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Review

Capillary electrophoresis in clinical chemistry

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Abstract

Since its introduction, capillary electrophoresis has diversified, spreading out into different specialized fields covering solutions for almost any analytical questions arising in research laboratories. In the context of clinical chemistry, results must be provided at low costs and in a clinically relevant time frame; however, the attributes which have made capillary electrophoresis such a successful tool in basic research are identical to those attracting clinical laboratories: speed (more efficient, less labor-intensive), low costs (minimal buffer consumption), small sample volume (reduced blood collection volume from patient), increased selectivity (determination of multiple solutes in one run), and versatility (detection of analytes over the wide range of molecular masses and chemical composition). Nevertheless, it should be mentioned that there are still some drawbacks at this stage to be solved in the near future, such as lack of sensitivity for many clinical applications or the constraint to measure in a sequential mode. The aim of this survey is to familiarize clinical chemists, as well as chemists, with a short introduction to capillary electrophoresis, followed by chapters reviewing prominent fields of applications and the latest developments in clinical chemistry. © 1997 Elsevier Science B.V.

Keywords: Reviews; Proteins; Peptides; Amino acids; Lipoproteins; Drugs; Hormones; Vitamins; Ions; DNA; Hemoglobin

Contents

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1. Introduction

Over the years, research in the field of clinical chemistry has experienced a continuous expansion of the number of diagnostic procedures available to physicians for evaluation of their patients. The state-of-the-art has progressed to the point that most laboratories on one hand utilize multi-channel auto-analyzers that routinely perform simultaneously 90 different chemical and immunological analyses on a single serum sample (up to 1200 tests/h). On the other hand, more sophisticated techniques such as high-performance liquid chromatography (HPLC), gel electrophoresis, special immunological assays, automatic amino acid analyzers, molecular biology methods, etc., are in use to solve the more difficult analytical problems. Many of these methods are either not generally available or have little clinical utility at present, but are expected to become necessary for optimal care in a not too distant future. Capillary electrophoresis (CE), the latest addition to the analytical instrumentation, will undoubtedly have a great impact on clinical chemistry (Fig. 1), because of its broad potential to separate a wide spectrum of biological molecules, including amino acids, peptides, proteins, glycoproteins, oligonucleotides, DNA fragments, as well as any number of small organic molecules such as drugs or even ions.

At present, one can distinguish three main classes of compounds being analyzed in the routine laboratory. The first includes substances like glucose, urea, creatinine, total protein, cholesterol, sodium, calcium, aminotransferases and others. Variations in their blood levels reflect a consequence rather than a disease. The development of suitable big autoanalyzers permits their analysis in large series. Another series of compounds are substances intimately connected with the cause of illness, e.g., hormones, vitamins, enzymes or distinct proteins, and recently DNA. Clinical genetics is approaching a more and more prominent position in medicine. The third group of compounds include external administered drugs. The determination of the second and third class components is often more complex, requiring

