Preparation of Ultrafiltration / Microfiltration Ceramic Composite Membranes for Biotechnology Applications

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Abstract

Ceramic membranes possess desirable properties for industrial separation and concentration processes where extreme conditions of heat, pH, ionic strength are present. Ceramic composite membranes consisting of a support and a thin membrane layer with finer pores were prepared in this work. Ceramic supports were prepared from fine alumina and zirconia powders by dry-pressing and slip-casting. These supports were sintered in the 1100-1200°C temperature range. These supports were dip-coated with ceramic sols prepared from zirconium propoxide and aluminium isopropoxide for the preparation of thin layers with finer pores. Average particle sizes of these sols were measured as 3-7 nm for zirconia sol and 30-40 nm for alumina sol by laser scattering technique. The dip-coated membranes were calcined in the 500-600°C temperature range. The thermal characterization of membrane layers was determined with DTA/TGA. The microstructure of the ceramic composite membranes was investigated with SEM.

The clean water permeability (CWP) of membranes was tested by using deionised water in a filtration set-up. Separation experiments were performed with bovine serum albumin (BSA, Stokes diameter: 7 nm) solution and whey to determine protein separation capacity of the composite membranes. The CWP of the dry pressed alumina supports heat treated at 1100°C was found to be higher than the permeability of the slip-cast zirconia supports heat treated at 1200°C. The protein retention of the slip-cast zirconia support was detected as 60% with UV-VIS spectrophotmeter analysis (Abs. at 280nm). After the modification of the support with dip-coating protein retention of 96% was achieved. Then the composite membranes were used in whey protein concentration experiments. Whey, which is a complex biological liquid, containing proteins, carbohydrate, minerals, was fed to the membrane without any clarification process. The retention of whey proteins with dry-pressed alumina support modified with 1/4 diluted zirconia sol (calcination temperature: 500°C) was found to be 96% with a permeate flux of 40 $Lm^{-2}hour^{-1}$.

Öz

Seramik süzekler, ısı, pH, iyonik kuvvet değerlerinin uç değerlerde bulunduğu sınai ayrıştırma ve yoğunlaştırma işlemleri için gerekli özelliklere sahiptirler. Bu çalışmada, bir destek ve daha ince gözeneklere sahip bir zar tabakasından oluşan seramik karma süzekler hazırlandı. Seramik destekler, ince alumina ve zirkonya tozlardan kuru baskılama ve alçı üzerine kalıba döküm yöntemleri kullanılarak hazırlandı. Bu destekler 1100–1200°C sıcaklık aralığında pişirildi. Pişirilen destekler daha ince gözeneklere sahip ince bir tabaka oluşumunu sağlamak için alüminyum izopropoksit ve zirkonyum propoksitten hazırlanan seramik sollerine daldırılarak kaplandı. Bu sollerin ortalama parçacık boyutları, lazer saçılımı tekniği kullanılarak, zirkonya solü için 3–7 nm ve alumina sol için 30–40 nm olarak ölçüldü. Daldırılıp kaplanan zar süzekler 500-600°C sıcaklık aralığında ısıl işleme tabi tutuldular. Zar süzek tabakanın ısıl nitelendirilmesi DTA (Diferansiyel Isıl Analiz)/TGA (Isıl Ağırlık Analizi) ile gerçekleştirildi. Seramik karma süzeklerin mikro yapıları SEM (Taramalı Elektron Mikroskobu) ile incelendi.

Süzeklerin saf su geçirgenliği (SSG) deiyonize su kullanılarak bir süzme düzeneğinde ölçüldü. Karma süzeklerin protein ayırım kapasitelerini belirlemek için sığır serum albumin (BSA, Stoke çapı = 7 nm) çözeltisi ve peynir altı suyu kullanılarak ayrıştırma denemeleri gerçekleştirildi. Kuru baskılanmış ve 1100°C' de ısıl işleme tabi tutulmuş alumina desteğin saf su geçirgenliği, alçı üzerine kalıba dökülen ve 1200°C' de ısıl işleme tabi tutulan zirkonya desteğin geçirgenliğinden daha yüksek olarak ölçülmüştür. Alçı üzerinde kalıplanan zirkonya desteğin protein tutma oranı UV-VIS spektrofotometre kullanılarak (280 nm dalgaboyundaki absorbanı) %60 olarak belirlendi. Desteğin daldırılıp kaplanarak değiştirilmesinden sonra %96' lık bir protein tutmaya ulaşıldı. Proteinler, karbonhidrat, mineraller içeren karmaşık bir biyolojik sıvı olan peynir altı suyu süzeğe hiçbir saflaştırma işlemi uygulanmadan beslendi. Kuru baskılanmış ve 1/4 seyreltilmiş zirkonya sol ile kaplanılarak 500°C' de ısıl işleme tabii tutulup değişime uğratılmış alumina desteğin peynir altı suyundaki proteinleri %96 oranında tuttuğu bulundu.

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Chapter 1

INTRODUCTION

In biotechnology, the downstream processes are important steps in production which affect the product quality and economics directly. The separation and purification of valuable organic materials (like enzymes, proteins, organic acids, amino acids, antibiotics, etc.) from aqueous media, with a reliable method is always in concern. The fragility of such organic products forces the producers to use non-destructive methods for separation and concentration. This prevents usage of evaporation, in which heat application takes place at high temperatures. The other alternative for obtaining product from aqueous media is lyophilization which is non-destructive but very expensive. The membrane processes are considered to be the best solution for separation and concentration since they are non-destructive and relatively economic. There is neither heat application which will deteriorate the product, nor any energy consumption for changing the phase of water.

In membrane processes, the separation is performed due to the difference between the sizes of the materials to be separated. For this purpose membranes which are selective to the target material aimed to be separated are needed. The first membranes were natural polymers. Nowadays membranes made of polymers or inorganic materials (ceramic) are used. The inorganic ones may be thought as better choices due to their higher thermal, chemical, physical, microbiological stability. Although the initial investment for a filtration system with ceramic membranes is higher than the ones with polymeric membranes, due to the long life and better operating opportunities of ceramic membranes, it becomes more economical to use ceramic membranes, in long term. The ceramic membranes are also good alternatives in biotechnology and food industries. Since thermal or chemical sterilization is needed in such industries, the membranes should be compatible to steam or chlorine sterilization. Ceramic membranes are compatible to such conditions and have a rigid structure which will not shrink under pressure application. They are also stable to microbiological attacks. Cot *et al* (2000) stated that "A connected microporous structure in supported ceramic films can be created from a non aggregated nanoparticulate sol with individual

nanoparticles of less than 10 nm. Such materials offer attractive properties when used as ceramic nanofilters. Ceramic nanofilters are being recognized as being of growing importance due to increasing demands for membrane systems able to separate ions and small molecules in harsh working conditions (high temperature, extreme pH, organic solvent media)".

In this study, ceramic membranes for biotechnological processes were prepared with dry-pressing or slip-casting of ceramic powders (alumina or zirconia) to get a porous supports which are mechanically strong enough for high pressure filtration processes, and modification of these supports with sol-gel methods, to have a thin membrane layer over these supports which has finer pores and enables separation of smaller molecules.

Chapter 2

MEMBRANES

A membrane is a thin barrier, which is selectively permeable. It allows passage of some components, which form the *permeate*, and retains the others, which form the *retentate*. The membrane may be solid, liquid or gas. Generally, solid membranes are widely used and the others are used for some specific processes. Solid membranes may be classified as inorganic and polymeric membranes. The latter ones have been used for a longer time.

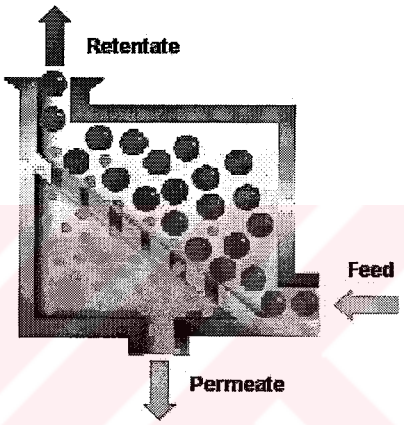

Figure 1. The separation process by filtration.

2.1. History of Membranes

Abbe Nolet, in 1748, observed that, when a bladder filled with brackish water is put into water, expands, although there is the tension of the bladder making it difficult. In 1854, Graham, with curiosity of observing the deflating of a toy balloon, made experiments with different gases and realised some gases leave the balloon faster. That was due to the osmotic pressure. (Howell *et al*, 1993)

Such experiments increased the curiosity and research on the topic. In 1855, Fick developed the first polymeric synthetic membrane made of nitrocellulose. Then Traube and Pfeffer also prepared artificial membranes. These were generally prepared by dissolving the polymer (nitrocellulose) with a suitable solvent (alcohol, ether or acetic acid) and by evaporating the solvent after pouring the solution on a flat plate. The first quantitative measurements about diffusion and osmotic pressure were performed with these membranes. Benchold developed methods to control the pore size of

membranes by controlling the evaporation rate in 1907. The research activities on membranes increased rapidly between 1870 and 1920. Especially, van't Hoff's theory about dilute solutions and Gibbs' work on the relationship between the osmotic pressure and other thermodynamic properties were important. (Cheryan, 1998)

In the light of such studies, the first commercial membrane was produced in 1927. These commercial membranes were being used for the removal of microorganisms and particles from fluid streams. In 1950s, Samuel Yuster, predicted that, it should be possible to produce potable water from brine, using Gibbs adsorption isotherm. Further experimental work showed that it was possible to get potable water from brine, but the efficiency was very low. Between 1958 and 1960 Sourirajan, tried to heat the membrane and predicted that, this would cause the enlargement of pores, and this enlargement will be preserved after cooling. He thought that, this enlargement would cause a higher flux. But in fact when the cellulose acetate ultrafiltration membranes were heated the pores over the surface shrank and this caused in an asymmetric structure throughout the membrane. But this new membrane, surprisingly, allowed a higher flux and a higher rejection of salts. This was a milestone in membrane technology. (Cheryan, 1998)

In 1970s ultrafiltration was performed widely in electropaint and dairy industries. Several polymeric membranes were produced from different polymers. The Journal of Membrane Science was started in 1973, indicating the increasing interest to membrane research. (Howell *et al*, 1993)

Another progress was the introduction of ceramic membranes, which were under investigation since 1940s in the industry. The energy crisis caused a great interest in nuclear power plants, which were in need of high concentrations (3%) of ^{235}U . ^{235}U is naturally present in Uranium with a quantity of 0.7%. The concentration was being increased by mass spectroscopy and this was an expensive method. The gas diffusion concentration process with ceramic membranes in the nuclear power plants has been using the same ceramic membranes for over 20 years in the industrialised countries (Bhave, 1991).

In 1980s gas separation and pervaporation units were taken into operation (Cheryan, 1998, Howell *et al*, 1993). In 1990s, a great progress was achieved in the membrane industry, with an increase in the number of entrepreneur firms and the variety of membrane process equipment (Howell *et al*, 1993).

2.2. Classification of Membranes

The filters can be classified into two sub groups, as depth and screen, according to their structure (Figure 1). The depth filters keep the retentate in its matrix of fibres or beads, made of cotton, fibreglass, asbestos, sintered metals, diatomaceous earth, etc. These filters are generally used as dead-end filters for retention of 0.01 μm particles. (Cheryan, 1998)

The screen filters are retaining target material on their surface like a sieve. Membrane filters are screen filters. The screen filters are more advantageous since there is a relatively high recovery due to the absence of losses in the depth of filter, and since they have a rigid structure, microbial growth is not a frequent problem. (Cheryan, 1998)

Screen filters may be classified as microporous and asymmetric. Microporous filters may also be classified as isotropic, in which the pores are uniform in size throughout the membrane, and anisotropic, in which the pore size change from one surface to the other. Asymmetric membranes have a relatively thin skin layer, which has

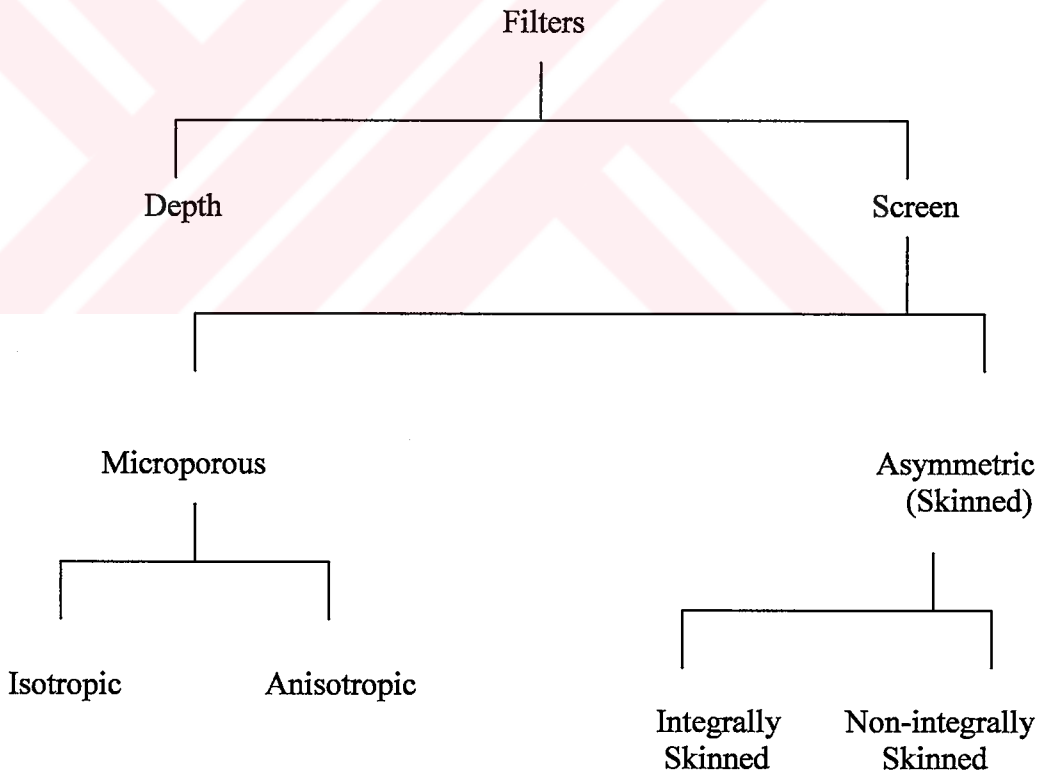

Figure 2. Classification of filters (Cheryan, 1998).

smaller pore sizes than the other layers of membrane and they may be classified into integrally and non-integrally skinned membranes according to the form of this skin layer. (Cheryan, 1998)

Microporous filters are much more susceptible to plugging than the asymmetric membranes, but they are both effected from fouling and concentration polarization (*plugging*: the plugging of pores with particles approximately the same size as the pores, *fouling*: deposition of solid particles over the membrane surface, *concentration polarization*: accumulation of material forming retentate over the membrane with a higher concentration than it is in the feed, resulting in a retentate concentration gradient which acts as a drawback for filtration) (Cheryan, 1998).

2.2.1. Membranes According to Their Material

Membranes may be manufactured from both organic and inorganic materials and/or combinations of these, which form *composite* membranes. The methods for manufacture of membranes are given in Table 1.

Table 1. Methods of manufacture of synthetic membranes (Cheryan, 1998).

Process	***Materials***
Phase inversion by	Polymers
Solvent evaporation	Cellulose acetate, polyamide
Temperature change	Polypropylene, polyamide
Precipitant addition	Polysulfone, nitrocellulose
Stretching sheets of partially crystalline polymers	Polymers: PTFE
Irradiation and etching	Polymers: Polycarbonate, polyesters
Molding and sintering of fine-grain powders	Ceramics, metal oxides, PTFE, PE

2.2.1.1. Polymeric Membranes

Organic membranes may be produced from polymers like cellulose acetate (CA), polyamide (PA), polysulfone (PS), polyethersulfone (PES), etc. (Cheryan, 1998).

Cellulose acetate is the most widely used membrane material due to a number of reasons like its hydrophilic nature which minimizes fouling, the possibility of the preparation of membranes with a wide pore size range (for MF, UF, RO), its low cost and relative easiness to handle. It has very important disadvantages besides the above advantages. Its operating pH range is only limited to 3-6. It is very susceptible to chlorine which is used very commonly for cleaning and sanitizing in the industry. Pressure causes compaction of the membrane. The upper operating temperature limit is 30°C, and it is very biodegradable. In spite of all these disadvantages CA is commonly used, especially for RO of the seawater to get potable water. (Cheryan, 1998)

Polyamide membranes contain – CONH – amide bond. Their operating pH range is between pH 3-11, but their chlorine resistance is lower and biofouling are higher than CA membranes. (Cheryan, 1998)

Polysulfone and polyethersulfone membranes have some considerable advantages. They may be used in the pH range of 1-13 and up to the temperature of 75°C. It has moderate chlorine resistance and it is easy to handle during membrane preparation in several configurations for a pore size range of 0.2 μm to 10°A, with which MF and UF may be performed. It has good chemical resistance against aliphatic hydrocarbons, fully halogenated hydrocarbons, alcohols and acids, but is affected by aromatic hydrocarbons, ketones, ethers and esters. The hydrophobicity of these materials is prone to fouling. Another disadvantage is the low operating pressure limits (Cheryan, 1998). The main polymers used for membrane preparation and their application processes are given in Table 2.

