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UTLIZATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY TO ASSESS THE CLEANLINESS OF ULTRAFILTRATION MEMBRANES

By

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ABSTRACT

UTILIZATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY TO ASSESS THE CLEANLINESS OF ULTRAFILTRATION MEMBRANES

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An immunological assay was developed to detect milk proteinaceous soils in ultrafiltration (UF) membranes. Specific antibodies to milk proteins were raised in rabbits and a titer of 1:10,000 was determined by an enzyme-linked immunosorbent assay. Of two procedures attempted to couple peroxidase to the antibodies, the glutaraldehyde method resulted in great loss of immunological activity and the m-periodate method yielded a conjugate with a titer of 1:500. The first trials were conducted using a commercial antibody conjugate as a marker on skimmilk soiled membranes removed from the thin-channel unit in a procedure referred to as dismantling. An insignificant correlation (-0.39) was found between cleaning time and cleanliness . Significant correlations were found, however, between the percentage of nitrogen on the membrane and both cleanliness measured by the ELISA method and flux restoration. A non-dismantling procedure was set up

based on the the disruption of antibody to protein bonds on the membrane in the UF unit , using a 2 M sodium chloride solution and distilled water as eluents. In first tests, conducted with virgin membranes, a nonspecific binding baseline was established as an absorbance value of 0.106, above which any reading would be related to the presence of soils in the membrane. When the test was carried out in membranes soiled with skimmilk and washed for diferent periods, the amount of nitrogen in the membranes varied from 0.098 to 0.314 % and a significant correlation (0.96) was found between these measurements and the absorbance values in the water eluent. Testing of the procedure in repeatedly soiled and washed membranes showed that flux restoration was a variable parameter and that the return of the absorbance value to the baseline typical of virgin membranes was possible only in clean membranes. When the non-dismantling method was carried out with the prepared anti-milk protein peroxidase conjugate, a significant correlation (0.96) was found between the absorbance values in the water eluent and the presence of soil in the membrane. Results indicated that ELISA could be successfuly used to detect proteins in the UF membrane, without dismantling of the unit.

To my wife Eliana

and our children Edelweiss,

Poliana and Bryan.

I love you all.

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INTRODUCTION

Ultrafiltration (UF) is a process associated with the filtration of components of solutions or suspensions based on the size of the components. This process has a wide range of applications in the dairy industry such as: 1) increase in cheese yield due to the recovery of high quality whey proteins, 2) decreased energy requirement for heating, cooling and transportation of the products, 3) reduction of the waste disposal problem in cheese manufacturing, 4) reduction in the amounts of cultures and coagulant, and 5) possibility of cheesemaking on a continuous basis.

Proper cleaning of UF membranes is important to the processor from two standpoints: 1) the products derived from the UF process must be acceptable for the safety and welfare of the consuming public and 2) poor cleaning leads to reduced performance of the UF unit, due to fouling of the membrane surface, which decreases the permeate flow rate.

Two main techniques have been used for testing the efficiency of an UF cleaning procedure: flux rate determinations and visual inspection by the U.S. Department of Agriculture. The first is a non-destructive method and is based on the return of water flux rate to levels equal those

in new membranes (Beaton,1979; Parkin and Marshall,1976). However, it has been shown that water permeate flux, despite being restored easily, does not correlate with efficacy of cleaning, as measured by other parameters (Smith and Bradley,Jr., 1987; Harper and Moody, 1981). Soils may still be bound to the membrane and go undetected and act as a substrate for baterial growth. The visual inspection of UF membranes, in most cases, requires the dismantling of the membrane which, in the case of spiral wound membranes, widely used in the dairy industry, may destroy them.

The great specificity and resolution of immunochemical techniques are responsible for their increasing application to problems in biochemistry and other areas (Cooper, 1977). Enzyme-labeled antibodies have been used cytochemically to detect antigens in tissues or for detection of toxins. One of the most important immunological methods currently used in the enzyme-linked immunosorbent assay (ELISA), which has a high level of sensitivity and reproducibility yet employing relatively simple and inexpensive equipment (Liu et al., 1985; Ceska, 1978).

The objective of this study was to apply an ELISA to the detection of minimum amounts of milk proteinaceous soils bound to the surface of UF membranes. The hypothesis was that specific antibody bound to a protein soil in the membrane could be released by means of adequate elution techniques and that utilization of this type of immunoassay

could eventually lead to the establishment of a non-dismantling indirect method to assess the cleanliness of UF membranes.

LITERATURE REVIEW

Ultrafiltration basic concepts

Ultrafiltration (UF) is a technique for the fractionation of liquids using a semipermeable membrane. The word "ultrafiltration" is very familiar to the world dairy industry where this membrane technique is continuously expanding. Some are claiming UF will be as common in a dairy plant as a separator or a heat exchanger (Maubois,1979). Membrane ultrafiltration is a hydraulic pressure activated process capable of separating solution components largely on the basis of molecular size and shape. Under the pressure gradient across the membrane,a portion of the solvent and smaller solute species pass through the membrane and are collected as the permeate. Larger solute species are retained by the membrane and recovered as a concentrated retentate (Porter and Michaels,1970). According to Cheryan (1986). The nature of the membrane controls which component permeates and which component is retained. Ultrafiltration membranes retain only macromolecules or particles larger than about 10-200 angstroms or from about 500-1,000,000 in molecular weight (Cheryan, 1986; Porter and Michaels,1970). Compared to other separation procedures like reverse osmosis

(a membrane process which retains all components other than the solvent and considered a dewatering technique) ultrafiltration offers the advantage of needing fairly low pressure for operation. Typical UF operating pressures range between 5 and 100 psi (compared to 500-2,000 psi required for reverse osmosis) reducing the pumping and equipment costs by a considerable margin (Porter and Michaels, 1970).

Ultrafiltration applications on milk and milk products began early and many large installations are in operation throughout the world (Kosikowski,1986). During ultrafiltration milk enters the system under pressure and flows across the membrane surface for fractionation. Water, lactose, soluble salts and nonprotein nitrogen pass through the membrane, producing a clear fluid known as permeate. Fat, true protein and insoluble salts are retained in a decreasing pool of milk serum to give a liquid retentate (Kosikowski,1986; Richter,1983). According to Maubois (1979) using UF techniques one can produce a precheese, which has about the same composition as drained cheese, thus eliminating the need for cutting and cooking the curd.

Ultrafiltration is also of considerable interest to other food processing industries (fruit juices,vegetable proteins, egg white, gelatin,etc) for the fractionation and purification of liquid foodstuffs and for the recovery of valuable components from food processing waste (Kosikowski,1986;Cheryan,1986;Porter and Michaels,1970). The

process is becoming particularly interesting for the dairy industry due to the following advantages : 1) yields of cheese from a given quantity of milk are increased due to recovery of high quality whey proteins. 2)Energy requirements for heating, cooling and transportation of the product are decreased. 3)Whey proteins may be recovered from whey, reducing the waste disposal problem in cheese manufacturing. 4)The quantities of coagulants and cultures needed for manufacture of cultured dairy products are reduced. 5)The manufacture of cheese on a continuous basis is possible.

Economic importance of UF processes

Ultrafiltration processes have found a variety of applications in the food industry, and the utilization in pilot plants or comercial manufacturing includes (Harper,1979):

1- Production of whey protein concentrate (WPC).
2- Preparation of precheese for cheese manufacturing.
3- Protein enrichment for fluid products.
4- Fractionation of raw milk on the farm to reduce transport costs.
5- Preparation of specialty products for geriatric feeding.
6- Manufacture of calf replacers.
7- Enzyme recovery from lactose syrup preparation.

8- Recovery of cleaning compounds,for reuse.

In the dairy industry, UF processes have been used mainly in the fractionation of whey and manufacturing of soft cheeses. In 1981, an estimated 15% of Danish-made and 20% of French-made fresh and ripened soft cheeses were produced by UF process (Kosikowski,1986).

A good example of the successful application of UF is the processing of cheese whey. Whey is a by product of the cheese industry. Typically 10 Kg of milk will yield 1-2 Kg of cheese and 8-9 Kg of whey. Whey disposal is a major problem for the dairy industry (Cheryan, 1986). More than 25 billion lb of cheese are produced annually throughout the world (Kosikowski,1980), resulting in approximately 88 billion lb of liquid whey, of which the United States produce one half (Cheryan,1986).

Despite significant gains in the amount of whey being processed, more than 40% of current U.S. whey production is still disposed as raw whey (Teixeira et al.,1982). By far the largest share of whey solids is processed into dry whey for use in human food and animal feed. About 5% of the whey produced is used to manufacture whey protein concentrate (WPC) by ultrafiltration.Whey protein concentrate is a highly functional concentrate and can be used for a wide variety of food ingredients applications as a substitute for non-fat dry milk (NFDM) and other protein ingredients (Teixeira et al.,1982). The annual U.S. production of WPC is

estimated at about 15.4 million lb (Morr,1984). There are about 50 companies in the U.S. manufacturing 35% WPCs and an additional 5 or 6 companies producing 70-80% WPCs (Morr,1984).

Cheese consumption in the U.S. is increasing at 4% per year (Cheryan,1986) and its principal by-product, whey, has experienced a steady 2-5% annual increase (Morr,1984) which indicates that the whey disposal problem is getting worse (Cheryan, 1986). Ultrafiltration has attracted the attention of cheese producers by affording a means of simultaneously fractionating the whey and purifying and concentrating the whey proteins, enhancing by-product utilization and reducing the pollution problem (Cheryan,1986).

The costs of UF whey processing has been compared to the costs of concentrating in 2-stage vacuum evaporation systems and has been found that the advantage of UF increases at low capacities. The modular construction of UF units keeps the equipment costs roughly proportional to size whereas small evaporators have higher unit costs than larger ones (Porter and Michaels,1970).

Ultrafiltration Membranes

Molecular membranes have evolved rapidly with distinctive features. They have a thin surface layer, or skin, where permeation occurs, and most have an open, porous

interior or backing to support the surface skin (Kosikowski,1986). Ultrafiltration membranes have anisotropic structure, where the pores change in size from one surface of the membrane to the other (Cheryan, 1986).

The original membrane used in the dairy industry was made of cellulose acetate (Harper,1979). The raw material for cellulose acetate membranes is cellulose which is a polymer of glucose units. The membrane is prepared from cellulose by acetylation, i.e., reaction with acetic anhydride, acetic acid and sulfuric acid (Cheryan,1986). Initially, cellulose acetate was pratically the only material used in fabricating membranes, but since 1975 complex polymers have been replacing cellulose acetate in membrane preparation. There are several disadvantages to the use of cellulose acetate and its derivatives as membranes materials (Harper,1979; Kosikowski,1986; Cheryan,1986):

1- A narrow temperature range (maximum 30 C).

2- A narrow pH range (pH 2.0 - 8.0) which is a problem in devellopingcleaning procedures.

3- Poor resistance to chlorine (This poses a special problem, since chlorine is almost a universal sanitizer in the food industry).

4- Cellulose acetate is also reported to undergo the "creep" or compaction phenomenon caused by fluid pressure to a slightly greater extent than other materials.

5- Cellulose acetate is also highly susceptible to microbial attack due to the nature of its cellulose backbone.

Due to these problems, other polymeric materials are now widely used in the manufaturing of UF membranes.Polysulfone is often used and is characterized by having in its structure diphenylene sulfone repeating units, which contribute to a high degree of molecular immobility producing high rigidity, strength, creep resistance, dimemsional stability and heat deflection temperature (Cheryan,1986).

Polysulfone was considered quite a breakthrough for UF applications due principally to the following characteristics (Cheryan,1986; Kosikowski,1986):

1- Wide temperature limit (up to 75 C).

2- Wide pH tolerances (1 to 13).

3- Fairly good chlorine resistance (up to 200 ppm).

4- Easy to fabricate membranes in a wide variety of configurations.

5- Wide range of pore size available for UF applications, ranging from 10 to 200 angstrons, or molecular weight cut-offs from 1000 up to 500,000 in commercial-size modules.

A third generation of membranes for UF , the so-called mineral or ceramic membranes,are formed by deposition of inorganic solutes (like zirconium oxide) on the microporous support. They, apparently, have improved

qualities over those of polysulfones (higher pressure limits and even wider pH tolerance) (Cheryan,1986; Kosikowski,1986).

Ultrafiltration configurations

Several types of UF systems have been used in the dairy industry. The most commom ones have been the tubular membrane, the spiral-wound membrane, and the plate and frame membrane systems.

Tubular modules were perhaps the earliest design of industrial scale ultrafiltration equipment using synthetic membranes (Cheryan,1986). The tubular system consists of tubular membranes attached to fiber supports enclosed in stainless steel housing. The liquid flows through the membrane and permeate is collected in the stainless steel housing. This system is easy to clean and presents no dead spaces. However,they require high floor space for installation and present a high hold-up volume (volume of feed solution necessary to fill up the UF module) per unit area, limiting the degree of concentration that can be achieved. They might also have a high pressure drop (variation between inlet and outlet pressure during operation) in the tube connections (Cheryan,1986; Richter,1983; Harper,1979).

The spiral-wound system is one of the most compact and

inexpensive designs available today. In this module, membrane and support materials are wrapped around a perforated stainless steal pipe and enclosed in stainless steel housing. The liquid enters one end of the module and flows across the membrane.Permeate, which passes through the membrane, is collected in the center pipe, while the retentate exits through the opposite end of the housing.While they are easy to change membranes, this system presents a dead space behind a product seal put around the module at one end to minimize feed by-pass , which may result in cleaning problems (Cheryan,1986 ;Richter,1983; Harper,1979).

The plate-and-frame membrane modules were one of the earliest configuration in the market. It consists of membranes sandwiched between support plates arranged in various stacked plates which are enclosed in a stainless steel housing. As the liquid enters the system it is forced through narrow channels formed when two support plates come together. The liquid is fractionated as it flows across each membrane plate. The permeate is channeled between the support structures to a collection point. This system is ideal for viscous solutions, has a low hold-up volume and low pressure drop. However, it is not easy to clean and presents several dead spaces (Cheryan,1986; Richter,1983; Harper,1979).

Another design, the hollow fiber configuration, is a

newer approach to UF module design. The membrane is in the form of a self-supporting tube and has a dense "skin" layer on the inside of the tube. It presents a large area of membrane per unit of volume (compact equipment), low hold up volumes, low pressure drops and a potentially low cost of operation. A big advantage with the hollow fibers is its "back-flushing" capability (pressurizing the permeate and forcing it back through the membrane into the lumen of the fibre), which vastly increases its cleanability. However, the system has a maximum pressure rating of 25 psi (1.8 atm), which is sometimes a disadvantage (Harper,1979; Cheryan,1986).

