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Formylated polysulfone membranes for cell immobilization

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Polysulfone membranes can be obtained in a great variety of morphologies by the classical phase inversion technique. Immersion precipitation is the most widely used phase inversion technique which involves the exchange of the solvent from the polymeric solution with a non-solvent for the polymer, but which is miscible with the solvent. Phase inversion takes place when the concentration of non-solvent in the system polymer/solvent/non-solvent has been increased to such a level that the solution is not thermodynamically stable anymore and demixing will occur. There was developed new phase inversion technique that is based on a chemical reaction which takes place in a specific formylated polysulfone/ solvent/non-solvent - with cell content system and which induces phase separation.

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1. Introduction

Membrane separations are almost exclusively applied to aqueous systems. The application in organic solvents offers a new and interesting field. Applications can be found in pressure driven processes such as ultra-filtration and reverse osmosis and activity driven processes such as pervaporation. The performance of the membranes was tested with ethanol/water solutions [1-5].

There are some products where the alcohol concentration has to be decreased for a specific group of consumers: drivers, children, and pregnant women. Among these products alcohol-free beer is well known, and other interesting products may be champagne for children and alcohol-free wine. The process industry slowly moves towards a sustainable technology in which raw materials and side products are recovered and reused and the energy consumption is reduced as much as possible. In a further stage this may result in closed systems in which the emission of polluting substances to air and water are reduced to a minimum level. In order to face these challenges, new separation technologies are required with a high separation performance at low energy consumption. Membrane technology is a relatively new separation process, which fulfils these criteria. It can be applied in process integrated systems and in this way waste problems are drastically reduced while valuable products are recovered.

One of the fundamental steps in many biological analyses is the separation of different cell types from a raw, heterogeneous sample such as blood. Often, the desired cell population must not only be separated but also enriched, or concentrated, well beyond original volumetric numbers denssities in the raw sample. Conventional methods for cell separation take advantage of differences in various physicochemical cell properties [6]. Separations by physical properties are usually based on differences in cell size and density [6,7] and have even included differences in cell stiffness [8,9]. However, these differences are often slight and difficult to discern among different cell types. Immunological techniques, such as fluorescence-based flow cytometry (FACS), magnetic cell labeling (MACS), and affinity chromatography, have therefore become mainstay in clinical laboratory due to their high specificity and their broad applicability. Such techniques have also been implementated succesfully in microfluidic devices [10-12]. The use of antibody-mediated immobilization or labeling, however, generally requires some preparatory and incubation time periods. Additionally, ancillary systems for actuation and detection, which are often large and elaborate, must accompany the central semple handling device. Then, following the separation step, cells must be post-processed, sometimes requiring the removal of labeling. These steps add additional processing time and reagents and may hurt process yield and can sometimes be harmful to the cell themselves. For microfluidic devices, one alternative separation technique is dielecrophoresis, used either alone alone or in conjunction with another method [13-16]. This technique, while effective in many cases, does adversely affect cell viability. The development of miniature bio-analytical systems will therefore continue to benefit from the exploration of new, elegant cell separation techniques that can serve to augment or even supplant cumbersome traditional techniques. This work introduces an alternative method for collecting cells from blood semples, technique based on formylated polysulfone membrane [17].

The properties required for polymeric micro filtration membranes are: the dimensions of the pores larger than 0.1 μm; high porosity and a narrow pore size distribution; mechanical resistance; chemical resistance towards the

components from the feed and the cleaning agents; good wettability; low fouling ability; thermostability; low costs. Two types of polymeric micro filtration membranes can be distinguished: (a) Membranes with a symmetrical structure - the diameter of the pores varying a little over the cross-section of the membrane and (b) asymmetric membranes - characterised by an asymmetrical structure. The dimension of pores at the feed side-the active surfaces-are small compared with the pore size at the bottom side. Such membranes can be obtained by a phase inverse technique. The membranes was characterised by FTIR spectrometry and the morfology was investigated by scanning electron microscopy [18].

