Table 9 Experimental conditions of the HPLC and of the SFC analyses of polymethoxylated flavones of sweet orange oil

	HPLC	*SFC*
Column	Zorbax silica, 25 cm × 4.6 mm internal diameter (7 μm)	S5W uncoated silica, 25 cm × 4.6 mm internal diameter (5 μm)
Eluent	Hexane–ethyl acetate, 95 : 5	CO_2 modified with small amounts of methanol
Flow rate	1.6 mL min^{-1}	2 mL min^{-1} for 4 min, then gradient of 2 up to 5 mL min^{-1} thereafter held constant
Programme	Isocratic	$P = 100$ atm, $T = 40°C$; modifier, 1.5% min^{-1} from 10% to 30% thereafter held constant
Injected amount	20 μL of a 5% solution of oil in ethyl acetate	100 μL of a solution obtained by diluting 0.71 g of oil to 20 mL of ethyl acetate
Detection	UV absorbance at 315 nm	UV absorbance at 315 nm

provide faster separations with the same resolution as that observed in longer columns packed with particles of larger diameter.

See also: **II/Chromatography: Liquid:** Detectors: Mass Spectrometry. **Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Modes of Development: Forced Flow, Over Pressured Layer Chromatography and Centrifugal; Preparative Thin-Layer (Planar) Chromatography. **III/Essential Oils:** Gas Chromatography; Thin-Layer (Planar) Chromatography; Distillation.

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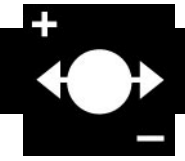
CLINICAL APPLICATIONS



Capillary Electrophoresis

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Introduction

The area of clinical applications of capillary electrophoresis (CZE) is such a rapidly growing field that it would be impossible here to cover it in detail. We thus offer a list of major reviews to which the reader is referred for a more comprehensive coverage of the literature. Such reviews can be divided into:

1. Broad-coverage reviews, such as those of Lehmann *et al.* (1997), Perrett (1999; CZE in clinical chemistry) and Guzman *et al.* (1997; dedicated also to on-line analyte concentration and microreaction). Also of interest are special issues of the *Journal of Chromatography B* dedicated to CZE in the life sciences (Krstulovic 1997) and of *Electrophoresis* devoted to CZE in the clinical sciences (Landers 1997) and in forensic science (McCord, 1998).
2. Specialized reviews, such as those of Thormann *et al.* (1996, 1997; drug analysis in body fluids); Lurie (1996; analysis of seized drugs), Hong and Baldwin (1997; metabolite profiling in human urine), Righetti and Gelfi (1997a,b, 1998; CZE of

DNA for molecular diagnostics), Jellum *et al.* (1996, analysis of urinary diagnostic metabolites and serum proteins), Lazaruk *et al.* (1998; genotyping of forensic short tandem repeat systems).

CZE: Some Basic Concepts Related to Clinical Chemistry

An important aspect of CZE relevant to clinical chemistry is the paradox by which one of the noted advantages of CZE, namely the small volume of the capillary, also leads to a significant drawback, i.e. the minute amount (3–10 nL) of sample introduced, which results in poor concentration limits of detection, one to two orders of magnitude lower than in high-performance liquid chromatography (HPLC). Thus, preconcentration techniques are often required for compounds present in very low concentrations in biological fluids. These include transient isotachophoresis, analyte stacking, field-amplified sample injection (all on-capillary techniques) or off-column preconcentration techniques, such as liquid–liquid or liquid–solid extraction. Alternative on-column methods include miniaturized solid-phase extraction with cartridges containing reversed-phase HPLC packing materials or with impregnated membranes. When analysing small analytes or drugs in sera, it is often necessary to deproteinize the sample. This can be efficiently achieved by extracting sera with 60% acetonitrile, which accomplishes two tasks: protein precipitation and analyte stacking upon electrokinetic injection because of the very low conductivity of such a solution. Some areas or interest where CZE offers unique resolution and sample quantification are discussed below.