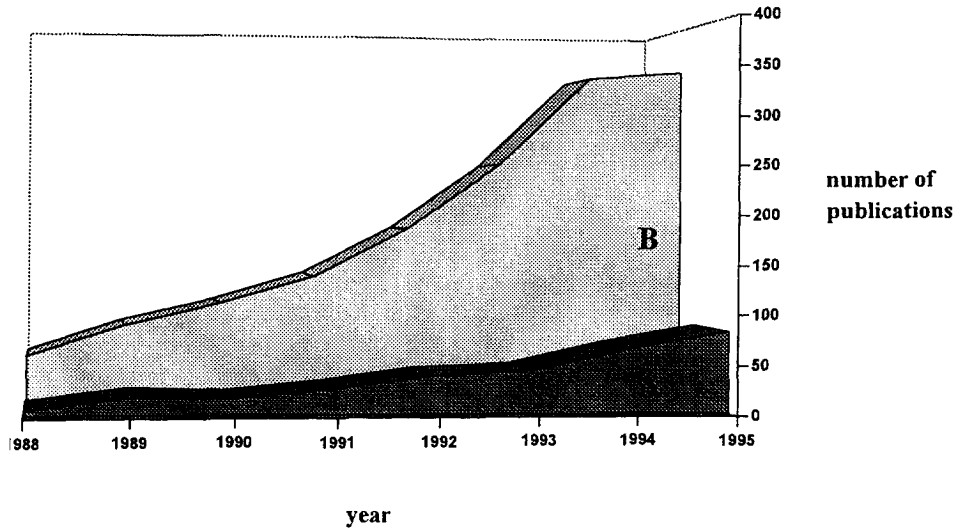

Fig. 1. (A) Growth in numbers of the CE publications dealing with applications in clinical chemistry since the introduction of the first commercially available instruments in 1988. (B) Total number of CE articles (based on WinSPIRS™ MEDLINE literature research).

the more sophisticated techniques mentioned above, to which capillary electrophoresis must now be added.

The first time that human serum protein fractions and other analytes were separated in an electric field can be settled in the early stage of electrophoretic method development. The technique was first described in 1930 in the doctoral thesis of Arne Tiselius and a publication appeared in the scientific literature in 1937 [1]. In 1967, Hjertén originally introduced CE [2]. In his communication, separations of serum proteins, also inorganic and organic ions, peptides, nucleic acids, viruses and bacteria are described. In the following 10 to 15 years, isotachophoresis (ITP) was the main method used in open tubes [3], but the acceptance was low. In the late 1970s [4] and early 1980s [5,6], free zone electrophoresis was remembered again. Mikkers et al. [4] used a PTFE capillary with 200 μm inner diameter (I.D.), adapted from ITP. After some essential modifications, especially by introducing fused-silica capillaries, high voltages and lower inner diameters (100 μm and less) for high-performance capillary zone electrophoresis (CZE), Jorgenson and Lukacs demonstrated the power of modern CE [5]. However, uncharged substances could not be separated. Another significant step forward followed in 1984 by the developments of Terabe et al. [7], describing the addition of surfactants to the electrophoretic buffer, thereby forming micelles, which allow separation of neutral and charged species: micellar electrokinetic capillary chromatography (MECC) was invented. Hjertén and his coworker Zhu adapted isoelectric focusing in 1985 to capillaries (CIEF) [8]. The development of gel-filled (capillary gel electrophoresis (CGE) [9]) and coated capillaries [6] further increased the separation techniques in the field of CE. Details about theory, principles, separation modes, method development, instrumentation and applications have been surveyed in a number of papers (for selected reviews refer to Refs. [10–18]) and books, e.g., Refs. [19–21].

In the late 1980s, the first commercial CE apparatus were offered on the market giving now a broader access to its perspectives like rapid, highly efficient separations of ionic species and biomacromolecules [22]. All commercially available instrumentations apply essentially the same principle to perform any mode of capillary electrophoretic separation, including CZE, MECC, CGE, CIEF and CITP (capillary isotachophoresis), but there is still the necessity for some essential improvements to guarantee an easy use in a clinical laboratory. For routine work, on-board sample preparation (dilution or concentration), automated buffer replenishment, sample-to-answer walk-away operation, automated loading of multiple samples (≥30), rapid multiple-capillary analyses for high-volume sample throughput, furthermore calculating and processing the data produced, are required to facilitate the analytical tasks [14].

One of the greatest challenges in the area of clinical CE is the development of on-capillary detectors that possess the required limits of detection in physiological specimens, e.g., analyzing components like hormones in the pmol/l range. On-column UV-detection is the standard equipment (sensitivity: 1–100 μmol/l), although many other modes have been used including fluorescence, UV–Vis multiwavelength, conductivity, electrochemical, or mass spectrometry [19,20]. Fluorescence detection offers the potential improving the detection limit 100–1000 times over UV absorption. Lasers can increase the flux of the excitation light, thereby extending the detection limits to about 1 pmol/l in favorable cases [23]. An interesting development in improved CE detection sensitivity is the recently evaluated extended light path capillary for use in CE–LIF detection [24]. CE–mass spectrometry (CE–MS) is a hyphenated technique, allowing to differentiate molecules on the basis of their electrophoretic mobilities and structure, making this combination to one of the most powerful techniques for the identification of unknown substances in complex matrices (reviewed in Ref. [25]).

