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Practical subminiature immunoanalysis

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Abstract
The development and use of miniaturized assay systems have many potential advantages, including small size *per se*, portability, ease of automation, and decreased volumes of reagents and samples. However, precision and the cost of adequately sensitive detection methods remain problems for the majority of clinically oriented systems. Several manufacturers are currently evaluating approaches to the miniaturization of electrophoresis and of immunoassays for proteins. This brief review discusses a few representative methods specifically related to testing of plasma proteins and, in particular, a subminiature protein assay system that is currently under development.

Introduction

Most analytical and clinical assay methods have been moving rapidly toward the development and use of smaller and even miniaturized systems. This trend began several years ago with such assays as cytofluorometry, microcolumn separations, micro- or submicroprobes for analysis of single analytes, and the use of solid surfaces for reaction and detection, as seen in many clinical routine chemistry analyzers currently in use. Miniaturization has several potential advantages, including down-sizing of instruments (in some cases permitting true portability for point-of-care testing), increased automation with decreased sample handling (of particular importance with infected samples), increased throughput and work flow efficiency, and the requirement of markedly decreased volumes of expensive reagents and of patient samples. One example of a such an instrument for general chemistry testing, including proteins, is the Piccolo® (Abaxis, Sunnydale, CA), which weighs only 15 pounds (<7 kg) and can perform a panel of 12 tests on 90 μL samples of blood, plasma, or serum.

Miniaturization of Plasma Protein Assays

Neither qualitative nor quantitative studies of plasma proteins have been neglected in the trend toward miniaturization. Among the former, both preparative and analytical methods have been substantially downsized over the past 2 decades. A major determinant of this change has been the development of more sensitive detection devices with better precision and lower cost. For example, mass spectrometric instruments can now be used for detection and analysis in separations by miniature or subminiature capillary zone electrophoresis (CZE) separations of protein mixtures (1). Not only can the substances be tentatively or definitively identified by MS, but molecular mass determinations can be made on attomoles (moles x 10^{-18}, or ~1-100 picograms) of proteins. At the same time, the cost of these MS instruments has dropped and continues to fall (1). In addition, recent electrochemical methods now permit sensitive detection of substances in eluates from CZE or from miniature high-performance liquid chromatography columns (2). Phase-contrast methods can be used to visualize proteins during preparative microelectrophoresis (3).

Clinical protein electrophoresis has likewise seen a drastic reduction in size with surprisingly little loss in definition. Since Tiselius' rather large and cumbersome "free boundary" electrophoresis apparatus in the 1930s, we have seen the introduction, in approximate temporal order, of filter paper strip, agar and agarose gel, cellulose acetate membrane, and acrylamide gel electrophoretic

procedures. Many clinical laboratories now use very small solid supports and short runs for even routine electrophoresis. The trend toward small solid support procedures is perhaps best illustrated by the Phast® system (Pharmacia, Sweden), which can be used for single and two-dimension electrophoresis and isoelectric focusing of serum, cerebrospinal fluid, and urine samples (4). More recently, micro-capillary electrophoresis has received increasing acceptance for both research and clinical applications (5,6). Development of miniature flat solid support systems is currently in progress as well; these may be in the range of 1-5 cm separations on microchips within the near future (7). For all of these, the availability of fast, inexpensive, computer-assisted detection and processing of data has, of course, been a boon.

Since this symposium is focusing primarily on quantitative aspects of plasma proteins as applied to the clinical laboratory, these will be discussed in a bit more detail. Early methods of assay utilized gel diffusion, in particular radial immunodiffusion, which required a few microliters of sample, relatively large volumes of antiserum, long periods of diffusion, visual measurement of ring size, and manual plotting of graphs for interpolation of results. The speed of assay and precision were improved dramatically with the introduction of electroimmuno assay by Laurell in 1972 (8); however, much technologist time and manual work were still required.

The introduction of nephelometric assays using the Technicon AutoAnalyzer II® (Technicon Corporation, NY) represented the first truly automated system for clinical immunoassay of proteins (9). However, this system was rather large and required a substantial investment of time for technologists' time for setup and maintenance. In addition, calculation of results was not performed by the instrument or system itself; the user had to calculate them manually or develop his/her own automatic reading system. Of interest to the discussion below is the fact that streamlining of flow in relatively small tubing--normally a detriment in assay systems--was utilized in the AutoAnalyzer as a means of mixing sample, diluent, and reagents (9), as shown in Fig. 1. Subsequently, several manufacturers have introduced automated nephelometric and turbidimetric assay systems which, although still fairly large and requiring relatively large volumes of reagent, were more precise and less demanding of technician time. Examples of these include the Beckman Array® (Beckman Instruments, Brea, CA) and the Behring Nephelometer® (Behringwerke, Marburg, Germany).