Laboratory Scale Ultrafiltration

Laboratory bench-top apparatus provide means of studying the UF process and the parameters which govern it. Industrial scale conditions can be reproduced using small amounts of foodstuff and greatly reduced membrane surfaces. Results can eventually be up scaled to pilot plant or industrial conditions.

There are three main types of laboratory UF units: dead-end cells, stirred cells and thin-channel cells (Cheryan,1986). Dead-end cells have control of accumulation of retained macrosolute above the membrane, restricting solvent and solute permeation.The best utilization has been for separating very dilute solutions of macromolecules in small volumes. Stirred UF cells have

agitation provided by a magnetic stirring bar placed as close as possible to the membrane surface, improving polarization control and increasing flux.

Thin channel devices are a further step-up on the ladder of polarization control (Cheryan,1986). In this UF unit, the retentate is pumped through narrow channels or slits on top of the membrane, at high shear rates. The retentate is returned to the feed reservoir and recycled, minimizing accumulations of retained species on the membrane surface and resulting in much higher flow rates than those obtained with stirred cells (Cheryan,1986).

Thin-channels have been used by researchers in laboratories to simulate UF plant operational conditions. Howell and Velicangil (1979) used a flat thin-channel spiral type cell to study membrane fouling during concentration of Cheddar cheese whey and the treatment of the membranes with immobilized proteases. The enzymes were pre-coated on the membrane surface, so that clogging proteins were hydrolysed as they were deposited.

Glover (1971) used a similar system to study the blocking of UF membranes by proteins during milk concentration and found out that proteins form a gel which adheres to the membrane and in effect adds another filtering layer.

Ultrafiltration modes of operation

There are three main modes of operating a UF system: 1) single-pass, 2) batch and 3) continuous flow processes. The single-pass system is designed to concentrate the product to the desired level in a single movement through the membrane system and involves no recycling of the retentate, which is a factor limiting the attainment of high concentrations (Richter,1983; Kosikowski,1986).

The batch systems recycle the retentate to the starting supply tank until the desired retentate concentration is obtained. Heat exchangers can be included in the recycling loop to control temperature.High concentration attainment is accompanied by long retentate residence time, which can lead to increased microbial growth. This is the fastest method of fractionating a given amount of material and it will also require the minimum membrane area (Cheryan,1986; Kosikowski,1986).

Continuous systems have been developed to provide uninterrupted fractionation of products. The retentate moves through a series of modules and before entering each module bypasses into a loop where pumps raise the pressure before it moves through the succeeding modules. The constant pressure established maintains the initial high flux. Modules can be arranged in series or parallel, and the retentate becomes concentrated to high levels as it moves

through the system, while residence time is held to a minimum. As the retentate is released from the system, fresh material is supplied to the feed tank (Richter,1983; Kosikowski,1986).

Applications of ultrafiltration in the dairy industry

Industrial ultrafiltration was initially developed primarily for the treatment of wastewaters and sewage to remove particulate and macromolecular materials. In 1969 UF was proposed for the first time for use in cheesemaking, in France (Cheryan,1986). From then on, the utilization of UF has greatly expanded to other dairy products. The two main applications are still in the fractionation of whey to produce WPC and fractionation of milk for the manufacturing of fresh and ripened soft cheeses.

If unfractionated whey is dried, the powder contains only 10-12% of the total solids as protein. If the whey is concentrated by ultrafiltration, powders with 35 , 50 or 80% protein may be manufactured, due to the elimination of lactose, salts and other low molecular weight compounds in the permeate (Richter,1983).

Anti-pollution legislation has forced many cheese manufacturers to turn to the utilization of whey rather than its disposal as waste (McDonough et al.,1971). Indeed, in the natural liquid state, whey contains 6.0 - 6.5 % total solids and many nutrients, and has a high biological oxygen demand (BOD),approximately 30,000, which creates a very

severe waste disposal problem (Cheryan,1986; Kosikowski,1986).

In 1984 the U.S. produced about 40 billion lb of sweet cheese whey and about 3.75 billion lb of cottage cheese acid whey, which contained about 360 million lb of whey proteins and 2.8 billion lb of whey solids (Morr,1984). About 5% of the total whey production is utilized for the manufacturing of WPC by ultrafiltration. The majority of WPC is dried and has a variety of uses in the food industry. The major whey proteins,(α-lactalbumin and β-lactoglobulin) are largely undenatured and have good functional and nutritional qualities. Dried WPC can be used as a partial replacement for skim milk powder in ice cream, and as enrichment agent for breadstuffs, confections, nutrient beverages,baby foods and comminuted meats (Richter,1983; Kosikowski,1986).

A serious problem associated with WPC production is to dispose of or utilize the resulting high-BOD permeate which is rich in lactose. Most of the permeate that is generated from current WPC production is used in the production of crude and refined lactose (Teixeira et al.,1982), but consumption levels often are not high enough to assure a steady market at an attractive price (Kosikowski,1986). For this reason a number of other applications for the permeate have been studied ,such as utilization in the production of galactose/glucose syrups (to be used in sugar confectionery, ice cream and beer production, etc) or in the production of methane, alcohol, yeast protein, lactic acid and vinegar,

among other products (Coton,1980; Teixeira et al.,1982; Richter,1983; Kosikowski,1986).

Traditional cheesemaking has been defined as a "fractionation process by which fat and casein are concentrated in the curd, while lactose, soluble proteins, minerals, and vitamins are lost in the whey fraction" (Cheryan,1986). The utilization of UF procedures in cheese manufacturing is somewhat changing this concept. The proteins of the retentate (pre-cheese) obtained through direct ultrafiltration of milk includes the whey proteins because they cannot permeate the membrane. Retentates produced from skim milk with present polysulfone membranes can attain a concentration of about 21% total protein (7:1), and from whole milk, 16% total protein (5:1) (Kosikowski,1986), which enables them to be converted into cheese with almost no production of whey.

Several advantages have been associated with cheesemaking by ultrafiltration, as stated by Cheryan (1986):

1- Increases in the yield of cheese, which is claimed as the primary advantage (up to 10-30% higher yields).

2- Lower overall energy consumption.

3- Enzyme (rennet) usage is reduced for a given amount of milk processed or cheese produced.

4- Waste disposal problems are reduced because there is less protein in the waste stream.

5- Space requirements are reduced due to the smaller volume of milk handled per unit weight of cheese.

In the literature there are several reports of successful utilization of UF procedures in the manufacturing of cheeses. One of the most successful applications has been in the manufacturing of Feta cheese, which was originally produced in Greece, from sheep's milk (Cheryan,1986). As of 1983, over 95% of the Danish Feta cheese production of 77,000 tons per year was produced in ultrafiltration plants (Olsen,1981; Cheryan,1986; Kosikowski,1986).

Cream cheese manufacturing by means of UF procedures was originally developed by Covacevich and Kosikowski (1977), who reported that the resulting cheese showed excellent shelf-life and smoothness comparable with standard commercial cream cheese, but with much greater hardness of body. By adjusting the mineralization of UF cheeses, Maubois (1979) reports that the texture problem of cream cheese was solved and resulting products were judged organoleptically identical to the best commercial cheeses. The same author describes a technique leading to the production of Ricotta cheese by ultrafiltration, which had higher yield and good organoleptic qualities.

Soft mold ripened cheeses, like Camembert, have been successfuly made in France using UF procedures (Kosikowski,1974; Maubois,1979; Kosikowski,1986). Higher

yields and good mold growth were observed in all experiments.

Saint-Paulin, a French variety of cheese related to Gouda or Edam cheeses, was made by Maubois (1979) using mineral membranes, the pre-cheese having a protein content higher than 21% .

Using a continuous UF system, Delbeke (1987) conducted experiments on the making of Saint-Paulin cheese by full concentration of milk. Cheeses obtained showed serious defects in numerous tests, but by combining some specific manufacturing parameters, these defects could be considerably reduced or avoided.

By manufacturing Colby and Brick cheeses from ultrafiltered milk, Bush et al. (1983) reported that reductions in cooking temperature and milk-clotting enzymes and elimination of curd washing were achieved. Sensory evaluation analysis indicated that there were no differences in overall preference between experimental and commercial cheeses, at an age of three months.

The developments of UF techniques to date have focused mainly on the soft and semi-hard cheeses. The production of hard cheeses (usually about 60% solids and above) have presented some problems. When produced by UF procedures hard cheeses like Swiss, Grana and Cheddar, whose protein levels are high (often above 25%) usually present textural and organoleptic properties different than when manufactured by traditional methods (Kosikowski,1986; Cheryan,1986).

Several investigators have reported problems in the production of Cheddar cheese using milk concentrated by ultrafiltration. These problems include difficulty in cutting due to the heavy texture, elevated loss of fines, problems in handling the curd, and slow loss of curdiness during ripening due to a reduced rate of proteolysis (Richter,1983; Cheryan,1986; Kosikowski,1986). Covacevich and Kosikowski (1978) found that retentate made by a single diafiltration and homogeneization gave the most acceptable Cheddar cheese of various UF treatments, but was crumbly and corky in body and lacked typical cheese flavor when compared to conventionally-made Cheddar. According to Sutherland and Jameson (1981) the body defects most frequently reported by graders judging UF-made Cheddar cheese are weak, soft, mealy,pasty and sticky . However, Kosikowski (1986) indicates that with the application of wide channel UF membranes, which permits higher concentration rates, acceptable Cheddar cheese could be made by this procedure.

Ultrafiltration procedures have also been used in the manufacturing of cultured milk products like yogurt, koumiss and kefir, with improvements in curd firmness and viscosity (Jepsen,1977; Kosikowski,1986).

Another interesting application of UF is in the manufacture of cheese from goat's milk. Goats produce very little milk in the winter and, traditionally, cheesemakers kept frozen acid curd from goat's milk obtained in the late summer for use in the winter. However, storage caused the

curd to become oxidized and accquire off-flavors. Ultrafiltration retentate of goat's milk, however, can be successfully stored with few problems (Cheryan,1986).

Fouling of ultrafiltration membranes

A major limiting step in the use of pressure driven membrane processes, is what is loosely termed as fouling of the membrane. Fouling manifests itself as a decline in flux (volumetric rate of flow of the permeate through the membrane)with time of operation when all operating parameters, such as pressure, flow rate, temperature and feed concentration, are kept constant (Cheryan,1986). Another cause of flux decline is the formation of a concentration polarization layer, which is an increase in the concentration of solute molecules close to the membrane. Ultimately this concentration at the membrane surface may exceed the solubility limit for a particular solute, which would then precipitate (Skudder et al.,1977).

However, Kun-Pei Kuo and Cheryan (1983) state that fouling differs from the concentration polarization phenomenon in that the latter occurs whenever any impermeable solute is ultrafiltered and is generally considered a reversible phenomenon. On the other hand, fouling is generally attributed to the accumulation of macromolecular or colloidal particles (such as proteins, lipids, microorganisms or inorganic salts) on the membrane surface and to the possible crystallization and

precipitation in the membrane pores of smaller solutes that are normally permeable.

The general consensus appears to be that fouling is due to the deposition of protein on the membrane surface and that the microenvironment, especially pH and type and concentration of salts, plays a very significant role (Skudder et al.,1977; Cheryan and Merin, 1979; Hill,1980; Kun-Pei Kuo and Cheryan,1983). According to Skudder et al. (1977), electron micrographs of membranes showed that the major component of the deposit was casein micelles linked by bridges to form a lattice. Chemical analysis of the deposit confirmed a high casein content and showed that calcium phosphate was precipitated in the deposit throughout fractionation. When fat globules were present in the feed solution they appeared to be caught up in the deposit, but did not affect its initial formation. Other studies (Tong et al.,1988) have shown that during the ultrafiltration of whole milk , whey proteins accounted for most of the proteinaceous membrane foulants.

Cheryan and Merin (1979), on studying the fouling of membranes during ultrafiltration of Cottage cheese whey, found the exact role of salts to be unclear. They speculate that salts could change the conformation of the proteins, making them more susceptible to precipitation on the membrane, or they could act as a salt bridge between membrane and orotein, leading to greater fouling .

According to Maubois (1980) any technological treatment affecting either the solubility of phosphocalcic salts or the quantity of these salts will decrease fouling and improve the permeation rate. The author suggests addition of 0.2% sodium citrate to the whey or demineralization by using ion exchange resins, prior to UF treatment. Adjusting the pH of pasteurized whey to 2.0 or 3.0, followed by conventional centrifugation (5,000 g for 30 sec), significantly minimized membrane fouling and improved flux during ultrafiltration of cottage cheese whey(Kun-Pei Kuo and Cheryan (1983).

A consequence of fouling is higher cleaning costs. In addition, depending on the nature and extent of fouling, restoring the flux may require stronger cleansing agents which may damage the membrane.(Cheryan,1986).

Cleaning of ultrafiltration equipment

Membrane equipment is fundamentally different from most equipment used in the dairy industry and presents special challenges to the sanitary design engineer. The requirements for smooth, non-absorbent materials, have to be waived for the membrane material and also for porous support materials, where employed. Disassembly of equipment for manual cleaning, which is not always possible ,is impractical because of the large surface areas involved. Therefore systems must be designed for clean-in-place (CIP) procedures (Beaton,1979). Cleaning of membranes after UF

operation is a very important problem, since the cleaning efficiency is closely related to the total productivity of the system. At present, choice of operating conditions in the cleaning process is largely based on experience (Nakanishi and Kessler,1985) . Despite the great interest of the dairy industry in the wide range of applications of UF, the ability to clean and sanitize these systems to meet regulatory requirements has received little attention (Smith and Bradley,Jr.,1986).

To develop an effective cleaning strategy one need to know what components of the feed stream are causing the fouling and whether the cleansers being used solubilize or disperse the foulants (Cheryan, 1986). Studies of the fouling material deposited on membranes have been performed using scanning electron microscopy and have concentrated on the organic components. However, inorganic material containing sulphur, phosphorus, chloride, sodium, potassium, calcium and magnesium has been found to deposit in significant amounts during daily ultrafiltration operations. Calcium phosphate in the "apatite" form has been indentified by Armishaw (1982).

Cleaning has two main objectives. The first is to eliminate microbial contamination and the second is to remove all soils and restore the flux rate of the system to its original level. A typical cleaning and sanitizing regime of UF equipment includes (Harper,1979; Cheryan,1986):

1- Preconditioned fresh water rinse to remove residual material (usually, performed at a temperature of 35-50 C, depending on the type of membrane).

2-Circulation of an alkaline detergent solution to remove protein and fat deposits. The detergent could contain complexing agents such as EDTA or hexametaphosphate, to help remove mineral constituents.

3- A water rinse to remove the detergent.

4- With some types of products being ultrafiltered, an acid cleaning cycle may be required, followed by another water rinse.