It is possible to enhance the properties of organic membranes by manufacturing them with combinations of materials to combine their desired properties while depressing undesired ones. The general methods for these purposes are:

- Casting the ultra thin barrier layer separately, followed by lamination to the support film,
- Dip-coating of a solution of the polymer onto a microporous support and drying, or dip-coating a reactive monomer or prepolymer solution followed by curing with heat or irradiation,
- Gas-phase deposition of the barrier layer from a glow-discharge plasma,

- Interfacial polymerisation of reactive monomers on the surface of the support film. (Cheryan, 1998)

Cellulose acetate and polyamide composite membranes were produced with higher range of operating pH, temperature and pressure, with higher fluxes by using such techniques. (Cheryan, 1998)

2.2.1.2. Inorganic Membranes

Inorganic membranes have presented great opportunities for the solution of separation problems, by enabling filtration in severe conditions and allowed development of several membrane processes. They are inert to common chemicals (especially chlorine), can be used at elevated temperatures (e.g. 350°C, but it must be considered that, to prevent from thermal shocks the membrane should be cooled carefully after usage at elevated temperatures) and in a wide pH range (pH 1-14). They are superior to organic polymeric membranes, and beside these superior properties, inorganic ceramic membranes enable *back flushing*; feeding fluid from the permeate side to clean up the accumulated solids over the membrane. This is done by the application of a greater pressure than the normal feed pressure but in the opposite direction. Their rigid structure prevents deformation under pressure. Beyond these advantages inorganic membranes have relatively longer operating lifetimes (The ceramic membranes produced for nuclear power plants are being used for over 20 years). They also have some disadvantages too. First of all, they (except stainless steel ones) are brittle and they should be handled carefully. While using them a large pumping capacity is needed to get the desired fluid velocity over the membrane. Currently there are inorganic membranes for micro, ultra and nanofiltration, but not for reverse osmosis. Another major disadvantage is their cost. A filtration system for MF/UF with organic membranes cost approximately 10-18 times less expensive than the one with ceramic membranes. However it should be kept in mind that, these are only the initial costs. Since the inorganic membranes have longer operating lifetimes, they are not needed to be changed often, that causes a decrease in labour cost, and since they have a wider range of operating parameters with generally higher fluxes, they may be a better choice in spite of their high initial cost. (Cheryan, 1998)

The inorganic membranes are classified into two main groups as dense (made of metals or conductive oxides) and porous membranes (Cheryan, 1998, Bhave, 1991, Cot *et al*, 2000). Pores can be due to the crystal structure (as in zeolites, clays minerals) or

due to void spaces formed by packing and consolidation of small particles (Cot *et al*, 2000). The IUPAC definition states that the pores with diameters above 50 nm are macropores, with diameters between 2-50 nm are mesopores and with diameters smaller than 2 nm are micropores (Howell *et al*, 1993, Cot *et al*, 2000).

Inorganic membranes may be manufactured from such materials like ceramics, metals, carbon and glasses, by using different methods;

- Particle dispersion and slip casting,
- Phase separation and leaching,
- Anodic oxidation,
- Thin-film deposition,
- Track-etching. (Cheryan, 1998)

The most common inorganic membranes are ceramic membranes in asymmetric structure (Lee and Pope, 1994). They are produced by forming thin selective layers, with smaller pore sizes over supportive structures, with larger pores as seen in Figure 3 (Bhave, 1991, Cot *et al*, 2000, Lee and Pope, 1994). The supports may be carbon, extruded ceramic or etched stainless steel.

The ceramic membranes suitable for microfiltration, with pore diameters of 0.1-1 μm may be prepared by coextrusion of small and big ceramic particles, simultaneously

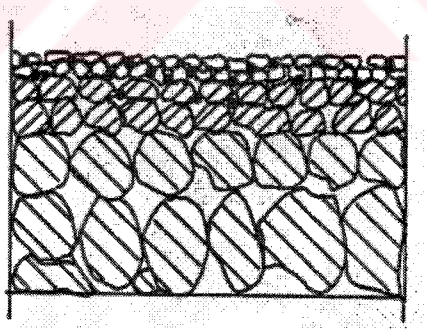

Figure 3. Porous structure formed by consolidation of suspensions (Bhave, 1991).

to form the support and permselective layer, or it may also be prepared by casting a well dispersed suspension of submicron ceramic powders on a support (Lee and Pope, 1994). The ceramic powder suspension may be prepared with ceramic powders, water and a binder like polyvinyl alcohol or ethylene glycol, which also acts a plasticizer, improving

the rheological properties of the slurry (Lee and Pope, 1994). After forming the desired green body, heat treatment is applied to get a stabilized membrane structure. The heat treatment conditions depend on the type of ceramic material.

For ultrafiltration, smaller pores are needed, so the voids between the particles must be smaller. In other words the particles forming the membrane should have a narrower size distribution than the ones in microfiltration membrane. Such small particles may not be formed by using powders, but may be formed by sol-gel processes. Sol-gel process enables production of well-dispersed colloidal structures, which may form porous structures after consolidation. There are two main routes in sol-gel processes, polymeric or colloidal (Bhave, 1991, Lee and Pope, 1994, Ward and Ko, 1995). These routes include dispersion of metal salt or metal-organic precursors, formation of gel layer by the formation of contacts between polymeric or colloidal particles, drying of the gel layer, and firing (Bhave, 1991, Lee and Pope, 1994, Ward and Ko, 1995). The routes may be either acid catalysed or base catalysed (Bhave, 1991, Lee and Pope, 1994, Ward and Ko, 1995). Smaller particles are formed by the acid catalysed route and which will allow formation of smaller pore sizes (Bhave, 1991, Ward and Ko, 1995). During sol-gel process the reactions of hydrolysis and condensation occur:

Hydrolysis: $- MOR + H_2O \longrightarrow - MOH + ROH$

Condensation: $- MOH + ROM - \longrightarrow - MOM - + ROH$ or

$- MOH + HOM - \longrightarrow - MOM - + H_2O$

Here $M(OR)_n$ represents the precursor alkoxide (M = Zr, Al, Ti, Si, etc.).(Ward and Ko, 1995, Loehman, 1993)

The size distribution of the particles in the sol depends on the lengths of the -MOM- chains, formed in hydrolysis and condensation reactions. The variation of experimental parameters for these reactions would make it possible to control particle size distribution, which maybe used to form a layer with desired pore size distribution. For this purpose, some additives (like acetyl acetone; acacH) are used,

$$M(OR)_n + acacH \longrightarrow M(OR)_{n-1}(acac) + ROH$$

Here acacH reacts with the alkoxide to form complexes with different physicochemical properties, with decreased hydrolysis tendencies. This prevents formation of long chains, thus precipitation does not occur. An acacH / M ratio higher than 1, allows preparation of a stable sol of nanoparticles. This may be achieved due to the organic shell formed by acacH as schematically shown for Zr in Figure 2. For a Zr / acacH ratio of 2, after heat treatment at 500°C, a mean grain size of 6 nm was determined. In this membrane, packing of nanoparticles resulted in 1.4 nm pore diameters and 18% porosity. (Cot *et al*, 2000)

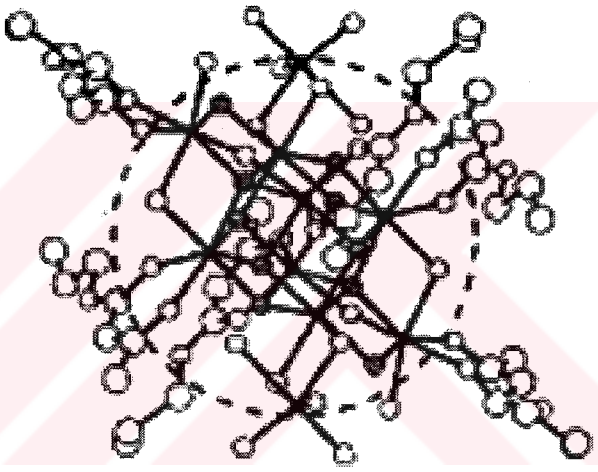

Figure 4. Organic shell formed by acacH (Black dots are Zr, grey dots are oxo, hydroxo bridges, chains of white dots are acacH group) ($Zr_{10}O_8(OPr)_{18}(acac)_6$) (Cot *et al*, 2000).

Table 2. Materials used in the manufacture of membranes (Cheryan, 1998).

Material	MF	UF	RO
Alumina	X		
Carbon – carbon composites	X		
Cellulose esters (mixed)	X		
Cellulose nitrate	X		
Polyamide, aliphatic (e.g. nylon)	X		
Polycarbonate (track-etch)	X		
Polyester (track-etch)	X		
Polypropylene	X		
Polytetrafluoroethylene (PTFE)	X		
Poly vinyl chloride (PVC)	X		
Polyvinylidene fluoride (PVDF)	X		
Sintered stainless steel	X		
Cellulose (regenerated)	X	X	
Ceramic composites (zirconia on alumina)	X	X	
Polyacrylonitrile (PAN)	X	X	
Polyvinyl alcohol (PVA)	X	X	
Polysulfone (PS)	X	X	
Polyethersulfone (PES)	X	X	
Cellulose acetate (CA)	X	X	X
Cellulose triacetate (CTA)	X	X	X
Polyamide, aromatic (PA)	X	X	X
Polyimide		X	X
CA/CTA blends			X
Composites (e.g. polyacrylic acid on zirconia)			X
Composites, polymeric thin film (e.g. PA on PS)			X
Polybenzimidazole (PBI)			X
Polyetherimide (PEI)			X

Chapter 3

MEMBRANE PROCESSES

The separation and purification of valuable organic materials from aqueous media has been an important challenge in engineering. Several methods like filtration (membrane processes), evaporation, freeze-drying, centrifugation, etc. are used for this purpose. Among these, filtration processes are found to be preferable, since there is no need for phase transformation during filtration, different than evaporation and freeze-drying, which is economically advantageous. The heat applied during evaporation, for phase transformation of liquid to gas may have severe effects on the heat sensitive materials like enzymes, vaccines, antibiotics, etc. Freeze-drying, which also contains a phase transformation, of solid to gas, may be used for heat sensitive materials, but it also has some economical drawbacks. The only competitive alternative of filtration seems to be centrifugation, with regards to versatility. But, a density difference between the materials to be separated is needed for centrifugation, and the materials must be immiscible. On contrary, the membrane processes allow even separation of dissolved molecules, when suitable membranes are used. But it should be kept in mind that absolute dryness can not be reached with membrane processes.

3.1. Classification of Membrane Processes

The classification of various membrane processes according to the driving force used is given in Table 3. The pressure driven membrane processes have the advantage of speeding up the transport process with increasing the hydraulic pressure. They cover a wide range of applications with which several target materials may be separated which is summarized in Table 4. Microfiltration (MF) is used generally for clarifying by the separation of suspended particles in the size range of 0.1-10μm. Ultrafiltration (UF) is used for separation of macromolecules like proteins from liquid media. Nanofiltration (NF) is a relatively new technique, which may be used for separation of smaller species than those separated by ultrafiltration and which utilizes lower operating pressures than those applied in reverse osmosis. It may be used for desalination of liquid media and separation of dissociated and undissociated salts. Reverse osmosis (RO), ideally, allow

total removal of dissolved/undissolved solids in the liquid media, with the application of very high pressures, over the osmotic pressure of the target material. (Cheryan, 1998)

Table 3. The membrane processes according to the driving force (Cheryan, 1998).

Process	***Driving Force***	***Retentate***	***Permeate***
Osmosis	Chemical potential	Solutes, water	Water
Dialysis	Concentration difference	Large molecules, water	Small molecules, water
MF (micro-filtration)	Pressure	Suspended particles, water	Dissolved solids, water
UF (ultra-filtration)	Pressure	Large molecules, water	Small molecules, water
NF (nano-filtration)	Pressure	Small molecules, divalent salts, dissociated acids, water	Monovalent ions, undissociated acids, water
RO (reverse osmosis)	Pressure	All solutes, water	Water
Electrodialysis	Voltage / current	Charged species	Oppositely charged species

Pressure driven membrane processes may be used with great versatility as mentioned above. The worldwide market of membrane processes, in 1998, was over 4 billion US $, with an annual growth rate of 8-10%. The share of pressure driven membrane processes was 1.8 billion US $ (Strathmann, 2001). These processes are used in several industries. There is extensive research on the exchange of other unit operations with the pressure driven membrane processes, and economical advantages of these exchanges (Mohr *et al*, 1989, Howell, 1990).

Table 4. The pressure driven membrane processes with working pressure and target material size (Krijgsman, 1992, Cheryan, 1998).

Process	*Target product size (nm ; 10^{-9} m)*	*Pressure (bar)*
Micro-filtration (MF)	10,000 – 100	0.5 - 2
Ultra-filtration (UF)	400 – 2	2 - 10
Nano-filtration (NF)	5 – 0.5	10 – 30
Reverse Osmosis (RO)	1 – 0.1	35 - 100

3.2. Membrane Processes in Food Industry and Biotechnology

The general applications of membrane processes are well described in literature (Cheryan, 1998, Strathmann, 2001, Mohr *et al*, 1989, Howell, 1990, Bhave, 1991).

In food industry;

- MF is used for removal of bacteria from milk (cold pasteurisation), clarification of fruit juices and clarification of fermented alcoholic beverages (wine &beer).
- UF is used for concentration of proteins (milk, soybean, egg, whey), cheese production, removal of pigments from fruit juices
- NF is used for simultaneous partial demineralisation and concentration of whey
- RO is used for concentration of whey and milk before evaporation.

Another application of membrane processes is separation of pyrogens, from water, by UF. Pyrogens are known as endotoxins with molecular weights of 10,000-200,000, which cannot be removed by MF or autoclaving (heat treatment) (Bhave, 1991).

3.2.1. Membrane Processes for Downstream Applications

Production of valuable organic materials like, antibiotics, alcohols, vaccines, organic acids, by fermentation is an important application in biotechnology. The fermentation process, which allows production of desired products with the aid of cultivation of microorganisms, has several parameters for optimum product efficiency.

The fermentation broth contains product, microorganisms, other materials produced by microorganisms, residual of nutrients which were fed into fermentor before and/or during the fermentation, etc. The desired product produced by microorganisms during fermentation must be separated from all the other components of fermentation broth successively by using downstream processes. The pressure driven membrane processes offer preferable choices due to the sensitivity of products to heat applications like evaporation for this purpose.

The membrane processes allow new configurations for fermentation. The increase in of product concentration in the fermentor generally causes an inhibition of microorganism growth, which adversely affects production efficiency. This problem may be solved by using a membrane process, by which the fermentation broth is fed to the membrane, which is permeable for the product, but not for microorganisms or nutrients. This configuration, called *bioreactor*, allowed an increase in the amount of microorganisms (*biomass*) by a factor of 12 and increase in alcohol production by a factor of 10, in Zymomonas mobilis fermentation, compared to batch configuration (Moo-Young, 1985). The positive effect of bioreactor configuration on ethanol and lactic acid fermentations can be seen on Figure 5 (Cheryan, 1998). The target material in downstream processes may be the biomass (e.g. for vaccine production), which may be achieved by using MF, too.

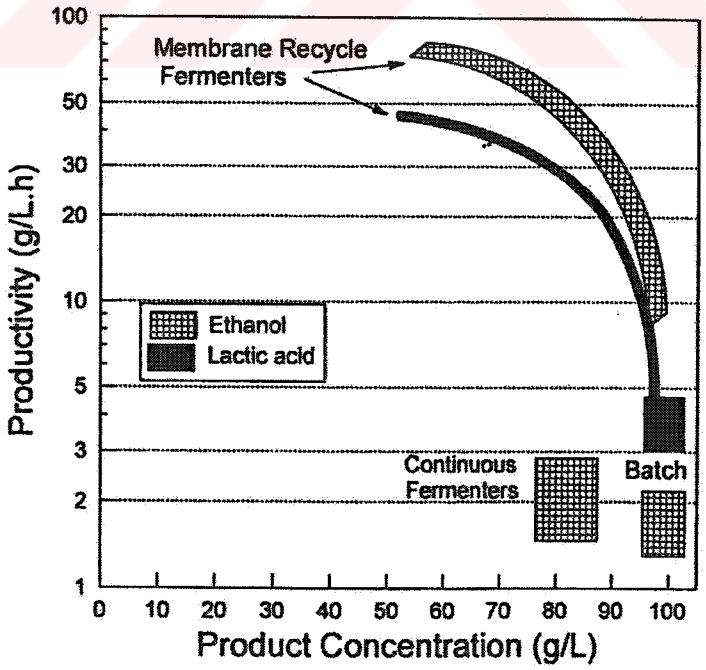

Figure 5. The comparison of productivities for the batch and bioreactor fermentation (Cheryan, 1998).

Lactic acid (CH_3-CHOH-COOH, molecular weight: 90) and lactates are used in food production (as acidulants, preservatives, or flavour enhancers), in pharmaceuticals, cosmetics, the textile and the leather industry (Vick Roy, 1985). The present annual production of lactate is 25-30 million kg and it is becoming a commodity chemical (Vick Roy, 1985, Lipinsky *et al*, 1986). There are recent applications of the polymers of L-lactic acid, in production of biomaterials, sutures and biodegradable packaging. It is possible to produce lactic acid chemically from petroleum, or by fermentation of lactose. Since the petroleum stocks are diminishing, and since it is possible to produce L isomer of lactic acid selectively by fermentation, the latter choice seems to be more advantageous. For fermentation, whey, which contains 5% lactose, is used as feed as seen in Table 5. As described above, fermentation must be followed by downstream process, for separation and concentration of lactic acid. The bioreactor configuration may be used for increasing efficiency (Smith *et al*, 1977), and further membrane processes may be used for lactic acid or lactate concentration (Schlicher and Cheryan, 1990). For these purposes organic (polymeric) NF and RO membranes are being used.

Table 5. The composition of whey.

Component	Weight % (Mohr *et al.*, 1989)	Weight % (Üçüncü, 1996)
Water	93 – 93.5	-
Total solids	6.5 – 7.0	5.8 – 7.0
Lactose	4.4 – 5.24	4.0 – 4.7
Protein	0.62 – 0.8	0.7 – 1
Minerals / ash	0.5 – 0.8	0.5 – 0.85
Lactic acid	0 – 0.5	0.2 – 0.8
Fat	0.02 – 0.2	0.1 – 0.8
Other	0.05 – 0.4	-
pH	-	4.4 – 6.4

3.2.2. Membrane Processes for Protein Separation and Fractionation

The milk proteins, given in Figure 6, have different functional properties such as solubility, foaming, whipping, emulsification, gelation and flavour entrapment ability (Punidadas and Rizvi, 1998). These proteins may be fractionated by MF and UF (Cheryan, 1998, Bhave, 1991, Punidadas and Rizvi, 1998). Punidadas and Rizvi tried to get the optimum conditions to separate skim milk into milk protein rich and whey

protein rich fractions, with ceramic membranes. They collected milk protein (casein) rich fraction as retentate, and whey protein (α-lactalbumin, β-lactoglobulin) rich fraction as permeate, with a 0.05 μm pore sized multichannel ceramic membrane, and reported the optimum conditions as 5.4 ms^{-1} feed velocity over the membrane, 138 kPa operating pressure and 50°C operating temperature (Punidadas and Rizvi, 1998).