Another way to overcome the low on-line sensitivity of CE or CE–MS are various sample pretreatment procedures, e.g., preconcentration strategies, where the concentrators are directly switched on-line to the fused-silica capillary [26]. On-line sample preparation, which can be used for multiple consecutive analyses after a short rinse to regenerate, may be an interesting goal for clinical capillary electrophoretic method development.

The first CE apparatus for routine clinical analysis is now available. The Paragon CZE 2000™ (Beckman, Munich, Germany) for serum protein electrophoresis (SPE) and immunofixation/subtraction, equipped with seven capillaries in parallel for a high

sample throughput has been introduced this year. The Paragon CZE 2000™ is the first CE-based autoanalyzer, conceived as a walk-away-system, well suited for clinical routine use of two different protein analytical methods. Moreover, in the field of genetic analysis a completely automated multi-color-fluorescent CE instrument, the ABI PRISM™ 310 (Perkin Elmer, Weiterstadt, Germany), was recently introduced. The system is capable performing nearly all kinds of DNA analyses, like DNA sequencing, DNA fingerprinting, single-stranded conformation polymorphism, etc. In the context with CE instrumentations for routine analysis, several aspects should be mentioned attracting clinical chemists to equip their laboratories with a CE apparatus in future times: (1) Only nanoliter of a patient sample are needed for a CE analysis, i.e., only a few microliters in the sample vial are necessary; (2) minute quantities of buffer (milliliter) are required for multiple analysis; (3) the capillary can be rinsed and regenerated for subsequent runs in seconds to minutes; (4) the potential to install fully automatic procedures. As a net result, the technique provides rapid, highly efficient, automatically controlled separations of low volume samples under native or denaturing conditions, analyzable with almost any kind of detection mode.

In the following sections references from all areas of clinical chemistry with special emphasis on analyses of proteins, peptides, amino acids, drugs, hormones, neuropeptides, biogenic amines, vitamins, ions, DNA, and sample pretreatment procedures are selected. It is anticipated that these topics will become also an integral part on the ongoing discussion about capillary electrophoresis.

2. Sample preparation

The sample specimen in clinical chemistry is a mixture of thousands of known and unknown components with more or less large individual variation in the concentration of each (from very low concentration in the pg/l up to the g/l range). Furthermore the sample matrices are very complex, e.g., high protein concentration or high content of triglycerides in serum samples, varying ionic strength and pH in urine, etc. These complex conditions may disturb the analytical procedures. Therefore, developing a method, especially for clinical CE, the line of sight has to be focused not only on a clear-cut separation profile of standard substances, but also on the characteristics of the matrices and the sample composition itself. A well separated standard is no guarantee for a successful quantitative and reproducible determination of the component in samples of various biological origin. Consequently, methods quantifying substances in biological specimens have to be sensitive, selective, reproducible, robust, and, for the use in clinical chemistry, able to allow a high sample throughput and yield reliable results.

As mentioned in the introduction, CE provides both advantages and limitations. The use of narrow bore, small volume capillaries (typically 10–100 μm I.D. and 20–100 cm in length) results in a total capillary volume in the microliter scale. Especially in clinical chemistry the necessity of blood quantities in the low microliter range for different analytical procedures is of enormous interest, not only for newborns. Vice versa, avoiding overloading by injecting volumes in the nanoliter range (≤0.1–50 nl) results in poor concentration limits of detection. If the concentration of the desired analyte is low, the capillary dimension becomes a significant drawback of the technique. Therefore, the sensitivity limits attainable with CE may restrict its utility in some areas of clinical chemistry until now, e.g., the measurement of hormones. Furthermore, the samples may include components which interfere with the analytical procedure. Rapid and simple ways to increase the concentration of the substances of interest (e.g., hormones or drugs) or remove (optional reduce) individual components with extreme concentration (e.g., proteins or ions), or direct derivatization of the sample have still to be overcome in some analytical areas of clinical interest for widespread adaptation of CE in laboratory diagnosis.