5- Sanitizing by circulation of 50-100 ppm chlorine solution .

6- A final water flush to remove the sanitizer.

7- Checking of the water flux under standard conditions.If water flux is not up to expected values, step 2 should be repeated.

8- Storing of the membrane in water or glycerine.

The objective of sanitary operation is to control the degree of contamination of product with microorganisms, principally bacteria. Sanitation of many UF membranes is commomly accomplished by use of solutions of sodium hypochlorite containing 50-100 ppm available chlorine; chlorine is freely permeable to the membranes and will also sanitize the downstream side of the system (Beaton,1979).

Bisulfite has been proposed as a sanitizer for UF systems, especially for control of yeasts and molds. However, Smith and Bradley,Jr. (1986) report that effectiveness of bisulfite is pH dependent. At pH 4.7 bisulfite solution was ineffective in controlling microorganisms and at pH 3.5 bisulfite was microstatic. Extensive corrosion of stainless steel housings was apparent following longterm contact (2 weeks) with bisulfite solution.

The chemical cleaner used in UF systems is frequently tailored to meet the requirements for removing specific foulants, e.g., non-ionic detergents (0.1%) to remove fat deposits, acids to remove inorganic precipitates, alkaline detergents to remove protein deposits. Proteases and lipases in enzyme detergents, which hydrolyse protein or fat, are sometimes used to supplement or replace alkaline detergent cleaning (Beaton,1979).

Smith and Bradley,Jr. (1987)evaluated the efficacy of four commercial enzyme-based cleaners (pH 7.0 to 8.4) of UF systems by means of microbiological analysis (swab and rinse water samples) and permeate flux restoration. They found that the four enzyme-based cleaners were unsatisfactory when microbiological criteria were considered. Loss of sanitizer strength and problems with yeast and especially mold growth over time also indicated lack of effective cleaning.

Assessing cleanliness of UF systems

Generally speaking, physical cleanliness is a level of sanitation at which no soil is visible to the naked eye (Maxcy, 1968). This is the traditional way of judging cleanliness and it is the basis for most of the criteria of cleanliness as used by both industry and regulatory agencies. With the naked eye one can see obvious defects, and minor accumulated defects can also been seen. The ultimate goals of inspections are to ensure that food residues are not adequate for the harborage of microorganisms . A more accurate system is needed for judging the level of cleanliness (Maxcy,1968).

Visual inspection is possible only where equipment surfaces can be observed easily such as vat pasteurizers. Modern methods of processing liquid products generally are closed systems and are cleaned by circulation. Therefore soils remaining on the interior of the equipment cannot be observed (Maxcy,1968; Arnold and Maxcy,1970). This is the case of UF equipment.

The visual inspection of UF membranes by regulatory agencies in most cases requires the dismantling of the membrane. No problems occur with tubular or plate-type membrane systems. However, spiral-wound membranes must be unrolled for visual inspection, which destroy them. Although the oldest module in the system is normally used for the evaluation (in hollow fiber systems), the cost of the

module replacement must be born by the processor (Semerad,1984).

The accepted non-destructive method for evaluating cleanliness has been the return of flux rate to levels equal to those in new membranes. The water flux measurements are generally taken after cleaning (Parkin and Marshall,1976; Beaton, 1979; Semerad,1984; Cheryan,1986).

However, it has been shown that water permeation flux, despite being restored easily, does not correlate with efficacy of cleaning, as measured by other parameters. Harper and Moody (1981) studied the enzymatic cleaning of cellulose acetate membrane reverse osmosis systems by comparing several methods of evaluating cleanliness, such as flux restoration, amount of dissolved soils in the permeate during cleaning and use of radioactive phosphorus. They found out that water rinsing alone provided over 95% flux restoration and that this method did not appear suitable for critical evaluations of cleanliness of reverse osmosis membranes.

Smith and Bradley,Jr. (1987) found similar results when evaluating the efficacy of enzyme-based cleaners in UF systems. Rinse water was collected after each cleaning and water recycling step, followed by swabbing of a small area of the stainless steel housing. Swab and rinse water samples were plated to determine numbers of bacteria, yeasts and molds. They found that water flux was easily restored but

did not correlate with efficacy of cleaning based on numbers of microorganisms remaining in the system.

Rinse and swab techniques are considered the most sensitive methods for assessing the efficiency of desinfection of cleaned milk equipment (McKinnon and Mansell,1981) but they are not always applicable to UF systems due to the required dismantling of some modules. If soils are left bound to the membrane, no sanitizer can be expected to work properly and sterile filth is not a desirable objective (Smith and Bradley,Jr., 1986).

The sanitation of reverse osmosis and ultrafiltration equipment was also studied by McDonough and Hargrove (1972). After cleaning and sanitization, the equipment was filled with sterile water and samples were taken immediately and periodically for an additional 48 hr and plated as a check on sterility. They found that complete flooding of the equipment was necessary and that could not be assured unless the modules were mounted in a vertical position.

Whittaker et al. (1984) evaluated cleaning strategies for removal of biofilms from reverse osmosis membranes. Cleaning effectiveness was scored by scanning electron microscopy, which required the cutting of sections of the membrane.No treatment or combination of treatments was completely effective at all stages of biofilm development.

Radioisotope tracing of soils was used by Harper and Moody (1981) to evaluate the efficacy of enzymatic cleaning

of a tubular reverse osmosis system. Radioactive labelled inorganic phosphate was added to whey, which was used to soil the membranes. Their conclusions were that permeate flux regeneration and permeate conductivity measurements were not accurate enough techniques for testing of cleaning efficiency, whereas radioisotope tracing of soils gave results consistent with microbiological evaluation. The radioactive tracer technique for testing cleaning efficiency is a useful research method, but suffers the drawback of not being suitable for use in food processing systems.

Uptake of chlorine by the oxidizable material left on non-cellulosic ultrafiltration membranes after cleaning was used as an indicator of cleanliness by van Altena (1975). The test membrane was fouled with a butter milk solution and then cleaned using an alkaline backflush. This cleaning procedure resulted in full recovery of the membrane water flux. Chlorine uptake by the cleaned membrane was determined as a function of cleaning time and compared to the chlorine uptake of a virgin membrane. The uptake of chlorine was greater in soil-containing membranes, but even in virgin membranes some dissipation of free chlorine could be observed, which decreases the accuracy of the technique. In addition, this method would not be suitable for assessing cleanliness of cellulose acetate membranes, which can tolerate only brief exposure to chlorine at 10-50 ppm level (McDonough and Hargrove,1972; Beaton,1979).

Enzyme immunoassays

The specificity that antibodies exhibit against antigens is one of the most remarkable achievements of biological evolution. Minor modifications of complex macromolecules can be recognized by the humoral immunological system. In addition, immunoglobulins can be used to measure small molecules (Harris, et al., 1982).

Enzyme immunoassay procedures are increasing in popularity for the detection of many antigens and antibodies. Although many of these assay systems are quite similar to radioimmunoassay procedures, their increasing usage can be attributed to the fact that the detector antibody is labeled with an enzyme rather than a radioisotope, eliminating the hazards and problems associated with the preparation, measurement and disposal of radioactive material (Parkinson et al.,1982).

Enzyme immunoassays are based on the labeling of an immunoreactant with an enzyme. Following a series of antigen-antibody reactions, the quantity of labeled reactant involved in the reaction is quantitated by the addition of enzyme substrate. The amount of substrate conversion is measured either by determining the accumulation of product or by kinetic measurements. The colorimetric magnification of the enzyme-substrate reaction allows for the measurement of small quantities of immunoreactants (Harris et al., 1982).

The enzyme selected as a marker should have a relatively high turnover rate. One of the fundamental benefits of using the enzyme as a marker is the amplifying ability of the enzyme resulting in a more sensitive detection method (Nakane,1982).

One of the most important assays with enzymes as a label is the enzyme-linked immunosorbent assay (ELISA). The first description of such technique appeared in 1971 and since then several hundred publications have appeared on the subject (Schuurs and van Weemer,1977). ELISA methods are analagous to those used in radioimmunoassay and immunofluorescence and can be used to assay both antibodies and antigens at a high level of sensitivity and reproducibility yet employing relatively simple and inexpensive equipment. Horse-raddish peroxidase and alkaline phosphatase have been predominantly used as enzyme labels (Ceska,1978).

The simplest noncompetitive indirect ELISA involves the coating of the antigen to the well of a microtiter plate, followed by reaction with an unconjugated antibody. The excess of unbound antibody is washed off and an enzyme-labeled antiglobulin directed against the immunoglobulin subclass of the animal source of the antibody is added. Following a wash, a chromogenic substrate is added and optical density is determined (Harris,1982).

Utilization of immunoassays in dairy products

. Due to their great sensitivity and high specificity immunoassays have been increasingly used as detection tools for the analysis of a variety of dairy products. ELISA was used by Lembke and Teuber (1979) for the detection of bacteriophages in whey. The assay was able to detect at least 10^7 plaque forming units of bacteriophages which were specific for lactic streptococci.

ELISA has been also used by different authors for the detection of aflatoxin M_1 in dairy products. Using a highly specific antibody against aflatoxin M_1 , a radioimmunoassay (RIA) and an ELISA were developed by Pestka et al. (1981) for the quantitation of the aflatoxin in milk. As low as 0.25 ng M_1/ml in artificially contaminated milk (raw, whole, skim) could be detected by ELISA in 3 hours without extraction or cleanup. Fremy and Chu (1984) used ELISA for determining aflatoxin M_1 levels in dairy products and found that the detection limits for M_1 in milk, yogurt,Cheddar and Brie cheeses, were 10, 10, 50 and 25 ppt (ng/Kg), respectively. The same assay was used by Jackman (1985) to determine aflatoxin M_1 in raw milk and detection at levels as low as 0.1 ng/ ml was achieved.

The detection of casein in milk and other foodstuffs has been done by means of immunoassays. Using rabbit anti-casein serum, in a radial immunodifusion assay, Klostermeyer and Offt (1978) were able to determine the total casein

content in insoluble foods or feed of complex composition. The Ouchterlony's radial double immunodifusion technique was used by Gombocz et al. (1981) for the detection of cow's milk caseins in ewe's milk. Their method could ensure reliable detection of as little as 5% cow's milk added to ewe's milk. The casein content of milk was estimated by ELISA by Rittenburg et al.(1984). Using a competitive assay in which peroxidase was covalently coupled to casein, the authors found that the lowest limit of sensitivity ocurred at a concentration of approximately 0.5 ug casein /ml of milk.

Heppell (1985) used ELISA for the detection of residual antigenic milk proteins after graded heat treatments. It was found that the assay was capable of detecting levels of purified β-lactoglobulin down to 10 ng/ml.

MATERIALS AND METHODS

Preparation of antigens

Milk proteins were used as antigens in the immunization of rabbits. Fresh, pasteurized skimmilk was dialysed overnight at 5 C against two changes of 0.03 M sodium citrate. After dialysis part of the milk was freeze-dried and part was precipitated at 40 C by adjusting the pH to 4.6 with N HCl, followed by filtration on Whatman No. 42 filter paper. The filtrate was freeze-dried, to be used for testing cross-reactivity of the antibodies prepared against milk proteins.

Discontinuous polyacrylamide gel electrophoresis

To assess the purity and identity of the milk and whey proteins to be used in the immunoassays, discontinuous polyacrylamide gel electrophoresis was performed according to the method of Ornstein (1964). All electrophoretic studies were performed in 6mm I.D., 2mm walled and 125 mm length glass tubes. Running gels contained 9% acrylamide and 7 M urea. Casein (α- casein and β-casein) and whey protein (α-lactalbumin and β-lactoglobulin) standards

were bought from Sigma Chemical Company (St. Louis, MO). All samples and standards were dissolved in 2 ml of 7 M urea extraction solution and made to 3 ml with distilled water, followed by application of an aliquote on top of the spacer gel. Bromophenol Blue was added to each sample as a marker. Electrophoresis was carried out for about 3 hours, at pH 8.3, in a water - cooled Bio-Rad Model 150-A electrophoresis apparatus (Bio-Rad Laboratories, Richmond, CA) and power was supplied by a Bio-Rad Model 500 power supply.Gels were stained for protein with Coomassie Brilliant Blue G250 (0.04%) for 15 hours and destained in 10% acetic acid solution, for 1 hour, in a Bio-Rad Model 1200 A electrophoretic destainer.

Gels were scanned at 550 nm using a Beckman DU spectrophotometer Model 2400 (Beckman Instruments, Inc., Fullerton, CA) equipped with a gel scanner Model 2520 and a Photomoter 252 (Gilford Instrument Laboratories, Inc., Oberlin, OH). This system was summoned to an HP Integrator Model 3380 S (Hewlett Packard, Avondale, PA), where individual protein peaks were recorded.

Rabbit immunization

Two New-Zealand White rabbits were immunized every 10 days for about 40 days; fifty milligrams of the freeze-dried milk proteins were mixed with 8 ml of saline-Freund adjuvant (Difco Laboratories, Detroit, Mi) 1:1 and from the resulting emulsion 0.4 ml was injected intramuscularly into the upper

thigh and 0.6 ml subcutaneously on the upper back of the rabbits. The first two boosters were emulsified with complete Freund adjuvant and the following ones with incomplete adjuvant. Before bleeding, the ear of each rabbit was cleaned, shaved and swabbed with xylene to dilate the vein. Approximately 25 ml of blood were drawn via marginal ear vein at regular intervals and kept at 5 C until red cells were removed by centrifugation at 5,000 x g.

Antisera purification

The antisera were purified by repeated precipitation in saturated ammonium sulfate solution (Herbert et al., 1973). With constant stirring, ammonium sulfate was dropwise added to the sera, equivalent to half the volume of the serum to be purified; the mixture was gently stirred for 20 minutes and centrifuged at 1,400 x g for 30 minutes to pack the precipitated protein. The clear supernatant was discarded and the pellet was resuspended and dissolved in PBS to a final volume equal to the volume of original serum. This initial procedure was repeated two more times and finally the purified sera were dialysed against 2 changes of 0.01 M PBS solution (3 liters), at pH 7.2 , at 5 C , for 24 hours.

