The Historical Development of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

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Electrophoresis is probably the most widely used method of biochemical analysis. Its use in protein separation, molecular weight determination, and peptide mapping has enabled the characterization of many complex mixtures, including body fluids and cell extracts. Many individuals have contributed much time to the development of functional electrophoretic techniques. The development of the state-of-the-art technique for sodium dodecyl sulphate-polyacrylamide gel electrophoresis did not occur overnight but has slowly evolved throughout the course of the twentieth century.

All electrophoretic techniques fall into one of two categories:[1] moving boundary electrophoresis[2] or zone electrophoresis.[3] Techniques of moving boundary electrophoresis exploit the fact that similar molecules have charge properties which are also very similar. These similar molecules will move close together as a band during electrophoresis, and boundaries will be formed between substances with slightly different electrophoretic mobilities. Competent techniques of moving boundary electrophoresis require much too complicated and expensive equipment for regular laboratory use.[4]

Zone electrophoresis techniques are based on the fact that charged particles which are supported on a relatively inert and homogeneous solid or gel framework will migrate as separate zones depending upon their specific characteristics.[5] Theoretically, zone electrophoresis can achieve complete separation of all of the electrophoretically different components of a mixture. Many different supporting media have been applied to zone electrophoretic techniques, all of which have specific advantages and disadvantages. Of the supporting media in use today, polyacrylamide gels are probably the most versatile and extensively used.

Electrophoresis has existed as a means of biochemical analysis for about fifty years. Experimentation with electrophoretic methods began around the turn of the century.[1,2,6,7] Attempts to develop an electrophoretic method for separating proteins were unsuccessful until 1937 when Tiselius separated proteins in a solution

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[1] O. Teague and B. H. Buxton, *J. Exp. Med.*, 1907, **9**, 254.
[2] C. W. Field and O. Teague, *J. Exp. Med.*, 1907, **9**, 86.
[3] R. J. Wieme, 'Agar Gel Electrophoresis,' American Elsevier Publishing Co., Inc., New York, 1965.
[4] 'A Biologist's Guide to Principles and Techniques of Practical Biochemistry', B. L. Williams and K. Wilson, American Elsevier Publishing Co., Inc., New York, 1975.
[5] D. J. Shaw, 'Electrophoresis', Academic Press, New York, 1969.
[6] C. W. Field and O. Teague, *J. Exp. Med.*, 1907, **9**, 222.
[7] H. Picton and S. E. Linder, *J. Chem. Soc.*, 1892, **61**, 148.

through electrophoresis.[8,9] Tiselius' method used a moving boundary technique that allowed for the formation of initial sharp boundaries.[8]

Zone electrophoresis, as an analytical method, was not used until 1952, when two methods of zone electrophoresis were proposed;[10,11] neither of these, however, provided a resolving power as great as that of the Tiselius method. In 1955, Smithies described a technique of zone electrophoresis which used a starch gel as the supporting medium.[12] The resolving power of the technique in many cases was found to be equal, if not superior, to that of the Tiselius method.

After the introduction of starch gels in 1955, experimentation began with other forms of gels as possible supporting media for zone electrophoresis. Raymond and Weintraub in 1959 introduced a polyacrylamide gel as a useful stable, flexible gel for zone electrophoresis.[13] The gelling agent present in the polyacrylamide gel was named Cyanogum, and was formed through a polymerization cross-linking reaction between two organic monomers, acrylamide and *N,N'*-methylenebisacrylamide. The gel was found to be best for electrophoresis when three- to five-percent Cyanogum was added to acid or alkaline buffers (0.3M to 0.01M). Raymond and Weintraub found the gel, once formed, to be optically clear and colourless, flexible and elastic, stable, and completely insoluble in water.[13]

Research with polyacrylamide gel advanced slowly after the introduction of Cyanogum gel. Along with co-workers, Raymond attempted to perfect his method of polyacrylamide gel electrophoresis (PAGE),[14,15] and by 1962 an apparatus which they designed was being produced by the E–C Apparatus Corporation of Swathmore, Pennsylvania.[16] The apparatus was a vertical gel electrophoresis cell which consisted of an upper and lower electrode chamber with cooling water channels embedded in the walls of the gel compartment.[17]

Raymond and his research group were not the only individuals performing research with PAGE. In 1962, Chang *et al.* were able to obtain clear resolution of at least twenty-five bands with a soluble protein preparation made from a mutant strain of *Neurospora crassa.*[18] Only eighteen bands were obtained using a starch-gel technique.[18] Davis and Ronstein also contributed much to the early techniques of PAGE by developing improved buffer systems which allowed high-resolution separations of proteins.[19]