Drug Analysis

With the more efficient therapeutic application of various drugs and the necessity for screening and confirmation of drugs in body fluids for diagnostic and research purposes, there has evolved a need for reliable analytical procedures. CZE is becoming the method of choice for drug monitoring in body fluids, including plasma, serum, saliva and urine. Some relevant data are given in **Tables 1–3**.

Profiling Clinically Important Metabolites in Urines

There are approximately 300 known metabolic disorders many of which, if not treated, have serious and sometimes life-threatening consequences. Generally, the diagnosis of these disorders is made or confirmed by identification and quantification of characteristic metabolite(s) occurring in body fluids as a result of the particular enzyme deficiency involved in each disease. In addition, once the diagnosis has been made, the effectiveness of subsequent therapy can also be evaluated by analogous monitoring of the levels of the same metabolic markers. Recently, CZE has become an attractive tool for monitoring clinically important metabolic markers, including alditols, carbohydrates and amino acids, which can be profiled directly in urine with electrochemical (EC) detection at a copper electrode. EC detection allows sensitivity down to the femtomolar level and can be performed

Table 1 Selected validated CZE/MEKC[a] assays for drugs[b]

Drug	*Body fluid*	*Therapeutic range ($\mu g\ mL^{-1}$)*	*Calibration range ($\mu g\ m\ L^{-1}$)*	*CE method*	*Sample preparation/ detection*[c]	*Reference assay*
Theophylline	Serum	8–20	2.0–28.3	MEKC	DSI/275	EMIT
Phenobarbital	Serum	15–40	5.3–52.6	MEKC	DSI/245	EMIT
Ethosuximide	Serum	40–100	19.8–99.2	MEKC	DSI/220	FPIA
Flucytosine	Serum	20–80	20–123	MEKC	DSI/210	Bioassay
Antipyrine	Plasma	—	1.0–40	MEKC	DSI/240	HPLC
Antipyrine	Saliva	—	1.9–66	MEKC	DSI/260	HPLC
Felbamate	Serum	15–135	5.0–160	MEKC	DSI/214	HPLC
Pentobarbital	Serum	—	10–100	CZE	PPI/253	HPLC
Thiopental	Serum	—	2.0–60	MEKC	EXI/290	HPLC
Bupivacaine	Drain	—	0.5–20	CZE	EXI/200	GC
Cicletanine	Plasma	—	0.01–1.0	MEKC	EXI/214	HPLC
Retinol	Serum	—	0.003–0.035	CZE	UF/LIF	HPLC

[a]MEKC, micellar electrokinetic capillary chromatography; CZE, capillary zone electrophoresis; DSI, direct sample injection; EXI, extract injection; PPI, injection of supernatant after protein precipitation; EMIT, enzyme-multiplied immunoassay technique; FPIA, fluorescence polarization immunoassay; GC, gas chromatography; HPLC, high-performance liquid chromatography; UF, ultrafiltration; LIF, laser-induced fluorescence detection with 325 nm excitation and 465 nm emission.
[b]Reprinted from Thormann W, Zhang CX and Schmutz A (1996) *Therapeutic Drug Monitor* 18, 506–520, with permission.
[c]The number represents the detection wavelength.

Table 2 Selected CZE/MEKC[a] screening confirmation assays for illicit, abused and banned drugs[b]

