HIGH PERFORMANCE ELECTROPHORESIS

S. Hjertén
Institute of Biochemistry, University of Uppsala, Biomedical Center, Box 576, S-751 23 Uppsala, Sweden

High performance liquid chromatography (HPLC) is characterized by the following features:

(1) High resolution

(2) Short duration of a run

(3) Only µg quantities of sample required (high sensitivity)

(4) Direct monitoring of the solutes as they leave the column, *i.e.* there is no delay in the recording caused by time-consuming derivatization (staining).

We will present an electrophoretic method which has the same attractive features and which therefore can be classified as the electrophoretic counterpart of HPLC. This technique, which by analogy to the term high performance liquid chromatography may be called high performance electrophoresis (HPE), can be used for analysis of both high- and low-molecular weight substances.

Memo:

MOVING BOUNDARY ELECTROPHORESIS: ANALYTICAL-PREPARATIVE USEFULNESS AND THEORETICAL DEVELOPMENTS.

A. Chrambach and L. M. Hjelmeland
NIH, Bethesda MD, USA.

The theoretical treatments of moving boundary electrophoresis (MBE) by Longsworth, Rilbe and Martin and their scientific offspring are equivalent. This allows one to treat MBE as a single field, thus ending the confusion in the choice of experimental and instrumental conditions caused by the previous multiple terms describing it. Even isoelectric focusing with buffer constituents is a special case of MBE. The dynamics of pH gradients are accurately predicted by the moving boundary equation, when the proton and hydroxyl ion become the sole counterions. The practical usefulness of MBE is a) automatic protein concentration to give highly concentrated starting zones in gel electrophoresis; b) provision of a sharply defined reference zone for the characterization of zones by relative mobility; c) preparative automatic protein extraction and concentration from gel slices; d) preparative charge fractionation of oligocomponent systems with the highest load capacity of any electrophoretic separation method.

Memo:

ISOELECTRIC FOCUSING IN STABLE PREFORMED BUFFER pH GRADIENTS

M. Bier, R.A. Mosher and W. Thormann
Biophysics Laboratory, Univ. of Arizona, Tucson, AZ 85721, USA

The conventional wisdom in isoelectric focusing is that stable pH gradients need to be formed 'naturally' through the focusing of suitable carrier ampholytes. A significant advance has been realized recently by the introduction of polyacrylamide gel systems in which the buffering components are copolymerized and thereby immobilized.

Computer simulation clearly shows that pH gradients arise in all forms of electrophoresis, being nearly ubiquitous. For instance, in isotachophoresis two pH gradients may be seen, a migrating one at the interface between leader and terminator, and a stationary one arising from Kohlrausch-readjustment of terminator concentration. It is well known that such stationary pH gradients can be formed by simple dilution of a system of weak electrolytes away from neutrality, but these are not useful as the pH range is narrow and the conductivity difference is large.

We now wish to report a new system of stable and useful pH gradients not arising from the passage of current, but formed by simple pouring of the desired pH gradient in free solution using binary mixtures of buffers. An example is the forming of a gradient ranging from 4mM cacodylic acid and 2mM TRIS to 2mM acid and 4mM base, spanning the pH region 6 to 8. The stability and usefulness of such a system is easily demonstrated by its ability to focus hemoglobins. Only binary systems are stable, preferably of monovalent buffers and at neutrality. At more extreme pH values, H^+ or OH^- ions assume the role of a third component, thereby destabilizing the system. Experimental data on preparative focusing in density stabilized columns will be shown, including the preforming of sinusoidal pH gradients, covering the pH range 6 to 8 repeatedly, the current producing a parallel row of focused hemoglobins. These data will be complemented with computer simulations illustrating the range of system stability.

Supported in part by NASA grant NSG-7333.

Memo: