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FORCED-FLOW ELECTROPHORESIS FOR SEPARATION OF PROTEINS AND VIRUSES

by

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Prepared under the direction of Professor R. E. Sparks

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ABSTRACT

FORCED-FLOW ELECTROPHORESIS FOR SEPARATION

OF PROTEINS AND VIRUSES

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May, 1985

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In a conventional filtration process, the movement of the liquid phase toward and through a filter causes particle transport in that direction due to bulk flow. The retained particles accumulate and form a layer on the surface of the filter causing a decrease in liquid flux. If the retained particles are charged (e.g., plasma proteins, viruses), an electric field with appropriate polarity will cause some of them to migrate away from the filter. The purpose of this work is to test such feasability in order to increase the filtration flux and to improve the separation of different biological species.

The equipment consists of two membranes and a filter, the electric field being imposed by means of external electrodes, and two fractions are obtainable. The nature of the filter is not critical and

this in sharp contrast with processes such as ultrafiltration and electrodialysis. The reason is that the main discriminating factors are not the pore sizes of the membrane but the relative solute ionization which depends on the pH and the ionic strength of the buffer solution.

Previous studies of forced-flow electrophoresis have not established an adequate mass balance of the different species in the system and have usually neglected the heat generation due to the Joule effect. This work describes a model considering the desired species fraction removal, other fraction removals, and outlet concentrations of all species present in the system. The model predicts the necessary inlet flow rate of the retentate chamber, the rate of filtration and the voltage gradient and also provides an appropriate heat balance permitting consideration of possible heat denaturation of the species. To estimate the mobility of the different species and to study their separation, a zonal density gradient electrophoresis method has been developed.

Serum proteins (albumin, γ-globulin) and bacteriophages (M13, MS2, øX174), have been used to characterize the separation process. A practical application of this research work would be the removal of viruses (e.g., hepatitis B virus) from plasma proteins (e.g., factor VIII complex).

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FORCED-FLOW ELECTROPHORESIS OF PROTEINS AND VIRUSES

1. INTRODUCTION

Blood components such as fresh frozen plasma, red blood cells, granulocytes and platelets are now used more frequently than the whole blood. Blood fractions or derivatives such as albumin, plasma protein fractions and antihemophilic factors are prepared commercially from plasma pools containing from 500 to 1000 or more donations. The clinical need for various blood fractions has resulted in large scale production of plasma components.

The possibility of transmission of disease from blood fractions is a serious consideration. Among the infectious diseases that could be transmitted by transfusion are malaria, syphilis, AIDS, and hepatitis, with hepatitis being the most important. The reduced use of commercial blood donors and routine screening of all donors units by sensitive tests(e.g., radioimmuno-assay) for hepatitis B surface antigen (HBsAg) has reduced the incidence of post-transfusion hepatitis, particularly type B.

However, despite screening for HBsAg, post transfusion hepatitis B still is observed in approximately 0.77% of recipients (1)*(2)(3). Risks of hepatitis are associated with transfusion of platelets and granulocytes, factor IX complex for treatment of hemophilia B, and factor VIII complex for treatment of hemophilia A (4). Hence viral hepatitis remains a major health hazard associated with clotting factor concentrates derived by current methods of preparation.

Due to the considerable demand for factor VIII complex in treatment of patients with hemophilia, a method for eliminating or reducing the risk of transmitting hepatitis from factor VIII complex would be an important contribution to the management of the large population of patients requiring frequent administration of this material. The incidence of hemophilia is 4 to 6 cases per 100,000 persons (5)(6). The average amount of factor VIII used to treat hemophilic patients given from NIH (1972) survey of hemophilic patients in the united states is about 14,000 units/patient/year (6). Thus about 1.71×10^8 units are needed for the United States alone. Since approximately 50 units can be collected from 200 ml of plasma (6), 700,000 liters of plasma are required.

Among the separation and analysis processes for proteins, electrophoresis, membrane ultrafiltration, chromatography and

* The numbers in parentheses in the text indicate references in the Bibliography.

centrifugation have become the most widely used and effective methods (7)(8)(9). However, which separation method is best for protein fractionation? There is no simple answer, the 'best' method depends on the particular separation problem. Gel electrophoresis is the analytical method having the highest resolving power. For preparative purposes, chromatography is more popular than electrophoresis. Preparative electrophoresis is more complicated than chromatography with respect to the operation of the apparatus. The preparative method which has enjoyed most interest is a continuous flow electrophoresis device developed originally by Hannig (10) and Strickler (11). An ideal preparative electrophoresis method should be easy to operate, rapid, sensitive, reliable and economical.

In a conventional filtration process, the movement of the liquid phase toward and through a filter causes particle transport in that direction due to bulk flow. The retained particles accumulate and form a layer on the surface of the filter causing a decrease in liquid flux. If the retained particles are charged, an electrical field with appropriate polarity will cause them to migrate away from the filter. The retained particles becomes concentrated on the feed side and any particles present having an opposite charge or lower electrophoretic mobility are carried through the membrane due to bulk flow. This separation process is therefore useful for concentration and fractionation. The major discriminating factor for particles is their ionization, which is a function of the pH and the ionic strength of the buffer solution. However, the selection of the electrophoretic medium is a compromise between the requirements for preserving the