Journal of Membrane Science 318 (2008) 280–287

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Dye deposition patterns obtained in line printing on macroporous membranes: Improvement of line sharpness by liquid redistribution

Jun Wang [1], Volkmar Thom [b,*], Markus Hollas [b], Diethelm Johannsmann [a,**]

[a] Institute of Physical Chemistry, Clausthal University of Technology, Arnold-Sommerfeld-Str. 4, D-38678 Clausthal-Zellerfeld, Germany
[b] Sartorius AG, Biotechnology Division, Weender Landstr. 94-108, D-37075 Göttingen, Germany

ARTICLE INFO

Article history:
Received 13 July 2007
Received in revised form 15 February 2008
Accepted 22 February 2008
Available online 7 March 2008

Keywords:
Imbibition
Printing
Rapid immunochromatographic assays
Capillary transport
Protein deposition patterns

ABSTRACT

We report on dye deposition patterns obtained when printing dye-labeled protein solutions onto a macroporous membrane. A typical area of application would be the manufacturing of immunochromatographic tests, but the results bear significance for a wide range of printing processes. As the liquid is taken up by the membrane, the protein adsorbs non-specifically to the wetted membrane surface. The transport of protein to the pore surface involves spreading of a drop on top of the membrane, imbibition of liquid into the membrane, adsorption of protein at the pore surface, liquid redistribution from large to small pores, and liquid redistribution from the center of the wetted area to the rim.

Two techniques were employed to monitor the transport of protein, which are, firstly, conventional optical imaging of the wet membrane, and, secondly, fluorescence microscopy on vertical cuts through dried samples. The latter technique visualizes protein deposition. It is conceptually related to the acquisition of breakthrough curves in chromatography. Comparison of the deposition patterns obtained with different amounts of proteins allows to reconstruct the flow pattern.

We frequently find a brightening of the rim, which can be traced back to redistribution of liquid from the center to the edge during drying and to a preferential uptake of liquid across the rim of the drop. Small pores and slow drying are beneficial for the achievement of sharp printing patterns.

1. Introduction

Printing is a process of intriguing complexity [1,2]. It involves the application of the ink, the wetting of the substrate, the penetration of the liquid into the pores (if pores are present), the adsorption of the dye molecules or the pigment particles to the surface, and finally, the evaporation of the solvent. Similar sequences of events also occur in the cleaning of textiles [3], in the separation of biomolecules [4], and in a number of geophysical phenomena [5].

The uptake of the liquid by a network of pores as well as adsorption to the pore surfaces has been intensely investigated with regard to applications in thin-film chromatography [6]. In the context of printing, the liquid uptake is also termed "imbibition" [7] or "wicking" [8]. Imbibition is driven by the Laplace pressure at the curved meniscus. Depending on the time scale, the capillary force is balanced by either inertia (fast processes or small, open-ended ducts) or by viscous drag (slow processes or long, slender capillaries). The second kind of dynamic equilibrium was first described by Lucas [9] and Washburn [10], who predicted that the depth of penetration, $x(t)$, should scale as the square root of time. More specifically, the Lucas–Washburn (LW) equation states that

$$x^2(t) = \frac{\gamma R \cos\theta}{\pi\eta} t \tag{1}$$

where γ is the surface tension, θ is the contact angle, R is the radius of the tube, and η is the viscosity. Early modifications of the LW equation were proposed by Rideal [11] and Bosanquet [12], who pointed out that the LW equation predicts infinite speed at $t \approx 0 (\mathrm{d}x/\mathrm{d}t \propto t^{-1/2})$. Including inertia into the force balance cures this paradox. While this may appear as a technical subtlety, at first glance, inertia is indeed of considerable relevance in porous networks containing small, open-ended ducts. In this case, the forces of inertia are stronger than viscous drag. The importance of inertia is impressively demonstrated by the fact that the small pores often fill first, when both small and large pores are exposed to a liquid [13]. This finding contradicts the LW equation. Lucas and Washburn argue that the force of capillarity scales with R^{-1}, while viscous drag scales as R^{-2}. Balancing the two, one finds that

* Corresponding author.
** Corresponding author. Tel.: +49 5323 72 3768; fax: +49 5323 72 4835.
E-mail addresses: Volkmar.Thom@SARTORIUS.com (V. Thom), Johannsmann@PC.TU-Clausthal.de (D. Johannsmann).
[1] Present address: Chair of Technical Chemistry II, University of Duisburg-Essen, 45117 Essen, Germany.

0376-7388/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.memsci.2008.02.060

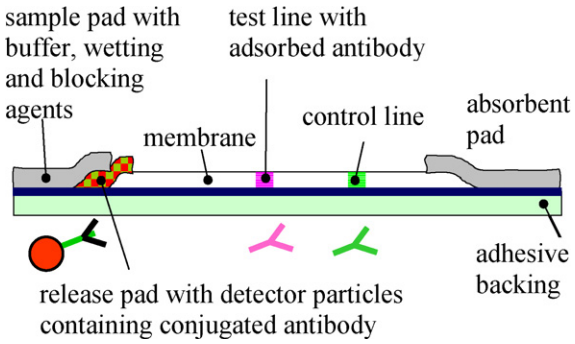

Fig. 1. Architecture of a typical lateral flow test.

the flow is faster for the wider pores. The situation reverses, when inertia dominates the dynamics.

