not complete, even under vacuum drying at room temperature for several days. The water/ethanol exchange procedure was then applied. Finally, the degree of ‘cleanliness’ was monitored by the disappearance of the strong aliphatic CH stretch near 2900 cm\(^{-1}\), which does not belong to PES. This control method has been reported for spectral studies of self-assembled monolayers [12]. The spectral features of our PES membranes in the area 4000–2000 cm\(^{-1}\), together with literature data for location and assignment of the bands [26], are given in Table 3.

The spectrum of the final washed membrane (Fig. 2) has no bands in the region below 3068 cm\(^{-1}\) (area of aliphatic CH stretching), but two characteristic intense bands associated with aromatic CH vibration are present at 3096 and 3068 cm\(^{-1}\). There are also two bands located at 3634 and 3553 cm\(^{-1}\) that, by our interpretation, are associated with the OH stretching vibration of water molecules, since porous materials may hold in their pores small amounts of water that, in practical terms, are almost impossible to remove. The phase-inversion method of preparation of the PES membranes may indeed result in the presence of entrapped water [25]. In contrast, in the spectrum of a nonporous ‘home-made’ PES film (not shown) prepared by casting from organic solution, there were no bands in the area of OH stretching.

Here, it should be remembered that in polymeric membranes water may exist in two distinctly different physical states — free and bound [28–30]. The ‘free’ water is present largely as monomeric H\(_2\)O molecules, and its fundamental OH stretching band was observed near 3650 cm\(^{-1}\), i.e. at higher frequencies than that of ‘liquid’ water. In liquid water, the OH stretching band of the hydrogen bridges is found near 3400 cm\(^{-1}\) as a result of overlapping asymmetric and the symmetric stretching vibrations [30]. The shift of this band toward lower frequencies and the location of bands below 3404 cm\(^{-1}\) characterize water molecules associated via hydrogen bonding with the surroundings (this water is known as ‘bound’ water). In water entrapped in polymers, the strength of the hydrogen bond may vary, depending on the nature of the polymer, the functional group and the porosity. By analyzing the positions of the stretching or bending bands, it is possible to elucidate the state of water; this has a direct effect on the transport (diffusion) and the sorption properties of membrane [31,32].

![Fig. 1. ATR-FTIR spectra of the Millipore PES membrane (a) as received (the bands that were removed by washing, are marked with arrows); (b) after washing with water.](image-url)
Table 3
Frequencies and assignment of the relevant IR bands in the area of 4000–2000 cm$^{-1}$

<table>
<thead>
<tr>
<th>IR band (cm$^{-1}$)</th>
<th>Literature data</th>
<th>Our data</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3650</td>
<td>OH stretch, non-hydrogen bonded (from H$_2$O residue)</td>
</tr>
<tr>
<td>3450</td>
<td></td>
<td>3450</td>
<td>OH stretch, slightly hydrogen bonded</td>
</tr>
<tr>
<td>3090</td>
<td></td>
<td>3096</td>
<td>CH-aromatic stretch</td>
</tr>
<tr>
<td>3067</td>
<td></td>
<td>3067</td>
<td>CH-aromatic stretch</td>
</tr>
<tr>
<td>2962</td>
<td></td>
<td>–</td>
<td>$\gamma$ CH$_3$ asymmetric aliphatic stretch</td>
</tr>
<tr>
<td>2919–2925</td>
<td></td>
<td>2925$^a$</td>
<td>$\gamma$ CH$_2$ asymmetric aliphatic stretch</td>
</tr>
<tr>
<td>2875</td>
<td></td>
<td>–</td>
<td>$\gamma$s CH$_3$ symmetric aliphatic stretch</td>
</tr>
<tr>
<td>2850–2856</td>
<td></td>
<td>2856$^a$</td>
<td>$\gamma$s CH$_2$ symmetric aliphatic stretch</td>
</tr>
</tbody>
</table>

$^a$ These bands are present in the PES samples designated Millipore 4020.

3.2. Surface modification by grafting

Before discussing the effect of grafting, it is worth emphasizing that the grafting of the chosen monomers results in the chemical attachment of new polymeric polyethylene main chains, e.g.,

$$\text{CH}_3 - \text{CH}_2 - \text{CH}_3$$

$$\text{COOR}$$

where R may be: H (methacrylic acid), or $-(\text{CH}_2)_3-\text{SO}_3\text{H}$– (sulfopropylmethacrylate or $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{OH}$– polyethyleneglycol).