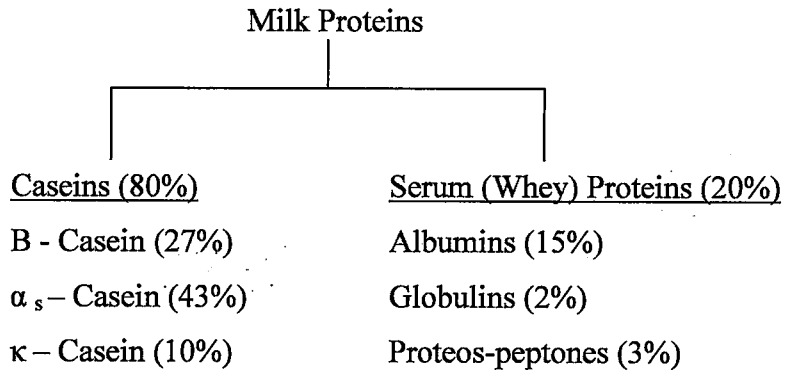

Figure 6. The classification of milk proteins (Metin, 1998).

The whey proteins are also, tried to be further fractionated with several methods including membrane processes. Konrad et al. worked on for isolation of native β-lactoglobulin by using several methods including membrane processes (Konrad *et al*, 2000). They reported that, according to the literature this fractionation can be performed by using salting-out procedure (selective solubility of β-lactoglobulin in the presence of 3% w/w trichloroacetic acid (TCA)), separation by ion-exchange chromatography or utilising the differences in thermal stability in acidic conditions. They used Kjeldahl method, SDS-Page (sodium dodecylsulfate - polyacrylamide gel electrophoresis), FPLC (Fast Protein Liquid Chromatography) for determination of the protein. They applied a novel method for the isolation of β-lactoglobulin, too. They exploited the resistance of β-lactoglobulin to pepsin and they applied a peptic treatment to whey, which is followed by a membrane process by using polymeric membranes with 0.1 μm pore size. This novel method had a better yield than the others where 67% of the protein was purified after drying (Konrad *et al*, 2000).

The isolation of the other whey protein α-lactalbumin, which is important in the production of infant food and in pharmaceuticals due to probable morphinomimetic and antitumour activities of some of its peptides, was also investigated by using membrane

processes. Muller et al. investigated the separation of α-lactalbumin and β-lactoglobulin from other whey proteins by using ceramic membranes with MWCO (molecular weight cut-off) range at 300 kg mol^{-1}. It was reported that zirconia membrane was chosen due to its less transmission to IgG (Immunoglobulin) and BSA (Bovine Serum Albumin), but acceptable transmission to α-lactalbumin and β-lactoglobulin (Muller *et al*, 1999).

The use of different types of membranes including unmodified inorganic membranes and inorganic membranes which were modified by PEI (polyethyleneimine) was investigated for the extraction of α-lactalbumin with the membrane processes (Lucas *et al*, 1998). This modification was performed for the investigation of the effect of the surface charge of the membrane on the retention of proteins. They reported some previous work and stated that the retention of whey proteins were not only dependent on the protein size but also on the charge of the protein and the membrane, pH and ionic strength of whey or the solution containing the proteins. The following observations were reported by various research groups on the effect of these parameters on the membrane protein separations;

- Clarified or dialyzed whey can be fractionated into the high molecular weight proteins (immunoglobulin Ig, lactoferrin, bovine serum albumin BSA) as retentate, and the low molecular weight proteins (β-lactoglobulin, α-lactalbumin) as permeate, with cellulose membranes (Mehra and Donelly, 1993),
- The retention of IgG (70-80%) was lower than the retention of another high molecular weight protein BSA (negatively charged) (100%), when filtration was performed at a pH close to the isoelectric point of IgG and a low ionic strength, with formed in place membrane of TiO_2 on stainless steel (Zhang and Spencer, 1993),
- The experiments with polyethersulfone membrane (with 300kg mol^{-1} cut-off) at low ionic strength have shown that there was a high transmission (low retention) of the protein with an isoelectric point close to the operating pH. (The other protein(s) had a charge at that pH.) This was predicted to be due to some electrostatic interactions (Saksena and Zydney, 1998),
- Higher retentions were observed for the proteins, having the same charge with the membrane; modified polycarbonate (MF) membrane by hydrophilic polyvinylpyrrolidone caused a higher retention of β-lactoglobulin compared to BSA, predicted to be due to the stronger interactions between β-lactoglobulin dimmers and polyvinylpyrrolidone tails at the membrane surface (Ko *et al*, 1993),

- α-Lactalbumin and β-lactoglobulin were separated from each other with a modified inorganic membrane (with positively charged polyvinylimidazole derivatives). The retention of β-lactoglobulin was 100% when the pH was 7 and the ionic strength was intermediate (0.2 mol L^{-1}) (Chaufer *et al*, 1993).

In another research paper on fractionation of whey protein hydrolysates by using charged UF/NF membranes, Pouliot *et al.* discussed the concept of *Donnan potential*; the potential formed by the concentration difference of co and counter ions in the charged membrane and in the solution. The concentration of co-ions (ions having same charge with membrane), is lower in the membrane, than the concentration of counter-ions (ions having opposite sign with the membrane), and their concentration in the solution is just the opposite. This concentration difference of charged ions causes a potential, called Donnan potential. Due to this potential, co-ions are repelled, while counter-ions are attracted, by the membrane. This potential is also effective on charged organic materials, like peptides (Pouliot *et al*, 1999).

The membranes show resistance (R) to fluid flow, like the resistance of a wire for electrical current; R=TMP / μ.J (TMP= Transmembrane Pressure, μ= viscosity of permeate, J= permeate flux) (Labbe *et al.*, 1990). This resistance (R) must be overcome to get a permeate flow. The membrane has its own resistance and the fouling of it adds up more resistance. That means much higher driving force (TMP) is needed to get the desired J (permeate flux), which increases energy consumption. Due to this reason extensive research on the prevention of fouling of membranes for increasing the efficiency of separation process was conducted (Su, *et al.*, 1999 and 2000, Labbe *et al.*, 1990, Taddei *et al.*, 1988 and 1989, Aimar *et al.*, 1988, Daufin *et al.*, 1993, 1991, Caric *et al.*, 2000). In some of these studies the research was performed with commercial ceramic membranes, named as Carbosep M4 (MWCO = 20,000 Da), M5 (MWCO = 10000) and M14 (average pore diameter = 0.14μm). These membranes are tubular ceramic membranes with a zirconia layer on carbon support. The fouling of the ceramic membranes (Carbosep M4) was found to be related with ZrO_2 – phospharte (Na-Ca) – proteins complex according to the analysis performed by using XPS (X-Ray Photoelectron Spectroscopy) (which gives information about fouling on the surface and IR (Infra Red Spectroscopy) (which gives information about fouling for whole membrane body) (Labbe *et al.*, 1990).

The fouling maybe classified as reversible fouling (which may be removed by rinsing with water) and irreversible fouling (which can be removed with special

cleaning solutions). Centrifugation (Taddei *et al.*, 1988b), calcium phosphate precipitation and MF (Daufin *et al.*, 1993) were the preliminary methods investigated for the prevention of fouling of membranes during UF of whey. Since there was a loss of proteins during these preliminary steps the progressive effect of them on the total process efficiency is questionable. Daufin *et al.* (1991) reported 100% BSA and immunoglobulin, 50% of lactoferrin and lactoperoxidase and around 30% of β-lactoglobulin and α-lactalbumin retention during clarification of whey by using MF after precipitation.

Another important criterion about fouling is the direction of fluid flow with respect to the position of the membrane. The fouling is much more severe if the fluid is fed to the membrane surface vertically (dead-end filtration) than when it is fed parallel to the membrane surface (cross-flow filtration). When the flow of feed is parallel to the membrane surface then the clogging of the pores of the membrane is less and this enables higher permeate fluxes.

Research on the UF of proteins showed that the protein retention (PR%) values were low at the beginning of filtration possibly due to the absence of protein layer formation which acts as a second membrane. Therefore it was suggested that filtration would start with low TMP and followed by higher TMP and also the fluid velocity to lower the extent of fouling (Taddei *et al.*, 1988a).

The adsorption of protein on the ceramic membrane is also effective on the efficiency of the separation process and fouling. It was reported that protein adsorption on alumina membranes was higher than it was on zirconia membrane and the adsorption to the inner parts of the porous structure is more effective on membranes with larger pores (200nm instead of 50nm) (pH=6.6, T=25°C) (Caric *et al.*, 2000).

Chapter 4

EXPERIMENTAL

4.1 Materials

The list of materials, chemicals and their specifications are given in Table 6. The whey used was supplied by Pınar Süt A.Ş., Kemalpaşa, İzmir, Türkiye.

4.2 Preparation and Characterization of Ceramic Composite Membranes

The membranes were prepared by two main steps; i) preparation of supports which will supply the mechanical strength to protect the unity of the membrane under pressure and ii) modification of supports to get a selective membrane layer over the support surface with finer pores.

4.2.1 Preparation and Characterization of Ceramic Supports

The ceramic supports were prepared by either cold-pressing or slip-casting of ceramic powders with uniform particle size distributions. The particle size distributions of ceramic powders were determined by using Micromeritics Sedigraph 5100. Partially hydrolysed (80%) polyvinyl alcohol (PVA) which is commonly used as a binder in ceramic processing, was added to these powders (~4 wt %) before cold pressing (Reed, 1995). After pressing the supports were placed into the high temperature furnace and heat-treated at 1100-1250°C. Suspensions of fine ceramic powders (Zirconia, TZ-3Y) were prepared for slip-casting of supports. For stabilizing the suspension at a considerable solids content (15% v/v), polyacrylic acid (PAA) was added and with the aid of ammonium hydroxide pH was increased and suspension was kept in an ultrasonic bath for 3 hours. Then the suspension was poured to the cylindrical dies on plaster to get slip-cast disc shaped supports. After the removal of water by the plaster mould the disc shaped supports were placed in the high temperature furnace for heat treatment which would allow the formation of a porous structure with considerable mechanical strength. The porosities of the supports were determined by using density tool-kit.

Table 6. The materials used in experimental work and their specifications.

Material	*Specification*
1-Propanol	High purity, FW: 60.10, d: 0.8, Riedel
Alumina Powder Al_2O_3	High purity, AKP-50, Sumitomo Chemical Co.
Aluminium isopropoxide	98%, FW: 204, Aldrich
Ammonium hydroxide NH_4OH	29 %, FW: 35, Aldrich
Bovine Serum Albumin (BSA)	Fraction V, Min. 96%, Sigma
Copper 2 sulphate pentahydrate $CuSO_4.5H_2O$	FW: 232.59, Aldrich
Ethanol	High purity, FW: 46.7, d: 0.81, Merck
Folin & Ciocalteu' s Phenol Agent	Sigma
Nitric Acid, HNO_3	65% FW: 63.01, d: 1.4, Aldrich
Polyacrylic acid (PAA)	M: 2000, Aldrich
Polyvinyl alcohol, PVA	80% hydrolysed, MW: 9000-10000, Aldrich
Potassium sodium tartarate	Merck
Sodium azide	Merck
Sodium carbonate Na_2CO_3	M: 105.99, Merck
Zirconia Powder ZrO_2	High purity, TZ-3Y, Tosoh Corp.
Zirconium Propoxide, $Zr(C_3H_7O_4)$	70% in alcohol, FW: 327.58, d: 1.044, Aldrich
Whey	Pınar Süt A.Ş.

4.2.2. Preparation and Characterization of Ceramic Sols

The supports were dip-coated with alumina and zirconia sols to obtain a thin top layer with nanometer sized pores with an asymmetric structure. Acid catalysis technique which is capable of forming smaller clusters/particles was used for the preparation of the sols.

Zirrconia sol was prepared from zirconium propoxide/propanol/nitric acid/deionised water with molar ratios of 1/13.31/0.826/2, respectively (Brodsky and Ko, 1995). The zirconium propoxide and approximately half of the propanol (~10 mL) were mixed in a glass bottle on a magnetic stirrer. In another glass bottle nitric acid and the rest of the predetermined amount of the alcohol and the water were mixed. The latter solution was added drop wise to the former solution very slowly (using a pipette in the form of drops) on the magnetic stirrer. Different amounts of acetylacetone (acac) were added to the propoxide solution to decrease the hydrolysis rate and to investigate its effect on sizes of sol particles. The effect of acid content on particle size distribution was investigated by using different amounts of acid.

Alumina sol was prepared by using aluminium isopropoxide/nitric acid/deionised water with molar ratios of 1/0.25/100, respectively. Hot water (80°C) was added to the aluminium isopropoxide powder and mixed for couple of hours. The acidic solution was further added and mixed for another couple of hours. The size distribution of the sol particles were analysed by light-scattering (Zetasizer 3000HSA, Malvern Co., UK). The volumetric contents of the alumina sol and zirconia sol are given in Table 7.

Table 7. The volumetric contents of the sols.

Zirconia sol	For 30 mL	**Alumina sol**	For 30 mL
Zirconium propoxide	7.89 mL	Aluminium isopropoxide	3.05 g
Nitric acid (65%)	1.44 mL	Nitric acid (65%)	0.259 mL
Deionized water	0.18 mL	Deionized water	26.79 mL
Propanol	20.49 mL		

4.2.3 Formation and Characterization of Thin Selective Layers on Supports

One of the surfaces of the supports were dipped in to the diluted sols (1:3 Alumina forming and 1:4 Zirconia forming sol) for 10 seconds and dried at 50°C before calcination.

The as prepared supports were potential membranes for MF. Dip-coating with ceramic sols would allow the preparation of different membranes with asymmetric structure which may be used for UF and NF. Unsupported membranes were prepared by drying sol samples at room temperature (RT) or 50°C overnight. These unsupported membranes were characterised by X-Ray Diffraction (XRD, Philips X'PERT PRO), for phase structure, Differential Thermal Analysis (DTA-50, Shimadzu Co.) and Thermal Gravimetric Analysis (TGA-51, Shimadzu Co.) for the thermal behaviour of sol with increasing temperature, and Nitrogen Adsorption/Desorption Analysis for the porosity/pore structure of heat-treated membrane structure. The sample weights were approximately 0.09 g. The samples were degassed at 90°Cfor 1h and 350°C for 24h. The analyses took approximately between 13-19h. The $(P/P_o)_{min}$ was approximately 0.00064±1 and $(P/P_o)_{max}$ was approximately 1±0.002. The microstructure (morphology/particle size/film thickness) of the unsupported/supported membranes and supports were examined by Scanning Electron Microscope (SEM, Philips XL30 SFEG).

4.3 Filtration Experiments

These membranes were tested in filtration experiments with deionised water to monitor the clean water permeability (CWP) with bovine serum albumin (BSA) model solutions and with whey which was supplied by Pınar Süt A.Ş..

4.3.1 Filtration Set-up

During this study a filtration set-up was designed and assembled. The picture of the filtration set-up is given in Figure 7 and it is shown schematically in Figure 8. The system consists of a feed tank, a positive pressure pump, pressure gauges, needle valves, a flow-meter, a membrane holder and piping system for the flow of feed, permeate and retentate streams. The temperature of the liquid in the feed tank can be kept constant by using a heater-stirrer and an immersed cooler. It was kept at 40°C during the experiments. The flow rate can be regulated with the frequency converter of the pump and was set at 5 LPM (litres per minute) for this work. The pressure was controlled by the needle valve on the exit of the retentate from piping system to feed tank to get a

transmembrane pressure (TMP) up to 100 bars. TMP values lower than 20 bars were mostly used in this work. The transmembrane pressure was defined as the difference between both sides of the membrane, as shown in the formula in Figure 8.

Figure 7. The filtration set-up.

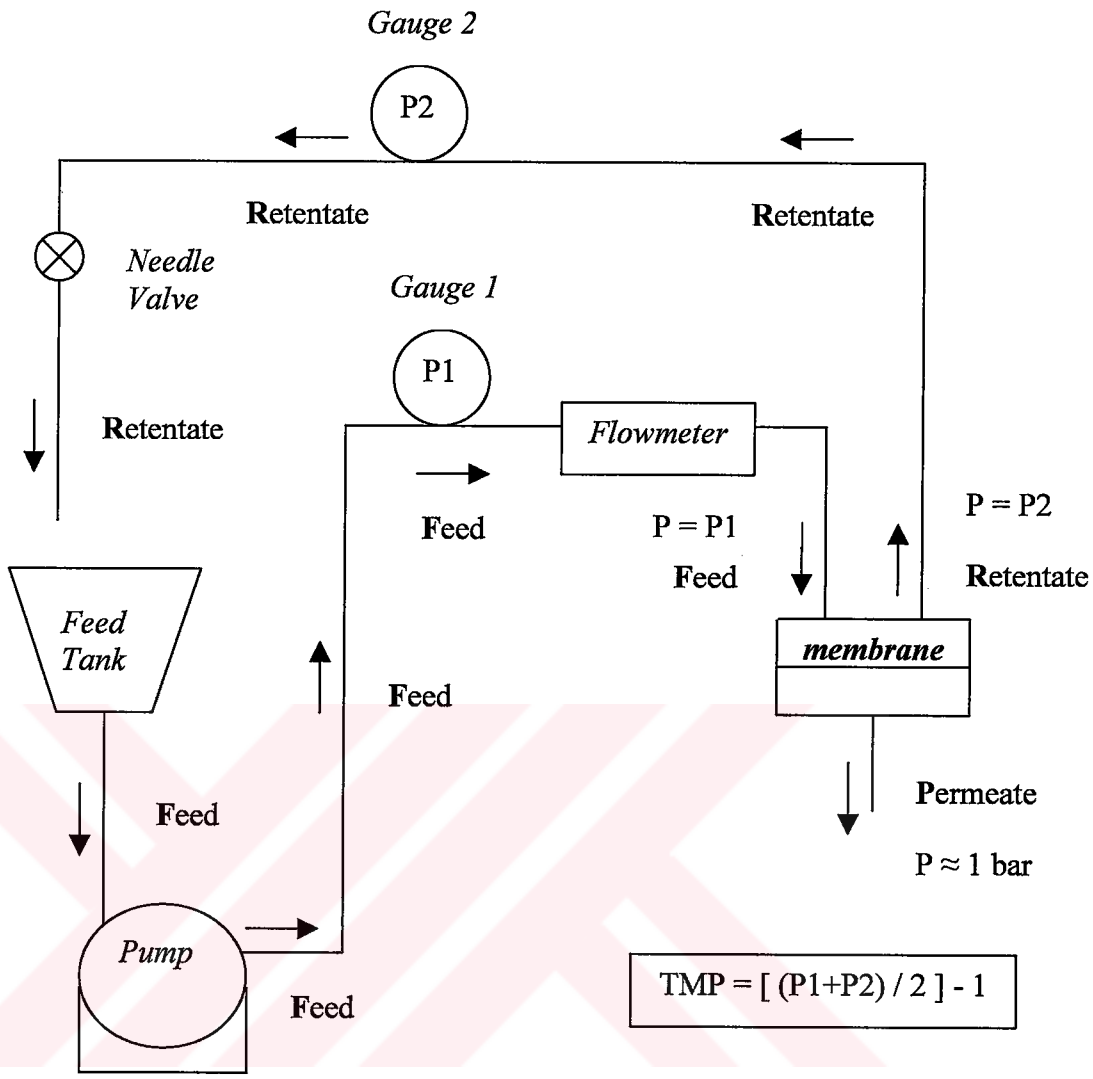

Figure 8. The diagram of filtration set-up.