2. Experimental

The formylated polysulfone was prepared in following way: 3g of polysulfone (Udel) was dissolved by stirring 7 hours at room temperature. After dissolving, 3ml of phosphorus oxychloride (Merck) are added at 0^{o}C (in a bath of ice) in 3 portions in half an hour. After this, the reaction is perfected by stirring 4 hours at 80-90^{o}C. Phase inversion membranes from a solution of formylated polysulfone/ cyclohexanone were prepared in two ways: (a) Immersion precipitation, in which the polymer solution is immersed in a non-solvent bath. The exchange of a solvent with a non-solvent is favoured by the miscibility of cyclohexanone with the majority of the organic solvents and with alcohol, as well. However, with alcohol solutions no membranes were obtained. The alcohol solutions extract too rapidly cyclohexanone from the system, resulting in polymeric grains, while the higher alcohols are not a good non-solvent (coagulation time approximately 30 minutes) and no usable membranes are obtained and (b) Chemical reaction, in which cell or more precisely the protein is extracted from the non-solvent bath solution. The membrane is hydrolyze in an deionized water with 5% sulfuric acid (Merck) at room temperature in 48 hours.

3. Results and discussion

The first system that has been investigated was based on formylated polysulfone. The experiments were carried out to prepare formylated polysulfone membranes by a chemical reaction that induces phase separation. The membranes were prepared from formylated Udel polysulfone6 with a molecular weight of 35000-45000 Dalton and cyclohexanone as solvent. Although cyclohexanone has a high toxicity and it is used because it present excellent solvent properties for formylated polysulfone, in order to give stable solutions and offer the possibility to use alcohols, ethers, esters, halogen derivatives and hydrocarbon compounds as non-solvents. Moreover, cyclohexanone represents a solvent that can be changed in a non-solvent by a reaction and consequently, membranes with a desired morphology could be prepared. Cell content of bath solutions directly give a polymeric membrane with immobilized cell (Fig. 2).

The formylated polysulfone was characterized by FTIR spectra (Spectrometer Bruker Tensor 37) –Fig. 1.

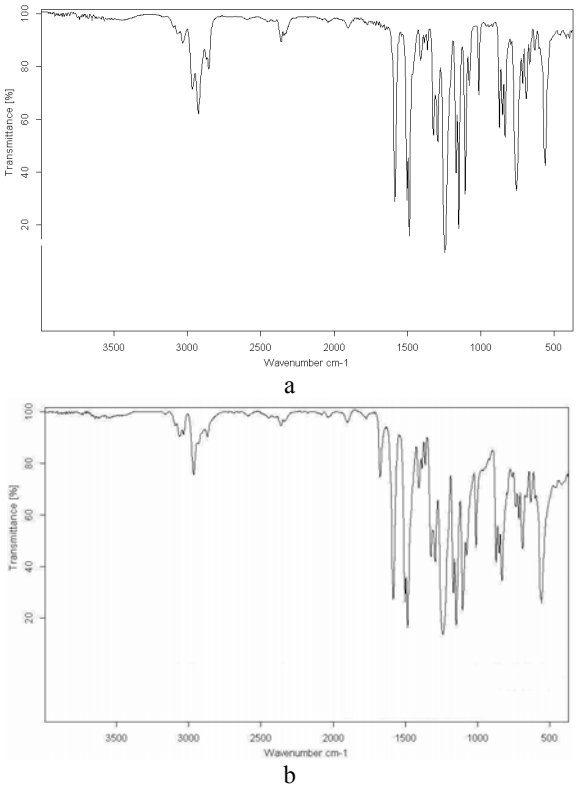
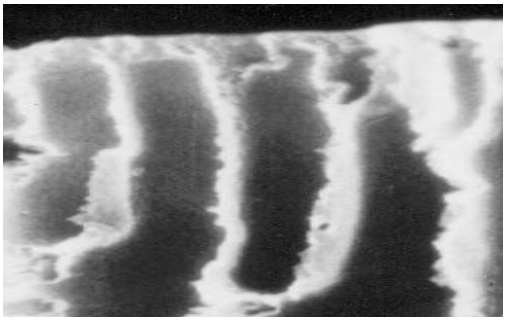


Fig.2. FTIR spectra of polysulfone (a) and formylated polysulfone membrane (b).