The significance of pore size in PAGE had not been fully realized by 1962, but this rapidly changed. Raymond and Nakamichi had noticed that changes in the gel concentration often resulted in changes in the separation of some bands, and even

[8] A. Tiselius, *Trans. Faraday Soc.*, 1937, **33**, 524.
[9] A. Tiselius, *Biochem. J.*, 1937, **31**, 313.
[10] H. G. Kunkel, and A. Tiselius, *J. Gen. Physiol.*, 1951, **35**, 89.
[11] H. G. Kunkel, and P. J. Slater, *Proc. Soc. Exp. Med., N.Y.*, 1952, **80**, 42.
[12] O. Smithies, *Biochem. J.*, 1955, **61**, 629.
[13] S. Raymond and L. Weintraub, *Science*, 1959, **130**, 711.
[14] S. Raymond and Yi-Ju, Wang, *Anal. Biochem.*, 1960, **1**, 391.
[15] S. Raymond and M. Nakamichi, *Anal. Biochem.*, 1962, **3**, 23.
[16] S. Raymond, M. Nakamichi, and B. Aursell, *Nature*, 1962, **195**, 697.
[17] S. Raymond, *Clin. Chem.*, 1962, **8**, 455.
[18] L. O. Chang, A. M. Srb, and F. C. Steward, *Nature*, 1962, **193**, 756.
[19] B. J. Davis and L. Ronstein, *Ann N.Y. Acad. Sci.*, 1964, **121**, 321.

the disappearance of others. He hypothesized that these effects were related to the pore size of the polyacrylamide gels.[16] In 1963, Hjerten proved that the migration velocity of a protein in polyacrylamide gels depended not only upon the charge and molecular size of the protein, but upon the pore size of the gel.[20] By 1964, polyacrylamide gels of variable pore sizes were readily being made by varying the gel concentration.[21]

Many variations and applications in the use of electrophoresis on polyacrylamide gels arose between 1964 and 1966. A majority of these fell into the area of discontinuous electrophoresis. Jovin *et al.* developed an apparatus which was suitable for preparative, temperature-regulated PAGE in discontinuous buffer systems.[22] Through the use of this apparatus, complete separation of haemoglobins A and S was obtained from loads of haemolysate as large as 40 mg.

Another apparatus for preparative, discontinuous electrophoresis on polyacrylamide gels[23] resulted from slight modifications in the conventional vertical system of Smithies.[24] This apparatus was capable of providing greater than seventy-percent recovery of proteins.[23] Many other pieces of apparatus for discontinuous electrophoresis on polyacrylamide gels were also developed during this two year period.[25–29]

The popularity of PAGE as an analytical method also grew rapidly between 1964 and 1966. Through the use of discontinuous zone electrophoresis on a ten-percent polyacrylamide gel researchers were able to separate soluble ribonucleic acid into four major fractions.[30] Another group of researchers found that polyacrylamide gels could be used for electrophoretic sieving of intracellular particles.[31] This finding came about through the separation of the 30S and 50S ribosomes of *Escherichia coli*.[31] Polyacrylamide gel was found to be a good medium for micro-immunoelectrophoresis after proteins in human urine and serum were identified by PAGE using 2.8% acrylamide gel plates.[32] PAGE also allowed the simultaneous determination of serum-haemoglobin binding capacity and haptoglobin type.[33] The previous procedure required two different analyses.

In 1966, Maizel published a description of a mechanical fractionator which produced electropharograms by extrusion of polyacrylamide gels through a narrow orifice in a continuous, sequential stream.[34] The main point of his publication was that his fractionator could with ease produce electropharograms of

[20] S. Hjerten, *J. Chromatogr.*, 1963, **11**, 66.
[21] J. Vos and H. J. van der Helm, *J. Neurochem.*, 1964, **11**, 208.
[22] T. Jovin, A. Chrambach, and M. A. Naughton, *Anal. Biochem.*, 1964, **9**, 351.
[23] E. J. Devillez, *Anal. Biochem.*, 1964, **9**, 485.
[24] O. Smithies, *Biochem. J.*, 1959, **71**, 585.
[25] C. Schwabe, *Anal. Biochem.*, 1966, **17**, 201.
[26] G. L. Wright and W. L. Mallmann, *Proc. Soc. Exptl. Biol. Med.*, 1966, **123**, 22.
[27] R. C. Allen and D. J. Moore, *Anal. Biochem.*, 1966, **16**, 457.
[28] G. R. Stepherd and L. R. Gurley, *Anal. Biochem.*, 1966, **14**, 356.
[29] L. J. Rogers, *Biochim. Biophys. Acta*, 1965, **94**, 324.
[30] E. G. Richards and W. B. Gratzer, *Nature*, 1964, **204**, 878.
[31] S. Hjerten, S. Jerstedt, and A. Tiselius, *Anal. Biochem.*, 1965, **11**, 211.
[32] H. J. Keutel, C. R. Ammons, Jr., and W. H. Boyce, *Invest. Urol.*, 1964, **2**, 22.
[33] T. G. Foris, R. E. Easterling, K. J. Nelson, and R. E. Budd, *Am. J. Clin. Pathol*, 1966, **46**, 385.
[34] J. V. Maizel, Jr., *Science*, 1966, **151**, 988.

