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Research Article

Enrichment and immobilization of macromolecular analytes on a porous membrane utilizing permeation drag

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ABSTRACT

Enrichment and immobilization of analytes by chemical bonding or physical adsorption is typically the first step in many commonly used analytical techniques. In this paper, we discuss a permeation drag based technique as an alternative approach for carrying out location-specific immobilization of macromolecular analytes. Fluorescein isothiocyanate (FITC) labeled macromolecules and their complexes were enriched near the surface of ultrafiltration membranes and detected by direct visual observation and fluorescence imaging. The level of macromolecule enrichment at the immobilization sites could be controlled by manipulating the filtration rate and thereby the magnitude of permeation drag. Higher enrichment as indicated by higher fluorescence intensity was observed at higher filtration rates. Also, larger macromolecules were more easily enriched. The feasibility of using this technique for detecting immunocomplexes was demonstrated by carrying out experiments with FITC labeled bovine serum albumin (FITC-BSA) and its corresponding antibody. This permeation drag based enrichment technique could potentially be developed further to suit a range of analytical applications involving more sophisticated detection methods.

1. Introduction

Attachment of biological macromolecules on diverse surfaces has direct implications on development of detection and analytical methods with application in bio-sensors and medical diagnosis [1,2]. Location-specific immobilization of analytes by chemical bond formation [3,4], or physical methods such as adsorption [5,6], is typically carried out as the first step in many analytical techniques such as immunoassays [7,8], surface plasmon resonance analysis [9], and Raman spectroscopy [10]. Once the molecules are immobilized at their desired locations, they are probed and analyzed using appropriate detection methods.

Permeation drag refers to the drag force exerted on solute molecules and particles towards the surface of a membrane by bulk medium during membrane filtration processes such as ultrafiltration and microfiltration [11–14]. Our proposition is that such permeation drag induced accumulation of macromolecules near the membrane surface of retaining ultrafiltration membranes could be utilized as an alternative physical approach for immobilizing macromolecular analytes. While various other techniques have been carried out to immobilize bio-macromolecules onto membranes [15,16], we demonstrate the feasibility of macromolecule immobilization by permeation drag. Ultrafiltration experiments were carried out using fluorescein isothiocyanate (FITC) labeled macromolecules. Location-specific immobilization was demonstrated by direct visual observation and fluorescent imaging. Film theory was used to explicate the permeation drag induced enrichment based on which the accumulation of retained macromolecules takes place within a stagnant film adjacent to the membrane surface. More or less corresponding to the hydrodynamic boundary layer, this is widely referred to as concentration polarization layer in membrane filtration processes. A large number of macromolecules are accumulated in a narrow region with two levels of concentration asymmetry: The concentration of the solutes is significantly higher in the polarized layer compared to the bulk solution; also, within the layer, the macromolecule concentration increases in an exponential manner from the bulk concentration (C_b) to the concentration at the membrane surface (C_w) [17]. If macromolecules are totally retained by a membrane, the two concentration terms are linked by the equation shown below:

$$C_w = C_b \exp(Q\delta/AD) \quad (1)$$

In this equation, Q represents the flow rate through the membrane having the area of A, δ represents the thickness of the concentration polarization layer, and D is the diffusivity of the solute. As a result, when $Q > 0$, C_w will be greater than C_b, and in a typical ultrafiltration experiment, C_w could be larger by more than

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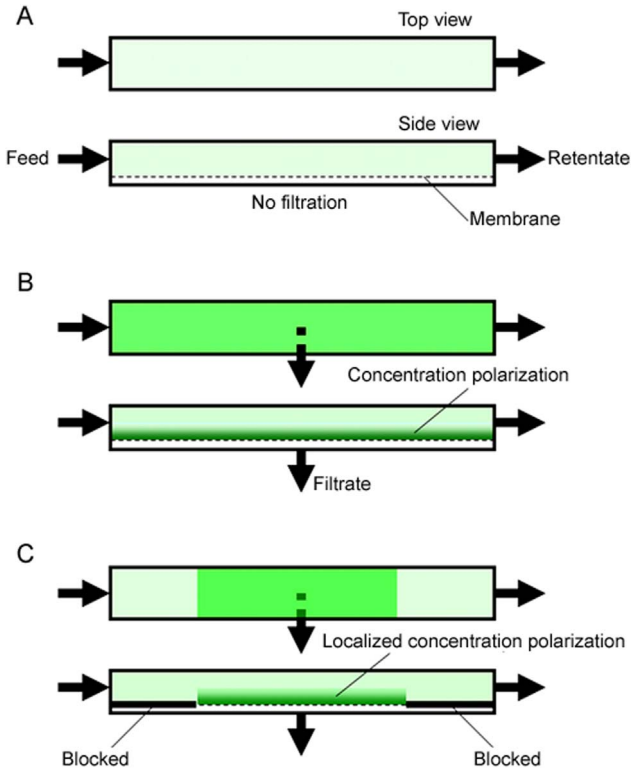

