ELSEVIER

Journal of Chromatography A, 698 (1995) 33–40

JOURNAL OF CHROMATOGRAPHY A

Review

Electrophoresis on cellulose acetate and Cellogel: current status and perspectives

Giovanni Destro-Bisol[a,b,*], Stefano A. Santini[c]

[a]*Istituto di Medicina Legale dell'Università Cattolica del Sacro Cuore, L.go F. Vito, 00168 Rome, Italy*
[b]*Dipartimento di Biologia Animale e dell'Uomo, Università di Rome "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy*
[c]*Istituto di Chimica Biologica dell'Università Cattolica del Sacro Cuore, L.go F. Vito, 00168 Rome, Italy*

Abstract

After introducing some general concepts of cellulose acetate membranes (CAMs) and gelled cellulose acetate membranes (GCAMs and their use for electrophoretic separations, an overview on CAM- and GCAM-based protocols for conventional electrophoresis, isoelectric focusing (IEF) and isotachophoresis (ITP) is given. Examples for each of the three methodologies are described and discussed. Further, some innovative applications of Cellogel in electrophoretic protocols are presented: (i) the reuse of GCAMs already used for the conventional electrophoretic analysis of some erythrocyte enzymes; and (ii) the transfer of electrophoretically separated proteins from an IEF polyacrylamide gel to a GCAM, without impairing the functioning of the gel.

Contents

1. Historical background and general concepts

In the last 40 years, electrophoretic methods have evolved intensely and now offer high resolution analytical and preparative separations in analytical chemistry, biochemistry and in biological sciences. This methodological renewal started after 1950 with the introduction of zone electrophoresis that replaced Tiselius's moving boundary electrophoresis [1] for many analytical

* Corresponding author. Address for correspondence: Dipartimento di Biologia Animale e dell'Uomo, Università di Roma "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy.

0021-9673/95/$29.00
SSDI 0021-9673(94)01279-2

and preparative purposes. In zone electrophoresis, components migrate as well defined and delimited zones in the free spaces of a supporting medium immersed in a single buffered solution that connects the anode and the cathode. Filter-paper was one of the first supporting media for zone electrophoresis and immediately received great attention from researchers and clinical chemists, also thanks to the contributions of Wieland and Fischer [2] and other researchers.

Cellulose acetate as supporting medium for zone electrophoresis was introduced later [3], as an improvement on filter-paper electrophoresis. Cellulose acetate is obtained by the action of carbonic anhydride on cellulose; the product of this reaction is then dissolved in an organic and volatile solvent which is left to evaporate under well controlled conditions of temperature and humidity. At the end of this procedure, a membrane with a uniformly distributed microporosity is obtained. The volume of the pores may account for 80% of the total, thus ensuring good absorption of electrolytic solution. Cellulose acetate membranes (CAMs), originally developed from bacteriological membrane filters, differ from each other in the degree of acetylation, thickness, pore size, texture, etc., but the separation patterns are essentially the same. The physical and chemical characteristics of the different CAMs are supplied by the manufacturers and will not be discussed here in detail. Gelled cellulose acetate strips (Cellogel, GCAM) represent a valid alternative to the classical CAMs for many applications, as gelification preserves membranes from dehydration due to the Joule effect during electrophoretic separation and makes them more resistant to inaccurate handling.

The principles of CAM and GCAM electrophoresis are essentially the same as those of filter-paper electrophoresis, whereas cellulose acetate has some properties that make this support more suitable than filter-paper for electrophoretic separations: (i) the material is homogeneous, microporous and chemically relatively pure, and adsorption of migrating molecules is minimal; (ii) neither cooling nor refrigeration is necessary during electrophoresis; (iii) the aliquots required for assays (usually much less than 1 mg of protein for analytical purposes), the amounts of various reagents used for the visualization of the bands and the incubation period during staining are minimal; (iv) the strips can be cleared using a suitable swelling agent or by immersion in an appropriate clearing fluid to glass-like transparency, thus reducing the background error, even for further scanning procedures.

For most purposes and particularly for routine clinical investigations, small-scale cellulose acetate electrophoresis (<10 cm long membranes) is still the method of choice. The large-scale method (usually 20 cm) is usually adopted for research analytical purposes and for micropreparative electrophoresis.