Drugs, drug classes	*Body fluid, tissue*	*CZE method*	*Sample preparation*	*Reference assay*
Barbiturates	Urine, serum	MEKC	DSI, EXI	EMIT
Salicylate, paracetamol, antiepileptics	Urine, serum	MEKC, CZE	DSI, EXI	FPIA, EMIT, UF
11-Nor-Δ-tetrahydro cannabinol-9-carboxylic acid	Urine	MEKC	EXI	FPIA
Methadone and its primary metabolite	Urine	CZE	DSI, EXI	EMIT, GC-MS
Benzodiazepines	Urine	MEKC	EXI	EMIT, GC-MS
Benzoylecgonine, opioids, methaqualone, amphetamines	Urine	MEKC	EXI	EMIT
Cocaine and all above mentioned classes	Urine	MEKC, CZE	EXI	EMIT, FPIA, GC-MS
Cocaine, morphine	Hair	MEKC	EXI	HPLC
β-Blockers	Serum	MEKC	EXI	—
Diuretics	Urine, serum	CZE	EXI	GC-MS

[a]For abbreviations, see Table 1.
[b]Reprinted from Thormann W, Zhang CX and Schmutz A (1996) *Therapeutic Drug Moniter* 18, 506–520, with permission.

directly on urine without extensive sample clean-up or analyte derivatization. Some examples are given in **Table 4**. Other interesting data on profiling the following metabolites: orotic acid, pyroglutamate, adenylosuccinate and propionic acid, for the following diseases: HHH-syndrome (hyperornithinemia-hyperammonemia-homocitrullinuria), glutathione deficiency, adenylosuccinase deficiency and propionyl CoA carboxylase deficiency, respectively, can be found in Jellum *et al.* (1997).

Table 3 Retention of anabolic steroids relative to testosterone[a]

Compound[b]	*MEKC*[c]	*HPLC*	*GC*
Fluoxymesterone	0.925	0.78	1.50
Boldenone	0.964	0.74	1.05
Nandrolone	0.979	0.84	0.91
Methandrostenolone	0.985	0.86	1.12
Testosterone	1.00	1.00	1.00
Methyltestosterone	1.02	1.17	1.05
Methandriol	1.06	1.25	0.89
Stanolone	1.07	1.25	0.89
Boldenone acetate	1.12	1.46	1.27
Stanozolol	1.16	1.69	1.68
Testosterone acetate	1.17	1.76	1.21
Nandrolone propionate	1.22	1.88	1.29
Danazol	1.23	1.52	—
Clostebol acetate	1.24	1.90	—
Testosterone propionate	1.26	2.01	1.43
Methandriol 3 acetate	1.26	2.13	1.10
Testosterone isobutyrate	1.35	2.17	1.54
Nandrolone phenylpropionate	1.44	2.25	2.28
Testosterone cypionate	1.64	2.63	2.19
Testosterone enanthate	1.69	2.60	1.92
Methandriol dipropionate	1.81	2.98	1.70
Nandrolone decanoate	2.06	2.87	2.26
Boldenone undecylenate	2.20	2.73	2.62
Testosterone undecanoate	2.36	3.18	2.56
Oxymetholone	—	—	1.28
Oxandrolone	—	—	1.17
Testosterone isocaproate	—	—	1.77
Testosterone decanoate	—	—	2.36

[a]Reprinted from Lurie IS (1996) *International Laboratory*, with permission.
[b]In order of increasing retention times.
[c]For abbreviations, see Table 1.

Profiling Proteins in Biological Matrices

Separation and quantification of distinct proteins from biological matrices is another goal now being accomplished by CZE. A list of some major proteins of importance for clinical diagnosis is given in **Table 5**. In some cases, immunosubtraction can be an efficient way of quantifying some protein families by CZE. A typical example is the quantification of specific immunoglobulin subclasses by sequential immunosubtraction. The sample is exposed to five different Sepharose supports, each containing an immunoglobulin-specific binder. Three of them are specific for the heavy chains IgG, IgA and IgM and two are specific for the light chains κ or λ. After incubation and sedimentation, the treated samples and an untreated control are separated by CZE. Six electropherograms are generated per sample. The class and type of monoclonal component can be determined by overlaying electropherograms from before and after immunosubtraction.