Antibody titration by ELISA

An indirect non-competitive ELISA was used for the determination of the antibody titer, and was based on procedures described by Warner et al. (1986) and Liu et al. (1985). In this assay, 100 ul of milk protein solution (10 ug of freeze-dried milk proteins in 1 ml of 0.1 M sodium carbonate buffer, pH 9.6) were added to each well of a 96-well microtiter plate (Microtest III, Becton Dickinson Labware, Oxnard, CA) and incubated overnight at 4 C. Wells were washed 3 times with 300 ul of 0.1M phosphate buffer in 0.15 M saline, pH 7.2 (PBS) containing 0.2% (vol/vol) Tween 20 (PBS-Tween). Non-specific binding (NSB) was decreased by incubating each well for 30 min at 37 C with 300 ul of 0.5 % (wt/vol) ovalbumin in 0.1M PBS, followed by four washes with PBS-Tween. Wells were then incubated for 1 h at 37 C with 50 ul of rabbit milk protein antiserum diluted serially in 0.1 M PBS. Wells were washed 6 times and reacted for 30 min at 37 C with 50 ul of goat anti-rabbit peroxidase conjugate (Accurate Chemical & Scientific Co., Westburg, NY), diluted 2:1,000 in 0.5 % ovalbumin in PBS. Wells were washed six more times with PBS-Tween and bound peroxidase was determined by the reaction for 3 min with 100 ul of substrate consisting of 22 ml citrate buffer (pH 4.0), 2ml 2,2'-azino-di-3-ethyl-benzothiazoline-6-sulfonate (ABTS) and 8 ul hydrogen peroxide (30%). The reaction was terminated by the addition of 100 ul of citric acid

(6.3 g/l) stopping reagent. Absorbance at 414 nm was then read and the results plotted to give the antibody titer.

To verify the reactivity of the anti-milk proteins antibodies against whey proteins, a similar procedure was carried out using a solution of the freeze-dried whey proteins (in PBS) to coat the microtiter plate wells.

After determination of titer, the antiserum was kept frozen at -80 C in aliquotes of 200 and 500 ul.

Preparation of enzyme-antibody conjugates

In order to simplify the immunoassay which would eventually be used to detect proteinaceous soils in UF membranes, the coupling of an enzyme-label to the antibody was tried, by two different procedures. The enzyme selected as a label was horse-radish peroxidase (HRP) (RZ 3.2, Type VI, No. P - 8375, Lot 15F-9535, from Sigma Chemical Company, St.Louis, MO).

Conjugation by the one-step glutaraldehyde method

The immunoglobulin content of antiserum was determined as described by Dixon (1985). A series of dilutions of the antiserum was prepared and absorbance was read at 280 nm. Immunoglobulin content was derived as follows:

$$A_{280}/1.40 = \text{mg IgG / ml of antiserum}$$

The coupling procedure was based on that described by Avrameas (1969). To 1 ml of 0.1M phosphate buffer, pH 7.2 (containing about 12 mg of IgG), 14.4 mg of HRP were added; while the solution was being gently stirred, 0.05 ml of a 1.0% aqueous solution of glutaraldehyde was added dropwise. The reaction mixture was allowed to stand for 2 h at room temperature and then was dialysed for 24 h , at 5 C , against 2 changes of 2.5 liters of PBS. The precipitate formed was removed by centrifugation for 40 min at 4 C, at 18,000 rpm.

Gel permeation chromatography, using Sephacryl S-200 Superfine (Pharmacia Fine Chemicals, Piscataway, NJ) was carried in a column measuring 30 x 0.9 cm, equilibrated with 0.1 M phosphate buffer, pH 6.8 . Fractions (120, of about 65 ul each) were collected in a ISCO Fraction Collector Model 1200 (Instrumentation Specialties Company, Lincoln, Nebraska). From each fraction an aliquote of 3 ul was added to the wells of a microtiter plate and 100 ul of a chromogenic substrate consisting of 11 ml of citrate buffer (pH 4.0), 1 ml of ABTS and 4 ul of hydrogen peroxide 30% were added. The reaction was terminated 30 sec later by the addition of 100 ul of a stopping reagent (citric acid, 6.3 g/l). The absorbance was read at 414 nm and the fractions corresponding to the first peak were pooled. The titer of the resulting conjugate was measured against freeze-dried milk proteins by the ELISA procedure previously described.

Conjugation by the m-periodate method

The procedure described by Wilson and Nakane (1978) was followed:

1) Six mg of HRP were dissolved in 1.5 ml of distilled water.

2) Three tenths of a freshly-prepared 0.1 M sodium m-periodate was added to the HRP and the solution was stirred for 20 minutes at room temperature.

3) The mixture was dialysed against 1mM sodium acetate buffer, pH 4.4, at 4 C, overnight.

4) The pH of the HRP-aldehyde solution was raised to 9.5 by the addition of 45 ul of 0.2 M sodium carbonate buffer, pH 9.5 .

5) Immediately, 1 ml of antiserum (containing about 12 mg of IgG) was added to the mixture, which was then stirred for 2 hours, at room temperature.

6) Fifteen tenths of a ml of sodium borohydride solution (4 mg/ml) was added and the mixture was left for 2 h at 4 C.

7) The mixture was then dialysed against PBS (pH 7.2) overnight, at 5 C.

8) The conjugate mixture was chromatographed in a column (30 x 2.5 cm) packed with Sephacryl S-200 superfine, equilibrated in PBS.

9) Seventy six fractions of 1.2 ml each were collected and the absorbance of each fraction was determined at 414 and 280 nm.

10) To test for HRP activity, a 25 ul aliquote of each fraction was diluted 1:20 in PBS and from this solution, 8 ul were applied to the wells in the microtiter plate. A chromogenic solution (as described in the glutaraldehyde procedure) was added and absorbance was read at 414 nm.

11) The fractions in the first peak area which presented an $A_{414} : A_{280}$ ratio of approximately 0.3 to 0.6 were pooled and submitted to titer determination by an ELISA (as previously described) against milk proteins and whey proteins.

12) After titer determination, the conjugate was freeze-dried.

In the procedure a ratio HRP:IgG of 1:2 was used. The same procedure was carried out again changing this ratio to 1:1.7 and using 30 % more sodium m-periodate, to increase the chances of a successful enzyme-antibody coupling.

Skimmilk and whey ultrafiltration

Ultrafiltration of skimmilk and whey was carried out in an Amicon TCF-10 (Amicon Co., Danvers, MA) thin-channeled spyral type UF unit, with 0.38 mm channel depth and 90 mm in diameter, with a maximum internal volume of 600 ml and

effective filtration area of 40 cm^2. The unit was equiped with Koch HFK-131 polysulfone membranes (Koch Membrane System,Inc., Wilmington,MA) with a nominal molecular weight cut-off of 5,000 and attached to a peristaltic pump which delivered the concentrating fluid at a speed of 0.45 l/min. The system was operated at partial recycle, only the retentate being returned to the feed reservoir. Fresh, pasteurized skimmilk (140 ml) was ultrafiltered at 30 C and under 40 psi of pressure, until a concentration factor of 2X was reached. This procedure reduced flux to about 63 % of the original value observed at the beginning of concentration . Fresh whey was filtered twice in gauze and concentrated in a similar way. One hundred and fifty milliliters of whey were ultrafiltered until a concentration factor of 7.5 X was reached. This procedure reduced permeate flux to about 50% of the original value. After removal of retentate, the system was flushed with distilled water, at 30 C, to remove loosely held soil, followed by the cleaning procedure.

Cleaning procedure

The unit was cleaned with Divosmose U.F.A. (Diversey-Wyandotte Co., MI) (0.9% v/v, pH 11); the cleaner (100 ml) was recycled at 30 C for different times (10, 20,40 or 60 min) to study the influence of cleaning time on flux recovery and the eventual correlation with residual

proteinaceous soils in the membrane; in some cases, after being soiled with skimmilk or whey, the membrane was not subjected to a cleaning cycle beyond the rinsing procedure with water. At the end of the cleaning procedure the permeate flux was determined with water (30 C, 12.5 psi) and compared to the original value obtained under the same conditions.

Application of ELISA (Method D)

In this assay a dismantling procedure, referred here as Method D, requiring the removal of the membrane from the system, was applied. The membrane was mounted on a Millipore filter holder (Millipore Filter Co., Bedford, MA) and 4 ml of rabbit milk protein antiserum (diluted 1:10,000 in PBS) were added. After 4 min ,the membrane was washed 5 times with 5 ml of PBS-Tween. Four milliliters of goat anti-rabbit peroxidase conjugate (diluted 1:10,000 in PBS) were then added to membrane ,which, after 4 min, was washed as previously described. Finally, 6 ml of a color developing solution (0.5 ml ABTS and 4 ul hydrogen peroxide in 5.5 ml of 0.05 M citrate buffer, pH 4) were added and allowed to react for 4 min. The solution absorbance was recorded at 414 nm.

Application of ELISA (Method ND)

In this assay a non-destructive, two-step procedure, referred here as Method ND (non-dismantling) was applied. The membrane was rinsed with water in the thin-channel unit, followed by circulation (4 min, 30 C, 10 psi) of 60 ml of rabbit milk-protein antiserum diluted 1:500 in PBS. Following flushing, the system was rinsed 3 times with 60 ml each of PBS-Tween to remove excess of unbound antibodies. Fifty milliliters of goat anti-rabbit peroxidase conjugate (diluted 1:1,250 in PBS) were circulated for 4 min (30 C, 10 psi) and flushed. The system was rinsed again with PBS-Tween as previously stated, followed by a pre-elution with 60 ml of sodium chloride 2M pH 8.5 (4 min, 30 C, 10 psi). Finally, the dissociation of the antibody-antigen complex was carried out by the circulation of distilled water adjusted to pH 7.4 with dilute sodium hydroxide. A 2.5 ml aliquote was collected from each solution circulated in the system, subsequent to the circulation of the rabbit milk-protein antiserum. To these aliquotes were added 200 ul of the chromogenic solution (0.5 ml of ABTS and 5 ul of hydrogen peroxide 30 % in 11 ml of 0.05 M citrate buffer, pH 4). Color was allowed to develop for 30 min, after which absorbance was recorded at 414 nm.

In order to simplify the operations, the technique described above was also carried out as a one-step procedure,using only the rabbit anti-milk protein peroxidase

conjugate (diluted 1 : 500 in 50 ml of PBS) obtained by the m-periodate conjugation procedure previously described, for the detection of proteinaceous soils eventually bound to the membrane. The technique adopted was the same as previously described, the only difference being the utilization of whey (150 ml , fractionated to a 7.5 X CF) to soil the membranes and the exclusion of the goat anti-rabbit peroxidase conjugate from the detection procedure.

Determination of a non-specific binding baseline

When the method ND was applied, as a two-step procedure, a non-specific binding (NSB) baseline was determined, based on the binding of antibodies to virgin membranes which were not soiled with skimmilk or whey. This value was recorded as absorbance at 414 nm and any value above that should then be atributed to the presence of proteinaceous soils in the membrane. When soiled membranes were submitted to the cleaning procedure, upon removal of all soils, the application of Method ND of detection should result in absorbance values similar to those established as NSB values.

In order to determine the influence of repeated skimmilk concentration and washing procedures on the NSB values, 3 membranes were subjected to a cycle of 5 skimmilk concentrations (as previously described) and washing procedures, followed by the application of the two-step

Method ND of detection of soils. The alkaline washing procedure previously described was complemented by an acidic cleaning carried out by circulation, for 20 min, of 100 ml of a phosphoric acid solution (0.35 % v/v, pH 2.0), at 30 C. Membrane original fluxes were recorded and compared to values obtained under the same conditions after each washing procedure.

Nitrogen determination in the membranes

In order to find out about any correlation between the absorbance values of both Methods D and ND and the amount of proteinaceous soils in the membranes, nitrogen determination was carried out in the latter. Analysis were performed using a micro-Kjeldahl apparatus. The digestion mixture consisted of 100 mg of membrane (dried for 18 hours, at 50 C), 1.95 g of catalyst mixture (40 mg of mercuric oxide added to 1.91 g of potassium sulfate), 2 ml of concentrated sulfuric acid, 2 ml of distilled water and 4 boiling chips. Samples were digested for 90 minutes and the digested mixture was neutralized with 10 ml of a sodium hydroxide (60%)- sodium thiosulfate (5%) solution and the ammonia released was steam distilled into 4% boric acid; the ammonium borate complex was titrated with 0.02 N hydrochloric acid using an indicator consisting of 0.1% methyl red and 0.03% methylene blue in 60% ethanol. For each sample the test was done in duplicate.

Experimental design

Results were analysed using one-way analysis of variance for a completely randomized design (Gill,1978).

To verify a possible relationship between two variables, the r coefficient was determined by simple regression analysis, along with a Student's T-test to check for the validity of the assumptions (Bhattacharyya and Johnson, 1977).

The data were analyzed by the following statistical model:

$$y = \beta_0 + \beta_1 x + E$$

where: y = random effects of treatment sample

β_0 = line intercept

β_1 = line slope

x = random effects of control sample

E = residual error

Whenever needed, differences among the means were analysed for significance by Duncan's Multiple Range Test (Gill, 1978). All statistical analysis were at 5% level of significance.

RESULTS AND DISCUSSION

Electrophoresis of the antigen

Electrophoresis on 9% acrylamide gels of the milk proteins sample, produced three distinct bands which are depicted in Figure 1. Both in the sample (B) and standard (A) densitometric patterns, the α-casein (α-CN) peak appears clearly as the leading band, followed in the sample by a smaller band partially representing the β - lactoglobulin (β-LG) fraction, which is overlapped by the β - casein (β-CN) peak. These are typical densitometer tracing of milk proteins, as shown by different authors (Ng - Kwai - Hang and Kroeker, 1984; Swaisgood, 1973; Yaguchi et al., 1968). Due to the presence of whey protein fractions in the sample (indicated by the β-LG band), one could expect that antiserum resulting from rabbit immunization with such antigen, would also show reactivity towards whey proteins, as was actually found.