2. Conventional electrophoresis on CAMs and GCAMs

Since their introduction as supports for zone electrophoresis [3], CAMs and, later, GCAMs have found a broad range of applications in conventional electrophoretic protocols. In fact, CAMs and GCAMs can be used for both analytical and preparative purposes, and the possibility of eluting or scanning the membranes also allows quantitative determinations. Finally, regarding biologically active substances, the relative rapidity of the electrophoretic separation considerably reduces the danger of loss of activity.

For preparative purposes, stained bands can be easily obtained from the membranes by elution with an appropriate buffer system (e.g., Tris or barbitone buffer for Ponceau Red-stained bands). Alternatively, the membrane can be dissolved by a solvent [e.g., chloroform–ethanol (9:1)]. Cellogel blocks (about 0.5 cm thick instead of the customary 0.5 mm) can be used to increase the recovery of purified substance [4].

Much wider is the range of applications of CAMs and GCAMs for analytical purposes, which parallels the variety of the different staining methods that can be successfully applied to the membranes: Coomassie Brilliant Blue

Fig. 1. EAP isozyme patterns by conventional electrophoresis on GCAMs [6]. Phenotypes from left to right: B, B, B and C. Anode on top.

(CBB), Ponceau Red, nigrosin, Schiff, gold stain, different types of immuno-staining and a great number of enzyme-specific staining methods.

In addition to some well established clinical routine applications such as haemoglobin (HB), serum proteins, lipoproteins and lactate dehydrogenase (LDH) [5], CAMs and GCAMs are currently used for the separation of the isoforms of many enzymes and proteins from different tissues. The latter applications are of particular interest for genetic screening in humans for anthropogenetic and forensic purposes [6] (Figs. 1 and 2) and for the biochemical characterization and classification of various pathogenic microorganisms such as *Leishmania* [7] and *Trypanosoma* [8]. In addition to the above examples of one-dimensional electrophoresis, two-dimensional CAM and GCAM protocols are also available [9,10]. Some recent applications of CAM electrophoresis are described in Table 1.

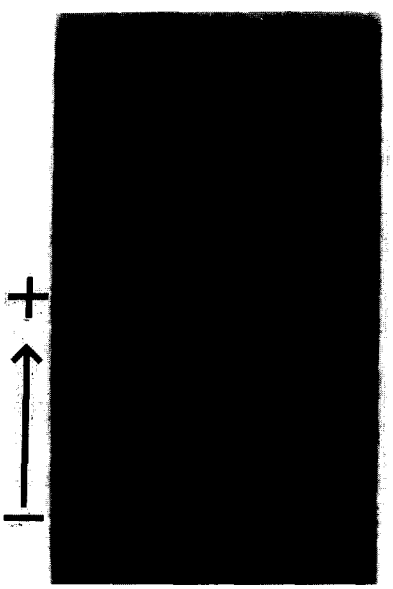

Fig. 2. GPX1 isozyme patterns by conventional electrophoresis on GCAMs [following Meera Khan et al. (1984), cited in Ref. [37]). Phenotypes from left to right: 2–1, 2, 2–1 and 1.

3. Isoelectric focusing on CAMs and GCAMs

Cellulose acetate has some properties that could make this support ideal for isoelectric focusing separations: (i) CAM is virtually a non-sieving matrix which allows unhindered migration of macromolecules to their p*I*s; (ii) CAM requires very little ampholines and is extremely convenient for staining and destaining, densitometry and storage. Unfortunately, CAM electroendosmotic flow (due to carboxyl groups) combined with the low ionic strength of focused carrier ampholytes makes it difficult to obtain good IEF results using CAM as a support. To overcome these drawbacks, CAMs and GCAMs have been treated with surface-active agents [22,23] or extensively methylated [24–26]. However, it has been claimed that also under these conditions the osmotic flow remains too high to allow reproducible separations [27], despite using high concentrations of carrier ampholytes for broad pH ranges [8% (v/v) instead of the customary 2% (v/v)] and low concentrations of electrolytes (such as 0.2 *M* lysine and 0.2 *M* acetic acid) for narrow pH ranges. Paradoxically, good results were obtained with untreated CAM strips [28], probably owing to the addition of 5% β-mercaptoethanol and 5 *M* urea as pH gradient stabilizing agents. Kane et al. [29] were able to improve CAM IEF (using Harada's membranes) for EAP and ESD analysis by shortening the