CZE Separations of Clinically Relevant Diagnostic DNA

A number of applications of CZE in sieving liquid polymers (notably linear polyacrylamides and

Table 4 Normal constituents of human urine and expected response for CZE at a Cu electrode[a]

Compound	*Related metabolic disorder*	*Normal concentrations (μM)[b]*	*Migration time in 0.1 N NaOH (min)*	*Detection limit (μM)*
		Alditols		
Erythritol	—	608	12.6	0.5
Inositol	Diabetes, renal failure	357	12.6	0.3
Ribitol	—	35	12.8	—
Xylitol	—	35	12.8	—
Arabitol	—	195	12.8	—
Glucitol	—	35	12.9	0.5
Mannitol	Diabetes	104	14.6	0.5
		Carbohydrates		
Sucrose	—	43	16.9	1
Lactose	—	15	19.6	1
Fucose	—	97	19.6	—
Galactose	Galactosaemia	29	21.3	1
Glucose	Diabetes	262	22.4	1
Rhamnose	—	115	22.8	—
Arabinose	Pentosuria	89	23.4	—
Fructose	Fructosuria	72	23.4	1
Xylose	Pentosuria	53	25.4	—
Ribose	Pentosuria	31	26.2	1
		Amino acids		
Lysine	Hyperlysinaemia	328	31.6	4
Threonine	Aminoaciduria	183	35.4	2
Histidine	Histidinaemia	860	43.1	1
		Others		
Creatinine	Muscle and renal disease	8800	12.8	80
Uric acid	Gout	2093	> 60	1.6

[a]Reprinted from Hong J and Baldwin RP (1997) *Journal of Capillary Electrophoresis*, 4, 65–71, with permission.
[b]Calculated assuming that the average urine volume for a 24-h period is 1.5 L.

celluloses) for the analysis of polymerase chain reaction (PCR) products of clinically relevant, diagnostic DNA have been reported. **Table 6** lists some major applications, divided into four classes: human genetics, quantitative gene dosage, microbiology/virology and forensic medicine.

Table 5 Survey of CZE separations of proteins in biological matrices[a]

Proteins	*Matrix*	*CZE[b] mode*	*Detection mode*
Immunoglobulin G, transferrin, albumin, prealbumin, β-trace proteins	CSF	CZE	UV 185 nm
Apolipoprotein A-I, A-II, B100, B48, C-III and E	Serum	MECC	UV 190 nm
Lipoprotein subfractions (HDL, VLDL, IDL, LDL)	Serum	CITP	Vis 570 nm
Leucine aminopeptidase	Serum, urine	CZE	LIF
Cerebrospinal fluid proteins	CSF	CZE	UF 200 nm
Myoglobin	Urine, tissue	CZE	Vis 405 nm
Albumin, α1-acidic glycoprotein, transferrin, β-microglobulin, immunoglobulin light chains	Urine	CZE	UV 200 nm
Monoclonal antibodies (anti-TNF, anti-CEA)	Serum containing culture medium	CZE, CIEF, CGE	UV 200 nm
Imidodipeptides (prolidase deficiency)	Urine	CZE	UV 269 nm
Cathepsin D	Breast tissue	CZE	UV 214 nm
Hemoglobin variants	Plasma	CZE, CIEF	UV 210 nm
36 low side M_r proteins, cut-off 30 and 5 kDa	Seminal, vaginal fluids, serum, saliva	CZE	UV 214 nm

[a]Reprinted from Lehmann R, Voelter W and Liebich HM (1997) *Journal of Chromatography B* 697, 37–66, with permission.
[b]Abbreviations: CIEF, capillary isoelectric focusing; CGE, capillary gel electrophoresis; CITP, capillary isotachophoresis; CSF, cerebrospinal fluid; CEA, carcino-embryonic antigen; TNF, tumour necrosis factor, HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

Table 6 Survey of selected capillary gel electrophoretic separations of clinically relevant diagnostic DNA[a]