equal quality to those obtained by laborious manual sectioning. In one step of his procedure 0.05% sodium dodecyl sulphate (SDS) was pumped into the carrier-fluid inlet at a rate of 4 ml min^{-1} in order to carry the crushed gel.[34] He used the SDS because of its powerful virus-dissociating ability, which he desired in his work with Adenoviruses.[34] Maizel only mentioned SDS four times in his publication.[34] He obviously did not realize the future effect that SDS would have on PAGE but none-the-less he was the first to use it in association with polyacrylamide gel electrophoresis.

SDS did not become prominent in electrophoresis until after 1970, but between 1966 and 1970 many PAGE techniques were developed. In 1967, refinements of electrophoresis on polyacrylamide gels, based upon the methods of Raymond and Weintraub,[13] and Davis and Ronstein,[19] allowed higher resolution of normal serum proteins.[35] The discontinuous electrophoretic method was modified so that large 22 mm gels could be used.[36] These large gels were desirable for preparative electrophoresis. At the same time, an apparatus for preparative electrophoresis was developed for a loading of one gram of protein.[37] Techniques for gel slicing[38,39] and a technique for the preservation of polyacrylamide gels[40] were discovered. Other new types of apparatus for PAGE were also developed.[41–44]

A method for estimating the molecular weights of proteins by PAGE was defined in 1967.[45] Through use of the vertical PAGE method of Raymond and Weinstraub,[13] calibration curves were set up comparing the ratio of the mobilities of known proteins in two different gel concentrations to the log of their molecular weights.[45] From this curve, crude estimations of molecular weights could be made. A year later, a new method was published for the estimation of the molecular weights of proteins with a precision of $\pm 4\%$.[46] In this work a calibration curve was prepared by plotting the molecular weights of well-characterized proteins against the slope of the line resulting from the log of the relative protein mobility *versus* the acrylamide gel concentration.[46] These methods were later perfected and give fairly accurate results.[47,48]

A technique for electrophoresis on a polyacrylamide concentration-gradient gel was devised to enable full separation of a mixture of proteins.[49] The theory was that the gradual, constant variation in pore size would separate all sizes of proteins, but the first attempt was not very successful.[49] In 1968, a pore-size gradient method

[35] R. F. Ritchie, J. G. Harter, and T. B. Bayles, *J. Lab. Clin. Med.*, 1966, **68**, 842.
[36] R. C. Peterson, *J. Pharm. Sci.*, 1967, **56**, 1489.
[37] S. Hjerten, S. Jerstedt, and A. Tiselius, *Anal. Biochem.*, 1969, **27**, 108.
[38] H. C. Birnboim, *Anal. Biochem.*, 1969, **29**, 498.
[39] B. Garfinkle, *Anal. Biochem.*, 1970, **38**, 552.
[40] I. Smith and J. B. Weiss, *J. Chromatogr.*, 1967, **28**, 494.
[41] M. DeMets, A. Lagosse, and M. Radaey, *J. Chromatogr.*, 1969, **43**, 145.
[42] K. Felgenhauer, *Biochim. Biophys. Acta*, 1967, **133**, 165.
[43] J. Wein, *Anal. Biochem.*, 1969, **31**, 405.
[44] W. S. Bont, J. Geels, and G. Rezelman, *Anal. Biochem.*, 1969, **27**, 99.
[45] J. Zwaan, *Anal. Biochem.*, 1967, **21**, 155.
[46] J. L. Hedrick and A. J. Smith, *Arch. Biochem. Biophys.*, 1968, **126**, 155.
[47] C. R. Parish and J. J. Marschalonis, *Anal. Biochem.*, 1970, **34**, 436.
[48] D. P. Blatter and F. J. Reithel, *J. Chromatogr.*, 1970, **46**, 283.
[49] J. Margolis and K. G. Kenrick, *Biochem. Biophys. Res. Commun.*, 1967, **27**, 68.

was developed which successfully enabled the separation into several bands of two highly concentrated proteins.[50] This method was further perfected using a polyacrylamide gel gradient ranging from 5% to 20%.[51] An additional variation in PAGE techniques between 1966 and 1970 allowed the preparation of mixed acrylamide and agrose gels.[52] These gels allowed for higher resolution and greater separation of protein fractions.