4.3.2 Analysis during Filtration

The permeate flux (L permeate / m^2 hour) which is the amount of liquid passing through a certain area of membrane at a certain time was determined by measuring the time at which permeate fills a container of 5 mL. The permeate flux of deionised water shows the clean water permeability.

The separation efficiencies of membranes were determined with model bovine serum albumin (BSA) solutions and whey. The protein concentrations of the BSA solutions were determined by using UV-VIS spectrophotometer (UV-1601, Shimadzu Co.) with direct absorbance readings at 280 nm wavelength. The Lowry method (Lowry *et al.*, 1951) was used for the protein concentration determination of the

permeate/retentate/feed streams of the whey separation experiments (Lowry *et al.*, 1951). Alkaline copper reactive (2.5 mL) was added on to the samples taken from these streams (0.5 mL), well-mixed and kept at room temperature for 10 minutes. The alkaline copper reactive consists of 1mL of R1 + 1 mL of R2 was brought to a volume of 100 mL with R3 (R1: 2% (w/v) copper sulphate, R2: 2% (w/v) potassium sodium tartarate, R3: 2% (w/v) sodium carbonate in 0.1 N NaOH). Folin reactive (0.25 mL of 1:1 diluted) was further added to this solution which was kept for half an hour at RT. Absorbance value was measured at 660 nm wavelength. The protein concentration was determined from the calibration curves prepared by using BSA solutions with known concentrations. Samples were diluted in order to obtain concentrations in the range of calibration. The calibration curves for direct absorbance reading and for the Lowry method are given in Figure 9 (absorbance at 280 nm) and Figure10 (absorbance at 660 nm), respectively.

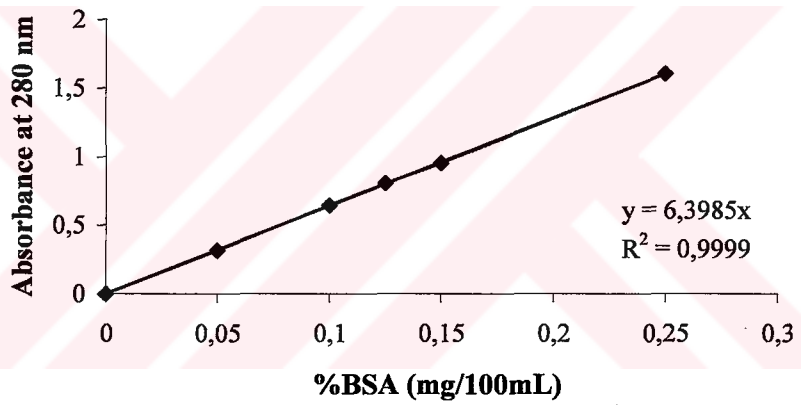

Figure 9. Calibration curve for direct absorbance reading at 280 nm.

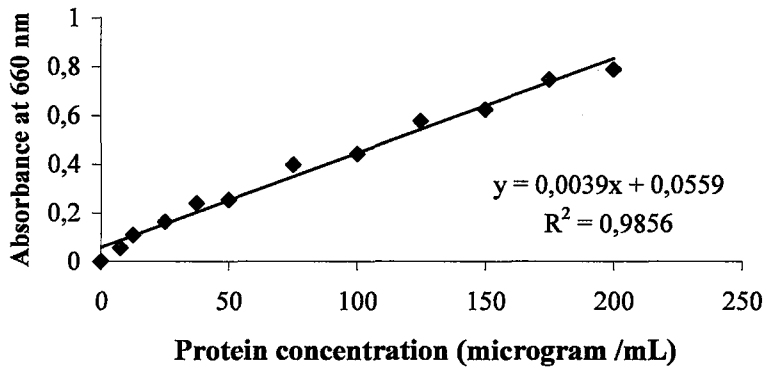

Figure 10. Calibration curve for whey proteins prepared with BSA for using in the Lowry Method (absorbance at 660 nm).

Chapter 5

RESULTS AND DISCUSSION

5.1 Membrane Preparation and Characterization

A number of supports (45 mm before heat treatment and 42 mm after heat treatment in the diameter) were prepared either by cold pressing or slip-casting, from alumina (AKP-50) and zirconia (TZ-3Y) powders which have fine submicron particle size distributions. The size distribution for the alumina powder given in Figure 11 shows that almost over 95% of the particles are smaller than 1 μm with a mean particle size of about 0.25 μm. The supports prepared with slip-casting were found to be mechanically stronger than the supports prepared with cold pressing due to the possible finer particle/agglomerate size distribution (since suspension was kept in an ultrasonic bath for couple of hours) and finer packing of powders while drying on plaster. The heat treatment temperature was also effective on mechanical strength. Increasing the temperature increases the mechanical strength while decreasing the porosity. The porosity values of the supports were between 40-60%. The maximum working transmembrane pressure of cold pressed supports (alumina heat treated at 1100°C) was 5 bars, while slip-cast supports (zirconia heat treated at 1200°C) could be used at 10 bars.

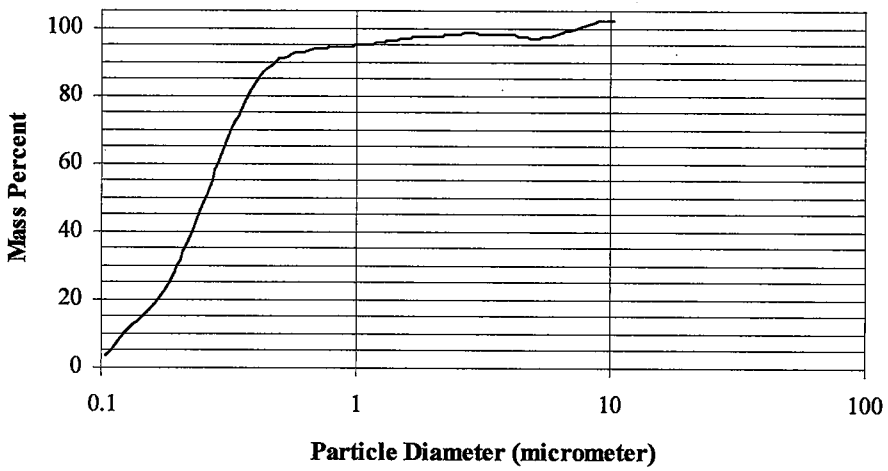

Figure 11. Particle size distribution of alumina powder (Micromeritics SediGraph 5100).

The supports have been investigated with SEM. The SEM picture of the alumina support heat treated at 1100°C is given in Figures 12 and 13. As it is seen in Figure 12 the alumina support heat treated at 1100°C has a uniform pore structure. The porosity was found to be approximately 45% by using the dry and soaked weight of the support. The SEM picture indicates that the pores are open. The SEM picture of slip-cast zirconia support heat treated at 1200°C is given in Figure 14. The picture shows that the support has a lower porosity which may be due to excessive heat treatment.

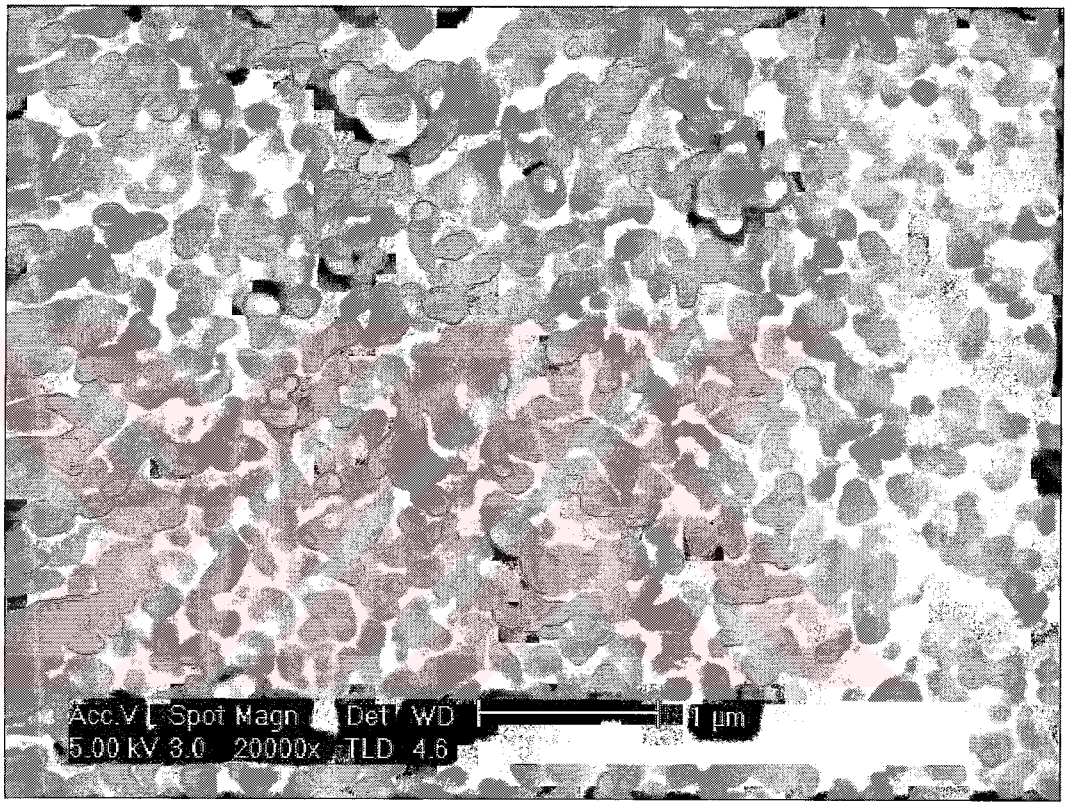

Figure 12. SEM picture of cold pressed alumina support surface heat treated at 1100°C.

Ceramic sols were prepared from zirconium propoxide and aluminium isopropoxide. Clear and stable zirconia sols were prepared with zirconium propoxide/propanol/nitric acid/deionised water with molar ratios of 1/13.31/0.826/2, respectively as described by Brodsky and Ko (1995). The particle size distributions of these sols which were determined by using light scattering (Zetasizer 3000HSA), were very narrow and the mean particle size was around 5 nm as seen in Figure 15. A series

Figure 13. SEM picture of cold pressed alumina support surface heat treated at 1100°C.

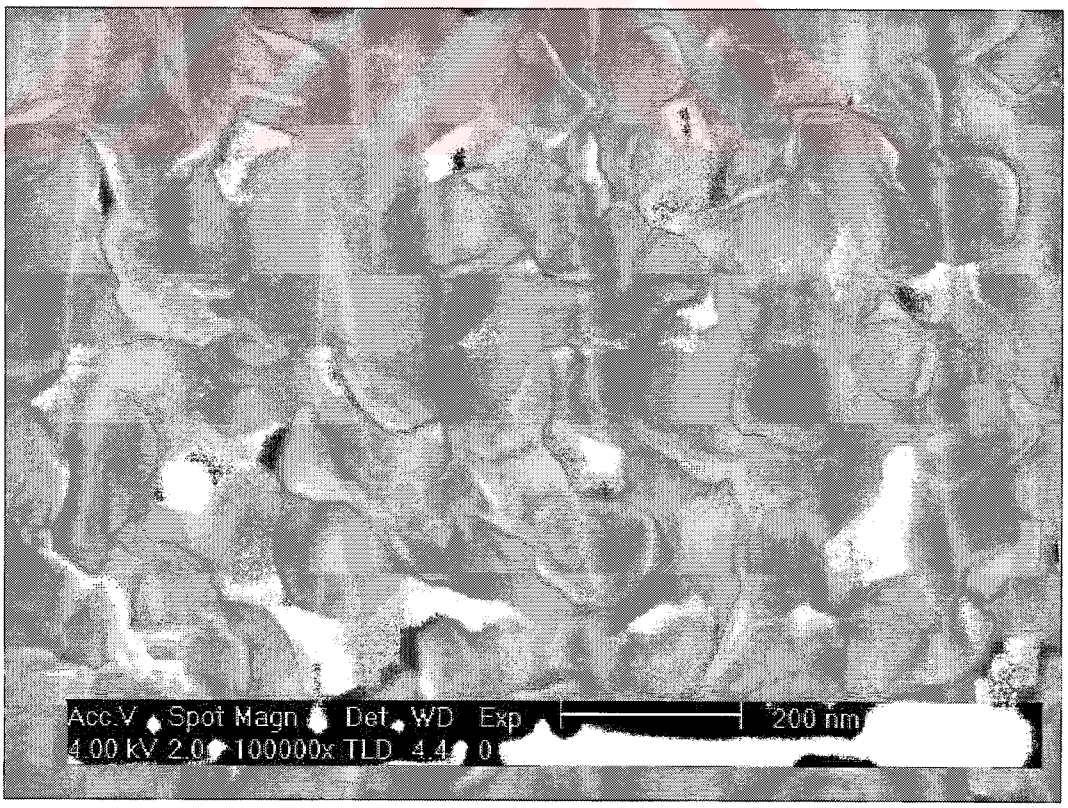

Figure 14. SEM picture of zirconia support surface heat treated at 1200°C.

of zirconium sols were prepared with various zirconium/acid molar ratio. A decrease in the mean particle size was observed with increasing acid ratio as shown in Table 8. Zirconia sols with 4.4 nm and 7.4 nm were prepared with Zirconium propoxide/acac molar ratio of 1 and 2, respectively, using acetyl acetone (acac) as a sol additive. This was probably due to the inhibitory effect of acac layer around the zirconia nanoparticles on their interactions which may cause the formation of larger clusters. Sols with smaller mean particle size, prepared either by increasing the acid ratio or addition of acac were observed to form gels relatively faster than the regular zirconia sol. These sols may be used for the preparation of membrane layers with much finer pores if process conditions are controlled closely during drying and heat treatment.

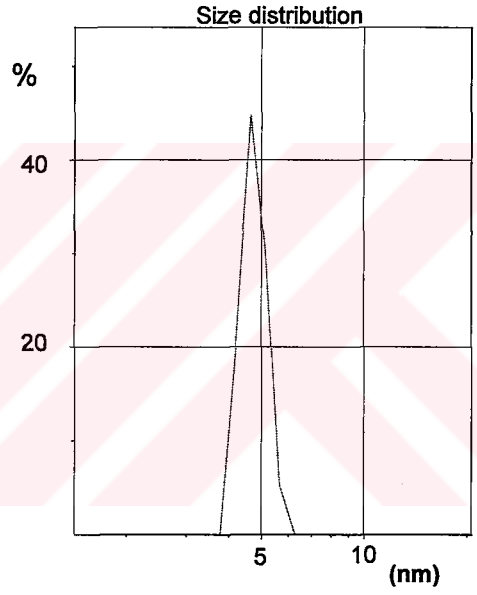

Figure 15. Particle size distribution of zirconia sol detected with Zetasizer (volume based distribution).

Alumina sol was prepared with water/alkoxide molar ratio of 100 and nitric acid/alkoxide molar ratio of 0.25 which had a mean particle size of 30 nm and the particle size distribution is given in Figure 16.

The TGA curve of dried zirconia gel consists of three main stages as given in Figure 17. It has shown that heat treatment at 500°C was enough to get a stable zirconia layer, since the weight loss approaches to a negligible level after that temperature. The first stage is from room temperature to approximately 270°C where 18% weight loss occurred which was probably due to water/alcohol removal.

Table 8. The mean particle sizes of zirconia sols with different acid contents.

Nitric Acid (mmol / mL propanol)	*Mean Particle Size (nm)*
0.826	5.7
0.926	4.7
1.026	3.1

The second stage is between 270° and 430°C during which 13% weight loss occurred. The bonded water and NO_x compounds might be removed during this stage. The third stage is between 430° and 480°C. There might be crystallization during this stage where a weight loss of 4.5% occurred. The DTA indicates an exothermic change during this stage.

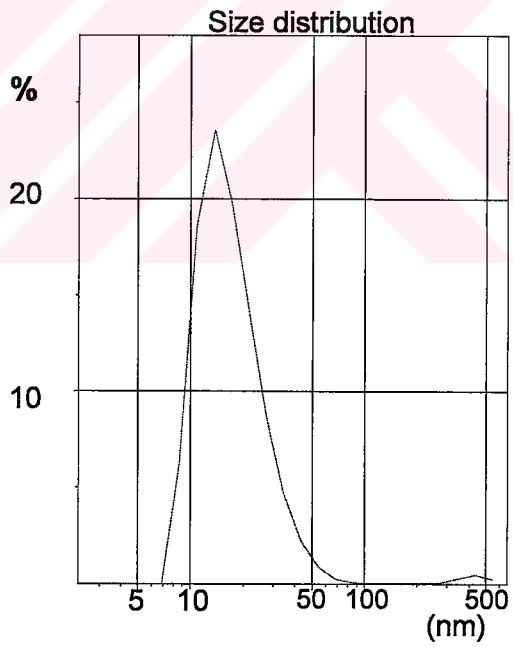

Figure 16. Particle size distribution of alumina sol detected with Zetasizer (volume based distribution).