Consequently, the absorbance of the main polyethylene chain may be expected in the region of CH aliphatic vibration for all three grafted polymer chains.

3.2.1. Modification with SPM

SPM has carbonyl and sulfogroups, which can be easily recognized in the IR spectrum. The asymmetric and symmetric vibrations of SO$_3^-$ are generally located in the regions of 1220 and 1040–1070 cm$^{-1}$, respectively [26,33], and the C=O vibration has a characteristic absorbance at 1730 cm$^{-1}$ [34]. Asymmetric SO$_3$ vibration is usually not readily observable (as in our case) due to overlapping bands [26]. The spectrum given in Fig. 3 shows a weak band of symmetric vibration at 1045 cm$^{-1}$ and the band assigned to the C=O vibration at 1724 cm$^{-1}$. The intensities of these two bands increased as the grafting proceeded, as it is seen from spectra of membranes modified 20 min (a) and 120 min (b).

Fig. 2. ATR-FTIR spectrum of dried membrane in the area of 4000–2500 cm$^{-1}$.

Fig. 3. ATR-FTIR spectrum of PES modified with SPM (20 min and 2 h) in the region of 1800–800 cm$^{-1}$. (a) modification for 20 min; (b) modification for 120 min.
In addition, we found that the area of aliphatic stretching vibration also provides essential information. The asymmetric stretching band at about 2925 cm$^{-1}$ becomes more intense as the degree of grafting increases (Fig. 4). The symmetric stretching vibration of CH$_2$ is clearly visible at 2852 cm$^{-1}$, in agreement with the literature data. [12,32,34] The intensities of bands at 2925 and 2852 cm$^{-1}$ increased compared with intensities of doublet at 3060 cm$^{-1}$ as we going from membrane modified for 20 min to that modified for 2 h.

Analysis of the area of OH stretching shows that in the spectrum of the membrane modified with SPM for 20 min the initial band at 3540 cm$^{-1}$ has split in two components, 3548 and 3412 cm$^{-1}$. The latter component has shifted slightly to a higher frequency (3439 cm$^{-1}$) in the spectrum of the membrane modified for 120 min (Fig. 4). In view of the intrusion of highly hydrophilic SO$_3$H groups, which could attract the water molecules surrounding the sulfogroups and hydrogen bridged, one might expect a shift towards lower frequencies. On the other hand, the monomer sulfopropyl methacrylate contains a hydrophobic part that might be an obstacle to hydrophilization. As a result of these opposing trends, the total shift is less significant than in the case of methacrylic acid grafting.

### 3.2.2. Modification with MA

The spectrum of the MA-grafted membrane (Fig. 5) is characterized by the appearance of a peak around 1709 cm$^{-1}$ (associated with the C=O group of dimers of carboxylic acids) [34]. The intensity of this peak increases as the grafting proceeds. Moreover, a slight but distinct shift towards lower frequencies (from 1712 to 1708 cm$^{-1}$) is observed. This might be the result of hydrogen bonding between the OH groups of acid carbonyl as their population increases. The bands associated with the aliphatic chain are evident at 2929 and 2860 cm$^{-1}$, almost in the same location as in the spectrum of the PES-SPM membrane. The spectrum of this particular MA-grafted polymer does not show any significant new bands in the region of OH stretching. (Fig. 6a). The picture changes dramatically with
prolonged grafting time. In that case, the hydroxyl stretching band shows the typical shape of self-associated polycrists — a very broad band that shifts significantly to low frequencies. At a higher degree of grafting, there appears to be massive intrusion of the carbonyl groups into the polymer backbone and the formation of large water associates. This is reflected in the change of shape of the peak associated with the OH stretching of carbonyl, which is dramatically broadened and shifted to lower frequencies with formation of a typical plateau (Fig. 6b,c). The formation of such a plateau was first observed by us in MA-modified polyamide RO membranes [2], and was correlated with changes in the properties of the membrane on the macromolecular level. The aliphatic vibration band is partly hidden behind this plateau, and the asymmetric stretch is evident at 2935 cm\(^{-1}\).

3.2.3. Modification with PEG-MA

As in the case with the two acrylic monomers mentioned above, attachment of PEG-MA to the PES membrane first becomes visible in the spectrum of the modified membrane by the appearance of a new band associated with the C=O vibration in the ester molecule, which is located at 1714 cm\(^{-1}\) (Fig. 7).