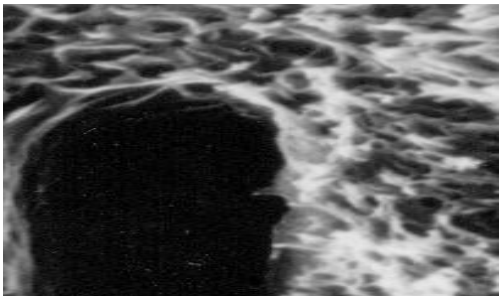
The obtaining membranes from the same system polymer / cyclohexanone / aqueous alcoholic solutions are compared. In the figure 1b, the essential modifications due to formylation reaction are very clear. The appearing of the band at 1670cm^{-1} (specified to an aromatic aldehyde functional group) and the enlargement of the band in the area from 3000-3600 cm^{-1} (specified to hydrogen bonds from the adduct alcohol-aldehyde) are the principal characteristics which appear. The obtaining of the polysulfone and formylated polysulfone membrane from the system polymer/cyclohexanone/aqueous alcoholic solution containing cells (Saccharomices cerevisiae) permit the evidence of the immobilization (Fig. 2) only in the case of the membrane from formylated polysulfone.

The obtaining of the polysulfone and formylated polysulfone membrane from the system polymer/cyclohexanone/aqueous alcoholic solution containing cells (Saccharomices cerevisiae) permit the evidence of the immobilization (Fig.3 and Fig.4) only in the case of the membrane from formylated polysulfone. The scanning electron microscopy indicate even at low resolutions (× 500) and high resolutions (× 5000) the polysulfone membranes have not cells and formylated membranes intercat strong with the cells. Practical, the experiments demonstrate the possibility of immobilization the cells on membranes if exist an activate polymer, in this

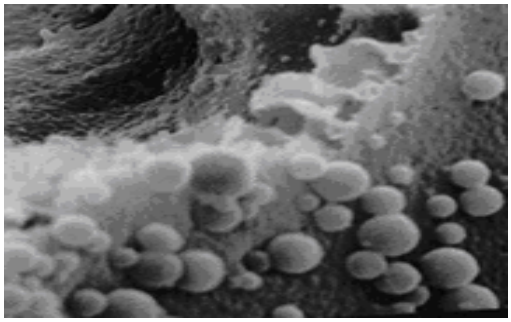
case, a formylated one and it is obtained the property of coagulation.



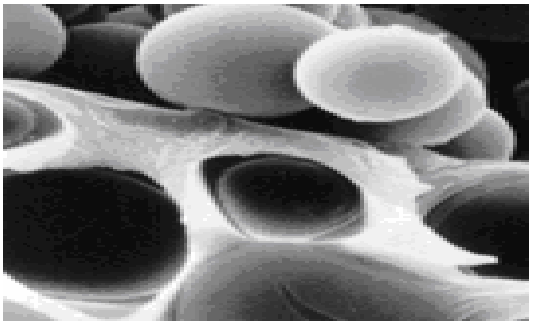
a: x500



b: x5000



c: x500



d: x5000

Fig.2. Scaning electron microscopy of unformylated (a and b) and formylated polysulfone membranes with cells (Saccharomices cerevisiae) (c and d)