After the first use of SDS in association with PAGE in 1966, Shapiro, Vinuela, and Maizel realized that it was possible to estimate the molecular sizes of polypeptides from the relative mobilities of their SDS-complexes on polyacrylamide gels. The method which they developed was rapid and versatile but had several flaws.[53] By 1969 the flaws were corrected and the method was used to determine the molecular weights of well-characterized proteins with an accuracy of at least 10%.[54] Later in the same year the method was further perfected to determine molecular weights of proteins with an error of less than $\pm 6\%$.[55]

Sodium dodecyl sulphate is a powerful, negatively charged detergent. It binds to the hydrophobic regions of protein molecules and causes them to unfold into extended polypeptide chains. This frees the individual protein molecules from their associations with other proteins or lipid molecules and renders them freely soluble in the detergent solution.[56] Whenever SDS is used, a reducing agent such as mercaptoethanol is usually added to break any S–S bonds present in the proteins.[56] The SDS-solubilized proteins are associated with many negatively charged detergent molecules and, as a result, each protein migrates toward the positive electrode when a voltage is applied across the gel.[56] Complete separation of all proteins occurs strictly according to size, regardless of their inherent solubilities in the aqueous solution.[56] The importance of this compound in biochemical analysis can not be stressed enough.

By using reducing agents, the correct molecular weights of proteins were determined by SDS-PAGE in 1972.[57] Indiscriminate use of non-reduced proteins and protein aggregates gave erroneous estimates for molecular weight determination by SDS-PAGE.[57] Later in the same year, the separation of human serum by SDS-PAGE was found to result in the resolution of twice as many component proteins.[58] Another research group[59] validated the molecular weight estimates of several membrane proteins through the use of SDS-PAGE. Confidence in the analytical accuracy of SDS-PAGE was growing.

During the following three years (1973—1975) SDS-PAGE expanded from isolated use by few researchers into one of the most common methods of

[50] E. Epstein, Y. Houvras, and B. Zak, *Clin. Chim. Acta*, 1968, **20**, 335.
[51] G. G. Slater, *Anal. Biochem.*, 1968, **24**, 215.
[52] U. Ringborg, E. Egyhazi, B. Daneholt, and B. Lambert, *Nature* 1968, **220**, 1037.
[53] A. L. Shapiro, E. Vinuela, and J. V. Maizel, *Biochem. Biophys. Res. Commun.*, 1967, **28**, 815.
[54] K. Weber and M. Osborn, *J. Biol. Chem.*, 1969, **244**, 4406.
[55] A. K. Dunker and R. R. Rueckert, *J. Biol. Chem.*, 1969, **244**, 5074.
[56] B. Alberts, D. Bray, D. Lewis, J. Raff, K. Roberts, and J. D. Watson, in 'Molecular Biology of the Cell', Garland Pub., Inc., New York, 1983.
[57] I. P. Griffith, *Biochem. J.*, 1972, **126**, 553.
[58] G. J. Brewer, E. Lopez-Corella, and M. E. Noelken, *Clin. Chim. Acta*, 1972, **36**, 574.
[59] G. A. Banker and C. W. Cotman, *J. Biol. Chem.*, 1972, **247**, 5856.

biochemical analysis. SDS-PAGE methods extend into all areas of electrophoresis. A technique for performing isoelectric focusing on proteins dissolved in SDS was developed.[60] Another SDS-PAGE technique allowed the determination of the molecular weights of proteins at the nanomole level.[61] These proteins had low molecular weights and were dissolved in a 0.1% SDS solution.[61]

Researchers isolated many proteins through the use of various SDS-PAGE methods during this same period, including the growth hormone (GH) and prolactin (PRL) from rat pituitary homogenates and cell fractions.[62] Quantitative determinations of GH and PRL were possible because SDS-gel systems allowed for such discrete separation.[62] The researchers involved were even able to estimate the degree of contamination present in GH and PRL separated by a PAGE method lacking SDS.[62] The molecular weight of human erythropoietin was also determined by SDS-PAGE.[63] SDS-PAGE allowed the preparative separation of milligram quantities of all of the major erythrocyte membrane proteins with a yield as great as 93%.[64]

Modifications of the SDS-PAGE methods were brought to near perfection by 1975. One simple modification eliminated the use of SDS in the gel and electrode compartment, limiting its use to the sample solution alone.[65] Another method allowed direct visualization of protein bands in SDS-polyacrylamide gels.[66] The procedure consisted of chilling the gels to 0—4 °C and observing the white opaque bands of proteins, thus eliminating the need for stains.[66] Another research group found that sodium octylbenzene-*p*-sulphonate could be used in place of SDS and PAGE, but the method was never extensively used.[67] Although it had limitations, by 1975 SDS-PAGE was the best means for protein analysis.