The XRD analysis (2Θ = 5-70°, step size = 0.05 (2Θ), counting time = 0.5 (s)) of zirconia unsupported membrane calcined at 500°C showed that, tetragonal zirconia crystals were formed during heat treatment as determined from the pattern in Figure 18.

The distinct sharp characteristic peaks of zirconia indicate the sample consists of pure zirconia crystals.

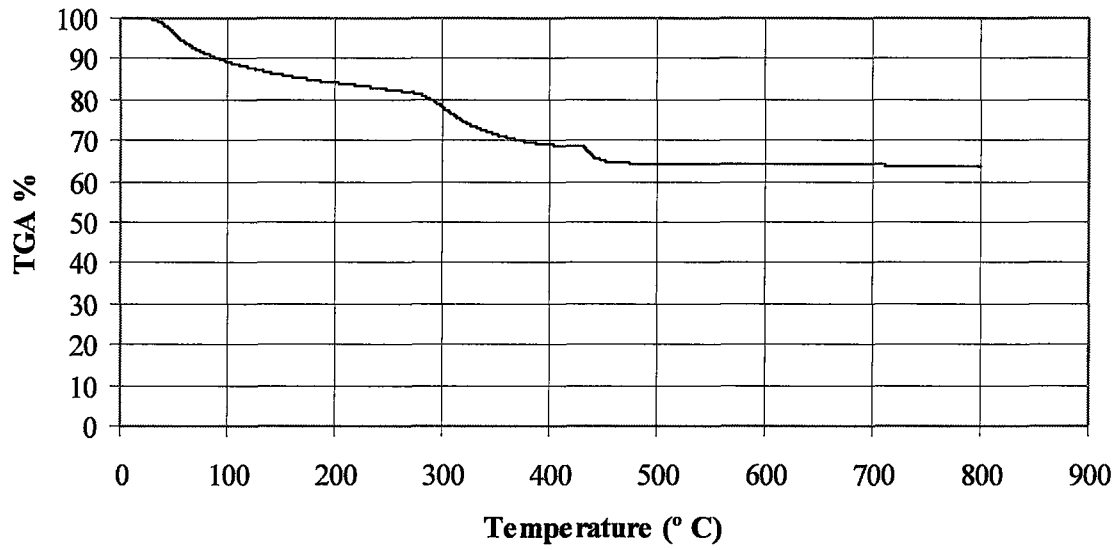

Figure 17. TGA of zirconia unsupported membrane (sample weight: 10.07 mg, atmosphere: nitrogen flow 40mL/min, heating regime: 2°C/min up to 800°C).

The TGA curve of dried alumina sol is given in Figure 19. The figure shows that the sample has reached a gravimetric stability approximately at 500°C after which the weight loss was negligible. This indicates a calcination temperature at or slightly above 500°C can be applied to get a stable structure. The TGA curve for dried alumina gel consists of three main stages which are between room temperature to 180°C, 180°C to 270°C, 270°C to 500°C. The third stage may be subdivided into two as 270°C to 330°C and 330°C to 500°C. There was approximately a 10% weight loss during the second sub-stage, which might be due to the transformation of boehmite into γ-alumina. Previous stages are probably due to the removal of free, bonded water and NO_x compounds.

The XRD results (2Θ = 10-80°, step size = 0.05 (2Θ), counting time = 0.5 (s)) of calcined alumina unsupported membranes are given in Figures 20 and 21 for 500°C and 600°C, respectively. The XRD peaks are not as sharp as in the case of zirconia. The phase transformation from boehmite to γ (Gama) phase in these alumina membranes should be complete and γ-peaks start to appear in the 600°C membrane pattern.

The pore sizes distributions of calcined alumina measured with nitrogen adsorption and desorption analysis are given in Figures 22 and 23. The calculations of these pore sizes were done according to the BJH (Barret-Johner-Halenda) model, which is commonly used for mesoporous structures. The results are tabulated in Table 9.

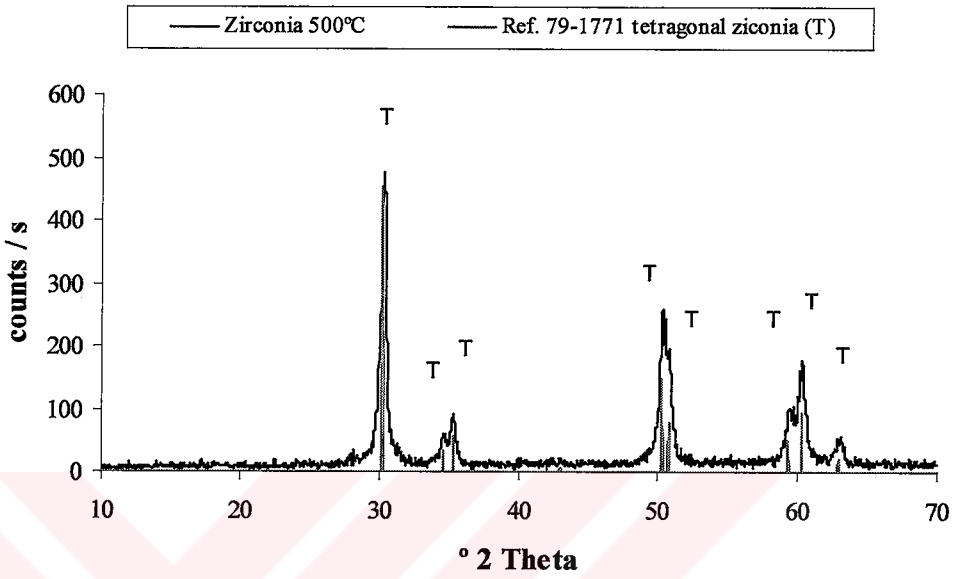

Figure 18. XRD analysis of zirconia unsupported membrane calcined at 500°C.

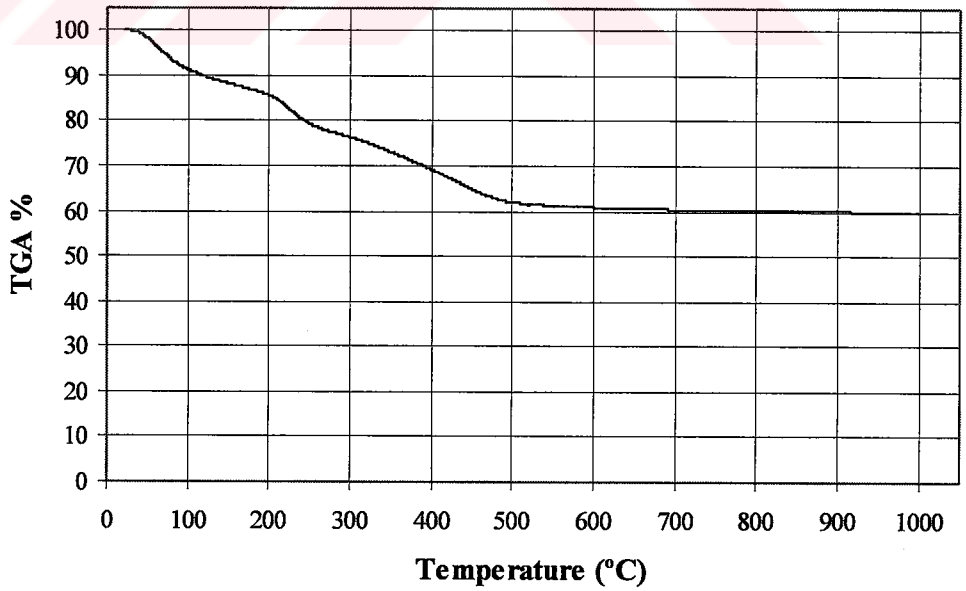

Figure 19. TGA of alumina unsupported membrane (sample weight: 10.38 mg, atmosphere: nitrogen flow 40 mL/min, heating regime: 10°C/min up to 1000°C).

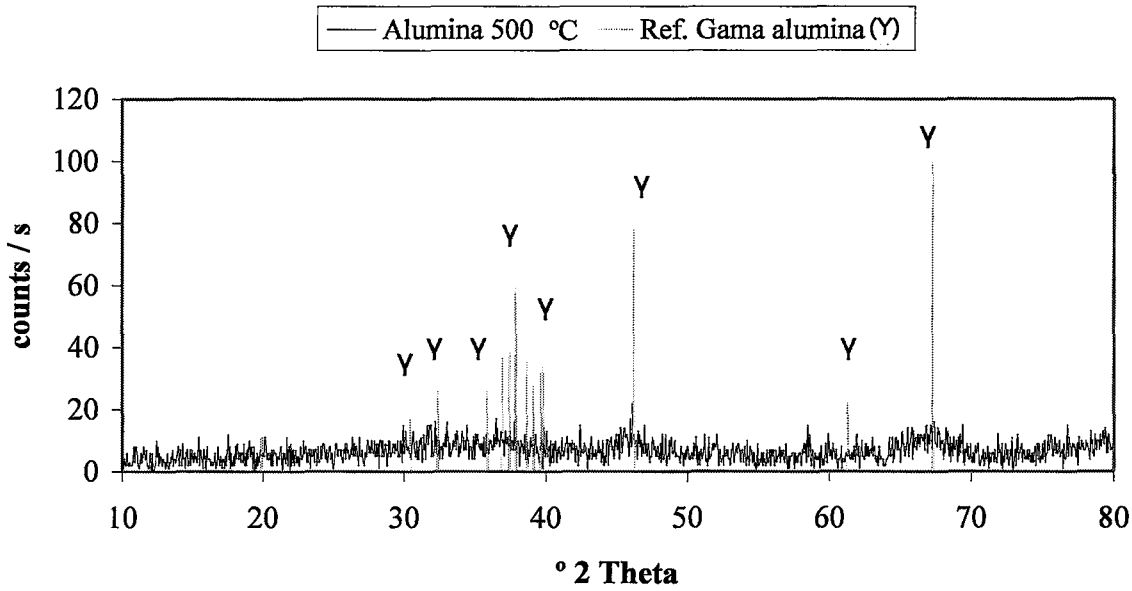

Figure 20. XRD analysis of alumina unsupported membrane calcined at 500°C.

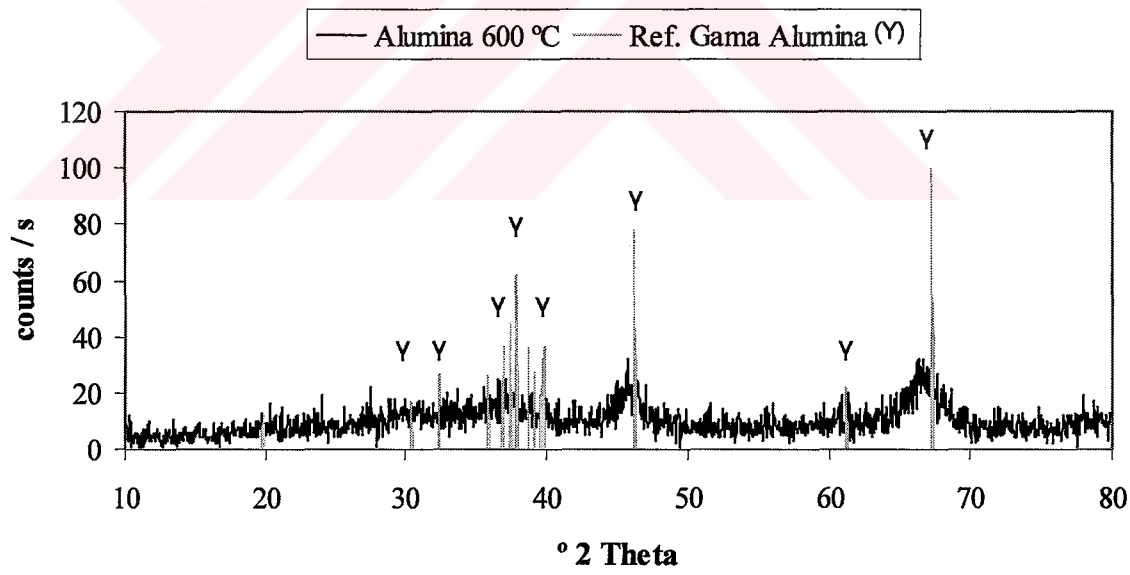

Figure 21. XRD analysis of alumina unsupported membrane calcined at 600°C.

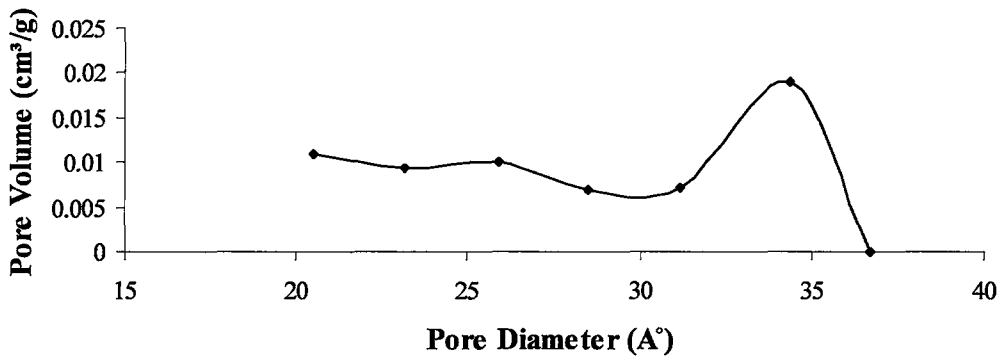

Figure 22. BJH pore size distribution of alumina unsupported membrane calcined at 500°C (from desorption data).

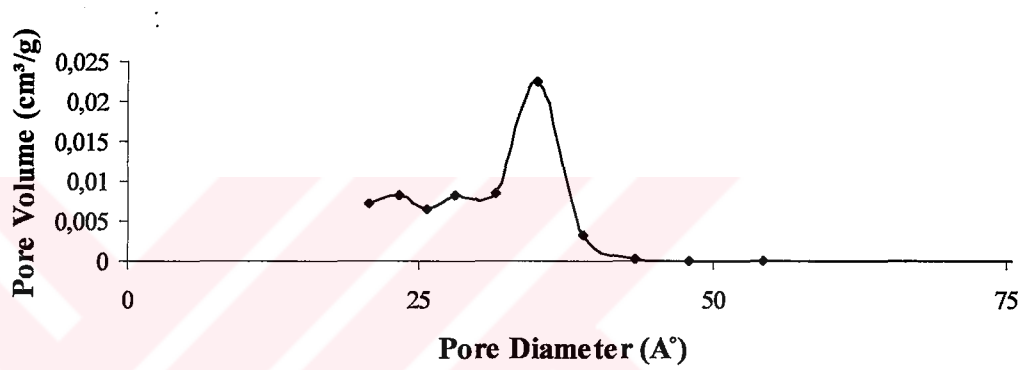

Figure 23. BJH pore size distribution of alumina unsupported membrane calcined at 600°C (from desorption data).

Table 9. Results from nitrogen adsorption/desorption analysis of alumina unsupported membranes.

	Alumina 500°C	**Alumina 600°C**
Single Point Surface Area (m^2/g)	296.7	246.5
BJH Desorption Cumulative Surface Area (m^2/g)	284.9	292.6
Average Pore Diameter (by single point) (A°)	27.5	31.6
BJH Desorption Average Pore Diameter (A°)	28.2	29.9

The supports which were dip-coated with diluted sols (1:4; for zirconia, and 1:3; for alumina) for 10 seconds, dried and calcined at 500°C or 600°C were investigated with SEM. The SEM pictures may be seen in Figures 24 to 35.

The SEM picture of the porous support and the layer formed on it by dip-coating is shown in Figure 24. When the magnification was increased while investigating the microstructure of the coated layer it was observed that it consists of small particles forming a porous structure with finer pores as presented in Figure 25. These small particles forming this structure with finer pores are approximately 10-15 nm in diameter.

The SEM picture of slip-cast zirconia support without sol coating is shown in Figure 26. The grains in the microstructure are 70-150 nm in diameter. The microstructure of a zirconia support coated with diluted (1:4) zirconia sol can be seen in Figures 27 and 28. First the dark and smooth surface was investigated for a closer look to the membrane formed by zirconia sol (Figures 29 and 30) with the idea that the other side was not coated with the sol and a slightly translucent layer formed by small particles (approximately 10 nm in diameter) were observed over the bigger grains of the support. When the other side which seems to mimic the support microstructure with submicron grains was investigated with increasing the magnification as given in Figures 31-35, it was observed that the surface of the support was coated with a thin zirconia layer which was formed from approximately again 10 nm zirconia particles. These observations confirm that the applied coating method is satisfactory to prepare a uniform membrane layer all over the surface of the ceramic support with a fine thickness.

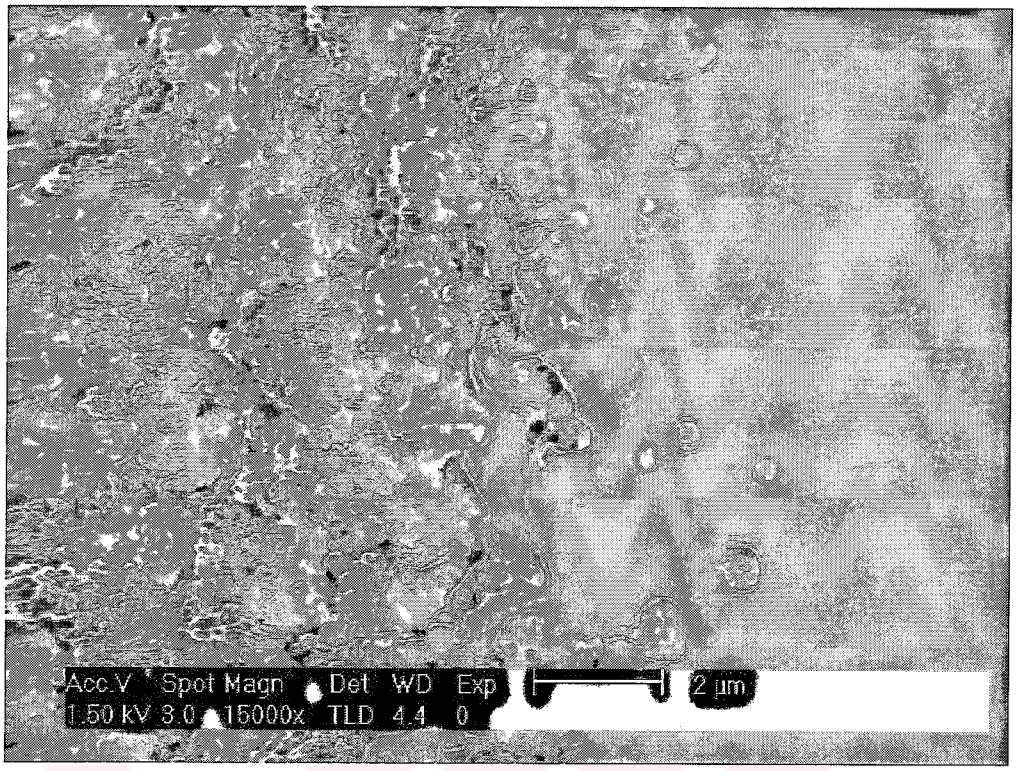

Figure 24. SEM picture of dry pressed alumina support surface heat treated at 1100°C and partially coated with alumina sol calcined at 600°C at 15 kX.