Table 1
Some recent applications of CAM electrophoresis

	Buffer composition and pH	Power supply settings and electrophoresis time	Ref.
Acidic glycoconjugates	0.1 *M* pyridine–0.47 *M* formic acid, pH 3.0	1 mA/cm, 30 min	Yoshihara et al. [11]
Bone and liver alkaline phosphatase	0.03 *M* Tris–barbital–sodium barbital, pH 8.8	180 V, 45 min	Nishikawa et al. [12]
Chondroitin sulfate E	0.1 *M* calcium acetate, pH 7.7	2.5 V/cm, 3 h	Kariya et al. [13]
Dermatan sulfate	0.2 *M* calcium acetate, pH 7.2	0.6 mA/cm, 6 h	Eggen et al. [14]
Glycosaminoglycans	0.2 *M* zinc sulfate, pH 5.1	1 mA/cm, 1 h	Horne et al. [15]
5′-Nucleotidase	0.03 *M* Tris–Barbital, pH 8.9	300 V, 12 min	Panteghini [16]
Pancreatic isolipase	0.1 *M* Tris, pH 7.8	230 V, 20 min	Panteghini [17]
Serum amylase	0.05 *M* Tris–HCl–barbitone, pH 8.6	250 V, 60 min	Ventrucci et al. [18]
Pyruvate kinase	0.02 *M* Tris–citrate–0.001 *M* EDTA, pH 7.8	12 V/cm 3 hr	Weernink et al. [19]
Serum and urine proteins	0.03 *M* Tris–barbital–sodium barbital, pH 8.8	200 V, 25 min	Pedraza-Chaverri et al. [20]
Sulfated glycosaminoglycans	0.2 *M* calcium acetate, pH 7.2	0.6 mA/cm, 6 h	Kirkham et al. [21]

electrode distance, so providing a higher field strength at low voltages, and using chemical spacers to flatten pH gradients. Some applications of CAM and GCAM IEF are listed in Table 2.

4. Counterflow affinity isotacophoresis on GCAMs

Isotachophoresis (ITP) or "displacement" electrophoresis permits the simultaneous concen-

Table 2
Some applications of IEF on CAMs and GCAMs

	Support	pH range and concentration (v/v) of carrier ampholytes	Power supply settings and electrophoresis time	Ref.
Lactate dehydrogenase	CAM	3.5–10 (3%)	500 V, 90 min	Harada et al. [23]
Globin chains	GCAM	6–8 (1.11%) and 3.5–10 (0.14%)	110 V (0.2 mA per strip), 1 h	Boussios and Bertles [28]
Arylsulfatase, hexosaminidase and β-galactosidase	CAM	3.5–9.5 (4%) and 4–6 (4%)	1000 V (4 mA per strip), 60–80 min	Farrell et al. [30]
Serum and urine proteins[a]	CAM	3.5–10 (5%)	800 V (max.), 1 mA per strip, 3 h	Toda et al. [31]
Erythrocyte acid phosphatase	CAM	5–8 (5–6.5 + 5–8, 5:1); 5–7 or 5–8	Max. 1200 V, constant 4 W, 30 min	Kane et al. [29]
Esterase D	CAM	5–6.5[b]	Max. 100 V, constant 4 W, 25 min	Kane et al. [29]
Phosphoglucomutase, locus 1	GCAM	5–8 or 5–7 (12.7%) and 3.5–10 (0.38%)	750 V, 1.5 mA per strip, 1.5 h	Dobosz and Koziol [32]

[a] Second dimension of a two-dimensional electrophoresis protocol
[b] N-(2-Hydroxyethyl)piperazine-N′-3-propanesulfonic acid separator is included here

tration and effective separation of different charged substances, including biological macromolecules [33]. In this method, the proteins are stacked as closely spaced narrow bands between the "leading" and the "trailing" ions. Since CAMs and GCAMs are not affected by the molecular mass of the separated molecules, ITP on this support may be performed for studying diverse sets of interacting biological macromolecules, such as antigen–antibody and glycoprotein–lectin systems. However, electroendosmosis affecting CAM and GCAM electrophoresis is in principle an obstacle to performing ITP separations on these supports. Abelev and co-workers [34,35] overcame this drawback, demonstrating that a cathodic counterflow combined with a constant flow of liquid through the membrane provides stable separation conditions; at the same time, the counterflow may be used as a "conveyer belt" which moves immunoreagents through antigens or antibodies immobilized on the membrane. In Abelev and co-workers' protocol [34,35], GCAM ITP is carried out in a discontinuous buffer system, with the buffer sharing the same cation and differing in the anions (chloride as leading ion and β-alanine as trailing ion); the mobilities of the separated macromolecules are between those of the two anions.