Lately, a number of experimental papers appeared, describing preliminary but promising results of different sample pretreatment and injection procedures, like on-line coupling sample extraction, head-column field-amplified sample stacking, microdialysis, cleanup, analyte preconcentration, and microreactor technology (recently reviewed by Tomlinson et al. [27]). Besides these complex procedures, also very simple work-up procedures have been developed, e.g., one dilution step only prior to the quantification

of apolipoprotein A-I in serum [28] or analyzing drugs like theophylline or ethosuximide directly in serum or urine without any pretreatment [29–31].

One problem that may occur are components with adequate concentrations, but high salt and/or protein content in the sample fluid. There are a number of papers dealing with on-line microdialysis [32–34] or off-line desalting and concentration of microliter volumes of protein solutions in one step using small pore polyacrylamide gels, which are suitable not only for CE but also for all kinds of conventional electrophoresis [35]. Acetonitrile deproteinization was found to be suitable for removal of serum proteins for the analysis of drugs and small molecules. As reported by Shihabi [36] many compounds exhibit a better peak shape and therefore an increased signal-to-noise ratio in the presence of 60% acetonitrile (demonstrated for theophylline in serum [36] and xanthine analysis in biological fluids [37]). Other very easy and rapid ways to separate small molecules in biological fluids from proteins in high concentration are ultrafiltration (provided that the substance is not protein-bound) [38], or micro size-exclusion chromatography [27].

If the amount of the analyte is near or below the detection limit, different possibilities are described to solve this analytical question. Besides a more sensitive detection mode or sample derivatization (if possible), simple sample stacking by salt mixtures [39,40] or by large-volume sample stacking [41], selective preconcentration of an antigen for CZE using protein G immunoaffinity capillary chromatography (on-line or off-line) [42], on-line preconcentration via membranes [26,43,44] or on-line solid-phase extraction [45–47] have been successfully tested.

Illustrated by the analysis of the hydrophobic antiarrhytmic drug amiodarone in serum, the highest sensitivity increase of all stacking techniques, the so-called head-column field-amplified sample stacking, has recently been introduced in the clinical context by Zhang and Thorman [48,49]. Following an organic extraction, the CE analysis of amiodarone starts with the hydrodynamic injection of a water plug followed by an electrokinetic loading of the charged molecules of the sample, thereby concentrating the components >1000-fold (nanomolar concentration level) compared to the highest sensitivity obtained by hydrodynamic injection or without sample stacking by electrokinetic injection. Removal of salts from biological samples is a prerequisite, and therefore prevents this stacking procedure becoming generally utilized.

Tomlinson et al. took C_{18} impregnated, styrene-divinyl benzene copolymer membranes for on-line preconcentration performing CE–MS in biomedical sciences [26,44]. Strausbauch et al. showed the utilization of on-line solid-phase extraction CE for the concentration of hypoglycemic drugs in human urine [45]. The concentrator tip for this study contains a 50–200 nl bed of reversed-phased C_{18} material and was suitable for multiple analyses. Selective sample work up of basic drugs in plasma prior to CZE can alternatively be achieved by supported liquid membrane technique [50].

On-line sample pretreatment shows a promising potential to be applied for assays of clinical relevance, suitable for a fully automatic microsample work up of components in complex biological matrices, but many of these techniques require rather expensive, complex hardware. In general, these procedures have been successfully applied to a specific analytical problem, or they are still in the experimental stage. Future developments will show if these first tendencies to realize or improve the analysis of complicated but clinically important components by CE will gain acceptance in praxi during the next few years.

3. Applications in clinical chemistry

3.1. Proteins, peptides and amino acids

Numerous different proteins are produced in the human body, soluble in fluids within or outside the cells or insoluble and structural. The methods used to separate the various groups of proteins from each other are based on their physical properties like shape, molecular mass, charge, isoelectric point, hydrophilicity or hydrophobicity.