Figure 2 shows the densitometric patterns of the whey proteins fraction prepared for testing the cross-reactivity of the antiserum obtained. The leading bands are represented by the β-LG fraction, followed by peaks representing the

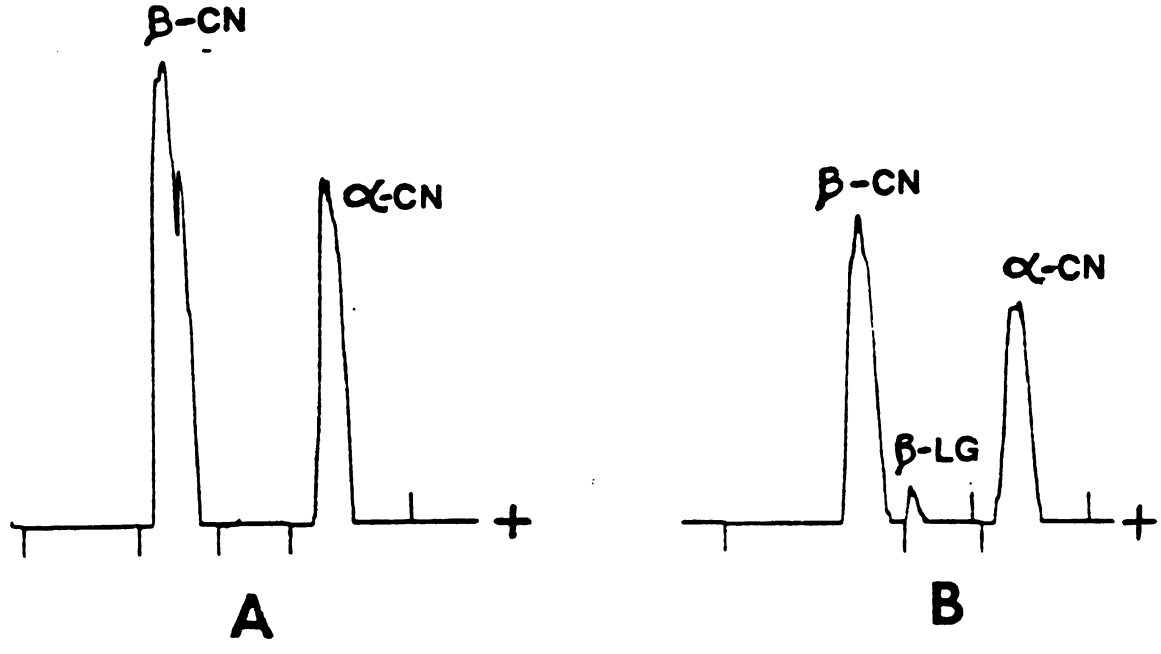


Figure 1-Densitometric traces of milk protein sample used as antigen (A = standard, B = sample).(Gels contained 9% acrylamide and 7 M urea and discontinuous electrophoresis was performed at pH 8.3 for about 3 hours).

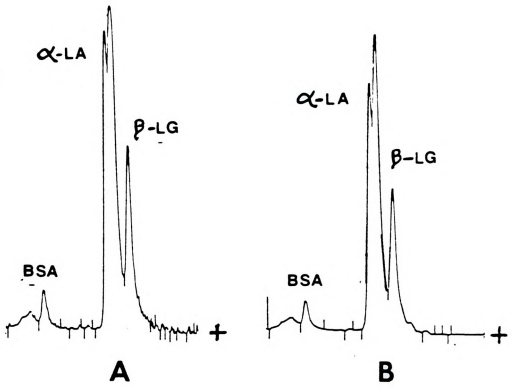


Figure 2-Densitometric traces of whey protein sample used in the immunological assay (A = standard , B = sample). (Gels contained 9 % acrylamide and 7 M urea, and electrophoresis was performed at pH 8.3 for about 3 hours).

α - lactalbumin (α-LA) and bovine serum albumin (BSA) fractions, both in the standard (A) and sample (B) tracings. These patterns are in agreement with those found by other authors (Ng-Kwai-Hang and Kroeker, 1984; Farah,1979; Hillier,1976). Due to presence of BSA in the sample, ovalbumin was used instead as a blocking agent to decrease non-specific binding (NSB) effects during the antibody titration by means of ELISA.

Antibody titration

Rabbits began to produce sera with anti-milk protein titer at approximately 13 weeks after the initial immunization. The results from a non-competitive indirect ELISA carried out by the incubation of milk and whey proteins with appropriate dilutions of rabbit anti-milk protein antiserum and the determination of specific interaction with a goat anti-rabbit peroxidase conjugate are shown in Figure 3. The last well in the dilution series to yield a distinct absorbance value (0.64) had a dilution of 1:10,000 and was designated as the titer endpoint. When tested against whey proteins, a similar titer was found. This result was already expected, considering that the antigen preparation contained also whey protein fractions (Figure 2), besides casein.One could expect that when skimmilk is ultrafiltered , both kind of proteins would be found as soils on the membrane, as described by Tong et al.

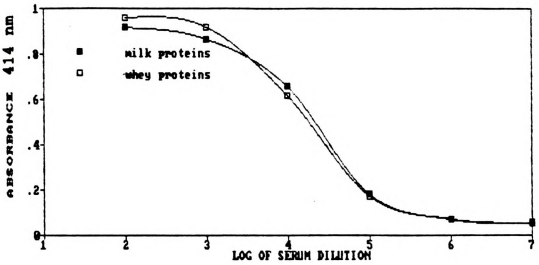


Figure 3 - ELISA titration curve for milk protein antiserum (serum from one rabbit, collected 9 weeks after beginning of immunization procedure. A titer of 1:10,000 was observed).

(1988),which characterizes the additional reactivity observed as an interesting tool in developing a general detection procedure. In addition, the same antiserum could eventually be used to assess cleanliness of ultrafiltration membranes soiled by the concentration of whey only. The technique would be of great interest, since the ultrafiltration of whey has increasingly attracted the attention of the U.S. dairy industry (Cheryan, 1986).

Antibody-HRP conjugation (Glutaraldehyde method)

Enzyme immunoassays are based on the labeling of an immunoreactant with an enzyme. Following a series of antigen-antibody reactions, the quantity of labeled reactant involved in the reaction is quantitated by the addition of enzyme substrate (Harris et al., 1982). Many immunoassays involve the use of an enzyme-labeled antibody directed against the unconjugated antigen-reactive antibody used in first place.

Horseradish peroxidase (HRP) is very commonly used as a marker in ELISA. It is readily available, presents good stability and has a high turnover rate, which increases the sensitivity of any detection method (Nakane, 1982).

Among different methods used for coupling enzymes to antibodies, the glutaraldehyde procedure is one of the most popular and can be used in a one-step method as well as in a two-step method. The action of glutaraldehyde is based on the following assumption: one of the two-aldehyde groups

reacts with an amino group in the first protein (antibody) and the second reacts with an amino-group in the other protein (enzyme marker) (Boorsma, 1983).

The results of titer determination of a conjugate obtained by the glutaraldehyde method are shown in Figure 4 and reveal a very poor yield with great loss of antibody activity. The titer obtained against milk proteins was 1:10, which is negligible. This is probably due to polymerization of the antibodies, forming high molecular weight agregates with great loss of activity.

This phenomenon has been reported by several authors. According to Boorsma (1983) using glutaraldehyde in a one-step method the conjugate is of heterogeneous composition, polymeric of structure and contains relatively few HRP molecules per antibody molecule. Blake and Gould (1984) states that when glutaraldehyde was added to a mixture of an enzyme and an immunoglobulin the conjugates produced were heterogeneous and of high relative molecular mass, which was due mainly to polymerisation of IgG with consequent loss of antibody activity.

When comparing different methods of enzyme-antibody coupling, Adams and Wisdom (1979) found that with the one-step glutaraldehyde method the enzyme and the immunoreactivity yields were of only 1.2 and 5.6% respectively, and polymers with molecular weight ranging from 400,000 to 1,000,000 were formed. These results are in agreement with those of Nakane and Kawaoi (1974) who

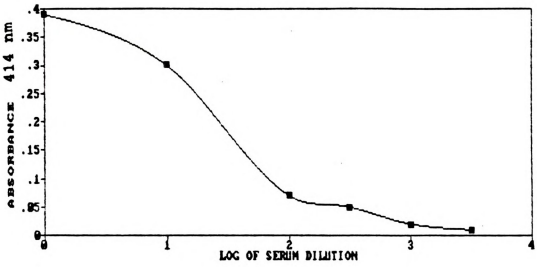


Figure 4-ELISA titration curve for antibody-HRP conjugate (conjugation was tried by a one-step glutaraldehyde method, using 12 mg of rabbit IgG and .05 ml of a 1.0% aqueous solution of glutaraldehyde.Conjugate showed a very low titer, of less than 1:100).

reported that using the same coupling procedure only about 2.0% of the added HRP was conjugated to IgG.

Schuurs and van Weemen (1977) indicate that, in general, conjugates prepared by the one-step method are of a high molecular weight and are assumed to be heterogeneous.

Antibody-HRP conjugation (m - periodate method)

Due to the unsuccessful results obtained with the glutaraldehyde method, a new approach to the coupling procedure was tried using the m-periodate method. This chemical is not an authentic coupling agent because it does not itself act as the bridge between HRP and the antibody. HRP has eight carbohydrate chains on its surface, which can be readily oxidised by m-periodate to yield aldehyde groups. The HRP aldehyde obtained reacts subsequently in a second step with the amino groups of the antibody and the enzyme conjugate will be formed. Self-coupling of the enzyme is prevented by the prior blocking of the amino groups with fluorodinitrobenzene or by carrying out the periodate oxidation at low pH (Blake and Gould, 1984; Boorsma, 1983; Wilson and Nakane, 1978).

The elution pattern of the antibody-HRP conjugate in a Sephacryl S-200 superfine chromatographic column is shown in Figure 5. Increasing the dilution rate of the aliquotes taken from each fraction (25 ul aliquotes diluted 1:20 in PBS) for the HRP activity test resulted in a better profile with a distinctive separation of the two main peaks.

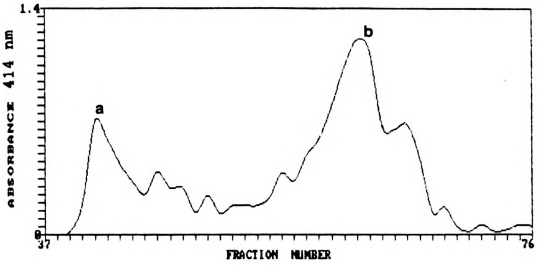


Figure 5-Elution profile of crude antibody-HRP conjugate on Sephacryl S-200 Superfine column (30 x 2.5 cm) equilibrated with 0.1 M phosphate buffer, pH 6.8 Peak A represents conjugated antibodies and peak B represents free peroxidase (conjugate was prepared by the m-periodate method).

Before pooling the fractions corresponding to the first peak, the absorbance of each fraction at 280 and 414 nm was determined, in order to establish a ratio between the content of total protein present (IgG) and the amount of bound HRP (mainly in the first peak area). According to Wilson and Nakane (1978) the HRP-IgG conjugate is the largest component (> 200,000 M.W.) of the conjugation mixture applied to the column and should be eluted first. The authors found that the best conjugates with respect to retention of antibody activity are those which have an $A_{414\ nm}$: $A_{280\ nm}$ ratio of aproximately 0.3 to 0.6. This ratio describes a conjugate having an average of 1 to 2 HRP molecules bound to each IgG.

Figure 6 shows the absorbance profile of the fractions eluted from the column. A ratio of 0.10 between the absorbances at 414 and 280 nm was observed in the region of the first peak. In the region of the second peak, a ratio of 1.16 was observed, but it does not characterize an extensive binding of HRP to the IgG and can be explained by the high content of unbound HRP (40,000 M.W.) coming out at the end of the elution procedure along with a low content of unconjugated IgG.

A very low titer (Figure 7) was found for the antiserum resulting from the pooling of the fractions which characterized the first peak in the elution profile (Figure 5). According to Wilson and Nakane (1978), HRP-IgG conjugates with A_{414} : A_{280} ratios less than 0.20

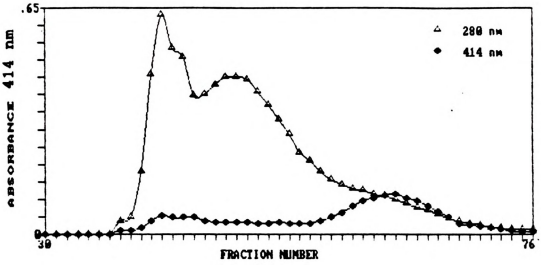


Figure 6 - Absorbance profile of antibody-HRP conjugate fractions (trial I) eluted from chromatographic column (a 30 x 2.5 cm column was packed with Sephacryl S-200 Superfine and equilibrated with 0.1 M phosphate buffer pH 6.8. Conjugates were prepared by the m-periodate method).

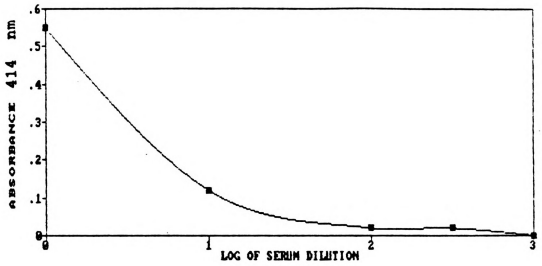


Figure 7 - ELISA titration curve for antibody-HRP conjugate prepared by the m-periodate method (conjugate showed a poor activity against milk proteins, with a titer of less than 1:100 , in trial I).

have low antibody activity, which is likely due to competition with unlabeled antibody.

A second trial, using the same basic procedure was then carried out, with some modifications; a ratio HRP:IgG of 1:1.7 was used (instead of 1:2 used in the first trial) and the amount of sodium m-periodate was increased by 30% . Wilson and Nakane (1978) observed that the activity of the conjugate can depend on the particular lot of HRP used and on the amount of sodium m-periodate, which should be increased if less than the optimal amount of conjugation is achieved in the first trials.

In Figure 8 the optical density profile of the eluted fractions in the second trial with the m-periodate method is shown. When compared to results shown in Figure 6, one can see that a much greater amount of HRP is present in the area of the first peak, which seems to indicate more binding of HRP to the IgG. An aborbance ratio ranging from 0.314 to 0.395 was found between fraction (of 1.25 ul each) numbers 40 and 55, which were pooled together and freeze-dried.

The results of titer determination for the conjugate are shown in Figure 9. When tested against milk and whey proteins, the conjugate showed a titer of about 1:500, which indicates a loss of 90% of the original activity. These results are in agreement with those found by Adams and Wisdom (1979) who reported that in a typical experiment using the m-periodate method, 8% of enzyme activity and 5.6%

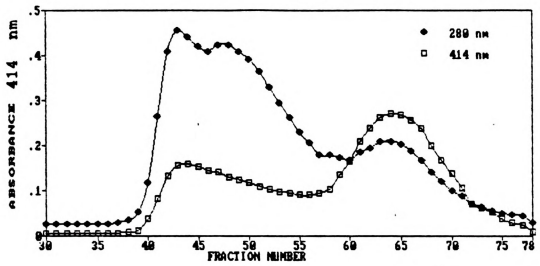


Figure 8 -Absorbance profile of antibody-HRP conjugate fractions (trial II) chromatographed on Sephacry S-200 Superfine column (30 x 2.5 cm) equilibrated with 0.1 M phosphate buffer, pH 6.8 (conjugates were prepared by the m-periodate method).