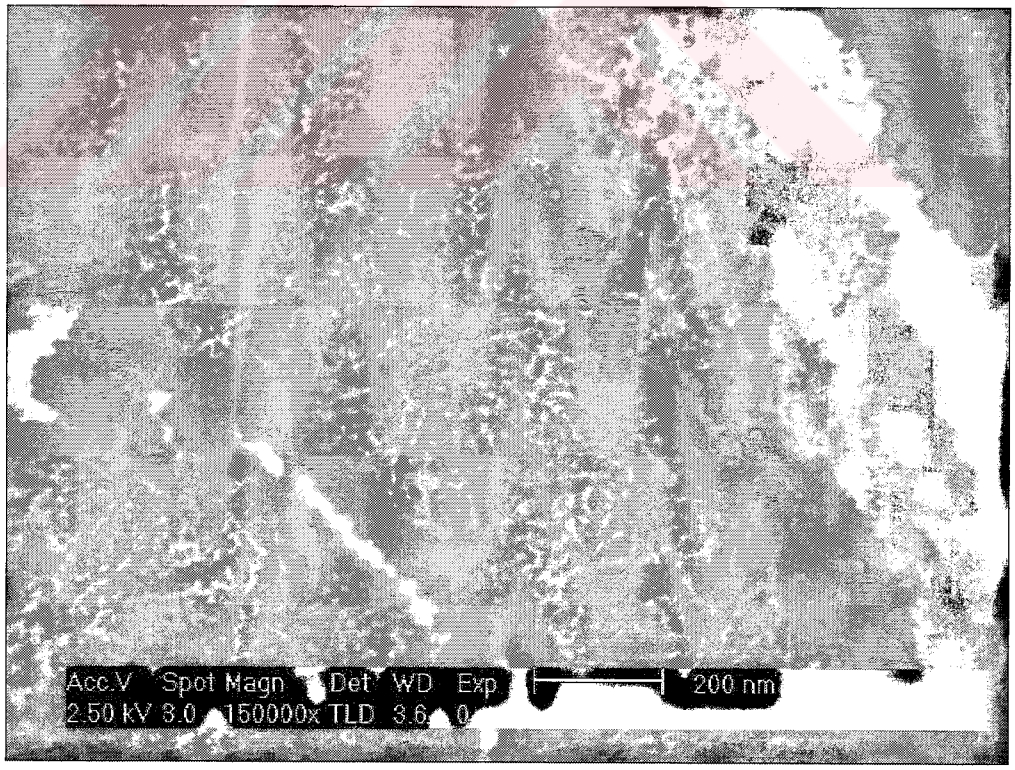

Figure 25. SEM picture of the alumina sol coated 600°C calcined membrane on dry pressed alumina support heat treated at 1100°C at 150 kX.

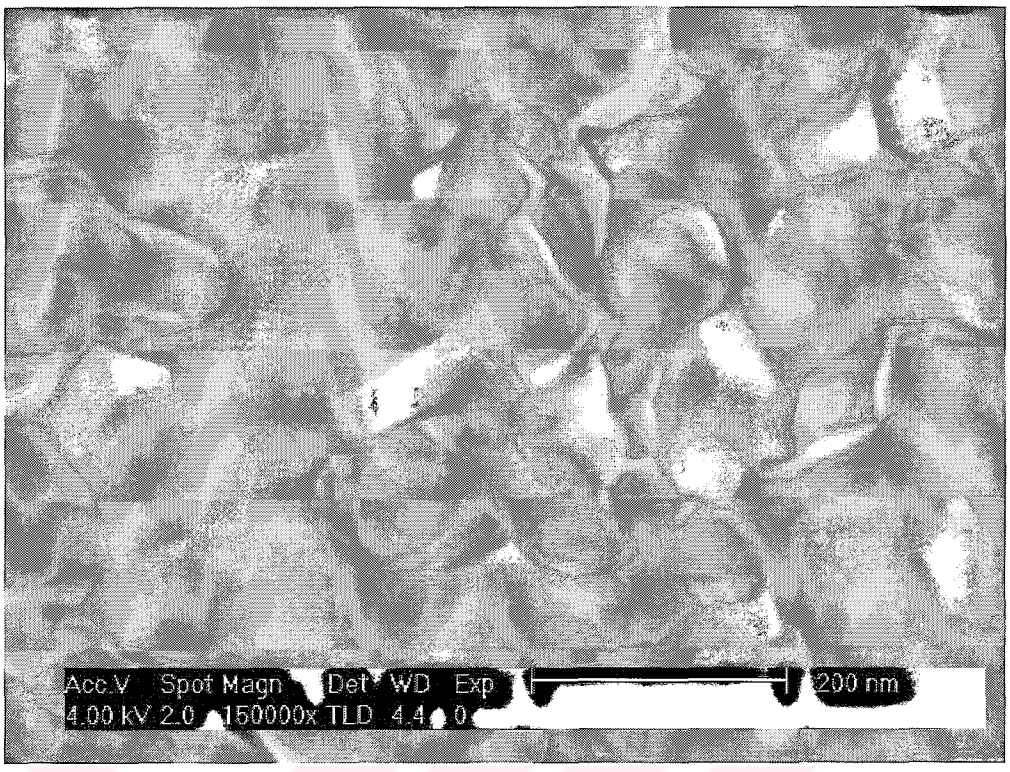

Figure 26. SEM picture of slip-cast zirconia support heat treated at 1200°C at 150 kX.

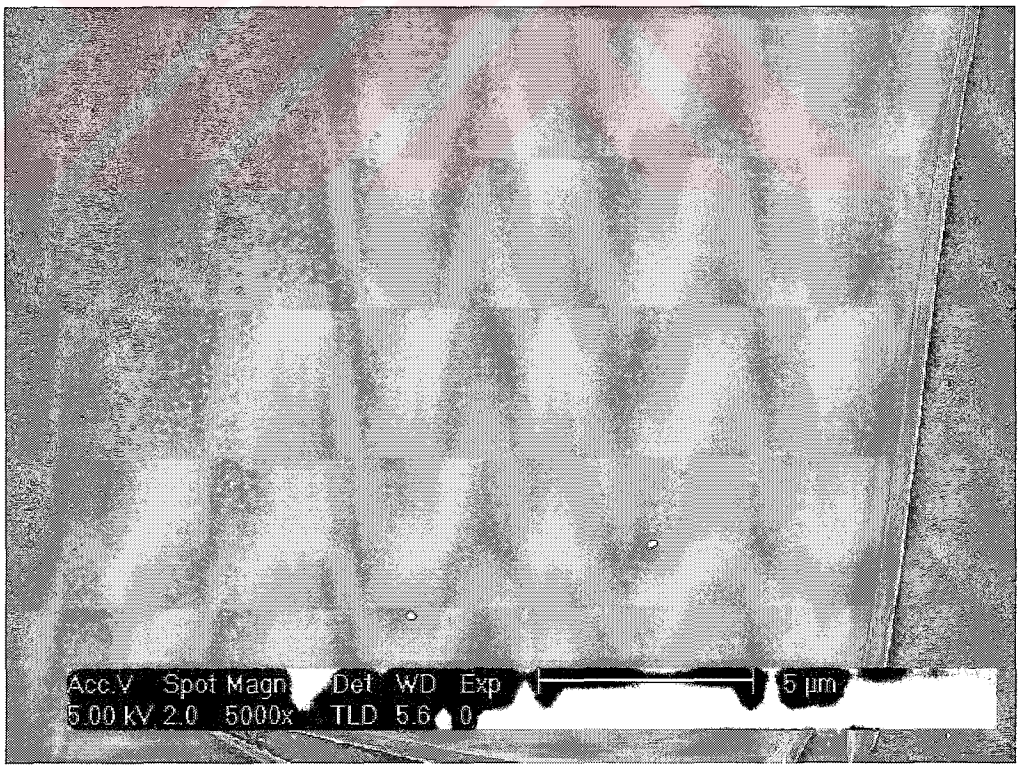

Figure 27. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 5 kX.

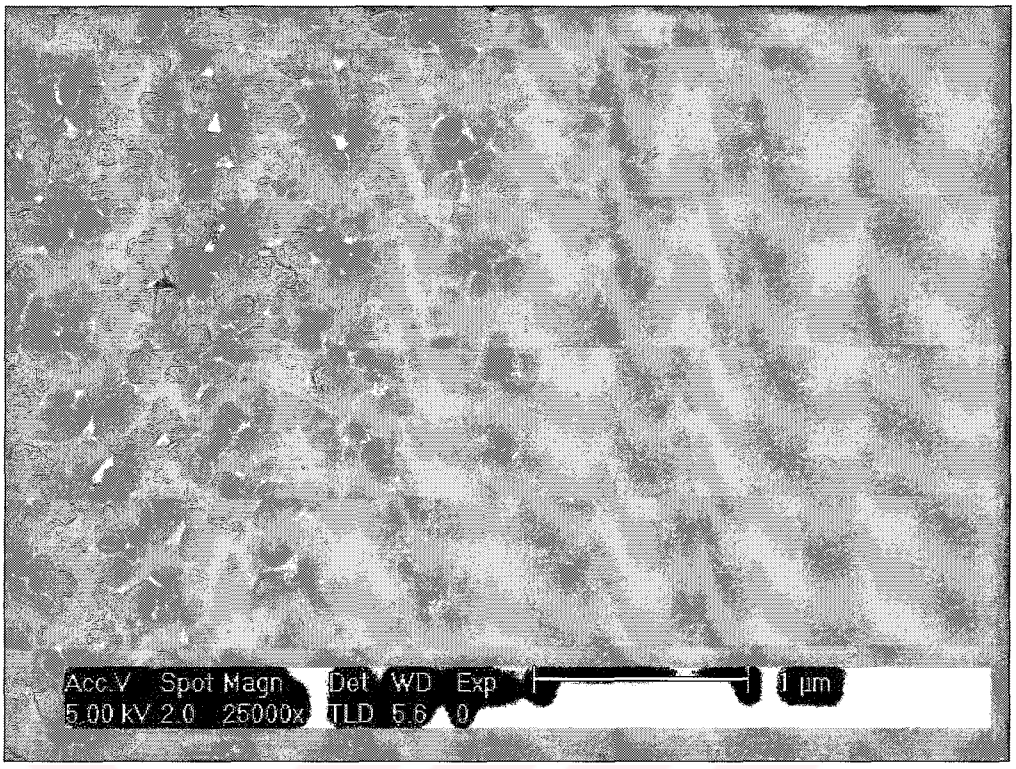

Figure 28. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 25 kX.

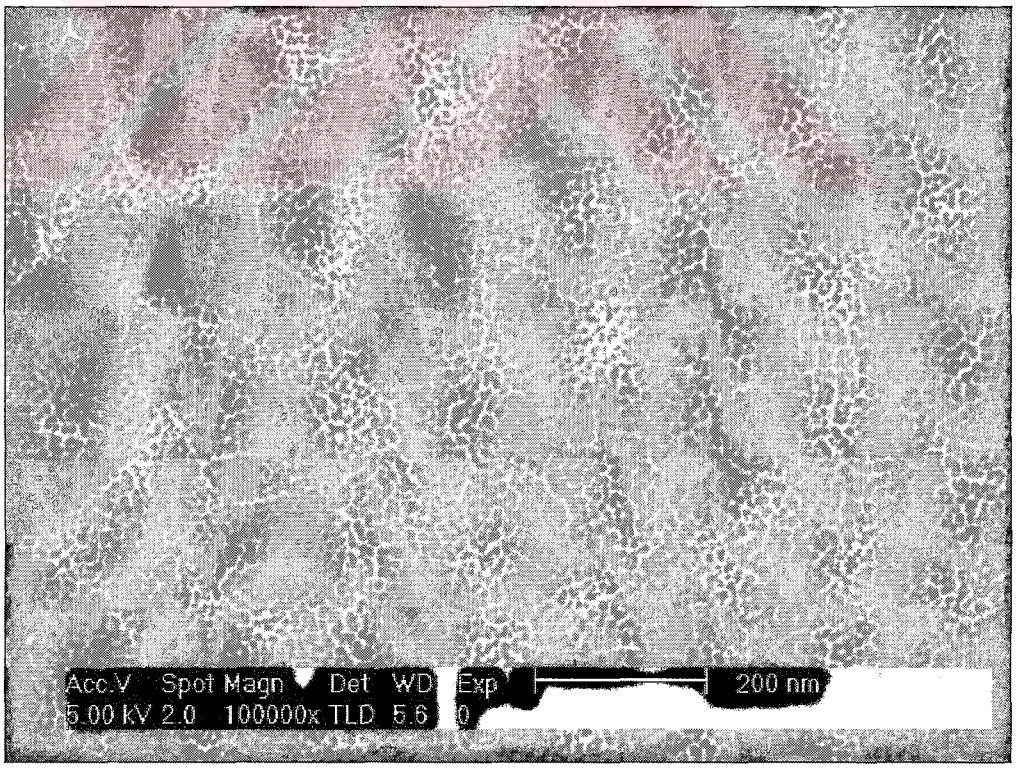

Figure 29. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 100 kX.

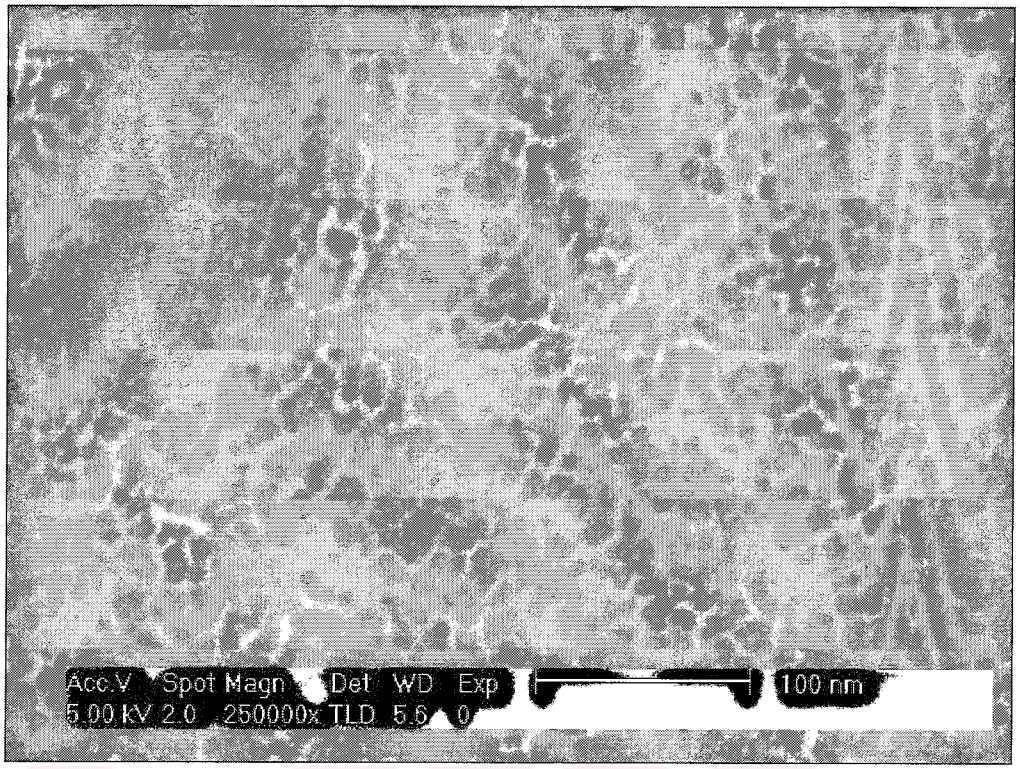

Figure 30. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 250 kX.

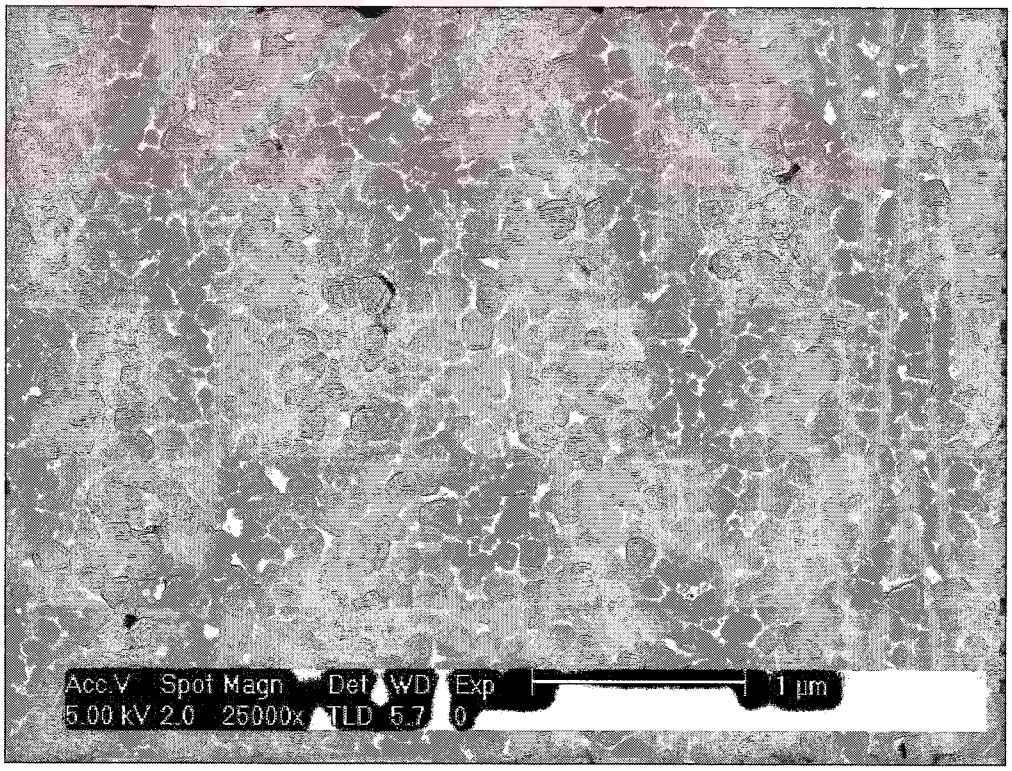

Figure 31. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 25 kX.