Disease	*DNA amplification*	*Sieving polymers*	*Detection*
Human genetics			
Cystic fibrosis	Allele specific PCR and restriction digest of PCR products (deletion)	6% linear PAA[b]	UV 254 nm
Cystic fibrosis	PCR, ΔF508	6% linear PAA	UV 254 nm
Cystic fibrosis	PCR/GATT microsatellites	6% linear PAA	UV 254 nm
Cystic fibrosis	Point mutants, TGCE	8% linear poly(AAEE)	UV 254 nm
Duchenne/Becker muscular dystrophy	PCR multiplex reaction	6–10% linear PAA	UV 260 nm
Dystrophin gene	RFLP	0.5% HPMC	UV 254 nm
Thalassaemia	Point mutants, TGCE	4% poly(AAP), 1.5% HEC	UV 254 nm
Congenital adrenal hyperplasia	PCR deletion	6% linear PAA	UV 254 nm
Androgen insensitivity syndrome	CAG triplet analysis	6% linear PAA	UV 254 nm
Kennedy's disease	CAG triplet expansion	8% poly(AAEE)	UV 254 nm
N-ras gene (human cancer)	Point mutants SSCP	8% PAA	UV 260 nm
ERBB2 oncogene	RFLP	0.5% HPMC	UV 260 nm
TX gene	PCR	3% PAA	LIF
P-53	SSCP	4% PAA	UV 260 nm
P-53	SSCP	2% PAA	LIF
Cancer (microsatellites instability)	PCR	Bio-Rad sieving polymer	UV 260 nm
Apolipoprotein B gene	PCR/VNTR	0.7% MC	UV 260 nm
Apolipoprotein E gene	PCR/RFLP	3% T PAA	UV 260 nm
Apolipoprotein E gene	PCR/RFLP	Beckman e/CAP	LIF
Medium chain AcylCoA dehydrogenase deficiency	PCR/allele specific	Polyacrylamide gel	LIF
von Willebrand Factor gene	PCR/VNTR	1% HEC	LIF
Fetal DNA (Y-chromosome)	PCR	Beckman dsDNA 1000 gel buffer	LIF
Quantitative gene dosage			
Down's Syndrome	Quantitative PCR	8% PAA	UV 254 nm
Rh D/d genotyping	Quantitative PCR	8% PAA	UV 254 nm
Follicular lymphomas	Competitive PCR	4% PAA	UV 260 nm
Basic fibroblast growth factor	Competitive RT-PCR	6% PAA	UV 254 nm
Microbiology/virology			
Mycobacterium tuberculosis	SSCP and ddF	1% HEC or 3% T, 0.5%C PAA gel	LIF
Hepatitis C virus	RT-PCR	1% HEC	LIF
Polio Virus	RT-PCR	3% T linear PAA	UV 254 nm
HIV-1	RT-PCR	3% linear PAA	LIF
Mitochondrial DNA	PCR	1% HEC	LIF
Mitochondrial DNA	PCR	0.5% MC	LIF
VNTRs at locus D1S80	PCR/VNTR	0.5% HEC	LIF
VNTRs at locus D1S80	PCR/VNTR	0.5% MC	LIF
VNTRs at locus HUMTH01	PCR/VNTR	1% HEC	LIF
VNTRs at locus HUMTH01	PCR/VNTR	3% T, 3% C gel	UV 260 nm
Therapeutic DNA			
Antisense oligonucleotides		18% PAA	MALDI-MS LIF
Antisense oligonucleotides		10% PAA, isoelectric His	UV 254 nm
Antisense oligonucleotides		10% T PAA, pH gradient	UV 254 nm

[a]Reprinted from Righetti PG and Gelfi C (1997), with permission.

