TWENTY YEARS OF ISOELECTRIC FOCUSING

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Before 1963 early versions of isoelectric focusing had been tried in only a few laboratories, however, with limited success. When I got the opportunity to work with Harry Svensson we started with carrier ampholytes made up of partial hydrolysates of proteins. Because these had many drawbacks it was challenging to find a better alternative. In 1964 I started developing a new system of synthetic polyamino polycarboxylic acids, which turned out very promising. During the following years I worked intensively with improving the synthetic procedures. The products were characterized and experiments made with several proteins. A very high degree of resolution was obtained at separation of proteins. The results were fascinating. These substances subsequently became commercially available (Ampholine(R)) and were adopted worldwide. The unique features of isoelectric focusing for both preparative and analytical purposes were increasingly recognized. Further important developments included procedures for focusing in gels and visualization of proteins at low concentrations. Isoelectric focusing used in two-dimensional electrophoresis procedures gives an unsurpassed resolution of proteins with respect to charge and size. This has a great impact in many areas of biological sciences. When combined with immunotechniques more information can be obtained. We are currently applying such techniques to reveal the effects of chemical exposure. To detect adverse effects is important. Different techniques depending upon isoelectric focusing are currently used in several branches of biological sciences and to detect malfunction at the molecular level.

Many have made important contributions to the development. More than twelve thousand reports have been published. It is estimated that the principle of focusing is now known by about one hundred thousand persons.

Memo:

ISOELECTRIC FOCUSING (IEF) ON SUPPORTED CELLULOSIC MEMBRANES

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Although cellulosic membranes have many advantages to the user, high electroendosmosis (EEO) prevented their use for IEF. The EEO is presumably caused by negative charges carried by free carboxyl groups. As previously reported, the carboxyl groups can be effectively esterified by MeOH with boron trifluoride as catalyst. Such treatment, however, impaired the mechanical properties of unsupported cellulose acetate membranes (CAM), so that they had to be inconveniently stored in wet form. A newly prepared Mylar supported CAM can be treated with BF_3 without adverse effects on their properties.

Using commercial amphoyltes (Servalytes) linear gradients are achieved. The focussing times are short ($<$1 h). The procedure is economical as only 1 mL of $<$5% ampholyte per about 70 cm^2 of CAM is used. Staining with Coomassie blue gives sharp intense bands on a white background; the CAM can also be cleared to transparency. The procedure has found several practical applications.

Memo:

A TEST FOR ARTEFACTUAL BINDING DURING ISOELECTRIC FOCUSING: BUFFERS VERSUS SYNTHETIC CARRIER AMPHOLYTE MIXTURES

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Two distinct types of binding artefacts in isoelectric focusing (IEF) have been described. One is related to ionic interactions and is quenched in 8 M urea, while the other is exemplified by the hydrophobic binding of NP-40.

An experimental test of binding became available with the introduction of a water-soluble macromolecular amphoteric dye, Poly R-480. IEF of this dye was conducted in non-restrictive polyacrylamide gel slab format. Three commercially available wide-range synthetic carrier ampholyte mixtures (SCAMs) were studied in comparison with a 47-component buffer mixture reported elsewhere.

In all of the SCAMs, Poly R-480 "focused" in a broad zone of pH 7-7.5, regardless of duration of focusing. By comparison, use of buffer carrier constituents resulted in prompt resolution of the dye into four discrete bands in the acid region.

When the study was repeated with the SCAMs in the presence of 8 M urea, binding in the neutral zone was effectively abolished. However, in each of the three preparations, the dye exhibited binding in complex patterns in the acid region. By comparison, IEF of the dye using buffer carrier constituents in the presence of 8 M urea effected the same rapid resolution as was observed in neat buffer gels.

These important findings represent the first demonstration of the persistence of artefactual binding in all of the SCAMs studied despite the use of 8 M urea. IEF in buffers, however, avoids these artefacts. Moreover, buffer electrofocusing serves as a useful test to define artefacual binding in SCAM systems.

Memo:

EVALUATION OF STAINED AND UNSTAINED ELECTROPHEROGRAMS BY MEANS OF PHOTOACOUSTIC SPECTROSCOPY

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It is demonstrated that photoacoustic spectroscopy is a superior tool in the evaluation of electropherograms.

The local distribution of proteins in ultra-thin layers of polyacrylamide gels used for isoelectric focusing (PAGE-IEF substrates) is mapped with a resolution equal or better than with conventional densitometers. Even unstained samples of colored proteins can be mapped with excellent sensitivity and resolution due to the fact that the photoacoustic effect is caused only by the absorbed fraction of the incident light; scattered light therefore does not cause a signal. Photoacoustic spectra of previously localized zones allow the identification of the prosthetic groups of these proteins.

Memo:

This abstract is the same as PS-3-7.