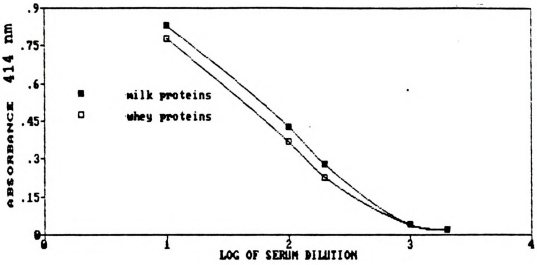


Figure 9 - ELISA titration curves for antibody-HRP conjugate prepared by the m-periodate method. (conjugate showed a titer of 1:500 against milk and whey proteins in trial II).

of the antibody activity were conjugated, although in some experiments as much as 20% of the enzyme activity was incorporated. The molecular weight of the conjugate was in excess of 500,000. Results reported by different workers vary somewhat, but the efficiency of this method seems to be higher than that of the glutaraldehyde methods (Adams and Wisdom, 1979; Wilson and Nakane, 1978; Nakane and Kawaoi,1974).

Other studies have revealed the HRP conjugate to be a highly heterogeneous mixture with molecular weights ranging from 200,000 to several million daltons. There seems to be an inverse relationship between retention of the HRP activity and the immunological activity of the protein to which the enzyme is coupled (Schuurs and van Weemen, 1977; Boorsma, 1983).

Application of adapted ELISA by method D

The main goal of this research was the development of a method able to detect the presence of proteinaceous soils in UF membranes. The analytical approach was based on ELISA procedures. All efforts were concentrated in trying to reproduce the steps usually carried out in the immunoassay, using the UF membrane as the solid support for the immunoreactions (see flow chart) . For that purpose, in this first approach, the membranes had to be removed from the thin-channel apparatus, which characterized the procedure as "dismantling" (D) .

METHOD D

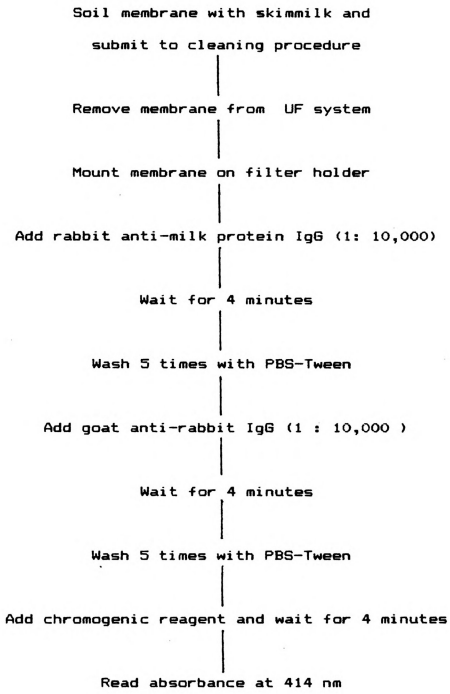

Soil membrane with skimmilk and
submit to cleaning procedure
Remove membrane from UF system
Mount membrane on filter holder
Add rabbit anti-milk protein IgG (1: 10,000)
Wait for 4 minutes
Wash 5 times with PBS-Tween
Add goat anti-rabbit IgG (1 : 10,000)
Wait for 4 minutes
Wash 5 times with PBS-Tween
Add chromogenic reagent and wait for 4 minutes
Read absorbance at 414 nm

Figure 10 shows the typical profile of changes in permeate volume and flux during ultrafiltration of 140 ml of skimmilk in the thin-channeled spiral type UF unit. A concentration factor of 2X was reached when 70 ml of permeate were collected, which usually required about 220 minutes of operation at 30 C under a pressure of 40 p.s.i. A steady decline in flux was observed, dropping from 25 ml/hour at the beginning to about 16 ml/hour at the end of the operation. Flux decline has been observed in all ultrafiltration procedures and was mainly attributed to the progressive fouling of the membrane by accumulation of macromolecular or colloidal particles such as proteins and lipids on the membrane surface (Kuo and Cheryan, 1983). Another cause of flux decline is the formation of a concentration polarization layer, which is an increase in the concentration of solute molecules close to the membrane. Precipitation occurs when the concentration exceeds the solubility limit for a particular solute (Skudder et al. 1977).

As shown in Figure 11, a very poor correlation ($r = -0.39$, $n = 24$, $P > 0.05$) was found between cleaning time and the optical density values. The data are shown in more details in Table 1. No significant difference ($P > 0.05$)was found between the average absorbances when membranes were cleaned for different periods of time, except when no cleaning procedure was applied at all.A great variability was observed in the means obtained and the data

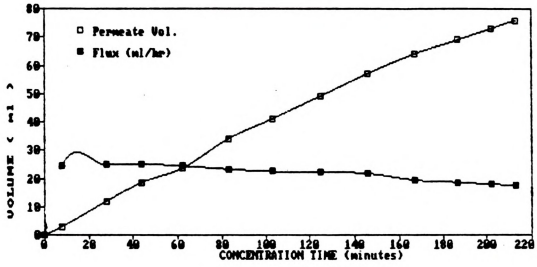


Figure 10 - Typical profile of trends in permeate volume and flux during ultrafiltration of skimmilk in a thin-channeled spiral type UF unit. As the volume of permeate increased, a decline in flux was observed.

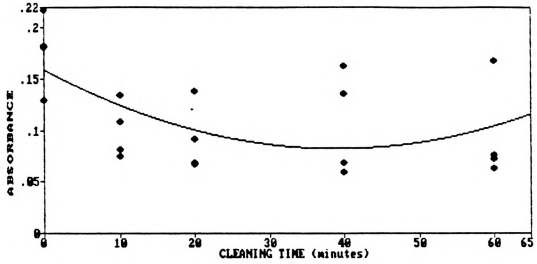


Figure 11 - Correlation between absorbance at 414 nm and membrane cleaning time as determined the dismantling method D (r = - 0.39 , P > .05). (Results indicate that soil removal is not related only to cleaning time).

Table 1- Absorbance (414 nm)values observed on the dismantling method D after membrane was submitted to different cleaning treatments.

Trial	Virgin membrane	Cleaning time (minutes) 60	40	20	10	0
1	.063	.168	.060	.092	.082	.217
2	.062	.063	.163	.068	.075	.130
3	.062	.077	.069	.067	.135	.181
4	.060	.072	.136	.138	.109	.182
Mean	.062$_b$	.095$_b$	.107$_b$	.091$_b$	.100$_b$	.178$_a$
S	.001	.049	.050	.033	.027	.036
CV	2.04	51.60	47.13	36.42	27.35	20.18
SEM	.0006	.025	.025	.016	.014	.018

S = Standard deviation

CV = coefficient of variation

SEM = standard error of mean

a,b = means with same letters show no significant difference ($P < .05$)

seemed to indicate that cleaning efficiency cannot rely solely upon time, even when all other conditions are kept the same. One possible explanation for this result may have been the variation of pore size in the membrane matrix (entrapment of soils). According to Cheryan (1986) different membrane materials with the same molecular weight cut-off will appear to give different solute rejection, which has to do with pore size distribution. Even membranes from the same material may present differences in retentivity and flux due to lot-to-lot variability. This may influence soil retention during ultrafiltration and later on, during the cleaning operation, may affect the ability of a detergent to remove soils from different parts of the membrane. It is well known that the porous materials used to construct UF membranes make cleansing more difficult than cleaning stainless steel materials (Smith and Bradley, 1987).

A somewhat better and significant ($P < 0.05$) correlation ($r = 0.77$, $n = 24$) was found between absorbance values and the amount of nitrogen in the membrane, as depicted in Figure 12. An absorbance value of 0.063 (0.062 $\pm$ 0.001) , found from the treatment of virgin membranes (see Table 1) was established as a treshold beyond which higher absorbance values might indicate the presence of proteinaceous soil. This treshold value can be atributed to non-specific binding (NSB) of immunoreactants to the membrane matrix and must be accounted for. According to Goding (1986) a certain degree of NSB is inevitable in

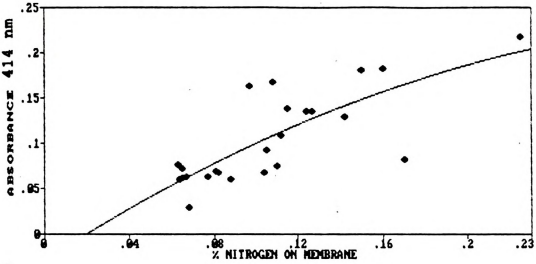


Figure 12 - Correlation between absorbance at 414 nm and the percentage of nitrogen found in the UF membrane, as determined by method D ($P < .05$).(results indicate an interaction between the amount of soil in the UF membrane and the observed absorbance values).

immunoassays. Some is due to properties of the matrix and some to properties of individual molecules in the antibody-containing mixture. Non-specific binding can be caused by interaction of charged groups on proteins with charged groups on the matrix or the presence of hydrophobic interactions. The results in Figure 12 indicate that by increasing the amount of proteinaceous soils, as nitrogen, in the membrane, a corresponding increase in the absorbance values could be observed, as determined by the adapted ELISA performed in method D.

When proteinaceous soils were left in the UF membrane, one could expect that to influence the percentage of flux recovery as determined by water circulation. Results in Figure 13 show that, indeed, a significant correlation ($r = -0.83$, $n = 24$) could be established between the percent return to the original membrane flux and the presence of soils, as nitrogen, bound to the membrane (0.063 to 0.225 % N). Despite being closely associated with the presence of soils in the membrane, as shown, flux recuperation cannot be used as a safe parameter to assess the cleanliness of UF membranes, as found by different authors (Smith and Bradley, 1987; Smith and Bradley, 1986; Harper and Moody,1981).

Indeed, when the absorbance values were correlated to the flux recuperation values, a greater variability was found ($r = -0.70$, $n = 24$, $P < 0.05$), as shown in Figure 14. These results could be due again to variations in the

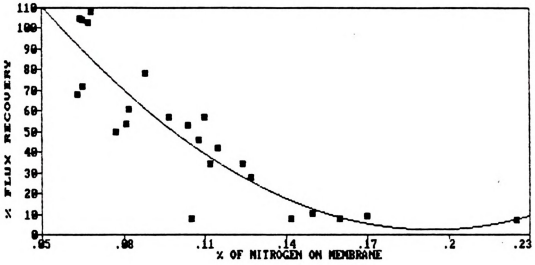


Figure 13 - Correlation between the percentage of flux recovery and the percentage of nitrogen found in the UF membrane, as determined by the dismantling method D. (Flux restoration seems to be related, to a certain extent, to the presence of proteinaceous soils on the membrane). ($r = -0.83$, $P < .05$).

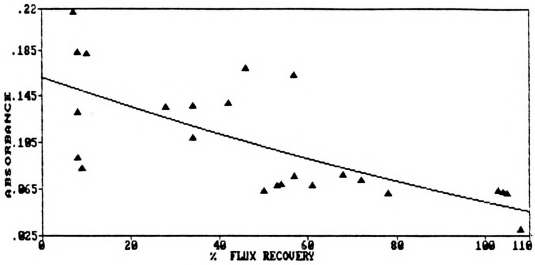


Figure 14 - Correlation between absorbance at 414 nm and the percentage of flux recovery in the UF membrane, as determined by the dismantling method D ($r = -0.70$ $P < .05$). Results indicate that flux recovery relates to soil on the membrane , but seems to be subjected to other factors, as well.

membrane pore size, which might affect flux of different pieces of the material, even if they have the same amount of soil bound to them. It has already been shown (Cheryan, 1986) that it is difficult to characterize the cut-off of a membrane by means of a precise molecular weight, since most membranes do not have completely uniform pores, but show a certain pore distribution.

In spite of a reasonable ability to detect soils in the UF membrane, method D carries a drawback which makes eventual upgrading to commercial usage questionable. Requiring the dismantling of the system prior to running the test would not be technically or economically feasible in most cases. Therefore a non-destructive approach, based on the same principles established for method D, would appear as the more promising approach to the problem of assessing cleanliness of UF membranes.

Application of adapted ELISA by method ND

Antibody molecules bind to antigens through a variety of forces, which may include electrostatic, hydrophobic or van der Waals forces, and hydrogen bonding (Kimball, 1983; Garvey et al, 1977). Antibody-antigen interactions characteristically have low dissociation constants, which can make the disruption of the complex a difficult task (Bureau and Daussant, 1981). One common approach to the desorption procedure is to change the environment of the complex to a point where it cannot be maintained, such as

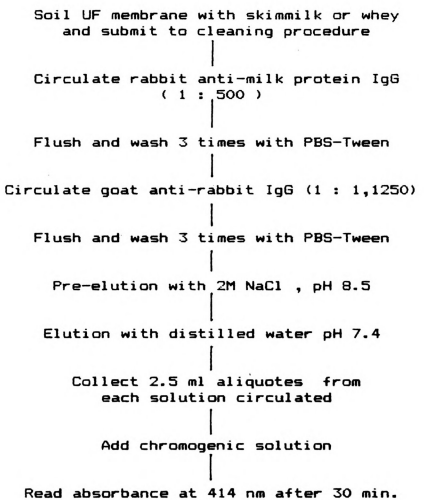
change in pH, temperature and ionic strength (Cooper, 1977).

Method ND was based on the assumption that the binding of an antibody to the appropriate antigenic determinant by the mentioned noncovalent forces, can be disrupted by changes in the environment surrounding the immunoreactants. However, when enzyme labeled antibodies are used in the procedure, extreme conditions of pH or reagent concentration must be avoided to prevent disturbing the stability and function of the enzyme used as a label (Bureau and Daussant, 1981). Loss of antibody activity could also occur in the elution procedure, but for the present study this should not interfere with the final results, since after being eluted from the system ,the complex will give results due to the presence of the enzyme label.

When dealing with systems involved in foodstuff treatment, great care must be exercised to avoid use of any compound which could be a potential contaminant to the product or harmful to the consumer. Therefore, the choice of chemical compounds was somewhat limited when setting up these experiments.

A gentle and simple procedure for desorbing antibodies was tried, as depicted in Figure 15. After skimmilk ultrafiltration, the membrane was cleaned with detergent as previously described, rinsed with water and submitted to the adapted ELISA in the thin-channel unit, without any desmantling whatsoever (see flow chart). The first

METHOD ND (two-steps)



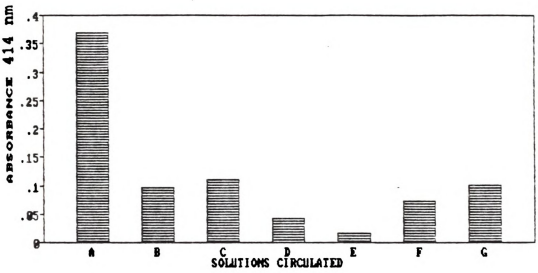


Figure 15 - Results from application of method ND to virgin membranes; solutions circulated in the thin-channel system are: A (goat anti-rabbit IgG before circulation), B (goat anti-rabbit IgG after circulation), C (first PBS-Tween wash), D (second PBS-Tween wash), E (third PBS-Tween wash), F (pre-elution with 2 M NaCl pH 8.5), G (elution with distilled water pH 7.4) (averages of 6 trials).

experiments were conducted using virgin membranes, in order to establish a NSB baseline. Following circulation of the rabbit milk-protein antiserum diluted 1:500 in PBS, the unit was rinsed 3 consecutive times with PBS-Tween to remove excess of unbound antibodies.The goat anti-rabbit IgG was then circulated, followed again by 3 consecutive washes with PBS-Tween. The average absorbance values were 0.111, 0.042 and 0.016 for the first, second and third washes (Table 2), respectivelly, showing clearly the continuous removal of unbound antibodies from the system.