The higher intensity of the peak for membranes modified for 2 h in comparison with 20 min (not shown) is not surprising and in agreement with published data. The spectral features in the area of OH and CH are most interesting. In the spectrum of the membrane modified for 20 min, the intensive broad band corresponding to aliphatic stretch has two components, one at 2923 cm\(^{-1}\) and the other at 2867 cm\(^{-1}\) (Fig. 8a). Although the location of this second peak is only slightly different to that obtained with MA (2860 cm\(^{-1}\)) and SPM (2852 cm\(^{-1}\)) modifications, the value falls within the normal range of 2852 \(\pm\) 15 cm\(^{-1}\) [34]. The high intensity of this peak could be due to the contribution of the (CH\(_2\)CH\(_2\)O) grouping in total absorbance of the main (polymeric) chain. Effect of the time of grafting is seen in that the intensities of CH stretching increase with the time of grafting, as expected.

3.3. Static absorption of albumin

The term ‘static’ means that the skin surface layer of membrane is in contact with protein solution within a non-stirred, no-flux, no-pressure situation [23,37].

3.3.1. Procedure A

The water flux was measured before and after exposure of the membrane to protein solution to confirm the protein deposition on the membrane. The significant decline of the initial water flux (not shown) indicated that adsorption of the protein did indeed take place on the surfaces of all the membranes, independent of modification.

After water flux measurements, membranes were removed from the UF cell, treated as described in
Fig. 9. ATR-FTIR spectra of CH-stretching region of unmodified (1) and SPM (2) and PEG-MA (3) modified membranes before (a) and after exposure to water vapour during 4 h (b) and 17 h (c).

Section 2, and examined by IR spectrometry. Generally, two characteristic areas are inspected as proof of the presence of albumin on solid surfaces — the areas of 1600–1700 and of 3000–4000 cm\(^{-1}\). The characteristic albumin bands that appear in these areas are the bands at 1650 cm\(^{-1}\) (amide I mode), 1540 cm\(^{-1}\) (amide II mode) and 3300 cm\(^{-1}\) (N–H stretch) [19,35,36]. The amide I band originates predominantly from the C= O stretching vibration of the peptide groups, and the amide II band is characteristic of the bending of NH groups in the plane and the C–N stretching modes of the polypeptide chains.

Careful examination of the spectra of three membranes reveals the presence of a very weak but broad band at 1650 cm\(^{-1}\) (not shown). It appears that the amide II band of 1540 cm\(^{-1}\) could not be easily resolved (this observation is in agreement with other works [16,20]). There are no significant changes in the area around 2900 cm\(^{-1}\). We thought it possible that treatment with an organic solvent, such as methanol and ethanol (see Section 2), may have influenced the conformation of the albumin and the resolution of its characteristic peaks. Therefore, the membranes were exposed to water vapor in a humidity chamber (88% RH). The process of hydration in time was followed by recording spectra after 4 and 17 h (Fig. 9). We can see that for the three types of membranes unmodified (1), SPM-modified (2) and PEG-MA-modified (3), sharp peaks appeared at 2922–2924 cm\(^{-1}\), with a shoulder at 2964 cm\(^{-1}\) and a small peak at 2875 cm\(^{-1}\). All these peaks correspond to CH stretching vibrations [12]. The position at 2964 cm\(^{-1}\) corresponds to CH\(_3\), while location 2922–2924 cm\(^{-1}\) belongs to a methylene (CH\(_2\)) group. In the region of OH vibration, a distinct shift of the band associated with the hydrogen-bonded OH of water (around 3400 cm\(^{-1}\)) to the higher frequency is also evident. In the region of 1200–1000 cm\(^{-1}\), the higher intensities of several peaks (1070, 1014 cm\(^{-1}\)) are evident upon hydration (not shown).

To explain these findings, we must address the theory of protein adsorption, which regarded albumin adsorption as an equilibrium reaction that takes place on the polymer surface in competition with the adsorption of water [36–38]. According to this theory, when protein is adsorbed on the surface, water molecules between the protein and the surface must be replaced. Protein adsorbed on the hydrophilic surface lost bound water of surface-contacting portion. If, however, the water state of the hydrophilic surface is similar to an aqueous solution, protein does not need to release bound water molecules.