4. Conclusions

There are many possibilities to immobilize cells. One commonly used class of immobilization matrices is photopolymers. Most photopolymers utilize visible or ultraviolet light to cross-linking the monomers used in the formation of the matrices. Some photopolymers utilize harsh chemical initiators to facilitate the polymerization. A photon from the light source breaks the photoinitiator into groups of highly energised radicals. The radicals then react with the resident monomer in solution and initiate the usually unstable thermoset polymerization. Photopolymers used as immobilization matrices are often in the form of micropatches formed by photolithographic techniques that are typically 50-500μm in critical dimension. The smaller microstructures result in a capture of 1-3 cells per structure. The result is a structure that can support cells for long periods; however, the small size does not allow the capture of many cells, lowering signal response. Another drawback to the photolithographic technique is a loss of cells. When the cells and photopolymer solution are place on a treated substrate, a mask is placed over the substrate and a light shone through the mask. Only the area of the solution that are exposed to the light source become gelled will be washed away.

Another technique of cell immobilization is based on thermally reversible gels. Thermally reversible gels, while most commonly used in drug delivery systems, are quickly being viewed as a potential matrix for immobilization. In aqueous solutions, thermally reversible gels undergo volume-phase transitions above a certain temperature. They formed collapsed, dehydrated, hydrophobic gels above a lower critical solution temperature (LCST) and swollen, hydrated, hydrophilic dispersed solutions below the LCST. Cells can be easily immobilized by mixing cells with aqueous thermally reversible gel solution at low temperatures. When the solution temperature is raised above its LCST, the cells still are immobilized within the hydrogel matrix. The most common thermally reversible gel is the poly(N-isopropylacrylamide) (PNIPA) gels. PNIPA is comprised of hydrophilic amide and hydrophobic isopropyl groups in its side chains. The change in phase of the hydrogel is due to a disruption of the hydrophilic and hydrophobic balance within the structure causing a macroscopic phase transition [19].

The two methods presented are good, but are very expensive and there are not very efficient in comparison with the separation of cells using the formylated membranes. The aromatic electrofile substitution is the most accessible way to functionalize the aromatic polymers. In the present stage, the polysulfones are an excellent support for liquid membranes and macromolecular and enzyme compounds. The immobilization of complex macro cyclic compounds or enzymes on the polysulfones is conditioned by the existing on the polymer network of several functional groups. Are well known reactions from obtained the nitrated, chloromethylated, halogenated, sulphonated, chloro-sulfonated, carbo-xylated and hydroxilated polymers in purpose to immobilize different compounds or changing the superficial properties of used membranes in separation processes. The method of obtaining these membranes is relatively cheap and accessible. The single bad point is representing by the using of phosphorus oxychloride in the reaction of synthesis of the membrane. The using of large

scale of polysulfones for preparing the micro-porous dense or composite membranes is justified by a large scale of material membranes characteristics: apart solubility in polar aprotic solvents, so exist the possibility of micro-, ultra- and hyper filtration membranes synthesis by phase inversion; thermal resistance in operation temperature domain (0-180^{o}C); chemical stability at all pH values; aseptic possibilities; mechanical resistance.

Chemically Modified Polymers (CMP) are currently used as supports for proteins, enzymes or other ligands in many biological and biotechnological applications. One the most important group which was introduced on polymers is –CHO function. There is a great possibility to modified heavy hydrolysable polymers. Vilsmeier-Haack on polysulfone is a good method to obtain activated polymers.

The preparation of a membrane by phase inversion is very much restricted by the limited number of solvents for the polymer. A new phase inversion mechanism has been developed - polymer precipitation was induced by a chemical reaction. In this work was obtained directly immobilized cell asymmetric polysulfone membranes through formylated polysulfone / solvent / non-solvent – with cell content system. Due to large variety in additives combined with a reaction system, various membrane morphologies can be prepared for different applications.

In the last years, membranes and membranes processes have improved from laboratory plants to industrial plants, with a considerable economical and commercial importance. The membranes processes have been removed the conventional separation techniques and they were used in the domains where classical methods were useless or very expensive.

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