Electrophoresis on polyacrylamide gels with SDS can separate proteins from relatively complex mixtures. However, when more complex mixtures (generally containing greater than fifty different proteins) are separated by this one-dimensional method, closely spaced protein bands tend to overlap. It was for this reason that two-dimensional electrophoresis was developed. The theory behind two-dimensional electrophoresis is defined by the dimensions. In the most advanced methods, proteins are first separated according to their isoelectric points and then further separated according to their molecular weights. Electrophoresis performed in this manner assures complete separation of all of the proteins in a complex mixture.

In 1969, Margolis and Kenrick published a method for two-dimensional electrophoresis which utilized polyacrylamide gels.[68] In the first stage a low-concentration polyacrylamide gel was used to perform discontinuous electro-

[60] D. W. Miller and S. C. R. Elgin, *Anal. Biochem.*, 1974, **60**, 142.
[61] T. Kato, M. Sasaki, and S. Kimura, *Anal. Biochem.*, 1975, **66**, 515.
[62] A. Zanini, G. Giannattasio, and J. Meldolesi, *Endocrinology*, 1974, **94**, 594.
[63] M. Dorado, J. Espada, A. A. Langton, and N. C. Brandan, *Biochem. Med.*, 1974, **10**, 1.
[64] H. Knuefermann, S. Bhakdi, and D. F. H. Wallach, *Biochim. Biophys. Acta*, 1975, **389**, 464.
[65] J. T. Stoklosa and H. W. Latz, *Biochem. Biophys. Res. Commun.*, 1974, **58**, 74.
[66] R. W. Wallace, P. H. Yu, P. J. Dieckert, and W. J. Dieckert, *Anal. Biochem.*, 1974, **61**, 86.
[67] K. Tsujii and T. Takagi, *J. Biochem. (Tokyo)*, 1975, **77**, 117.
[68] J. Margolis and K. G. Kenrick, *Nature*, 1969, **221**, 1056.

phoresis, thus allowing the proteins to migrate with very little resistance.[68] The second stage consisted of a gradient-gel electrophoresis.[68] Separation resulting by this method was only slightly greater than that resulting from one-dimensional PAGE.

A year later, researchers[69] proposed a two-dimensional polyacrylamide gel system which greatly improved the analytical separation of complex protein mixtures obtained from ribosomes. Through this method the protein mixtures of *Escherichia coli* ribosomes were separated into about fifty components.[69] The procedure was rather widely used over the next five years in spite of its limited applicability.

DeWachter and Fiers[70] published in 1972 a method of preparative two-dimensional PAGE which was capable of separating complex mixtures of RNA molecules. The first dimension separation was executed on polyacrylamide acid gels in the presence of a high concentration of urea, and the second dimensional separation was performed on more highly concentrated polyacrylamide gels buffered at pH 8.[70] Another two-dimensional PAGE technique was developed in 1973 by Orrick *et al.*[71] to separate extracted rat liver nuclei into about one hundred distinct proteins.

In 1974, a two-dimensional system of electrophoresis for the analysis of ribosomal proteins was described.[72] This system had several advantages over the previous systems. The first dimensional separation was based on the mobility of ribosomal proteins at pH 5 in 8M urea and 4% polyacrylamide. The second dimensional separation occurred on the basis of molecular weight, in the presence of SDS.[72] High resolution of ribosomal proteins resulted from use of this method. In the same year, the two-dimensional PAGE system developed by Orrick[71] was modified to improve the separation of proteins from rat liver.[73] The modifications included reducing the polyacrylamide concentration in the first dimension from 10% to 6% and substituting 8% polyacrylamide for a linear 8—10% polyacrylamide gradient in the second dimension.[73] These modifications resulted in improved resolution of the protein bands.

O'Farrell[74] in 1975 published a new method for high-resolution two-dimensional SDS-PAGE. In first dimensional separation the proteins were separated according to their isoelectric points, on isoelectric focusing gels made with polyacrylamide. In the second dimensional separation the proteins were further separated according to their molecular weights by means of a discontinuous SDS polyacrylamide gel system. With this technique, O'Farrell was able to resolve 1 100 different components from *Escherichia coli*. Proteins differing by only a single charge could be resolved and proteins comprising 10^{-4} to 10^{-5}% of the total protein content could be detected. The implications of this powerful method were obvious.

