Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **41 (1)** (**2008**), Timişoara

SURVEY REGARDING THE ULTRAFILTRATION OF PROTEINES THROUGH MEMBRANE BASED PROCEDURES

SRUDIUL PRIVIND ULTRAFILTRAREA PROTEINELOR PRIN PROCESE MEMBRANARE

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This work is based on examples that emphasize the complexity of the proteins ultrafiltration process, pointing out the first 10-15 minutes of ultrafiltration. The knowledgement of the factors that influence the separation through ultrafiltration of proteins will allow to choose the right type of membrane, the frequent use of the same membrane and the operation in mechanical and chemical conditions adequate to the ultrafiltration system, when it is separated a protein with certain molecular weight.
Key words: ultrafiltration, membrane, proteins

Introduction

In the last two decades some reasons have influenced the researches in the field of ultrafiltration through membrane of proteins solutions. The most important researches from the ultrafiltration field of proteins solutions refered to the role of concentration or gel polarization and the growth of solvent flux.

Also, in the case of proteins ultrafiltration shouldn't be neglected some factors like: the adsorbtion of proteins on membrane, the effect of pH and of proteins aggregation, the initial behaviour of membranes with a limited permeability for solutions, the ionic intensity and the hysteresis curve of the membrane. Another aspect that affect the determinations, is represented by these reproductability, because this firstly depends on the cleaning process of the membrane.

Materials and Methods

The proteins ultrafiltration can be achieved continuous or in a work cell with well delimited space, using different configurations of membrane and cell. In the case of laboratory measurements for very small sample volumes it is used a cell provided with movement or with very thin ultrfiltration tubes, while for separations at high scale are used fibre with high porousness or membranes with different

shapes. In any of these cases, protein solution comes in contact, in the separation cell, with a new membrane or with one previously cleaned and the experiment develops in the settled conditions.

After the experiment, the membrane is cleaned and another experiment is initiated in different or similar conditions. Usualy the worn-out membrane is replaced with a new one.

The used system presents some advantages comparing the ultrafiltration in closed system, where the solution volume subside while the ultrafiltration process advances so that the solution concentration in sample and the agitation conditions vary in time.

Another aspect that should be taken into account, in the case of cell with agitation, represents the posibility of turbionare agitation appearance.

The work procedure adopted for ultrafiltration is the following: it is used a ultrafiltration cell (UF) AND a tank half filled with buffer solution of citric acid 0,1M- phosphate disodic 0,2 M .

Tabel 1.

Buffer Mc Ilvain's: citric acid-fosfat disodic

pH	8,0	6,8	6,0	4,8	4,6 3,0
x	2,75	22,75	36,85	50,70	53,25 79,45

x cm³ citric acid 0.1+(100-x)cm³ 0.2 M fosfat disodic

In the case of serical albumin separation (BSA) the adsorbtion on the diaphragm surface XM 100 A (Amicon) is great. That is why after the usage the membrane is submerged, for 30 min., in a NaOH 0.1 N solution that contains ce 0.5% pepsin. If after rinsing with double distiled water and its filtartion at 20 atm., it is reached a solvent flux of at least 95 % from the initial flux value of a new membrane, this can be used for a new set of estimations. If the result is different the membrane is submerged again for another15 min. in the cleaning solution and the process is reiterated.

The samples concentration with proteins is spectometric determined using the Lowry method for protein solutions diluted and Biuret method modified for solutions relatively concentrated.

Results and Discussions

After the experiment it was observed that the solvent flux declines fast in time at the begining of ultrafiltration, simultaneous with the solut retention growth. After a while the modification speed of the solvent flux, respectively the solution retention declines substantialy and in almost 10-15 min. Both processes devolves practicaly with constant speed. This behaviour it was noticed in the case of serical albumin ultrafiltration with diaphragms PM 30, XM 100A and XM 300 (Amicon) at pressures of 10 - 35 atm. And a variation af solution variation between 0.05-0.5%.

It was studied the behaviour of a PM 30 membrane from the retention time evolution point of view of a 0.1% albumin solution, using a cell with agitation (800 rpm).

The same behaviour was noticed for other values of PH.

For the ultrafiltration of 0.05% albumin on a XM 100A membrane in a cell with agitation, it was noticed that the albumin retention vary significantly according with the time for all three values of PH, respectively 7.4; 3.0 si 4.8.

The retention percent of albumin from 20-30% at the begining of the ultrafiltration process was modified until 90% and even more until the end. Because the used XM 100 membrane has a separation index of 100.000 and the albumin has the molecular weight of 67.000 Da, the retention percent will be smaller than 100%.

When it is used a PM 30 membrane, with a bigger retention coefficient, one can observe that the albumin retention grade, in the first minutes, is about 70-85%, in time the retention grade adequate to the waiting state (after 10-15min.) vary between 90-98%, according with the applied pressure and the initial solution concentration.

As a rule, the proteins retention grade, in the balance phase, grows easy together with the concentration growth of the protein solution, and decline when the applied pressure grows.

Another aspect that should be mentioned is represented by the necessary time to reach equilibrium phase for the solvent flux, which is always smaller than the one necessary to reach the equilibrium state for the solut retention. For an induced period of time, even if the solvent flow capacity is constant, the solut retention rate grows. This phenomenon ows to membrane freckles which are obturated by the spheroidal proteins which affects significantly the retention grade of the solut.

The instability of such an ultrafiltration system ows to:

(i) the polarized section agglomeration in time (and of gel section);

(ii) the interaction between the membrane and solut that lead to the proteins adsorbtion on the membrane surface and to the possible obturation of its freckles.

The characteristic time for the first type of process it is proportional with the report between the effective diffusion coefficient of the solut in the feeding solution and the diffusion speed quadrant of the solvent through diaphragm.

Thus, for solutions with great concentations of proteins (smaller diffusion coefficients) and for great pessures (bigger initial solvent flux) the charateristic time of the polarized stratum agglomeration (and of the gel stratum) is smaller. In some researches was found that for a concentration of proteins between 0.05-0.5% and an applied pressure not great, the initial time until the equilibrium reach is of 10-15 min.

When the concentration of the initial potein solution and the apllied presure is great the time until the equilibrium reach may decline until 60 sec. and because of this its effect may be ignored.

The fast formation of the polarized stratum (and of the gel statum) will lead in time to the solvent flux declining. If this stratum will present adequate properties for a second membrane able to retain the solut, in time, the retaintion grade of the solut will be modified. If the protein adsorbtion takes place on the diaphragm surface, the adsorbted stratum will certainly influence the solut retaintion grade.

In this work was studied the ultrafiltration of a solution that contains a single protein.If it is used a mixture of proteins it should be take into account: the proteins association phenomenon, the competitive adsorbtion on the membrane surface etc.

Conclusions

C1 The membrane techniques have numerous applications in the biological compounds separation, a special importance represents the proteins adsorbtion in the case of ultrafiltration through membrane processes.

C2 The usage of ultrafiltration system with agitation leads to the pronunciation of the adsorbtion effect on the diaphragm surface.

C3 In the membrane processes, the solution agitation reduce the polaization and the flow capacity of the solvent through diaphragm grows.

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