Abelev and co-workers' approach, devised for determining low-concentration proteins in biological fluids such as urine, tears and cerebrospinal and amniotic fluid, turned out to be particularly useful for detecting low levels of urinary monoclonal Ig light chains (Bence–Jones protein) and α-fetoprotein in various pathological conditions [35,36].

5. Other applications of GCAMs in electrophoretic protocols

5.1. GCAMs as reusable electrophoretic support

Separations provided by CAM and GCAM electrophoresis are usually more rapid than those on starch, agar, agarose and polyacrylamide gels and their analytical power is in many instances comparable to that provided by the other electrophoretic substrates. However, the cost of CAM and derived products, much higher than that of alternative substrates, still remains a problem for their widespread adoption.

We have recently proposed a simple method [37] that makes it possible to restore Cellogel strips already used for the conventional electrophoretic analysis of some erythrocyte enzymes, including adenosine deaminase (ADA), adenilate kynase (AK), carbonic anhydrase (CAII), erythrocyte acid phospatase (EAP), esterase D (ESD), glutathione peroxidase (GPX1), glyoxalase (GLOI), phosphoglucomutase (PGM1) and 6-phosphogluconate dehydrogenase (6-PGD). After electrophoretic separation and visualization of enzyme activity, stained Cellogel strips (Chemetron, Milan, Italy) are soaked with water and methanol soakings (Table 3). In double blind trials, it was observed that the differences in the sharpness and resolution of bands between new and reused Cellogel strips are not significant. The procedure may be repeated two or three times, care being taken to avoid alterations of the strips due to dehydration following absolute methanol soaking and any kind of physical damage due to inaccurate handling.

5.2. GCAMs for protein transfer

Different electrophoretic patterns may be obtained in the same gel with the same power

Table 3
Protocol for reusing electrophoresed and stained cellogel strip

1. After electrophoresis and band visualization, soak the Cellogel strip in doubly distilled water for 2 h with three changes.
2. Gently blot the strip and soak it for 1 h in absolute methanol.
3. Store in aqueous methanol solution (300 ml/l).
4. Prior to further electrophoresis, gently blot the strip and soak it in tank buffer for 30 min.

In addition to CAMs stained for ADA, AK, CAII, GPX1, GLOI and PGM1 [37], this method can be applied to ESD- and EAP-stained membranes.

supply settings, according to the duration of electrophoresis. This is particularly evident in IEF where, during the same experiment, non-equilibrium isoelectric focusing (NEPHGE) is followed by true equilibrium focusing, and each of these phases makes it possible to obtain very different separations. Unfortunately, these opportunities offered by conventional electrophoresis and IEF cannot be exploited at present. This is due to a loss of separation capacity of the running gel which unavoidably follows the application of the current methods for visualizing electrophoretically separated proteins. Consequently, one or more copies of only one separation may be obtained from every electrophoretic experiment [38–40].

To fill this gap, we have developed a simple method for transfering electrophoretically separated proteins from an IEF polyacrylamide gel to a GCAM. The replica obtained can be stained with a convenient solution and the proteins are thus made visible directly on the membrane. The most important and innovative feature introduced here is that the procedure does not impair the functioning of the gel, thus permitting one to obtain more replicas from a gel at different stages of the same electrophoretic run (Fig. 3).

In its first presentation, the method was successfully applied to the sequential typing of the erythrocyte polymorphisms of EAP, ESD, HBβ and PGM1 [41,42]. We have recently found (unpublished data) that this method also works with serum polymorphisms such such as α-1-antitrypsin (PI) and group-specific component (Gc) (Fig. 4 and Table 4).

This new approach to protein visualization has some important features. The detection of a set of allele products at different points in an IEF process by a GCAM (Fig. 3) allows one to optimize the results in the following ways.