[b]Abbreviations: PAA, polyacrylamide; VNMTR, variable number of tandem repeats; HEC, hydroxyethyl cellulose; MC, methyl cellulose; SSCP, single strand chain polymorphism; RT, reverse transcription; HPMS, hydroxypropyl methyl cellulose; AAP, acryloyl amino propanol; AAEE, acryloyl amino ethoxy ethanol, TGCE, temperature gradient capillary electrophoresis; RFLP, restriction fragment length polymorphism; LIF, laser induced fluorescence.

Examples of Some Separations

It is quite difficult to compress in such a few pages the vast literature in the field of clinico-chemical applications of CZE. From our experience in DNA separations, we offer here a few examples pertaining to the screening for human genetic diseases. **Figure 1** displays the CZE analysis of a multiplex PCR for the

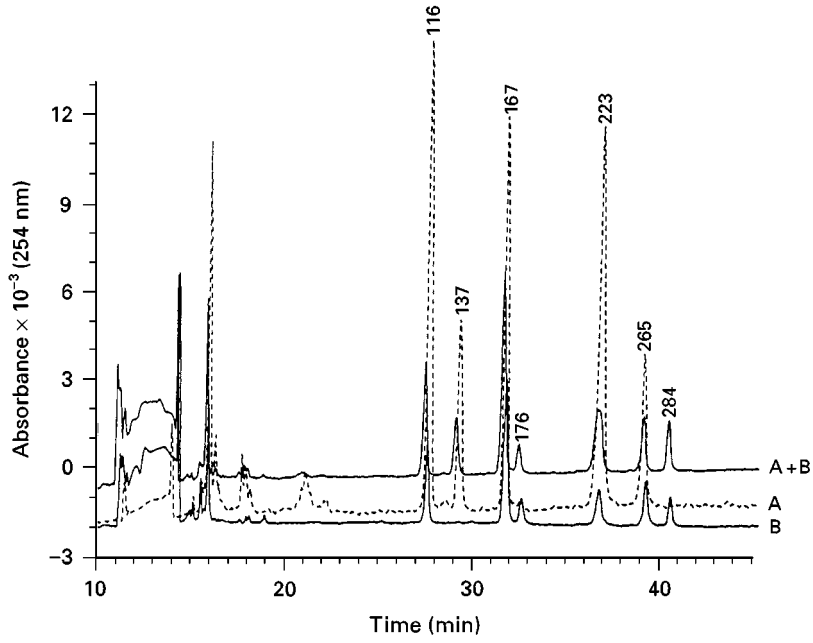

Figure 1 Simultaneous detection of ΔF508, G542X, N1303K and 1717-1G → A mutations in cystic fibrosis by CZE in polymer networks. Traces: A, patient carrying the mutations 1717-1G → A and ΔF508; B, patient affected by the G542X/N1303K mutations; A + B, artificial mixture of the amplified DNA fragments of patients A and B. Conditions: 100 μm ID, 37 cm long capillary, filled with a viscous solution of linear 6% T polyacrylamide in 100 mmol L^{-1} TBE (Tris-borate-EDTA) buffer, 10 μmol L^{-1} ethidium bromide, pH 8.3; Run at 165 V cm^{-1} with detection at 254 nm; electrophoretic sample injection at 165 V cm^{-1} for 8 s. Reprinted from Gelfi C, Righetti PG, Magnani C, Cremonesi L and Ferrari M (1994) *Clinica Chimica Acta* 229, 181–189, with permission from Elsevier Science.