Fig. 1. Simplified top- and side-view representation of localized permeation drag induced immobilization taking place within a membrane module (A: no filtration; B: enrichment on entire membrane; C: localized enrichment).

two orders of magnitude [13,18]. Clearly, concentration polarization caused by permeation drag could be utilized for quite significant enrichment of macromolecules on the surface of a membrane. Such enrichment would also be dynamic in nature, i.e. the accumulated layer of macromolecules would largely disappear if the filtration process is stopped. Eq. (1) suggests that the extent of such enrichment could be manipulated by adjusting the values of Q, δ, and D. While Q could be controlled by adjusting the transmembrane pressure, the value of δ depends on the flow behaviour adjacent to the membrane and D depends on the size of the macromolecule.

Fig. 1 shows a dilute solution of an FITC-labeled macromolecule flowing through a channel having an ultrafiltration membrane on one side. In the absence of permeation drag, i.e. when filtration rate is zero, concentration polarization does not occur (Fig. 1A). If filtrate was drawn through the membrane either using positive pressure or suction, the enriched layer of macromolecules adjacent to the membrane would be evident from the enhanced fluorescence intensity (Fig. 1B). Further, if a part of the membrane was blocked, concentration polarization would occur in a localized manner only in the non-blocked part (Fig. 1C). The enhanced fluorescence due to enrichment of macromolecules would now be easier to observe due to the contrast between regions with and without polarization. Based on Eq. (1), it may be predicted that higher enrichment would occur at higher filtration rates, and larger macromolecules and macromolecular complexes (which have lower diffusivity) would be easier to enrich. Accordingly, specific regions of rectangular flat sheet ultrafiltration membranes were blocked by applying polyurethane glue. Fluorescent patterns and features were generated on these membranes by localized concentration polarization of FITC-labeled dextran. The effect of filtration rate and molecular weight of macromolecules on intensity of fluorescence was examined.

The interactions between antigens and corresponding antibody molecules lead to the formation of macromolecular complexes called immunocomplexes, the ability to recognize which is widely exploited to carry out immunoassays [19,20]. Such immunocomplexes would be fairly easy to enrich as they are larger in size. The working principle of the localized concentration polarization based immunocomplex detection method is outlined in Fig. 2, which shows the polarization of a fluorescence-labeled antigen (Fig. 2A), the polarization of a mixture of the antigen and non-specific antibody (Fig. 2B), and the polarization of the immunocomplex (Fig. 2C). As indicated in the figure, the highest intensity could be expected in (Fig. 2C). The difference in intensity could therefore be utilized for immunocomplex detection. Proof-of-concept of such immunocomplex detection was obtained by using FITC-labeled bovine serum albumin (FITC-BSA) as model antigen and rabbit anti-BSA as corresponding antibody. The Effect of filtration rate, antigen concentration and antibody concentration on intensity was examined.

2. Experimental

2.1. Materials

FITC-dextran of different molecular weights (40 kDa, FD40S; 70 kDa, FD70S; 500 kDa, 46947; and 2000 kDa, FD2000S), FITC-BSA (A9771), anti-BSA (B1520), and whole antiserum polyclonal antibody raised in rabbit were purchased from Sigma-Aldrich

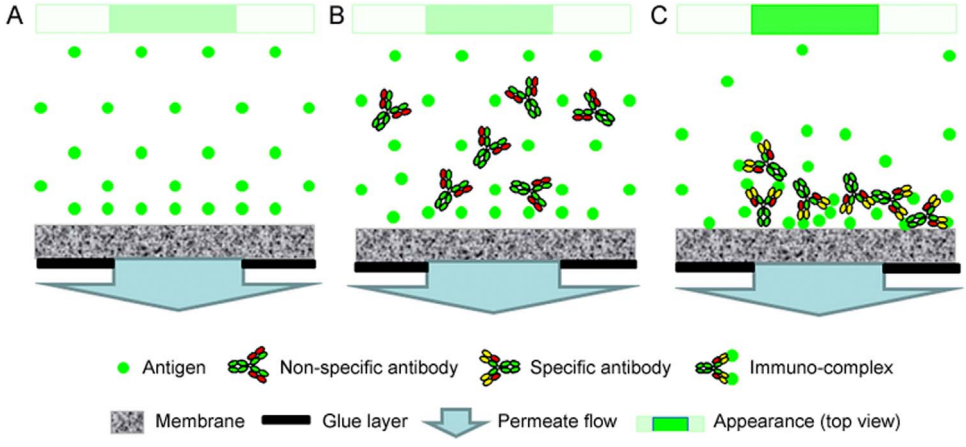

Fig. 2. Schematic diagram for immunocomplex detection by localized permeation drag induced immobilization explaining the basis for difference in fluorescent intensity (A: antigen only; B: antigen with non-specific antibody; C: antigen with specific antibody).

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