An important difference between chromatography and imprinting is the coexistence of the wetted porous interior with a liquid drop on top of the surface. A second difference arises because imbibition is eventually superseded by drying. Drying is not at all the reverse of imbibition. Drying is much slower than imbibition and depends on the rate of evaporation, which in turn depends on temperature, convection, diffusion, and the local curvature of the meniscus. Since evaporation is faster at the edge of the drop than in the center, material is drawn from the center to the edge, leading to the coffee-stain effect [14,15]. Also, the smallest pores dry last because of capillary wetting.

The study presented below was partly motivated by a practical question, namely the determination of optimal printing parameters for the deposition of capture lines in rapid immunochromatographic assays [16–18]. In the following, we briefly elaborate on this particular application. Membrane-based immunoassays can be categorized into normal and lateral flow formats, defined by the direction of flow relative to the plane of the membrane. Lateral flow tests for pregnancy or fertility have become high volume, over-the-counter commodities. Fig. 1 depicts the geometry. The test liquid is deposited on the sample pad. As the liquid is pulled towards the adsorbent pad by the forces of capillarity, it passes the release pad, the test line, and the control line. At the release pad certain labels (colloidal gold or fluorescent latex spheres to which antibodies have been conjugated) are picked up, which specifically bind to the analyte. At the test line, the analyte and the label adsorb to capture proteins and thereby colorize the line.

Most immunoassays make use of cellulose nitrate (CN) membranes due to the unique protein binding properties of this material and its ability to form a range of macroporous membranes through an economical phase inversion processes [19]. In order to build a membrane-based assay, an analyte-specific antibody is printed onto the membrane out of buffered aqueous solution, forming a signal-generating zone, often in the shape of a "test line" or "capture line." The conjugated colored particles used for labeling of the analyte couple to the antibodies printed at the test line. Presence or absence of the label at the test line indicates the outcome of a test, usually in a few minutes. Simple tests like the pregnancy test only require yes/no information, which is easily obtained by visual inspection. Quantitative read-out is also pursued. For quantification as well as for detection of small amounts of an analyte, it is essential to print sharp test lines, which can be well distinguished from a noisy background by automated image analysis. The achievement of sharp patterns depends on materials parameters (such as the contact angle and the constitution of the buffer) and on printing parameters (such as the speed of printing, the protein concentration, the width of the nozzle, and the speed of drying).

In order to identify critical parameters governing the final protein distribution at the capture line, we have adopted a procedure commonly applied in the characterization of chromatographic columns. Staining experiments were carried out, where the liquid was loaded with varying amounts of fluorescently labeled antibodies. Assuming that the adsorption of the antibodies to the pore surface is fast (see below), one can reconstruct the flow history from the stained portions of the column obtained with different amounts of antibody. If the concentration of the antibody is below the saturation concentration ("saturation" implying full coverage of the pore surface, as opposed to the limit of miscibility in the bulk), the boundary between the stained and the unstained part of the membrane as a function of time constitutes the "breakthrough curve" [20,21]. The breakthrough curve reflects the flow pattern.

When analyzing a printing process in the same way, the situation is slightly more complicated: firstly, liquid flows both into the vertical and the lateral direction and secondly, the liquid flow arrests at some point. When working with a dye concentration beyond the saturation concentration (which we did in some cases) the stain pattern obtained after complete drying is influenced by the drying process in addition to the imbibition process. The deposition pattern then reflects the redistribution of liquid in the late stages of drying, rather than the breakthrough curve, alone.

2. Experimental

2.1. Membranes

Two types of membranes were employed, the parameters of which are summarized in Table 1. Both are flat sheet, unbacked CN membranes manufactured by Sartorius AG, Germany. They mainly differ in their average pore sizes, which are 8 and 0.2 μm. The 8 μm-membrane (type 11301, termed "coarse", in the following) is a lateral flow diagnostic membrane, whereas the 0.2 μm-membrane (type 11327, termed "fine", in the following) would typically be used for normal flow diagnostic immunoassays. Micrographs obtained by scanning electron microscopy (SEM) are shown in Fig. 2.

The membrane thicknesses were determined by means of a mechanical thickness gauge (Hahn & Kolb, Germany, type 33197) as 140 ± 1 and 120 ± 1 μm, for the coarse and the fine membrane, respectively. The membrane porosities were calculated as 85% and 75% from the weight of membrane discs of known volume. For the density of the solid fraction, we used a literature value for nitrocellulose with a similar degree of nitration, which is $\rho = 1.6\,\mathrm{g/cm^3}$ [19]. Since CN is hydrophobic by nature, CN membranes used for immunoassays are usually impregnated with surfactants. In order to ensure identical wettability, the two membranes were washed two times for 30 min on a shaker with deionized water, re-impregnated for 15 min on a shaker with a 0.05% aqueous solution of a proprietary anionic surfactant, and dried under ambient conditions for 20 h.

Table 1
Membrane properties

Membrane type	Coarse	Fine
Nominal pore size (μm)	8	0.2
Thickness (μm)	140	120
Porosity (%)	85	75
CFP-width at 2 μL/cm (mm)	1.7	2.2
Maximum binding capacity Γ_{max} (mg/mL)	2.3	7.9
Langmuir constant K (L/g)	30	Not measured
Saturating conc. (mg/mL)	2.6	10.4