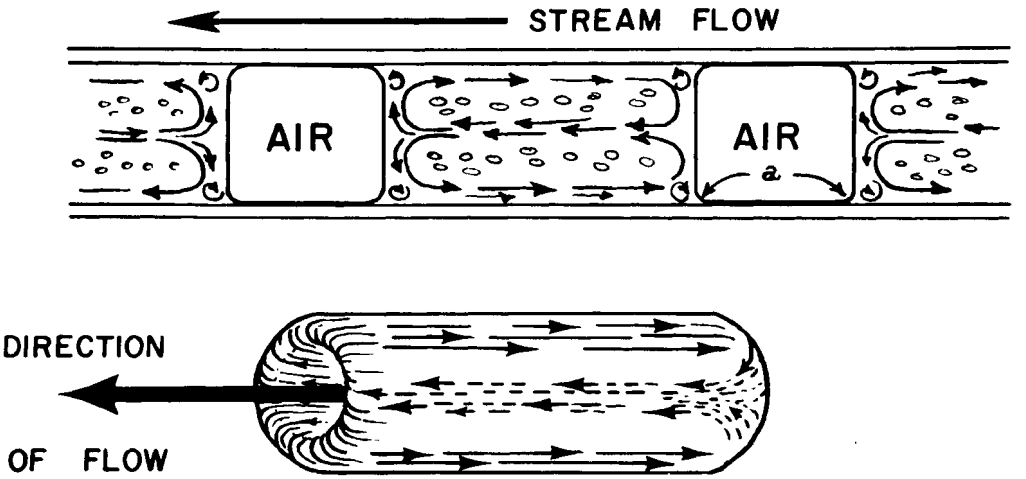

Fig. 1 Top: Sagittal schematic representation of air-segmented fluid movement within tubing. Angle (a) is angle of contact. Bottom: Three-dimensional representation of segment between bubbles illustrating toroidal configuration of fluid movement with rapid streaming of core in the direction of actual flow. (From Ref. 9, with permission)

Other semi-micro or micro assay systems using enhanced detection methods have been in use for several years by many clinical, as well as research, laboratories. Examples of these include time-resolved immunofluorescence assays such as the Delfia® system (Pharmacia), more traditional forms of enzyme immunoassay using microtiter trays, and chemi- or bioluminescence assays, using photon

counting (10). Because of their increased sensitivity, these methods can be used with smaller sample volumes or with samples containing much lower concentrations of proteins. Overall instrument size is not substantially smaller in most cases, however. More recently, assays based on solid supports, such as membranes (e.g., dot blots) and slides have been developed and are increasingly in use in clinical laboratories.

A truly subminiaturized immunoassay system, using flow and detection in solution rather than on a solid support, is currently under development by Chemcore (Malvern, PA, USA). The system is shown diagramatically in Fig. 2. The filters, flow channels, and separation "columns", depicted in the diagram, are all etched into a chip approximately 1 x 1 cm (Fig. 3). Nanoliter volumes of sample, diluent, and reagents are added via the input ports, using tuberculin syringes and a precision micropressure system. Following incubation by passage through the chambers, detection is performed in the large "cuvette" at the bottom of the diagram. The system, with modifications, theoretically permits the use of turbidimetry plus immunochemiluminescence and related methods of detection and quantification, if appropriately labeled antisera are used.