(a) In terms of reliability, as different separations of common variants may be monitorized from a single experiment and the point corresponding to their optimum resolution may be established; this may prevent a decrease in reproducibility due to accidental variations in the experimental conditions (slight alterations in the amount and/or purity of substances contained in electrolytes and gels, temperature and humidity, power supply settings) and/or to diversity of pH gradients created by different batches of carrier ampholytes in the same range of pH [27].

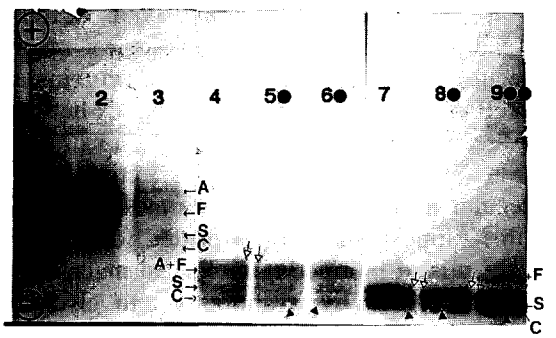

Fig. 3. GCAMs replica of HB taken at different times in the same IEF run. The first replica (lanes 1–3) was taken after 1 h of separation at 700 V, 2 W and unlimited mA: HB A, F, S and C are well separated but the bands are too diffuse for a reliable phenotyping. The second replica (lanes 4–6) was taken after a further 30 min at 700 V, 2 W and unlimited mA: HB bands are now readable and they are still separated each from the other with the exception of HB A and F. The third replica (lanes 7–9) was taken after a further 40 min at 1200 V, 3 W and unlimited mA: substantial focusing of the bands is accompanied by a general decrease in separation, while HBA and F remain indistinguishable from each other. Gel and electrode solutions were according to Destro-Bisol et al. [41]. Sample, an HB AFSC standard from Isolab Drawer (Akron, OH, USA). Small triangles indicate incomplete transfer due to the presence of air bubbles trapped between the GCAM and polyacrylamide gel during the transfer. Black spots indicate the number of the replica previously taken on the lane.

(b) In terms of analytical power: (1) obtaining an increased separation between the bands makes the detection of unusual variants easier; (2) within a single run, each set of allele products may be discriminated by different parameters, such as isotacophoretic mechanism (in NEPHGE) and isoelectric point (in true equilibrium focusing). This is important for optimizing information supplied by markers which can show a different allelic variability depending on non-equilibrium or true equilibrium focusing such as ESD [43].

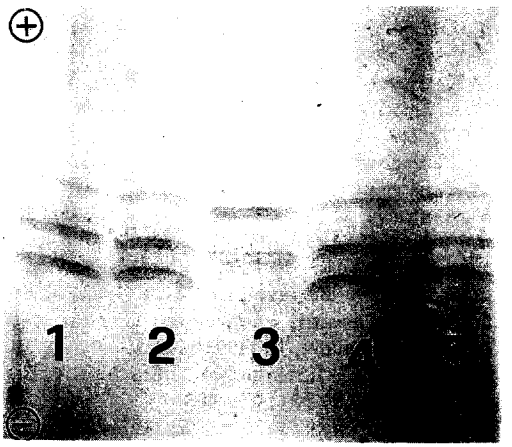

Fig. 4. GCAMs replica of Gc treated with monospecific antisera and stained with R-250 CBB. Lanes 1, 2, 4 and 5, GC 2–1S; lane 3, GC 1S. Protein transfer was performed as follows: GCAMs were removed from the methanol solution [30% (v/v) in water] in which they were stored and soaked in doubly distilled water for 1 h. At the moment of the replica, the electrophoretic run was suspended and the strips were gently blotted with filter-paper and then carefully laid over the surface of the gel, avoiding trapping air bubbles. At the end of the transfer, the membrane was peeled off and the electrophoretic run was resumed. Replicas for GC were incubated for 15 min at 37°C with the corresponding monospecific antisera (Behringwerke, Marburg, Germany), and then thoroughly washed overnight with saline. Visualization of immuno-precipitates was accomplished using R-250 CBB [0.5% (w/v) in 30% (v/v) ethanol–10% (v/v) acetic acid solution; destaining in 20% (v/v) ethanol–20% (v/v) acetic acid solution].