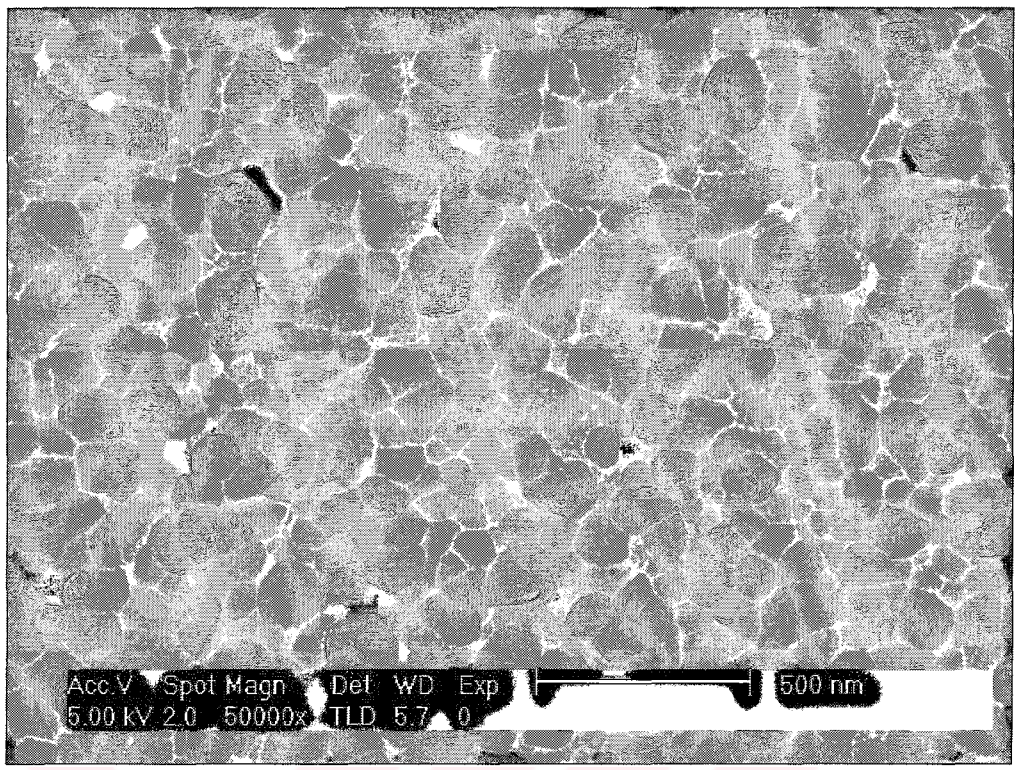

Figure 32. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 50 kX.

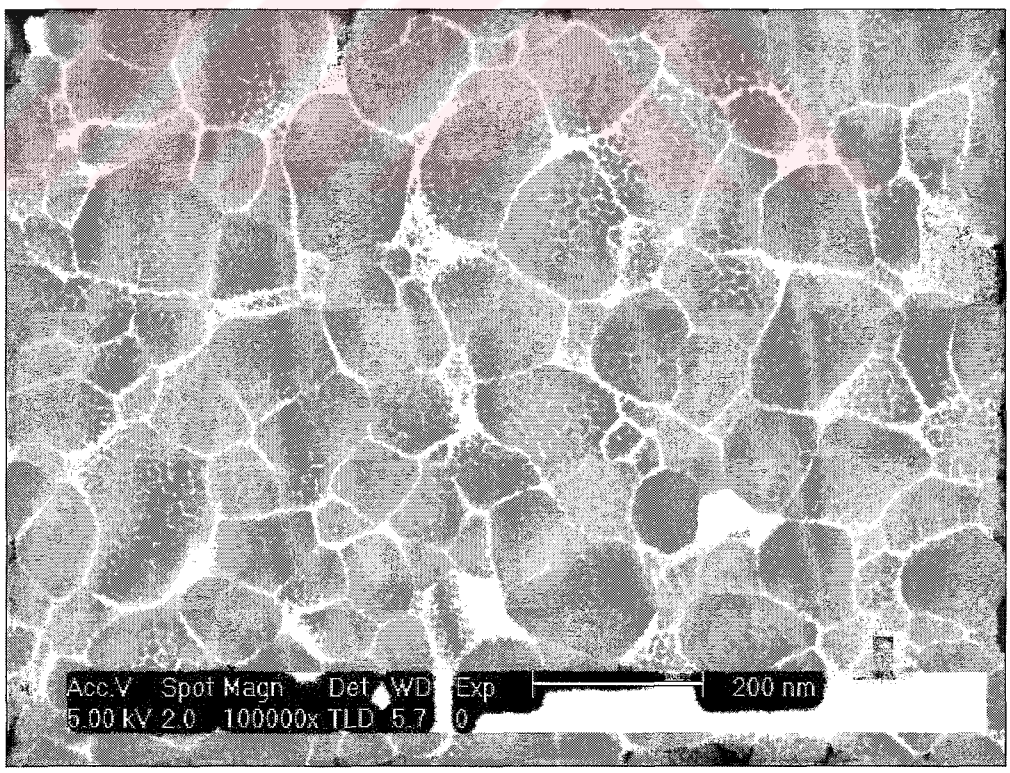

Figure 33. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 100 kX.

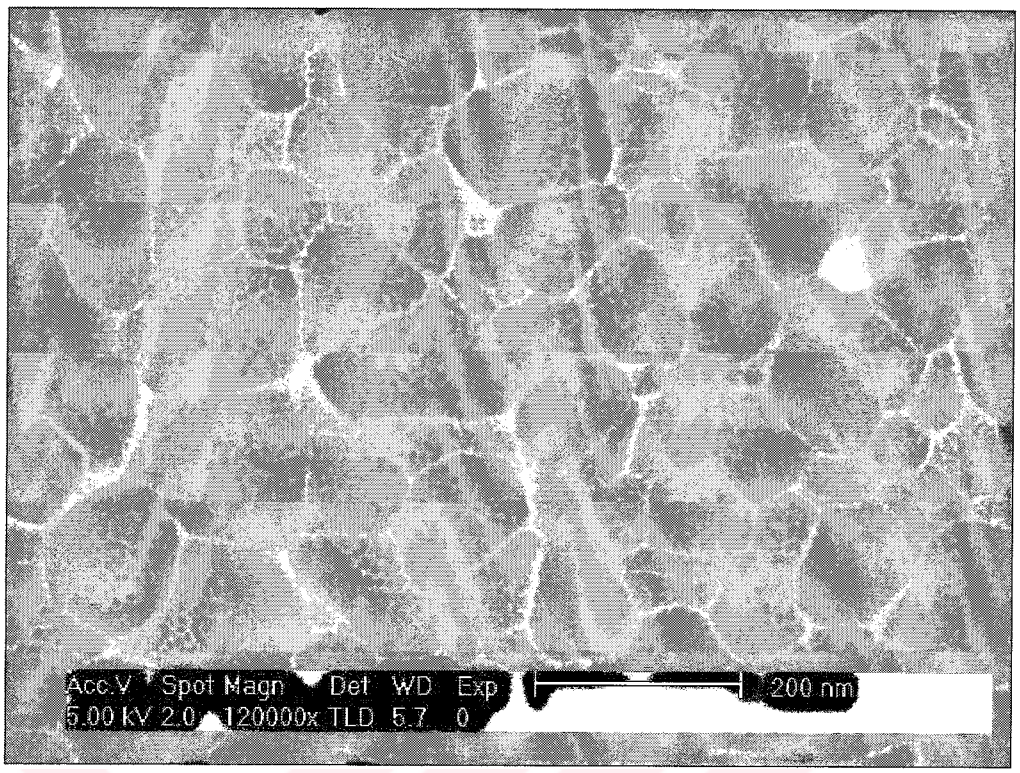

Figure 34. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 120 kX.

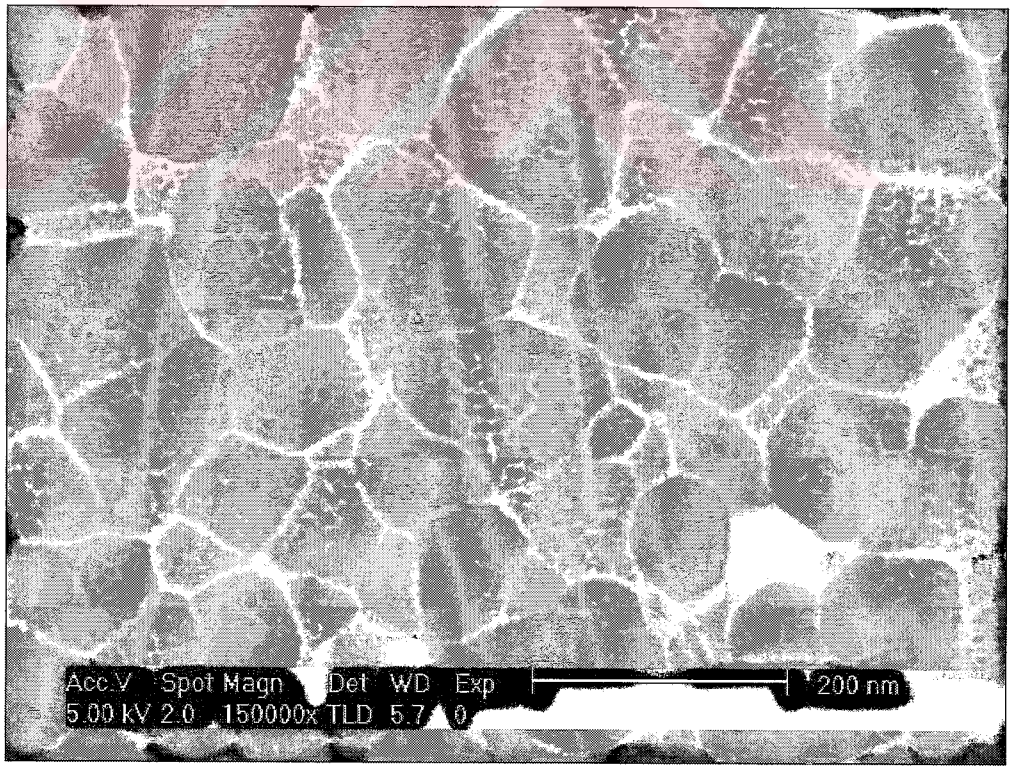

Figure 35. SEM picture of slip-cast 1200°C heat treated zirconia support coated with 500°C calcined zirconia sol at 150 kX.

5.2 Separation Experiments

These well characterized ceramic membranes were subjected to a series of filtration experiments in order to evaluate their separation efficiency and concentration of proteins as representative biotechnological products. Clean water (deionised), bovine serum albumin (BSA) model solution (2.5 g/L), and whey without any pre-treatment were used for this purpose.

5.2.1. The Clean Water Permeability (CWP)

The results about clean water permeability (CWP) of 1200°C heat treated slip-cast zirconia and 1100°C heat treated dry pressed alumina supports are given in Figures 36 and 37. The water permeability of dry-pressed alumina support was determined to be higher than the water permeability of slip-cast zirconia support (water flux: 5 L/min, water temperature: 40°C). The water permeability of both supports increased with increasing transmembrane pressure, but the dry-pressed alumina (heat treated at 1100°C) and zirconia (heat treated at 1175°C) supports were broken at 5 bars while it was possible to work up to 10 bars with slip-cast 1200° C heat treated zirconia support. Vacassy *et al.*(1998) reported a CWP of 32 L/m^2 h bar for a 15 kDa MWCO membrane (Kerasep support) and a CWP of 7.5 L/m^2 h bar for a zirconia nanofilter (2 μm thick, consist of 5nm zirconia particles) they formed on this support. Caric *et al.* (2000) reported CWP values for low TMP values for zirconia coated (50 nm average pore size) and alumina coated (200 nm average pore diameter) on alumina support. If extrapolation is done with the values from the plot of TMP (bar) vs. flux (L/m^2 h) the flux values will be calculated as 560 L/m^2 h and 900 L/m^2 h for TMP = 2 bar, for zirconia coated and alumina coated membrane, respectively. Yang *et al.* (1998) reported CWP values for inorganic MF membranes with average pore diameters 4 – 200 nm as 45 – 1500 L/m^2 h bar. Tsuru *et al.* (1998) reported CWP values between 0.54 – 5.4 L/m^2 h bar for the silica-zirconia nanofiltration (NF) membranes of which MWCO values are between 200 – 1000 Da. Larbot *et al.* (1994) reported CWP values of three NF membranes between 6 - 9 L/m^2 h for TMP = 5 bar. These membranes were able to separate a molecule with molecular weight of 375 g with 95% retention. The CWP value of the slip-cast zirconia (heat treated at 1200°C) and the dry pressed alumina (heat treated at 1100°C) supports, prepared in this work were 23 L/m^2 h and 72 L/m^2 h for TMP = 5 bars, respectively. These values can be considered to be relatively low for a ceramic support when compared with the results given in the literature, but these

supports also can be considered as thick MF/UF membranes with relatively finer pores with diameters lower than 40-50 nm.

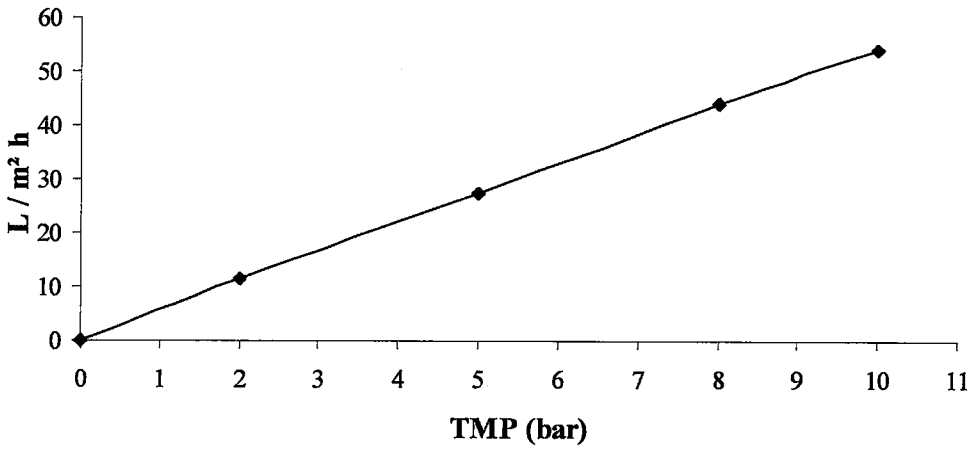

Figure 36. The clean water permeability of slip-cast zirconia membrane heat treated at 1200°C at different transmembrane pressures (TMP).

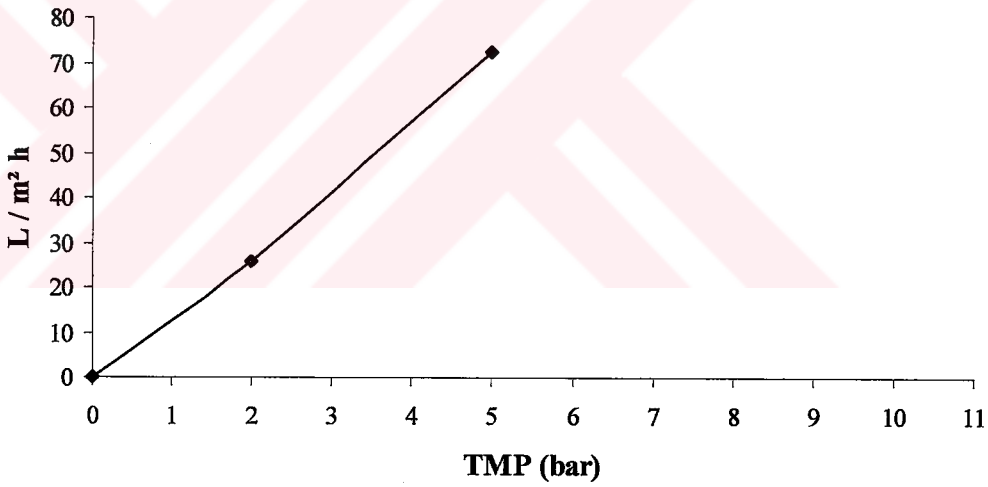

Figure 37. The clean water permeability of dry pressed alumina membrane heat treated at 1100°C at different transmembrane pressures (TMP).

5.2.2. The Protein Retention (PR)

The protein retention (PR) values of membranes were calculated using the following equation:

$$PR\ (\%) = 100 * (1 - (C_p / C_r))$$

where C_p and C_r are protein concentrations in the permeate and retentate streams, respectively (Daufin *et al.*, 1991).

The slip-cast zirconia support heat treated at 1200°C had a protein retention value of 60%, while working with 2.5 g/L BSA solution at a feed flux of 5 L/min and 40°C as given in Figure 38. This moderate protein concentration (2.5 g/L) which is higher than concentrations of total proteins in whey was chosen for preventing the instantaneous fouling of the membranes to be tested at high protein concentrations and for examining the effect of fouling after concentration of whey with lower protein content.