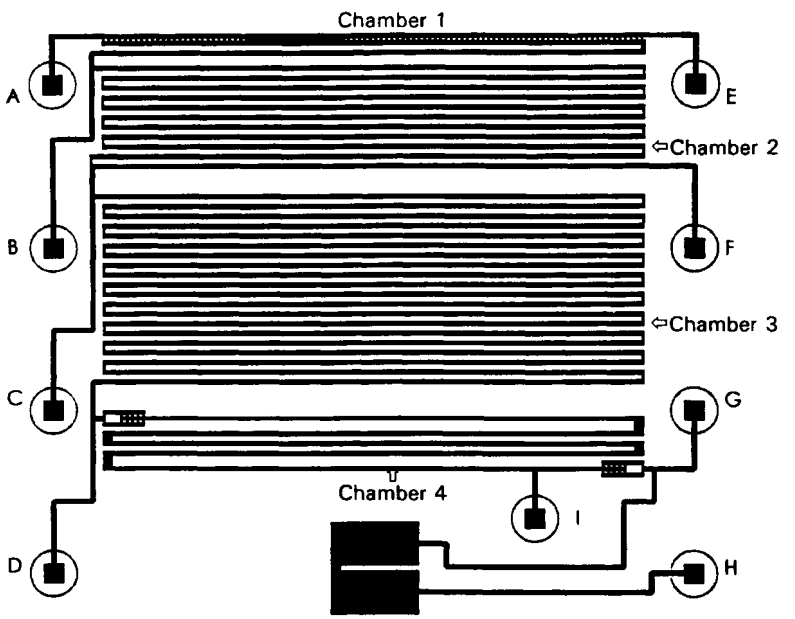

Fig 2 Diagram of the Chemcore chip. *A-D:* inputs for sample, diluent, antiserum, and buffer; *E-I:* effluent ports; *Chamber 1:* filter for whole blood; *Chambers 2-4:* incubation and mixing chambers. The large "U" shaped chamber (bottom) is the cuvette for readings. (Courtesy of AR Muir, Chemcore)

Because of the small calibers of the channels and tubing used in miniature flow systems, required pressures are extremely high. Containing liquids under these pressures is very difficult. Among other problems associated with miniaturization in general are the necessity for miniature yet sensitive detection methods, which are often very expensive, and difficulty in obtaining adequate precision. Of these, the latter is clinically the more significant. Precision is affected by several factors, including the following:

1. Difficulty in assuring precise bores of flow channels and tubing (5)
2. Difficulty in precise delivery of nanoliter volumes of sample and reagents
3. Problems related to the purely physical characteristics of flow
    a. Adhesion of proteins or reagents to tubing or channels (11)
    b. Streamlining of flow (8; *vide supra*)
4. Difficulty in assuring complete mixing of sample and reagents
5. Difficulty in obtaining adequate flatness of the walls of detection chambers

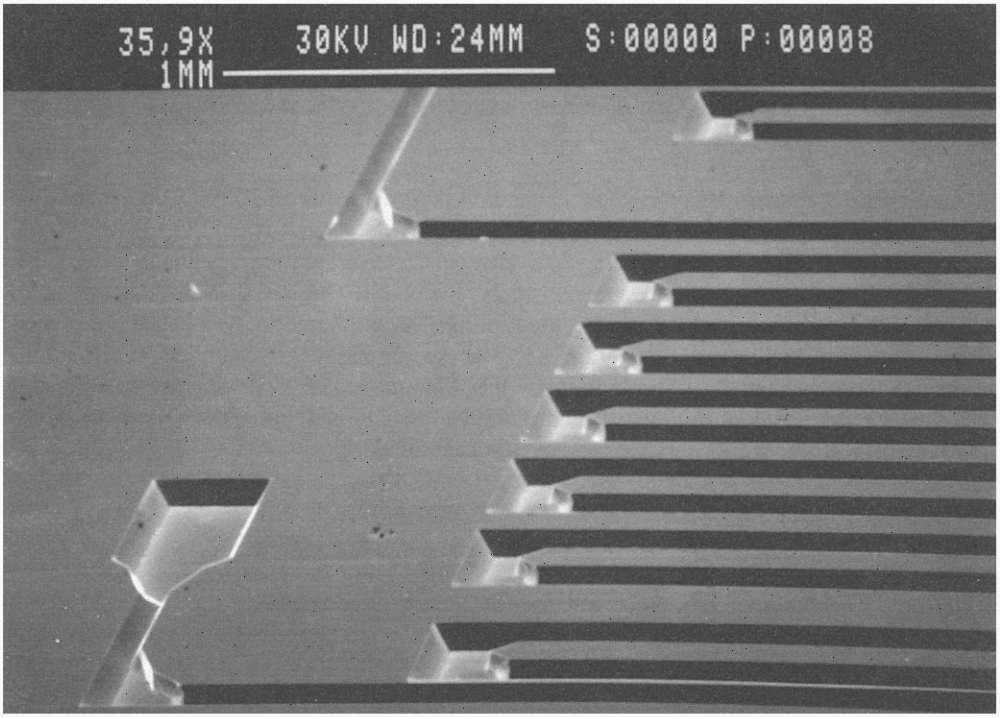

Fig 3 Scanning electron photomicrograph of flow channels from one of the mixing chambers (with cover removed). (Courtesy of AR Muir, Chemcore)

If multiple patient samples are run sequentially in the same flow system, the physical characteristics (item 3 above)--and especially adhesion--also increase the likelihood of sample cross-contamination. Practical solutions to all of these problems are currently under intensive development and evaluation.

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