Table 4
Application of the described method to the typing of some erythrocyte and serum genetic markers

Marker	Adopted contact time of gel with Cellogel strip (s)
EAP	90
ESD	40
GC	60
Hbβ	30
PI	60

The adopted contact times allowed reliable visualization of the isoforms under our experimental conditions. The original values [41] have been reduced in order to minimize collapse of the gel and alternation of the pH gradient following loss of water and ampholytes in the gel during the contact between the Cellogel strip and polyacrylamide matrix.

6. Conclusions

Since their introduction, CAMs and, later, GCAMs have found a wide range of applications in analytical chemistry, biochemistry and biological sciences. Many electrophoretic protocols for the rapid characterization of macromolecules have been based on CAM and GCAM as supporting media. However, whereas polyacrylamide and, to a lesser extent, agarose matrices have been subjected to a great number of modifications and improvements that have widened their range of applications and increased their analytical power, CAM and GCAM electrophoresis has retained its characteristics of a rapid but irreproducible and low-resolution method. Hence at present CAM and GCAM electrophoresis remains the method of choice for some routine clinical investigations, but is an ancillary method for many research applications.

Waiting for the introduction of improved and more controlled CAMs and GCAMs by manufacturers, it seems reasonable to assert that, in the near future, interest in CAM and GCAM electrophoresis will depend on the accomplishment of an optimum cost–benefit relationship, i.e., the amount of information vs. economics and time required. Consequently, the development of methods aimed at reducing the costs (e.g., the above protocol for reusing electrophoresed and stained membranes [37]) may contribute to revitalizing interest in CAM and GCAM electrophoresis.

Finally, as an area of potential development, the innovative use of GCAMs as a support for protein transfer [41,42] should be thoroughly explored, in the light of the peculiar property of allowing transfer without impairing the separation capacity of the gels.

Abbreviations

ADA	Adenosine deaminase
AK	Adenylate kynase
CAII	Carbonic anhydrase, locus II
CAM	Cellulose acetate membrane
CBB	Comassie Brilliant Blue
EAP	Erythrocyte acid phosphatase
ESD	Esterase D
GC	Group-specific component
GCAM	Gelled cellulose acetate membrane
GLOI	Glyoxalase, locus I
GPX1	Glutathione peroxidase, locus 1
HB	Haemoglobin
IEF	Isoelectric focusing
Ig	Immunoglobulin
ITP	Isotacophoresis
NEPHGE	Non-equilibrium isoelectric focusing
6-PGD	6-Phosphogluconate dehydrogenase
PGM1	Phosphoglucomutase, locus 1
p*I*	Isoelectric point
PI	α_1-Antitrypsin