In these initial trials virgin membranes were used and therefore any antibody binding to the membrane could be attributed to NSB forces, since no milk proteins were present in the system. According to Goding (1986) NSB is due basically to interactions of charged groups on antibodies with charged groups on the matrix and hydrophobic interactions between nonpolar groups in the binding site of antibodies and others in the solid support. One could expect those kinds of interactions to occur between the antibodies and the UF membranes, because the latter is electronically charged due to presence of SO_2 groups in the polymeric sulfone (Cheryan, 1986).

It was observed that by circulation of a 2 M sodium chloride solution, at pH 8.5, antibodies were indeed removed from the system, as shown in Table 2. The average absorbance value in the eluent was 0.074. Because the net charge of proteins varies with pH (protons are removed when the pH is

raised), NSB due to electrostatic effects are often pH-sensitive. Also, increasing the salt concentration of the reaction medium reduces the strength of the binding by providing competing ions for the sites (Goding, 1986; Kimball, 1983; Scopes, 1982).

High salt concentration may be successful if the interaction happens to be largely electrostatic, but it would strength any hydrophobic bonds. The binding of an antibody to the appropriate antigenic determinant is achieved by several noncovalent forces (Kimball, 1983). Since milk proteins containing different fractions of caseins and whey proteins (see Figure 1) were used in the immunization procedure, the resulting antiserum would contain antibodies with different specificities for each of the antigenic determinants present. One could expect, therefore, that other forces would still cause NSB to the matrix and that by breaking only ionic interactions would not be enough to displace all antibodies nonspecifically bound.

For that reason, another elution procedure was set up, aimed at the disruption of hydrophobic interactions, under relatively mild conditions. Since the strength of hydrophobic interactions is not appreciably affected by changes in pH (Kimbal, 1983), distilled water at pH adjusted to 7.4 was used as the final eluent. An average absorbance value of 0.101 was recorded in the eluent, indicating the capability of water as a desorbing agent (Table 2). This

Table 2 - Absorbance (414 nm) values observed in the solutions circulated in the UF unit during the treatment of virgin membranes by the non-dismantling method ND (two-steps).

	Solutions circulated (*)						
Trial	A	B	C	D	E	F	G
1	.363	.101	.110	.034	.017	.069	.101
2	.382	.084	.124	.033	.018	.071	.101
3	.389	.103	.112	.046	.014	.086	.108
4	.362	.094	.112	.048	.014	.066	.094
5	.376	.074	.098	.041	.016	.069	.104
6	.343	.091	.108	.050	.018	.087	.098
Mean	.369	.096	.111	.042	.016	.074	.101
S	.016	.011	.008	.007	.002	.009	.005
CV	4.50	11.92	7.55	17.23	11.35	12.46	4.77
SEM	.006	.004	.003	.003	.001	.004	.002

S = standard deviation, CV = coefficient of variation, SEM = standard error of mean

* A = goat anti-rabbit IgG before circulation
B = goat anti-rabbit IgG after circulation
C = first PBS-Tween wash
D = Second PBS-Tween wash
E = third PBS-Tween wash
F = pre-elution with 2M Nacl pH 8.5
G = elution with distilled water pH 7.4

role was confirmed by Scopes (1982), who indicates that the principle behind this elution system is the capacity of water for considerably weakening hydrophobic forces. Based on these results, an absorbance value of 0.106 (0.101 ± 0.005) was established as a NSB baseline, beyond which higher readings could be atributed to the presence of proteinaceous soils in the UF membrane. As it can be seen on Table 2, this method showed smaller mean variability compared to results obtained by method D (see Table 1).

When the method ND was applied to soiled UF membranes, the results indicated its ability to detect proteinaceous soils adhering to the membrane (Figure 16 and Table 3). An average absorbance value of 0.264 was found for the final water elution.These results were compared to those obtained with clean membranes and a significant difference ($P < 0.05$) was found between the absorbance values and nitrogen contents observed in membranes treated by both procedures, as shown in Table 4. In order to check the validity of this assumption, regression analysis was conducted to evaluate the eventual correlation between the percentage of nitrogen in the UF membrane and the absorbance values found in the elution with distilled water. As shown in Figure 17 a strong and significant correlation ($r = 0.96$, $P < 0.05$, $n = 15$) was found between the two parameters. The present results indicate that distilled water at pH 7.4, after a pre-elution with 2 M sodium chloride pH 8.5 , is able to displace the goat anti-rabbit

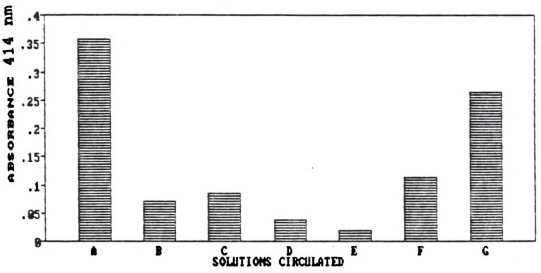


Figure 16 - Results from application of method ND to soiled membranes; solutions circulated in the thin-channel system are: A (goat anti-rabbit IgG before circulation), B (goat anti-rabbit IgG after circulation), C (first PBS-Tween wash), D (second PBS-Tween wash), E (third PBS-Tween wash), F (pre-elution with 2 M NaCl pH 8.5), G (elution with distilled water pH 7.4) (averages of 6 trials).

Table 3 - Absorbance (414 nm) values observed in the solutions circulated in the UF unit during the treatment of soiled membranes by the non-dismantling method ND (two-steps).

	Solutions circulated (*)						
Trial	A	B	C	D	E	F	G
1	.361	.095	.067	.035	.024	.119	.234
2	.384	.085	.084	.035	.021	.131	.197
3	.339	.045	.096	.033	.015	.119	.240
4	.368	.055	.091	.038	.012	.104	.296
5	.349	.071	.088	.044	.018	.102	.314
6	.349	.074	.079	.042	.017	.096	.304
Mean	.358	.071	.084	.038	.018	.112	.264
S	.016	.019	.010	.004	.004	.013	.047
CV	4.47	26.76	12.15	11.51	23.90	11.85	17.83
SEM	.006	.007	.004	.001	.001	.005	.019

S = standard deviation, CV = coefficient of variation, SEM = standard error of mean

* A = goat anti-rabbit IgG before circulation
B = goat anti-rabbit IgG after circulation
C = first PBS-Tween wash
D = Second PBS-Tween wash
E = third PBS-Tween wash
F = pre-elution with 2M Nacl pH 8.5
G = elution with distilled water pH 7.4

Table 4 - Comparison of percentages of nitrogen and water eluent absorbance (414 nm) values observed in the application of the non-dismantling method ND (two-steps) to clean (virgin) and soiled UF membranes.

	Absorbance		% Nitrogen	
Trial	Clean	Soiled	Clean	Soiled
1	.101	.234	.069	.218
2	.101	.197	.063	.162
3	.108	.240	.066	.227
4	.094	.296	.062	.262
5	.104	.314	.066	.291
6	.098	.304	.062	.287
Mean	.101	.264	.065	.241
S	.005	.047	.003	.049
CV	4.77	17.83	4.34	20.33
SEM	.002	.019	.001	.020
	P < .05		P < .05	

S = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

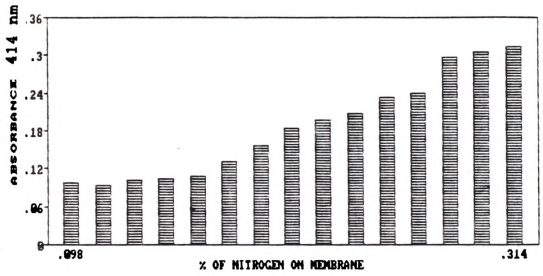


Figure 17- Correlation between the absorbance values of the final eluent (distilled water at pH 7.4) in method ND and the percentage of nitrogen on the UF membrane ($P < 0.05$). Results show that absorbance values found by this method are strongly correlated ($r = 0.96$) to the presence of milk proteinaceous soils on the membrane.

HRP-labelled antibody from its complex with the anti-milk protein antibody or the whole complex from its binding to the proteinaceous soil on the membrane system.

Effects of repeated cleaning cycles on the NSB baseline

The NSB baseline was determined, as previously described, using virgin membranes. However, one could speculate whether the cleaning regime would have an effect on that baseline, assuming that after skimmilk ultrafiltration and cleaning procedure, the membrane would be free of proteinaceous soils. To find out about any eventual interference, three virgin membranes had their original fluxes determined with water and were then submitted to skimmilk concentration five consecutive times, followed each time by a cleaning procedure, flux determination and an ELISA by the method ND.

The original flux in membranes 1, 2 and 3 were 847, 568 and 415 ml/h , respectively. Particularly noteworthy was the variation in flux observed in these 3 pieces of membranes which came from the same lot. These observations confirm the existence of variation in pore size distribution, whose effects seemed to be upgraded by the use of small sections of a unique piece of membrane. The absorbance values for the water elution in the virgin membranes were 0.096, 0.102 and 0.106, for membranes 1,2 and 3, respectively. Theses values were within the standard deviation limits found for

the mean O.D. value taken as NSB baseline (0.101 $\pm$ 0.005), as despicted in Table 2.

As shown in Figure 18, after each cleaning treatment, flux measurements were taken, and considerable variations were observed. In membrane 1 the percentage of return to the original flux ranged from 94 to 96.6 % ; in membrane 2, from 88.4 to 95.1 % and in membrane 3, from 96.4 to 99.5 % . In no case was a total flux restoration observed, which might lead one to think that membranes were not completely clean. However ,as it has been shown before,flux restoration has been found to be an unreliable tool to assess the cleanliness of UF membranes, due to variations in membrane pore size and distribution. Nitrogen determinations could not, of course, be carried out in the membranes after each cleaning treatment, but determinations made after the last treatment did indicate that the nitrogen content had increased from an average of 0.065 % found for virgin membranes to 0.097, 0.114 and 0.116 % in membranes 1,2 and 3 respectivelly. Based on these results, one could not indeed expect that, at least after this last treatment,the absorbance values of the water elution would return to a value within the limits of the NSB baseline found for perfectly clean (virgin) membranes.

The variations observed in the absorbance values of the water elution system after each cleaning treatment, are shown in Table 5 and graphically depicted in Figure 19.When compared to values found for virgin membranes ,no

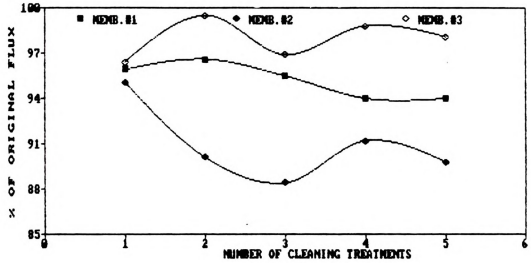


Figure 18 - Variations in water flux restoration after cleaning treatment of three UF membranes. In no case flux was restored to the original level observed in virgin membranes.

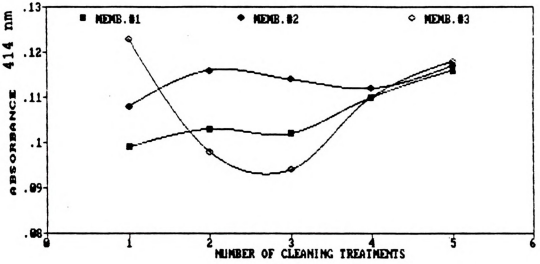


Figure 19- Variations in aborbance values of the water eluent in the non-dismantling method ND after each cleaning treatment of three UF membranes . Despite variations observed , no statistically significant difference was obseved between the results when compared to values found in virgin membranes ($P > 0.05$).

Table 5 - Absorbance (414 nm) values observed in the water eluent when the dismantling method ND was applied to UF membranes which were repeatedly soiled and cleaned.

Trial	Membrane 1	Membrane 2	Membrane 3
1	.099	.108	.123
2	.103	.116	.098
3	.102	.114	.094
4	.110	.112	.110
5	.116	.117	.118
Mean	.106$_a$	.113$_a$	.109$_a$
S	.007	.004	.012
CV	6.50	3.15	11.94
SEM	.003	.002	.005

S = standard deviation, CV = coefficient of variation, SEM = standard error of mean

a = means with same latter showed no significant difference ($P < .05$)

statistical difference was found ($P < 0.05$). The average results shown in Table 5 are very close indeed to values expected for a clean membrane (0.106) despite higher absorbance readings observed in the last treatment, when nitrogen content was also found to be higher. One can speculate that during the other trials nitrogen residues were kept low on the membrane and therefore generated lower absorbance readings, which contributed to lower the overall means shown in Table 5. In membrane 1 the absorbance values varied from 0.099 to 0.116 and presented a significant correlation ($r = -0.81$, $P < 0.05$, $n = 5$) with the water flux measurements, as depicted in Figure 20. A similar trend was observed in membrane 2, whose absorbance values varied from 0.108 to 0.117 and also showed a significant correlation ($r = -0.82$, $P < 0.05$, $n = 5$) with the membrane water flux measurements after each cleaning cycle, as shown in Figure 21. These results seem to indicate that flux may not have been totally restored due solely to the presence of soils in the membrane and that other factors such as membrane type , pore size and pore distribution may have played a role ,as well.

Indeed, great variations were observed in the flux restoration measurements of membrane 3, (see Figure 18) especially if they are compared to the absorbance values of the water elution system after each cleaning treatment. This membrane, before being used for the first time, had the lowest original flux (415 ml/h) and the highest absorbance

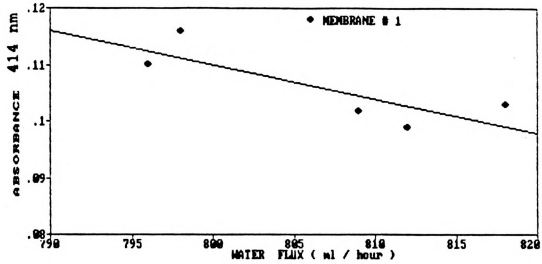


Figure 20-Correlation between the water elution absorbance values in method ND and the water flux measurements after each cleaning treatment of membrane 1. Correlation coefficient was -0.81 ($P < 0.05$).

