

## Chemistry of carrier ampholytes and Immobilines - Artefacts in 2D maps

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This lecture will be divided into two parts. Here are the highlights of both of them. Part one: The birth and evolution of IEF in conventional carrier ampholyte buffers is reviewed here, from a shaky start during World War II, via desperate attempts of Svensson to create pH gradients by stationary electrolysis of salts, to the development of the IEF theory and the solution of the steady-state equation. The remarkable synthetic process of Ampholines, as ingeniously devised by Vesterberg, is additionally recalled, with a thorough description of the fundamental properties of these amphoteric buffers, creating and maintaining the pH gradient under strong electric fields. The symptoms of decay are here presented through the simulations of Mosher and Thormann, clearly indicating an isotachophoretic mechanism for pH gradient decay with time. The decay of IEF was the birth of IPGs [1]. I will next highlight the developmental steps of the IPG technology, from a nebulous start limiting the technique to just 1 pH unit intervals up to the description of extended pH gradients, encompassing as much as 8.5 pH units. Although computer algorithms had been developed for optimizing recipes so as to obtain the most precise and most linear pH gradients, it was also realized that nonlinear pH intervals, covering the pH 3-10 range, would be extremely beneficial in 2-D map analysis, since they would follow the pI distribution of proteins in living systems. The synthesis of a number of Immobiline chemicals (the acrylamido weak acids and bases meant to be incorporated into the nascent polyacrylamide chains) is also reported. This survey will end with preparative aspects of IPGs, with the introduction of multicompartiment electrolyzers with Immobiline membranes [2]. Part two: In this section, I will touch upon a long-lasting debate on possible artefacts (i.e. generation of spurious spots, not belonging to the biological sample under analysis) induced by the separation technique (in this case, two-dimensional mapping) per se. It is shown here that some of the biggest offenders, always blamed in the past (at least since 1970, i.e. since the inception of gel-base isoelectric focusing protocols), namely deamidation (of Asn and Gln residues) and carbamylation (due to cyanate produced in urea solution), simply do not occur in properly handled samples and have never indeed been demonstrated in real samples,

except when forced in purpose. Conversely, two unexpected major artefacts have been recently shown to plague 2D mapping. One is formation of homo- and hetero-oligomers in samples that have been reduced but not alkylated prior to entering the electric field. The phenomenon is highly aggravated in alkaline pH regions and can lead to an impressive number of spurious spots not existing in the original sample. Thus, alkylation (best if performed with acrylamide or vinylpyridines) is a must for avoiding such spurious spots, as well as sample streaking and smearing in the alkaline gel region, and for maintaining sample integrity. In fact, the other unexpected artefact is desulfuration (?-elimination) by which, upon prolonged electrophoresis, the sample loses an -SH group from Cys residues. This loss, in the long run, is accompanied by massive protein degradation due to lysis of a C N bond along the polypeptide chain. Here too, alkylation of -SH groups of Cys almost completely prevents this noxious degradation phenomenon [3].

1. Righetti, P.G.: The Alpher, Bethe, Gamow of isoelectric focusing, the alpha-Centauri of electrokinetic methodologies. Part I. Electrophoresis 27 (2006) 923-938.
2. Righetti, P.G.: The Alpher, Bethe and Gamow of IEF, the alpha-Centauri of electrokinetic methodologies. Part II: Immobilized pH gradients. Electrophoresis 28 (2007) 545-555.
3. Righetti, P.G., Real and imaginary artefacts in proteome analysis via two-dimensional maps, J. Chromatogr. B, 841 (2006) 14-22