

This Week's Citation Classic

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Vesterberg O & Svensson H. Isoelectric fractionation, analysis, and characterization of ampholytes in natural pH gradients. IV. Further studies on the resolving power in connection with separation of myoglobins. *Acta Chem. Scand.* 20:820-34, 1966. [Biochem. Dept., Nobel Med. Inst., Stockholm, and Dept. Phys. Chem., Chalmers Inst. Technol., Gothenburg, Sweden]

With a new system of buffer substances it was possible to create pH gradients suitable for isoelectric focusing of proteins. The method offered outstanding properties at separation for preparative and analytical purposes. The isoelectric point of proteins could be determined easily and was reproducible. [The SCI® indicates that this paper has been cited over 1,130 times since 1966.]

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"The techniques for analytical and preparative separation of proteins have taken great strides during the last two decades. Seventeen years ago, the principle of isoelectric focusing was known by just a few scientists. At that time, I was lucky to start working with Harry Svensson at the Karolinska Institute, Stockholm. He had just worked out the theoretical basis of isoelectric focusing.¹ However, suitable buffer substances (carrier ampholytes) were lacking. Specialists in organic synthesis were contacted, but without success. One day it became obvious to me that it should be possible to make a large number of buffer substances with a suitable distribution of acidic and basic dissociation constants (pK values). During my studies I learned that pK values of polyvalent protolytes could be influenced by various substituents in the molecules, including the protolytic groups themselves. Thus, by coupling carboxylic acids to polyvalent amines, it should be possible to synthesize ampholytes useful in isoelectric focusing. After having considered various ways to make such buffer substances, I was able to prepare the first synthetic carrier ampholytes. They were shown to give the

predicted pH course on electrolysis and also to effect protein separation by isoelectric focusing. After these successful initial results, I worked very hard. Myoglobin, cytochrome c, and lactoperoxidase were now intensively examined by isoelectric focusing. Their isoelectric points were directly determined, and their heterogeneity was confirmed.

"Very interesting calculations were made concerning the resolving power of the method and also about the relation between differences in charge and in isoelectric points of very similar multiple molecular forms.¹ It was concluded that a charge difference of a few tenths of an electronic unit, or a difference in isoelectric point of about 0.01 pH unit, was sufficient to allow separation by isoelectric focusing. This implied a theretofore unattained resolving power. Isoelectric points of proteins could be determined in a very simple direct way (much easier than with other methods) and with a high degree of repeatability.² Thus a very valuable physico-chemical characteristic of proteins became easily available.

"More than 1,000 requests for reprints were received after publication of this article. I believe that the above text and mentioned advantages can explain this unusual interest. LKB Produkter's (Sweden) action to make apparatus and chemicals commercially available shortly after the article appeared also contributed to the success of the new method. The article became, so to speak, the definitive breakthrough of isoelectric focusing since it in all details substantiated theoretical predictions and gave clear evidence of an unsurpassed resolving power.

"In cooperation with others I have up till now published almost 60 papers in which isoelectric focusing has been used, and a review was recently published.³ Internationally, there are presently about 5,000 papers dealing with the method.

"Recently, I have been developing a new sensitive method for quantification of proteins by immunoelectrophoresis in tubes.⁴ Due to a very wide applicability it is also expected to attract the interest of many."

1. Svensson H. Isoelectric fractionation, analysis, and characterization of ampholytes in natural pH gradients. I. The differential equation of solute concentrations at a steady state and its solution for simple cases. *Acta Chem. Scand.* 15:325-41, 1961.
2. Vesttrberg O. Isoelectric fractionation, analysis, and characterisation of ampholytes in natural pH gradients. V. Separation of myoglobins and studies on their electro-chemical differences. *Acta Chem. Scand.* 21:206-16, 1967.
3.Isoelectric focusing. A review of analytical technique and applications. *Int. Lab.* 1978:61-8, 1978.
4.Quantification of proteins with a new sensitive method—zone immunoelectrophoresis assay. *Hoppe-Seylers Z. Physiol. Chem.* 361:617-24, 1980.