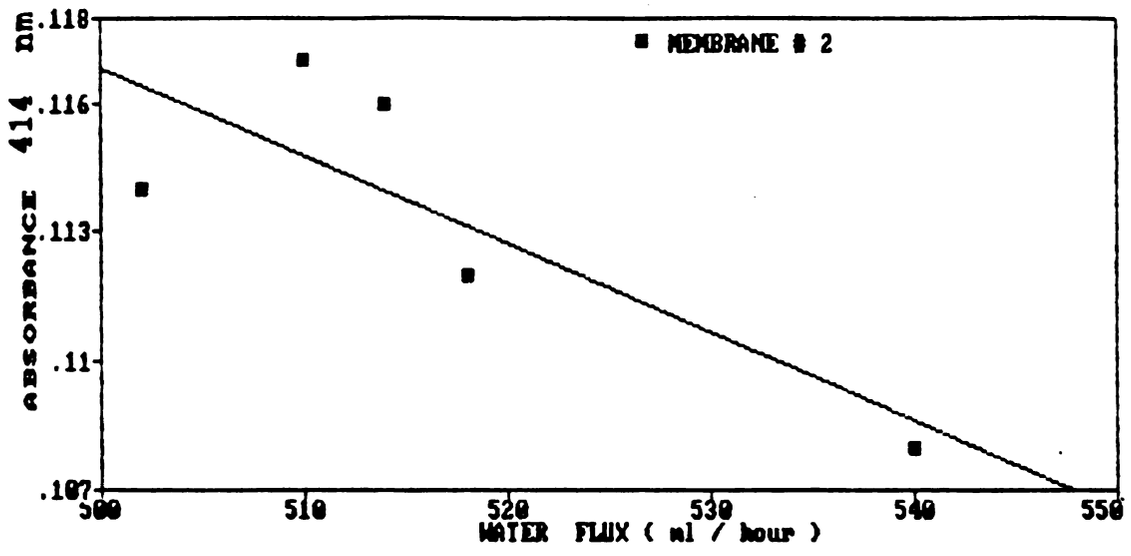


Figure 21- Correlation between the water elution absorbance values in method ND and the water flux measurements after each cleaning treatment of membrane 2. Correlation coeffiecient was -0.82 ($P < 0.05$).

value (0.106) when submitted to the adapted ELISA by method ND. A statistically insignificant correlation ($r = -0.33$, $P > 0.05$, $n = 5$) was found between the water elution absorbance values and the water flux measurements taken after each cleaning cycle, as ilustrated in Figure 22. The membrane presented 0.116 % of nitrogen after the last treatment, which indicated that it had soils stuck to it. That was apparently detected by the last ELISA applied, whose water elution absorbance value found was 0.118. The flux variations might be caused by membrane pore size and distribution, which may also affect the way a proteinaceous soil is entrapped in the matrix. The results seem to confirm the assumption that flux restoration is not a reliable parameter to assess the cleanliness of UF membranes.

Overall the results seem to indicate that a return to the NSB baseline absorbance value is possible, and should only occur if the membrane is completely clean and free of soils. In the present study, this goal may not have been entirely achieved by the cleaning regime adopted since an increase in the nitrogen content fo the membrane was observed after the last cleaning cycle. However, these results showed on the other hand, that method ND is able to detect even small amounts of proteinaceous soils in the UF membrane, as shown in Figure 23. When comparing the amount of nitrogen found in virgin membranes (average) and in the 3 membranes studied, after the last cleaning cycle. to the absorbance values of the water elution system in each

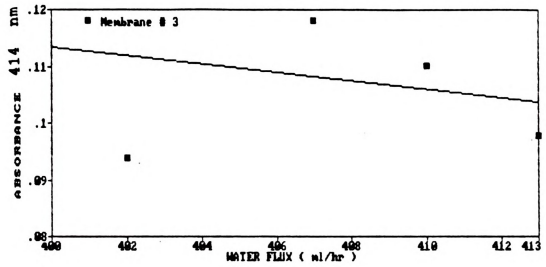


Figure 22-Correlation between the water elution absorbance values in method ND and the water flux measurements after each cleaning treatment of membrane 3. Correlation coefficient was -0.33 (P > 0.05).

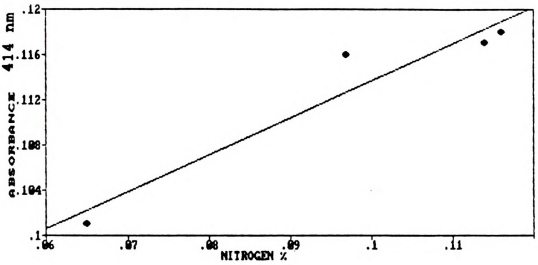


Figure 23 - Correlation between the percentage of nitrogen on the UF membranes and the average water eluent absorbance values observed after the last cleaning cycle, as determined by method ND ($P < 0.05$). Results indicate the ability of this method to respond to small amounts of proteinaceous soils on the UF membrane.

respective membrane, a strong and significant correlation ($r = 0.96$, $P < 0.05$, $n = 4$) was found, confirming the ability of this method to assess the cleanliness of UF membranes in a bench-scale unit.

Application of method ND as a one-step procedure

In the method ND previously described, antibodies raised in rabbits against milk proteins were first circulated in the system followed by a goat anti-rabbit peroxidase conjugate circulated to provide an adequate assessment of the presence of antibodies bound to proteinaceous soils adhering to the UF membrane. This was an adaptation of a classic indirect non-competitive immunoassay. A direct non-competitive assay could be performed by directly labeling the rabbit anti-milk proteins antibodies with horseradish peroxidase, which would mean the simplification of method ND, converted then to a one-step procedure. The coupling procedure was succesfully achieved (see Figures 8 and 10) and a conjugate with a working titer of 1:500 was obtained. This conjugate was able to recognize also whey proteins (Figure 10) and to test it, the UF membranes were soiled with filtered fresh whey from Cheddar cheese manufacturing. It has been shown that during the ultrafiltration of whole milk, whey proteins accounted for 95 % of the proteinaceous membrane foulants, very little

casein being identified as membrane foulant (Tong et al.,1988).

Whey (150 ml) was concentrated to a 7.5 X concentration factor in the bench-scale, thin-channel ultrafiltration unit. Figure 24 shows a typical profile of the fractionation procedure, during which permeate flux decreased from 310 ml/h at the beginning of the operation to about 155 ml/h at the end . One hundred thirty five milliliters of permeate were collected. Compared to skimmilk fractionation (Figure 10), whey fractionation achieved a much higher concentration factor in less time, which can be explained by differences in composition, which affects the formation of the polarization layer and the fouling of the membrane , such as the lower content of proteins and salts (Kosikowski,1986; Maubois, 1980).

A NSB baseline was first determined by running the test in virgin membranes followed by soiling with whey, rinsing and washing for periods ranging from 0 to 60 minutes.The one-step method ND was then applied as previously described, the only differences being the exclusion of the goat anti-rabbit peroxidase conjugate and the utilization of the anti-milk protein peroxidase-labeled conjugate obtained by the m-periodate coupling procedure (see flow chart). The average results concerning the absorbance values found for the final water elution system and the percentage of nitrogen in the membranes are shown in Tables 6 and 7.

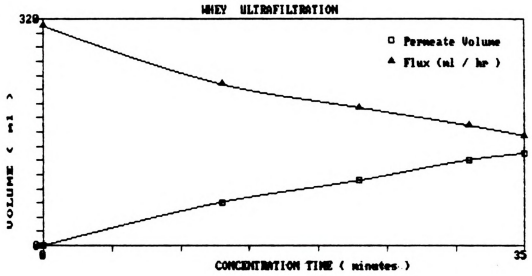


Figure 24 - Typical profile of trends in permeate volume and flux during ultrafiltration of whey in a thin-channeled spiral type UF unit.An increase in permeate volume corresponded to a gradual decline in permeate flux during operation.

METHOD ND (one-step)

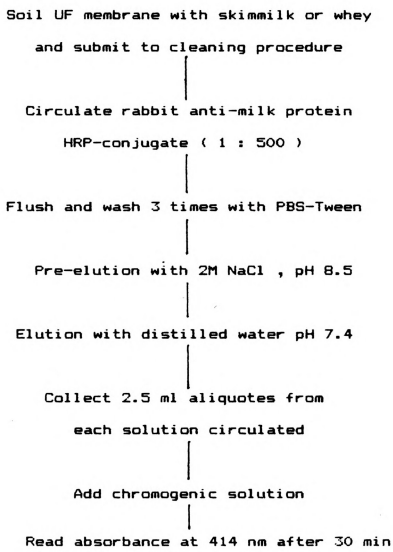


Table 6 - Absorbance (414 nm) values observed in the water pH 7.4 eluent when UF membranes cleaned for different times were submitted to the non-dismantling method ND (one-step).

Trial	Virgin membrane	Cleaning time (minutes) 60	20	0
1	.414	.381	.465	.552
2	.406	.387	.514	.582
3	.403	.401	.548	.535
Mean	$.408_c$	$.390_c$	$.509_b$	$.556_a$
S	.006	.010	.042	.024
CV	1.39	2.63	8.20	4.27
SEM	.003	.006	.024	.013

S = standard deviation, CV = coefficient of variation, SEM = standard error of mean
a,b,c = means with same letter showed no significant difference ($P < .05$)

Nitrogen residues in the membranes varied from 0.061 to 0.317 % and absorbance values in the respective water eluents ranged from 0.381 to 0.582 % . The NSB baseline found for virgin membranes had an average absorbance value of 0.414 (0.408 $\pm$ 0.006). Soiled membranes presented an average absorbance value of 0.556 and 0.299 % of nitrogen. These values decreased as the membranes were washed for longer periods. As shown in Table 6, the average absorbance values of membranes soiled and cleaned for 0 and 20 minutes were different ($P < 0.05$) from values obtained for virgin membranes and membranes washed for 60 minutes. These differences can be atributted to the presence of proteins , as nitrogen, on the membrane, as presented in Table 7. Membranes which were not washed at all or washed for just 20 minutes had an average nitrogen content of 0.299 and 0.211% respectivelly, which were found to be statistically greater ($P < 0.05$)than the values observed for virgin membranes and membranes washed for 60 minutes (0.71 and 0.068 %, respectivelly).

When compared to results obtained by method D and the two-step method ND , the absorbance values found here are considerably higher, as shown in Table 8. The absorbance value for the NSB baseline in the two-step procedure was found to be 0.106 as compared to 0.414 for the one-step method. These differences might be due to conformational changes in the antibody molecule caused by the enzyme coupling, altering its binding site and changing its NSB

Table 7 - Percentage of nitrogen observed in UF membranes cleaned for different times and submitted to the non-dismantling method ND (one-step).

	Virgin	Cleaning time (minutes)		
Trial	Membrane	60	20	0
1	.061	.104	.246	.286
2	.079	.094	.202	.317
3	.071	.068	.186	.294
Mean	.070$_c$	.089$_c$	.211$_b$	.299$_a$
S	.009	.019	.031	.016
CV	12.82	20.95	14.70	5.38
SEM	.005	.011	.018	.009

S = standard deviation, CV = coefficient of variation, SEM = standard error of mean
a,b,c = means with same letter showed no significant difference ($P < .05$)

Table 8 - Mean absorbance (414 nm) values obtained from the application of three soil detection procedures to clean UF membranes, to establish a non-specific binding (NSB) baseline.

	Method D	Method ND	
		two-step	one-step
Mean	.062	.101	.408
N	4	6	3
S	.001	.005	.006
CV	2.04	4.77	1.39
SEM	.0006	.002	.003
NSB baseline	.063	.106	.414

N = number of trials, S = standard deviation
CV = coefficient of variation
SEM = standard error of mean.
Method D = dismantling.
Method ND = non-dismantling.

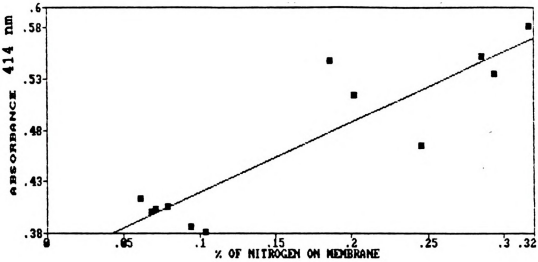


Figure 25 - Correlation between water elution absorbance values and nitrogen percentage on membranes, as determined by the one-step method ND ($P < .05$). Results indicate that the conjugate was able to recognize whey protein soils bound to the UF membrane.

ability. More probably, the differences could be explained by binding of several HRP molecules to one antibody, forming heavily-labeled antibodies with higher background staining properties when tested immunohistochemically (Wilson and Nakane, 1978). Furthermore, conjugation by the m-periodate method may lead to self-coupling of IgG to some extent, forming a heterogeneous mixture with molecular weights ranging from 200,000 to several million daltons (Boorsma, 1983; Schuurs and van Weemen, 1977).

Despite a possible formation of polymers, the conjugate obtained showed a good sensitivity to the presence of whey protein soils in the UF membranes, as illustrated in Figure 25. A statistically significant ($r = 0.89$, $n = 12$, $P < 0.05$) correlation was found between the water elution absorbance values and the percentage of nitrogen in the UF membranes.

CONCLUSIONS

Experimental evidence presented in this study shows that immunological procedures, such as ELISA, can be adapted to assess the cleanliness of ultrafiltration membranes on a bench-scale system. By immunization of rabbits with freeze-dried milk proteins, antibodies of high titer were obtained. These antibodies were able to recognize not only milk caseins but also whey proteins, which increases their application in these types of immunoassays, despite decreasing their specificity. Results obtained when a two-step, non-dismantling procedure was applied, showed the desired sensitivity for non-destructive assessment of milk protein soils on bench-scale ultrafiltration systems. When trying to conjugate an enzyme marker to the antibody, better results were observed using the m-periodate method than using the one-step glutaraldehyde procedure. However, a considerable loss of immunological activity was observed in the resulting conjugate, possibly due to the formation of high molecular weight polymers which increased the nonspecific binding baseline when the non-dismantling method was tried as a one-step procedure. Neverthless, this procedure also allowed for the detection of proteinaceous soils in the UF membranes. Results from this study indicate

that cleaning time does not correlate properly with cleaning efficiency and that the flux restoration can be a misleading parameter concerning the cleanliness of the membranes.

Finally, in performing a study of this nature, one must realize that there is a possibility of conflicting results when the conditions of the experiments are changed. The strength of antibody-antigen reactions is dependent on the closeness with which the interacting groups can approach each other or on the topography of the surface of the antigenic determinants. If the matrix material or performing characteristics are changed, it may affect the immunological reactions to some extent.

Further studies are necessary to learn the effects of system upscaling as well as different ultrafiltration systems, on the methods discussed here. It is believed, however, based on the present results, that immunological assays can be successfuly used as a tool to assess the cleanliness of ultrafiltration membranes.

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