[69] E. Kaltschmidt and H. G. Witmann, *Anal. Biochem.*, 1970, **36**, 401.
[70] R. DeWachter and W. Fiers, *Anal. Biochem.*, 1972, **49**, 184.
[71] L. R. Orrick, M. O. J. Olson, and H. Busch, *Proc. Nat. Acad. Sci. USA*, 1973, **70**, 1316.
[72] L. J. Mets and L. Bogorad, *Anal. Biochem.*, 1974, **57**, 200.
[73] G. I. Busch, L. C. Yeoman, C. W. Taylor, and H. Busch, *Physiol. Chem. Phys.*, 1974, **6**, 1.
[74] P. H. O'Farrell, *J. Biol. Chem.*, 1975, **250**, 4007.

Lambin *et al.* devised a new method of SDS-PAGE useful for the determination of large molecular weight proteins in 1976.[75] The electrophoresis was performed on a 20 to 30% polyacrylamide gradient gel; and proteins, with molecular weights ranging from 10 000 to 1 000 000, could be determined with good accuracy.

In 1977, a micro-gel-electrophoresis technique, which permitted the routine analysis of proteins in levels as low as 10^{-9} g was developed using SDS in the polyacrylamide gel system.[76] It allowed the use of micro-samples to determine the molecular weights of proteins.[76] Another technique was developed at the same time for the quantitative analysis of proteins on SDS-PAGE system.[77] Olden and Yamada developed a similar technique for the direct detection of antigens on SDS-polyacrylamide gels.[78] In 1978, a new system (which was entirely self-contained and highly efficient) was described for the preparative electrophoresis on polyacrylamide gels.[79]

Modifications developed in 1980 by preparative SDS-PAGE allowed improved separation of high molecular-weight proteins.[80] In the next year, a new method was developed for the detection of nanogram quantities of proteins on SDS-polyacrylamide gels.[81] The method employed an ^{125}I-labelled reagent.[81] In 1983, an improved method for the separation of low molecular-weight polypeptides was described[82] using a 10% to 18% linear gradient polyacrylamide gel containing 7M urea. With this method peptides whose molecular weights ranged from 1 500 to 100 000 were highly resolved.[82] In the same year, picogram amounts of *Escherichia coli* DNA-polymerase were successfully detected through the use of a modified technique of SDS-PAGE.[83] The modifications consisted of embedding fibrinogen in the gels and washing the detergents from the gels with aqueous isopropyl alcohol after electrophoresis.[83]

After the introduction of O'Farrell's astounding technique in 1975,[74] two-dimensional SDS-PAGE became popular for it required very few modifications to function in most situations. O'Farrell's technique was put to use in 1976, less than one year after it was published. Slight modifications were made in order to allow protein analysis of *Escherichia coli* and *Salmonella typhimurium* cell envelopes.[84] The same researchers used the technique to separate plasma membrane proteins from HeLa cells.[84] Another research group used the O'Farrell technique to analyse the non-histone chromosomal proteins of the HeLa cells.[85] More than 450 components were revealed, most of which were rare (less than 10 000 copies per cell) and were not previously detectable in the cytoplasm.[85]

[75] P. Lambin, D. Rochu, and J. M. Fine, *Anal. Biochem.*, 1976, **74**, 567.
[76] J. S. Condeelis, *Anal. Biochem.*, 1977, **77**, 195.
[77] T. Asao, *Anal. Biochem.*, 1977, **77**, 321.
[78] K. Olden and K. M. Yamada, *Anal. Biochem.*, 1977, **78**, 483.
[79] J. J. Koziarz, H. Koehler, and T. L. Steck, *Anal. Biochem.*, 1978, **86**, 78.
[80] D. A. Hardman and J. P. Kane, *Anal. Biochem.*, 1980, **105**, **174.**
[81] Y. W. Shing and A. Ruoho, *Anal. Biochem.*, 1981, **110**, 171.
[82] F. Hashimoto, T. Hosigome, M. Kanbayashi, K. Yoshida, and H. Sugano, *Anal. Biochem.*, 1983, **129**, 192.
[83] A. Blank, J. R. Silber, M. P. Thelen, and C. A. Dekker, *Anal. Biochem.*, 1983, **135**, 423.
[84] G. Ferro-Luzzi-Ames and K. Nikaido, *Biochemistry*, 1976, **15**, 616.
[85] J. L. Peterson and E. H. McConkey, *J. Biol. Chem.*, 1976, **251**, 548.