simultaneous detection of four mutations, ΔF508, G542X, N1303K and 1717-1G → A in cystic fibrosis (CF). This is an interesting example, in that it offers a fast and reliable method for the simultaneous detection of mutations which are predominant in a given population. In Italy, a survey of 391 CF patients, originating from all geographical regions, revealed that ΔF508 (53% of CF chromosomes), G542X (4%), 1717-1G → A (4%) and N1303K (4%) are the most frequent mutations, accounting for 65% of all molecular defects pertaining to CF. **Figure 2** gives the CZE analysis for the Duchenne (DMD) and Becker (BMD) muscular dystrophies, which represent the two most common myopathies described to date. They are given the names of Chamberlain and Beggs since these two scientists proposed two PCR assays (each based on co-amplification of nine dystrophin gene exons) allowing for the detection of over 98% DMD/BMD deletions. Thus, a method attempting simultaneous analysis of DMD/BMD should offer unambiguous resolution and identification of 18 fragments ranging in size from *c.* 100–500 bp. This is in fact achieved in the lower trace of **Figure 2** (representing a healthy individual). The upper trace shows a patient affected by muscular dystrophy, in which four fragments (196, 202, 331 and 357 bp) are missing. We hope that these two examples, albeit limited, provide an insight on the unique resolving power and capability of CZE as applied to problem solving in clinical chemistry.

Conclusions and Future Horizons

Compared with HPLC and gas chromatography, CZE has some distinct advantages, such as small sample size, minimal sample preparation, use of very small amounts of organic solvents and inexpensive chemicals, ease of buffer change and method development and low cost of capillary columns. Electrokinetic capillary assays are complementary to the widely employed immunoassays. For the widespread adoption of CE in routine laboratories, a number of improvements should be made. With the availability of instrumentation comprising multiple capillaries in parallel, sample throughputs comparable to those obtained in automated immunoassays should be possible. The same goal will be reached with the availability of chip-based instrumentation, i.e. CZE on a glass chip on which separation channels, a picolitre sample injector and solute detection are combined on an area of a few cm^2. In this approach, fluid flow is driven electrokinetically (thus via a plug, not a laminar flow) through a network of intersecting small channels fabricated on planar glass substrates