References

[1] A. Tiselius, *Trans. Faraday Soc.*, 33 (1937) 524.
[2] T. Wieland and E. Fischer, *Naturwissenschaften*, 35 (1948) 29.
[3] J. Kohn, *Clin. Chim. Acta*, 2 (1957) 297.
[4] C.M. Luz, I. Konig, R. Schirmer-Heiner and F. Frank, *Biochim. Biophys. Acta*, 1038 (1990) 80.
[5] T.L. Golias, *Helena Laboratories Electrophoresis Manual*, Helena Laboratories, Beaumont, TX, 1971.
[6] B.J. Grunbaum, in B.J. Grunbaum (Editor), *Handbook for Forensic Individualization of Human Blood and Bloodstrains*, Sartorius, Göttingen, 1980, p. 51.
[7] Y.B. Mebrahtu, P.G. Lawyer, H. Pamba, D. Koech, P.V. Perkins, C.R. Roberts, J.B. Were and L.D. Hendricks, *Am. J. Trop. Med. Hyg.*, 47 (1992) 852.
[8] Z.R. Lun, R. Allingham, R. Brun and S.M. Lanham, *Ann. Trop. Med. Parasitol.*, 86 (1992) 333.
[9] T. Fujita, K. Uchida and N. Maruyama, *Biochim. Biophys. Acta*, 1116 (1992) 122.
[10] S. Yamaguchi, T. Yoshioka, M. Utsunomiya, T. Koide, M. Osafune, A. Okuyama and T. Sonoda, *Urol. Res.*, 21 (1993) 187.
[11] S. Yoshihara, M. Sasaki, H. Kawasaki, M. Yokoyama, M. Endo and M. Konn, *Int. J. Pancreatol.*, 14 (1993) 219.
[12] Nishikawa, T. Kanda, H. Yoshihara, K. Fukumoto and I. Uematsu, *Clin. Chim. Acta*, 210 (1992) 13.
[13] Y. Kariya, S. Watanabe, K. Hashimoto and K. Yoshida, *J. Biol. Chem.*, 265 (1990) 5081.
[14] K.H. Eggen, A. Malmstrom and S.O. Kolset, *Biochim. Biophys. Acta*, 1024 (1994) 287.
[15] M.K. Horne, E.S. Chao, O.J. Wilson, S.J. Scialla, M.A. Lynch and P.J. Kragel, *J. Lab. Clin. Med.*, 118 (1991) 250.
[16] M. Panteghini, *Clin. Chem.*, 38 (1992) 1712.
[17] M. Panteghini, *Clin. Chem.*, 40 (1994) 190.
[18] M. Ventrucci, R. Pezzilli and L. Gullo, *Am. J. Gastroenterol.*, 85 (1990) 1381.
[19] P.A. Weernink, G. Rijksen, E.M. Mascini and G.E. Staal, *Biochim. Biophys. Acta*, 1121 (1992) 61.
[20] J. Pedraza-Chaverri, P. Calderon, C. Cruz and J.C. Pena, *Renal Failure*, 15 (1993) 149.
[21] J. Kirkham, C. Robinson, A.J. Smith, J.A. Spence, *Arch. Oral Biol.*, 37 (1992) 1031.
[22] H. Harada, *Clin. Chim. Acta*, 63 (1975) 275.
[23] H. Harada, D.P. Agarwal and H.W. Goedde, in B.J. Radola (Editor), *Electrophoresis '79*, Walter de Gruyter, Berlin, 1979, p. 687.
[24] J. Ambler, *Clin. Chim. Acta*, 85 (1978) 183.
[25] J. Ambler, *Clin. Chim. Acta*, 88 (1978) 63.
[26] J. Ambler and G. Walker, *Clin. Chem.*, 25 (1979) 1320.
[27] P.G. Righetti, *Isoelectric Focusing—Theory, Methods and Applications*, Elsevier, Amsterdam, 1976, p. 171.
[28] T. Boussios and J.F. Bertles, in N. Castimpoolas (Editor), *Electrophoresis '78*, Walter de Gruyter, Berlin, 1978, p. 137.
[29] M. Kane, T. Fukunaga, Y. Yamamoto, M. Yamada and Y. Tatsuno, *J. Chromatogr.*, 569 (1991) 297.
[30] D.F. Farrell, AM.P. Macmartin and A.F. Clark, *Clin. Chim. Acta*, 89 (1978) 145.
[31] T. Toda, K. Sano-Shiba, H. Cho, P. Soon, M. Nakao and M. Ohashi, *Electrophoresis*, 9 (1988) 149.
[32] T. Dobosz and P. Koziol, *Hum. Genet.*, 56 (1980) 119.
[33] H. Haglund, *Sci. Tools*, 17 (1970) 2.
[34] G.I. Abelev and E.R. Karamova, *Anal. Biochem.*, 142 (1984) 437.
[35] G.I. Abelev, E.R. Karamova, E.G. Bragina, N.E. Andreeva and G.V. Kruglova, *Int. J. Cancer*, 46 (1990) 351.
[36] D. Schranz, S. Morkokski, E.B. Karamova and G.I. Abelev, *Electrophoresis*, 12 (1991) 414.
[37] G. Destro-Bisol, *Electrophoresis*, 14 (1993) 238.
[38] T. McLellan and J.A.M. Ramshaw, *Biochem. Genet.*, 19 (1981) 647.
[39] R.P. Legocki and D.P. Verma, *Anal. Biochem.*, 111 (1981) 385.
[40] F.B. Desvaux, B. David and G. Peltre, *Electrophoresis*, 11 (1990) 37.
[41] G. Destro-Bisol, C. Battaggia and S.A. Santini, *Clin. Chem.*, 38 (1992) 592.
[42] G. Destro-Bisol, *Trib. Biol. Med.*, 4 (1993) 9.
[43] G.B. Divall, *Forensic Sci. Int.*, 26 (1984) 255.