We speculate that our spectral features may reveal, on a molecular level, that a water/protein connection does indeed exist, and might even be a dominant factor in fouling. As we mentioned above, when membranes with adsorbed albumin were exposed to water vapor, the effect of hydration was seen first in an increase of the intensities of the bands corresponding to the membrane itself (1070, 1014 cm\(^{-1}\)), the highest intensity being found for the SPM-modified membrane. The most striking finding, however, was that the longer time of exposure to water vapor (17 h) leads to the appearance of a sharp intense peak at 2961 cm\(^{-1}\), with a simultaneous drop of the peak at 2925 cm\(^{-1}\) in the spectra of virgin and both, PEG-MA and SPM modified membranes. This peak at 2961 cm\(^{-1}\) belongs to the asymmetric vibration of CH\(_3\) and may be found in the spectrum of albumin in KBr. (Note, that in the spectrum of original dry PES there is no band in this region). Considering the universality of the peak at 2961 cm\(^{-1}\) for all three membranes, it was thought that this might be due to the ordered character of some fragments of albumin when the hydration takes place (in accordance with mentioned above). Additional support for this idea may be found in the sharp contour of the peak at 2961 cm\(^{-1}\). The high intensities and the sharpness of the contour of the CH\(_3\) stretching band at
2961 cm\(^{-1}\) has been recently recognized as indication of the ordering character of aliphatic chains in such self-assembly structures as polypeptides, surfactants and Langmuir–Blogett films [14–16,39]. In order to determine whether absorbed and hydrated albumin fragments display an ordered character, dry samples was examined using polarized IR radiation. If the alkyl segments are oriented parallel to the substrate plane [14] then changes associated with CH vibration would not be visible in the parallel polarized IR. For this purpose the original membrane with adsorbed albumin (dry state) was taken. The spectra of albumin adsorbed on original membrane were obtained using both parallel and perpendicular polarized radiation (Fig. 10). From Fig. 10 it is evident that the bands around 2910–2920 cm\(^{-1}\) are almost not visible in the spectra obtained with parallel polarized radiation, but they have the highest intensities in the spectra obtained at perpendicular polarization. This might indicate that some segments of albumin molecules are oriented in a certain way, caused by conditions of adsorption, resulting in the tight and often irreversible bonding of the protein on the surface [23]. Finally, it is not certain whether the IR polarized experiments were sufficiently precise to detect orientation effects. However, our data agree in general with the picture that might be expected if such orientation effects are indeed present.

### 3.3.2. Procedure B

The membrane with the 100,000 cut off was employed to probe the albumin adsorption from a more concentrated solution. The spectrum of unmodified membrane is given in Fig. 11. Inspection of the spectra of membrane with adsorbed albumin shows a well-resolved amide I band (Fig. 11), although the amide band II was not well resolved. The modern IR spectroscopy in the internal reflection mode and with computerized software can be used for subtraction manipulation, where the spectrum of original membrane is subtracted from that of membrane with adsorbed albumin. This manipulation can eliminate all IR peaks from origin material. This requires a trial and error procedure using standard FTIR software until a best subtraction is reached.

The subtraction ‘result’ for albumin absorbed on the original polysulfone membrane is presented in Fig. 12.
Application of the subtraction technique for the modified membranes was not straightforward and we are now developing this procedure as well as method of Fourier self-deconvolution for better resolution of overlapping peaks comprising the broad amide I band. [21] This method will provide information on the structural conformers of albumin on the membrane surface.

4. Conclusions

It was shown that redox initiation grafting could be successfully applied to PES, as was done earlier for polyamide membranes. The FTIR-ATR technique provides a necessary information on functionalization as it depends on monomer’s nature and time. This technique was also used for studying protein adsorption on the surfaces of the membranes.

Polarized radiation was used to show that alkyl segments of adsorbed protein may be arranged in certain order. The peak at 2961 cm$^{-1}$ was detected in the spectra of the hydrated membranes.

Polarization-radiation mode was applied for dry membranes. This was possibly the reason that the latter spectra show no peak at 2961 cm$^{-1}$. Taking into account that this peak is associated with a CH$_3$ group, one may assume, that hydration of the membrane with adsorbed albumin results in the extrusion of methyl containing fragments. Water-protein relationship as a mechanism for protein adsorption was suggested on the base of these spectral results.

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