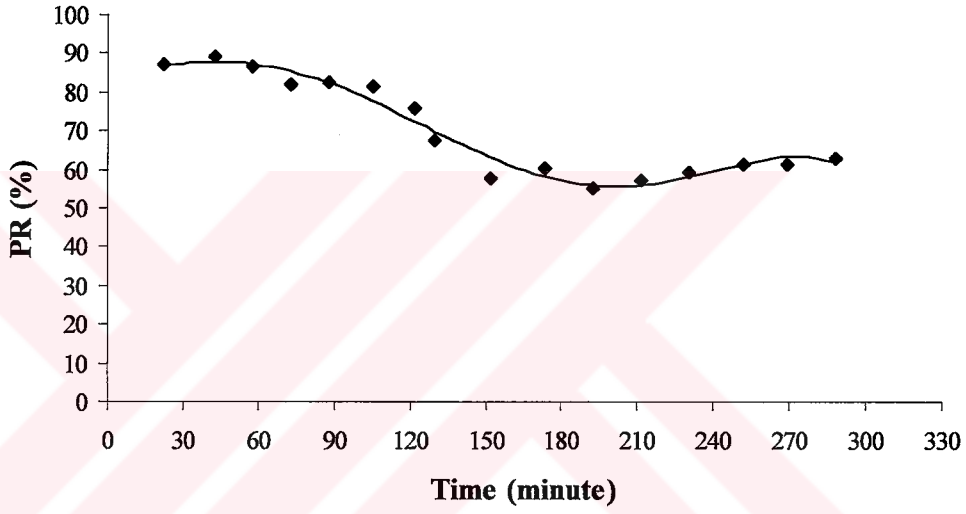

Figure 38. Protein (BSA) retention of slip-cast zirconia support (heat treated at 1200°C).

Protein retention of alumina sol coated membranes is given in Figures 39 and 40. Surprisingly the protein retention of alumina coated membrane calcined at 500°C was lower than the protein retention of 600°C heat treated membrane, although it had a finer microstructure. This may be explained by the presence of defects/microcracks in its finer microstructure.

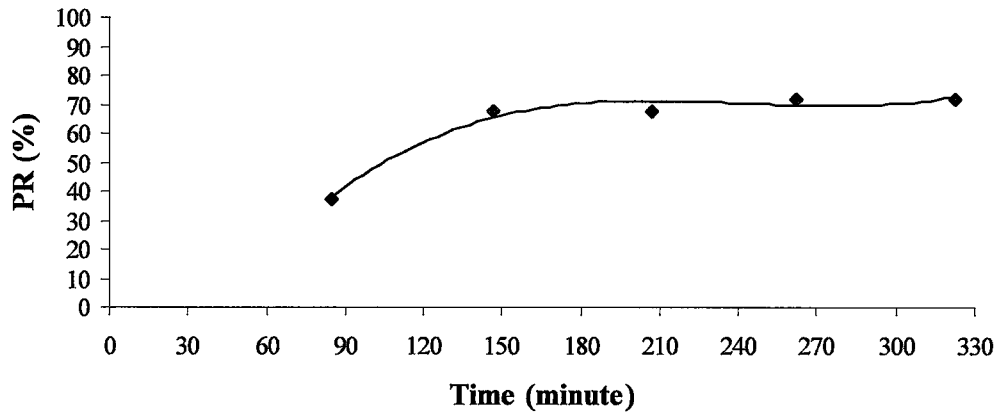

Figure 39. Protein (BSA) retention of 500°C calcined alumina coated slip-cast zirconia support (feed flux: 5 L/min, feed temperature: 40°C).

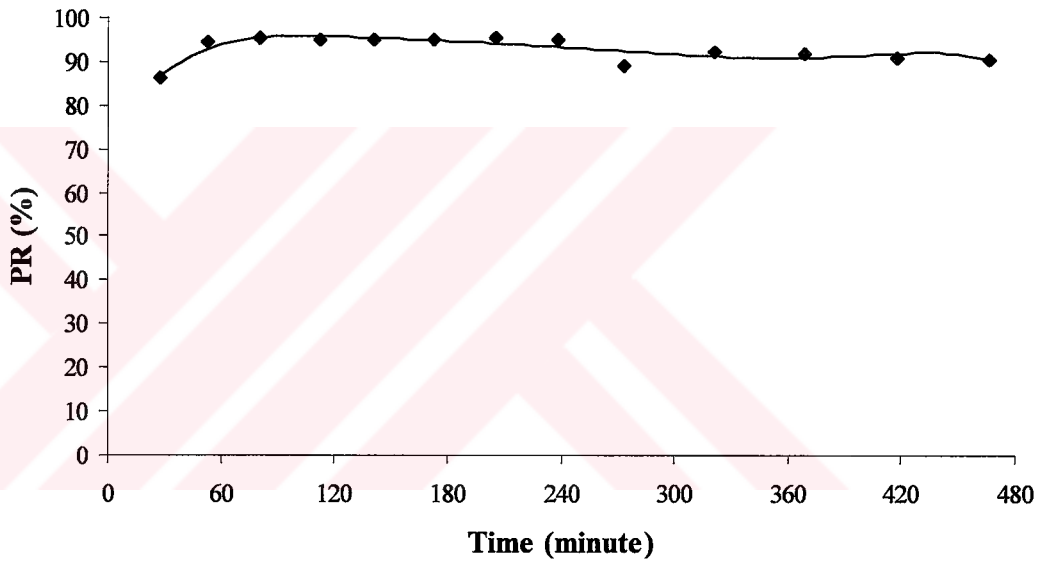

Figure 40. Protein (BSA) retention of 600°C calcined alumina coated slip-cast zirconia support (feed flux: 5 L/min, feed temperature: 40°C).

Zirconia sol was coated and calcined at 500°C on slip-cast zirconia support heat treated at 1200°C. The protein retention behaviour of this membrane was found to be close to the uncoated support as shown in Figure 41. This sol was further diluted with propanol at a ratio of 1:4 and the membrane formed with this diluted sol had a considerably higher protein retention capacity as shown in Figure 42. This observation showed the importance of selective layer thickness on the support. Relatively thick layers may contain significant levels of cracks/defects after heat treatment.

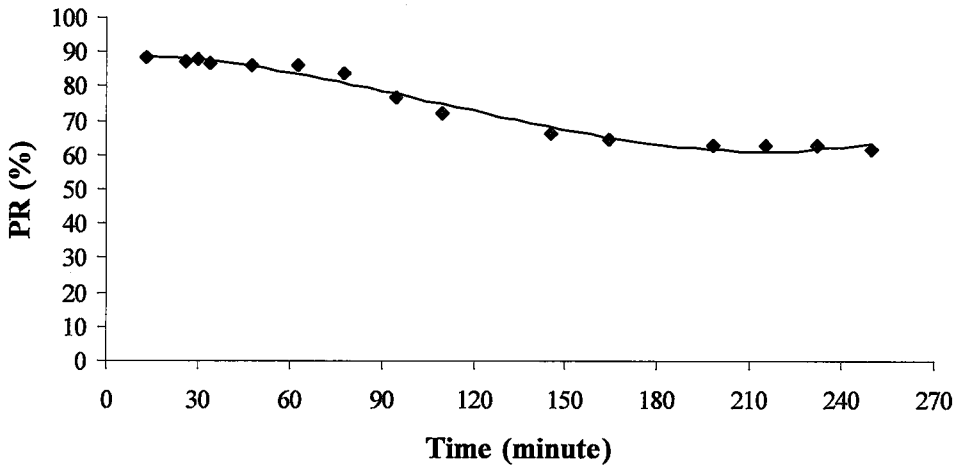

Figure 41. Protein (BSA) retention of 500°C calcined zirconia coated slip-cast zirconia support (feed flux: 5 L/min, feed temperature: 40°C).

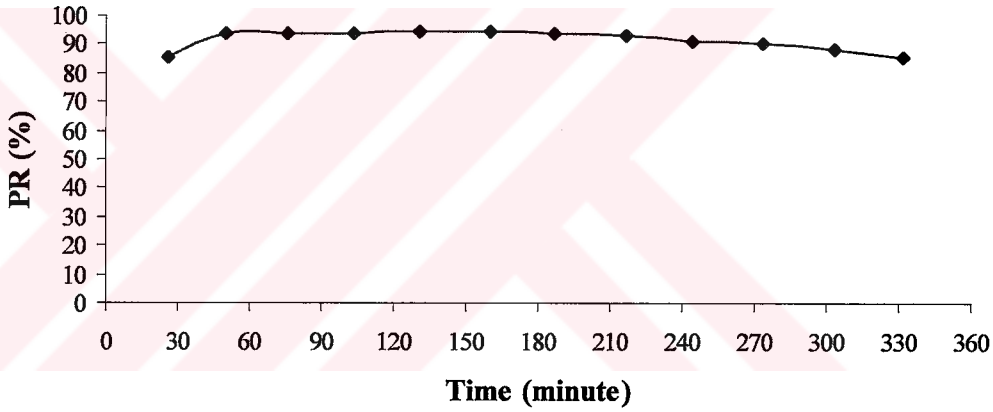

Figure 42. Protein (BSA) retention of 500°C calcined diluted zirconia sol coated slip-cast zirconia support (feed flux: 5 L/min, feed temperature: 40°C).

Both the flux and PR% values increased with increasing transmembrane pressure as shown in Figures 43 and 44, respectively.

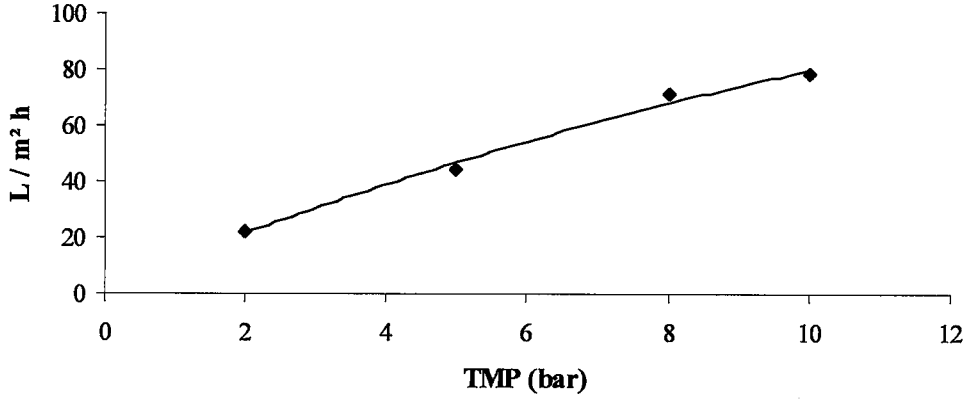

Figure 43. The change of flux with transmembrane pressure during BSA filtration with 1:4 diluted zirconia sol coated 500°C calcined selective layer/1200°C heat treated zirconia slip-cast support composite membrane.

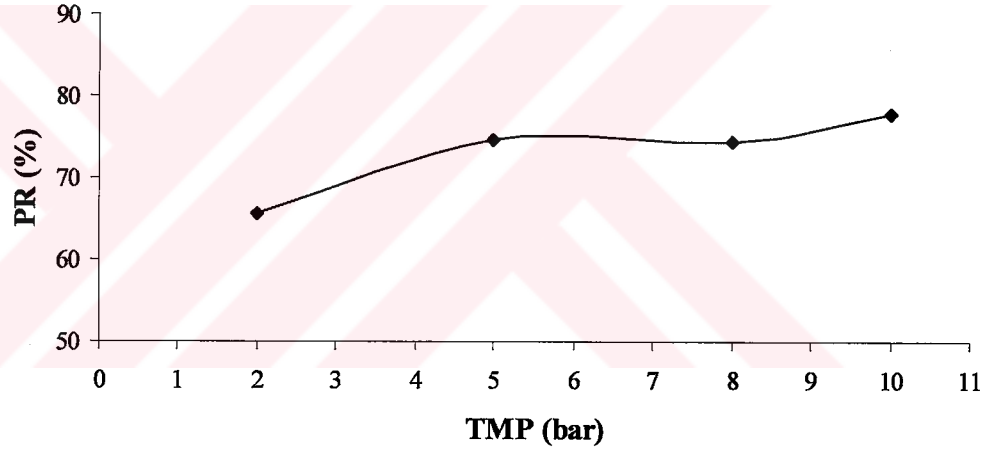

Figure 44. The change of protein retention with transmembrane pressure during BSA filtration with 1:4 diluted zirconia sol coated 500°C calcined selective layer/1200°C heat treated zirconia slip-cast support composite membrane.

The membrane formed with 1:4 diluted zirconia sol after calcination at 500°C on dry pressed alumina support was used for the concentration of proteins in whey. The retention of proteins in this complex biological liquid was at a rate of 96% with a flux value of around 40 L/m^2 h as given in Figures 45 and 46, respectively.

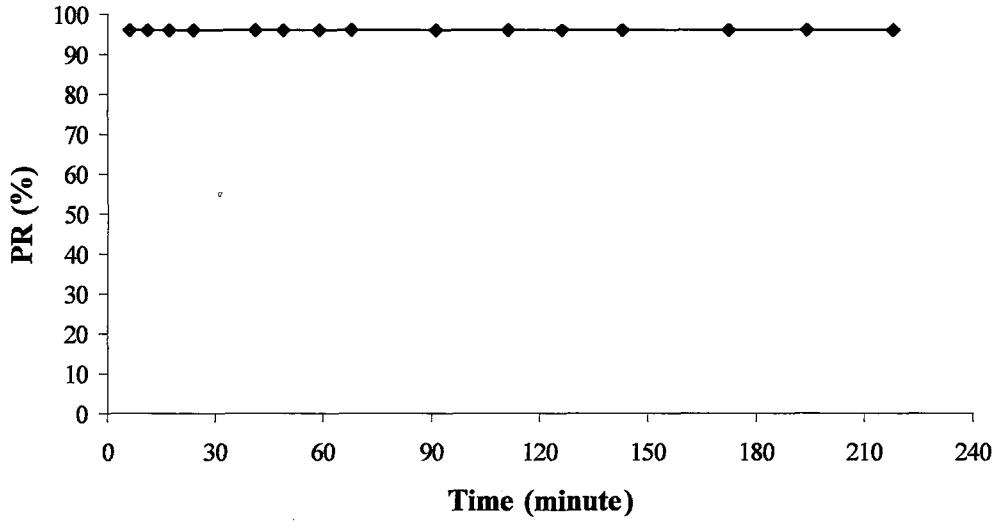

Figure 45. Whey protein retention vs. time with 500°C calcined 1:4 diluted zirconia sol coated 1100°C heat treated dry-pressed alumina support.

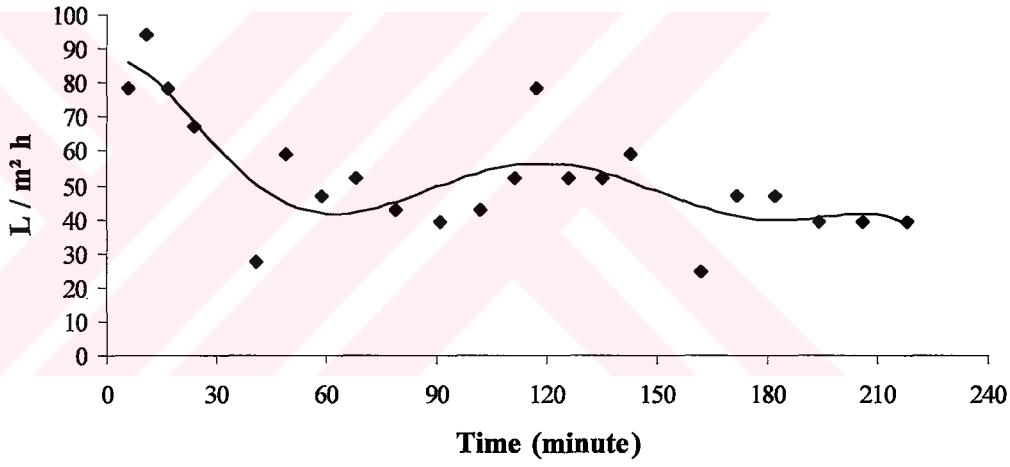

Figure 46. Permeate flux vs. time with 500°C calcined 1:4 diluted zirconia sol coated 1100°C heat treated dry-pressed alumina support.

As shown in Figure 46 the permeate flux was initially approximately 94 L/m^2 h then decreased to 40 L/m^2 h with fluctuations. This value is in the range of values reported in the literature for different inorganic membranes and working parameters as 25-70 L/m^2 h. The protein retention value given in Figure 45 is also in agreement with the values between 84-100% reported in the literature. Flux/retention levels should be optimized together for an efficient membrane separation process.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

Ceramic composite membranes for biotechnological applications were prepared in this work. Ceramic supports from two different powders (alumina, zirconia) were prepared for this purpose. These supports were dip-coated to get a thin selective layer with finer pores by using alumina and zirconia sols. Filtration tests with water, model protein solution (BSA) and untreated whey were performed by using these membranes in order to determine their protein separation efficiencies and fluxes.

The particle size distribution of ceramic powders, the particle size distributions and concentrations of ceramic sols and the heat treatment applied to the ceramics were found to be important and effective parameters during preparation of these ceramic composite membranes.

Zirconia sols with average particle size of a few nanometers (3.1 - 7.4 nm), and alumina sol with average particle size of 30 nm were prepared by using sol-gel method. These sols were used for dip-coating of supports with dilutions to get a crack free thin membrane layer with finer pores (1:3 diluted alumina sol and 1:4 zirconia sol). The calcination temperatures were 500°C for zirconia and 600°C for alumina layer in line with the TGA results.

The ceramic supports were able to separate proteins with a protein retention ratio of 60% without dip-coating. After coating with ceramic sols and calcination this value increased up to 96%. Same efficiency could be achieved also with such a complex biological mixture; whey, with a permeate flux of 40 $Lm^{-2}hour^{-1}$.

Increasing the transmembrane pressure also increased the permeate flux and protein retention. For membrane formed by the calcination of 1:4 diluted zirconia sol coated, dry-pressed alumina support (heat treated at 1100°C) the increases of these values were determined as 7.5 $Lm^{-2}hour^{-1}$ and 1.6% per bar, respectively.

Ceramic supports may be prepared with coarser ceramic powders for increasing the permeate flux. This may yield a support with larger pores (submicron rather than nanosized pores) and a much higher flux.

Ceramic sols with different solids contents may be further prepared and characterized to synthesize membranes with various pore sizes to use in different biotechnological applications.

The heat treatment and its effects on the microstructure of both ceramic supports and selective membrane layers can be further investigated.

Since the ceramic materials are amphoteric, their behaviour as a membrane, at different pH values can be investigated. The efficiency of the separation process may be severely affected with the interaction of solutes in the feed with the membrane surface due to the adsorption and also electrical attraction/repulsion forces.

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