Addition of SDS to the sample preparation enhanced the reproducibility of gel patterns in the O'Farrell technique.[86] The glass plates, which hold the second-dimension polyacrylamide gel, were slightly modified and ultracentrifugation was used to reduce clogging at the top of the isoelectric focusing gel.[86] In 1978, a procedure was developed for the determination of similar amino-acid composition among cellular proteins separated by two-dimensional gel electrophoresis.[87]

A differential two-dimensional PAGE method was devised in 1979 for peptide mapping of heterogeneous protein samples.[88] In the first dimension a mixture of denatured and the reduced proteins was separated on a SDS-polyacrylamide gel slab. In the second dimension each of the separated proteins was subjected to partial proteolysis and resolved into a characteristic pattern of peptides by a stacking technique. By means of this method, up to twenty individual proteins could be analysed at once.[88] In the same year the polypeptide turnover rates in

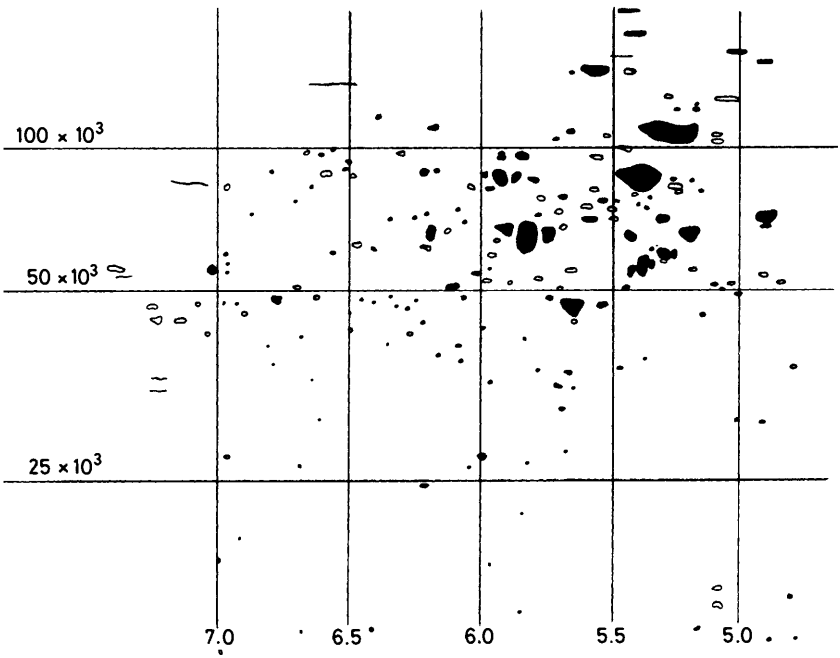

Figure 1 *Schematic representation of CHO plasma membrane polypeptide 'map.' Vertical bars dividing the map are based upon approximate pH values, while horizontal lines designate molecular weight. Major proteins of the CHO cell plasma membrane map are shown as black spots, while minor polypeptides or variable spots are shown in outline. Each one of the proteins was further identified using the radioactive isotopes*
(Reproduced by permission from *ref.* 89)

[86] D. L. Wilson, M. E. Hall, G. C. Stone, and R. W. Rubin, *Anal. Biochem.*, 1977, **83**, 33.
[87] F. Cabral and M. M. Gottesman, *Anal. Biochem.*, 1978, **91**, 548.
[88] C. Bordier and A. Crettol-Jarvinen, *J. Biol. Chem.*, 1979, **254**, 2565.

Chinese hamster ovary cell plasma membranes were correctly determined through the use of the O'Farrell technique (see Figure 1).[89] Previously, turnover rates were determined through the use of one-dimensional systems of electrophoresis which resulted in considerable overlap of the bands.[89]

One dimensional discontinuous SDS-PAGE slab was successfully used to give linear molecular weight separations from 2 500—90 000.[90] Slab SDS-PAGE was a strong improvement over the normal one-dimensional PAGE. Using two adjacent SDS-PAGE slabs, one coated with 2-mercaptoethanol and the other uncoated, showed the mobility of the reduced and non-reduced disulphide bonds in the protein.[91]

Chao in 1980 described a technique which allowed high-precision comparisons of complex protein patterns from two different cell lines.[92] The technique combined