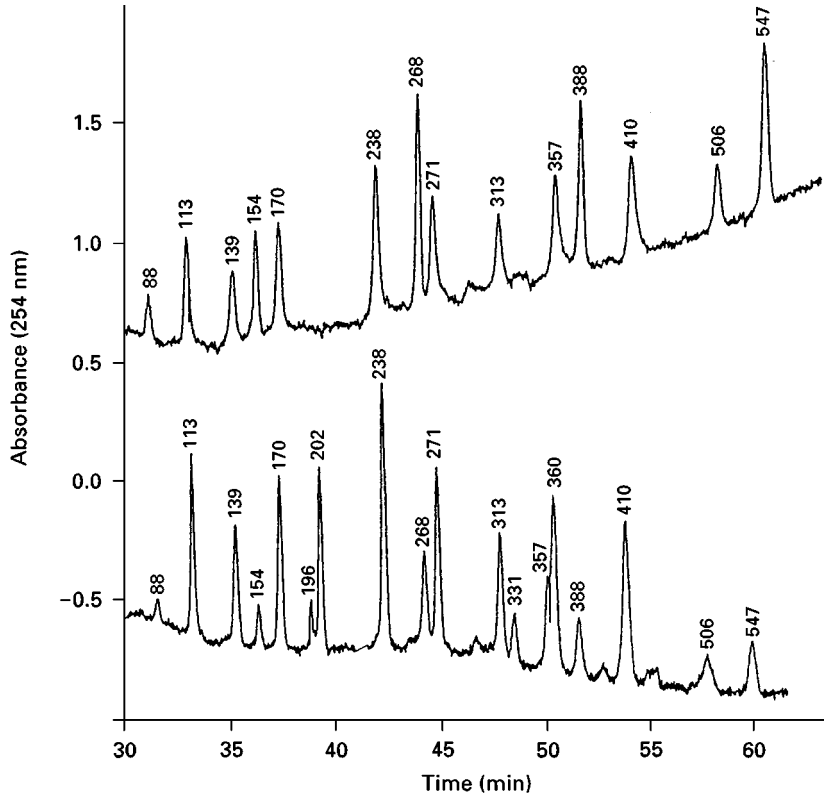

Figure 2 Screening for Duchenne (DMD) and Becker (BMD) muscular dystrophies by CZE in sieving liquid polymers. The upper trace represents the separation of 14 exons of modified deleted Chamberlains' and Beggs' mixed multiplex. The lower electropherogram shows the separation of 18 exons of modified nondeleted Chamberlains' and Beggs' multiplex. All runs were in a 32 cm long, 75 μm internal diameter capillary, filled with short-chain polyacrylamide, obtained by chain transfer at 70°C, in 89 mM TBE buffer, pH 8.3. Run: 165 V cm^{-1} with detection at 254 nm. Sample injection: 100 V cm^{-1} for 25 s. Reprinted from Gelfi C, Orsi A, Leoncini F, Righetti PG, Spiga I, Carrera P and Ferrari M (1995) *BioTechniques* 19, 254–263, with permission from Elsevier Science.

by photolithographic masking and chemical etching techniques and formed by bonding the etched substrate to a plain glass plate. Capillaries 30–70 μm wide, about 10 μm high and a few centimetres long have been shown to provide analytical runs in a few seconds.

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Electrophoresis

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Introduction

It is somewhat arbitrary to outline electrophoretic applications that are used in a routine clinical laboratory because they are highly dependent on the specificity of each application. Nowadays, a multitude of electrophoretic methods are routinely used in various clinical laboratories, according to the specific research being developed. More and more sophisticated methods are needed to resolve specific clinical problems, the reason why specialization of laboratories is mandatory in order to provide accurate results at the lowest possible cost. In addition, quality control is of major importance. Therefore, automation is progressively introduced in all the steps involved in the analytical process, and only a few manual methods will survive in the future. However, highly sophisticated electrophoretic techniques should be maintained and developed in a limited number of specialized laboratories, in order to resolve the different problems that are encountered in clinical medicine. Here, we present selected examples of electrophoretic techniques that are employed in the clinical laboratory.

Serum Protein Electrophoresis

Electrophoretic techniques for the separation of human serum proteins have been used for fifty years. Resolution has been improved by the use of support media such as paper, starch gel, cellulose acetate, agarose, and polyacrylamide gels, which have rendered electrophoretic methods very popular in the diagnostic area. However, many of those methods have remained labour intensive, being difficult to automate. Serum protein electrophoresis is widely used in clinical laboratories, especially for the evaluation of changes in proteins associated with inflammation, liver or kidney diseases as well as for the detection and identification of paraproteins. Traditional clinical electrophoretic procedures are manual methods that use agarose gels or cellulose acetate membranes as the separation bed. Quantitation of the five major serum fractions is done by densitometric scanning of the gel or the membrane. Clinical interpretation is based on the alteration of the content of one or more of the five fractions. Agarose, as supporting medium for protein electrophoresis, has been reported to give better resolution as well as to allow better detection of paraproteins than is cellulose acetate. Semiautomated agarose electrophoresis and immunofixation can be performed with various commercially available systems. No differences between manual and semiautomated methods have been seen with respect to paraprotein identification.

Over the last few years, capillary zone electrophoresis (CZE) has emerged as a powerful new tool for rapid separation of various biopolymers, including proteins. Separation by this technique depends on the electrophoretic mobility of the analyte and the electroosmotic flow of the bulk solution, and can be easily automated. Direct quantitation of proteins via peptide bonds is possible using UV detection at 214 nm. Separation patterns obtained by CZE are similar to those obtained after densitometric scanning of cellulose acetate membrane electrophoresis or agarose gel electrophoresis. Recently, dedicated automated systems for the routine analysis of human serum proteins in clinical laboratories have become commercially available. High sample throughput is attained due to the presence of several fused-silica capillaries, which allow the simultaneous analysis of different samples. Several studies have clearly shown that the electrophoretic patterns and the clinical information obtained by CZE are comparable with the data obtained by classical methods. In addition, the method is also suited to detect monoclonal gammopathies. Multicapillary instruments have been