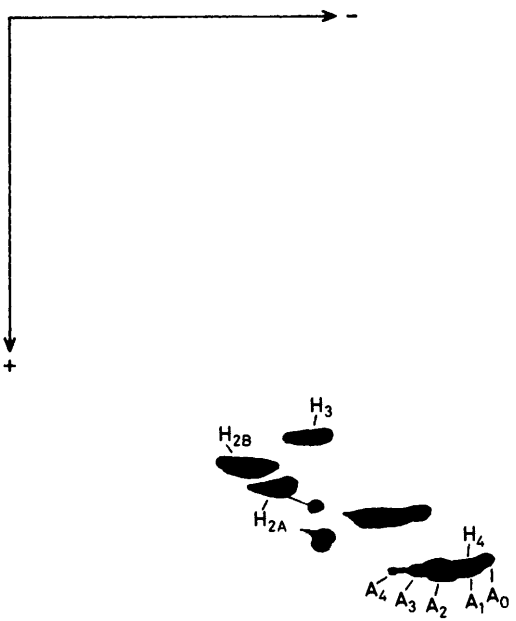

Figure 2 *Two-dimensional minislab gel containing yeast histones. Nine micrograms of yeast histone were electrophoresed on an acetic acid–urea minislab gel. After staining and destaining of the minislab gel, the gel lane was excised; prepared for the electrophoresis and electrophoresed on a 15% polyacrylamide–sodium dodecyl sulphate minislab gel. The results are shown above.*
A one-dimensional electrophoresis did not definitely separate the yeast histone. The two-dimensional run adequately separated them into their four acetylated species (H_2A, H_2B, H_3, *and* H_4) *and the* H_4 *species into its various protein species* (A_0–A_4).
(Reproduced by permission from *ref.* 93)

[89] M. N. Horst and R. M. Roberts, *J. Biol. Chem.*, 1979, **254**, 5000.
[90] B. L. Anderson and R. W. Berry, *Anal. Biochem.*, 1983, **132**, 365.
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a double isotope labelling procedure with two-dimensional PAGE.[92] Lam and Kasper developed a procedure for examining sequence homology between two or more proteins in a heterogeneous protein mixture (see Figure 2).[93] In the same year, two-dimensional PAGE was utilized to analyse human pancreatic fluid.[94] This analytical procedure separated pancreatic fluid into thirteen individual proteins. Even the α1 and α2 chains of rat tail tendon collagen were separated.[95] This occurred because the α2 chain binds appreciably to the SDS while the α1 has negligible binding.

In 1982, Davie devised two two-dimensional polyacrylamide gel systems for the rapid analysis of histones.[96] In the first system, an acetic acid–urea or acetic acid–urea–Triton X-100 minislab gel made up the first dimension and a polyacrylamide SDS minislab gel made up the second dimension. In the second system the first and second dimensional separations were simply switched. Both systems allowed for rapid, high-resolution analysis of modified histone species and variants.

Heating the peptides with SDS buffer prior to electrophoresis is very popular. Hodges and Hirata[97] showed that it hydrolyses the protein and leads to many new spots which show greater intensity with a silver staining solution. A similar enhanced mapping of protease after partial hydrolysis for samples as small as 50 mg of protein was reported by Cleveland *et al.*[98] However, these authors were probably unfamiliar with work by Rittenhouse and Marcus,[99] who showed that heating polypeptides to 110 °C in SDS buffer prior to electrophoresis preferentially cleaved the aspartyl–prolyl peptide bond. Although the latter authors performed most of their work on fructose-1,6-bisphosphatase, the cleavage was specific and exhibited distinctly different PAGE patterns than proteins containing no aspartyl–prolyl peptide bonds. It is possible that either Hodges and Hirata or Cleveland's success might be due to this cleavage.

In 1983, Tijssen and Kurstak developed an efficient two-dimensional SDS-PAGE method for the simultaneous peptide mapping of proteins contained in a mixture.[100] These two researchers found that the previous methods of peptide mapping[88,93] gave non-consistent results.[100] Their technique consisted of first separating the polypeptides by SDS-PAGE, then embedding the strip of gel obtained from SDS-PAGE perpendicular to the direction of electrophoresis in the stacking gel of a second gel system, into which proteolytic enzymes were loaded. The technique could also be performed in the Laemmli gel system[101] with modifications.[100]

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Since electrophoresis was first put into practical use in 1937 by Tiselius[8,9] it has constantly been under modification and improvement and has been used to identify all types of proteins and protein residues. However, with smaller and smaller samples, contamination also becomes more of a problem and Ochs[102] discovered that many erroneous bands in SDS-PAGE work were due to skin proteins. Her work indicated that extreme care is essential for good analytical biochemistry.

The proliferation of literature is an indication of the maturity of a method and specific reviews have started to appear. One by Spanos and Huebscher[103] reviews the enzyme catalytic activity after SDS-PAGE and another by Sano[104] covers the specific analyses of proteins.

Raymond and Weintraub[13] introduced polyacrylamide as a stabilizing medium for zone electrophoresis and in the years since their discovery great advances have been made in the techniques. It is obvious that many more unique techniques can be expected in the future.

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