Proteins from plasma, ascites fluid or tissue extracts are relatively difficult to separate, due to the presence of lipids and other associated proteins. Plasma proteins form an extraordinary complex mixture, and though in the past decade, mainly 1-

and 2-D electrophoresis separations in combination with amino acid sequencing or immunological methods increased considerably our knowledge on its constituents.

Separations of blood proteins by CE have been performed in coated or uncoated capillaries, whereby adsorption of positively charged protein segments to the negatively charged uncoated capillary wall reduces resolution and decreases reproducibility. This adhesion of peptides and proteins to the wall can be reduced or eliminated by the use of buffers of pH≥ 9.0, pH≤2.5, buffer additives or coated capillaries [51–54].

3.1.1. Clinical capillary electrophoretic routine analysis

The first successful attempt to analyze human serum samples under electrophoretic conditions was achieved in the 1930s by A. Tiselius, separating albumin from α, β and γ globulins [1]. Clinical routine analysis of serum samples started in the 1950s using paper strips [55], replaced a few years later by microporous acetate membranes [56], still in use in many clinical laboratories. Since the 1970s, gel-supported media were also introduced in diagnostic laboratories.

After membrane- or gel-supported electrophoretic separations, followed by a staining and destaining procedure, five distinct zones (albumin, α_1-, α_2-, β-, and γ-globulins) can be scanned by a densitometer for quantitative percentage estimation. The clinical interpretation of the electropherograms is based on variation in their content. Each fraction is composed of many individual proteins, except the single peak of albumin.

Since the introduction of commercial CE instruments in the late 1980s, quite a few research groups published their results on CZE separations of serum proteins with liquid-filled capillaries [28,57–69]. These results demonstrate that CZE profiles show higher resolution, thus yielding a more detailed diagnostic information, compared to the conventional and still officially adapted agarose gel or cellulose acetate membrane electrophoresis. Most of these reports are dealing only with optimization procedures of the separation profile [59,63,64] or qualitative comparisons [28,58–60,62,63,68]. One communication compared CZE with agarose gel electrophoresis using a very low number of samples [61], and another one took 1000 specimens but evaluated only paraprotein correlations in the β-, and γ-region and not all five protein fractions [66]. Recently the first clinical evaluations testing the performance of the CE method under routine conditions appeared in the literature [67,69]. In our investigation [69], analyzing 102 clinical samples, we found acceptable coefficients of variation and correlations with the routine method for all five fractions; the results are in good agreement with Ref. [67].

Fig. 2 demonstrates the power of CE supplying additional information of valuable diagnostic use: Fig. 2A) shows an electropherogram obtained in our daily routine electrophoresis laboratory on cellulose acetate membrane, with an inconspicuous pattern, except the shoulder in the peak of the γ-fraction. Also, the visual control on the membrane did not give any pathological indication. From the CE pattern, shown in Fig. 2B), a monoclonal gammopathy is indicated, based on the separation observed for the γ-globulins, as shown by two prominent and one minor plus the C_3-complement peaks in the electropherogram. The diagnosis, suggested from the CE results, could be confirmed by agarose gel immunofixation electrophoresis to be a monoclonal gammopathy of type IgG λ. Similarly, the CE electropherograms of sera from patients suffering from other diseases allow a far more unequivocal diagnosis compared to the conventional electropherograms due to the much better resolved globulin fractions. Furthermore, it is worthwhile mentioning, that the CE separations, traced at a wavelength in the UV range, are recorded by the intrinsic absorbance of the protein polypeptide bonds, while staining properties of the proteins are used in traditional electrophoresis. Therefore, quantitation errors, resulting from the staining procedure (time, temperature, stain concentration and age, destaining time and solution, differences in the stainability of the proteins, etc.) are avoided with CE.

A consequence of the promising results is the Paragon CZE 2000™ (Beckman, Munich, Germany) the first fully automatic walk-away capillary electrophoresis system for clinical utilization, with automatic dilution, separation and detection of the samples, performing serum protein electrophoresis and immunofixation electrophoresis/subtraction [68,70].

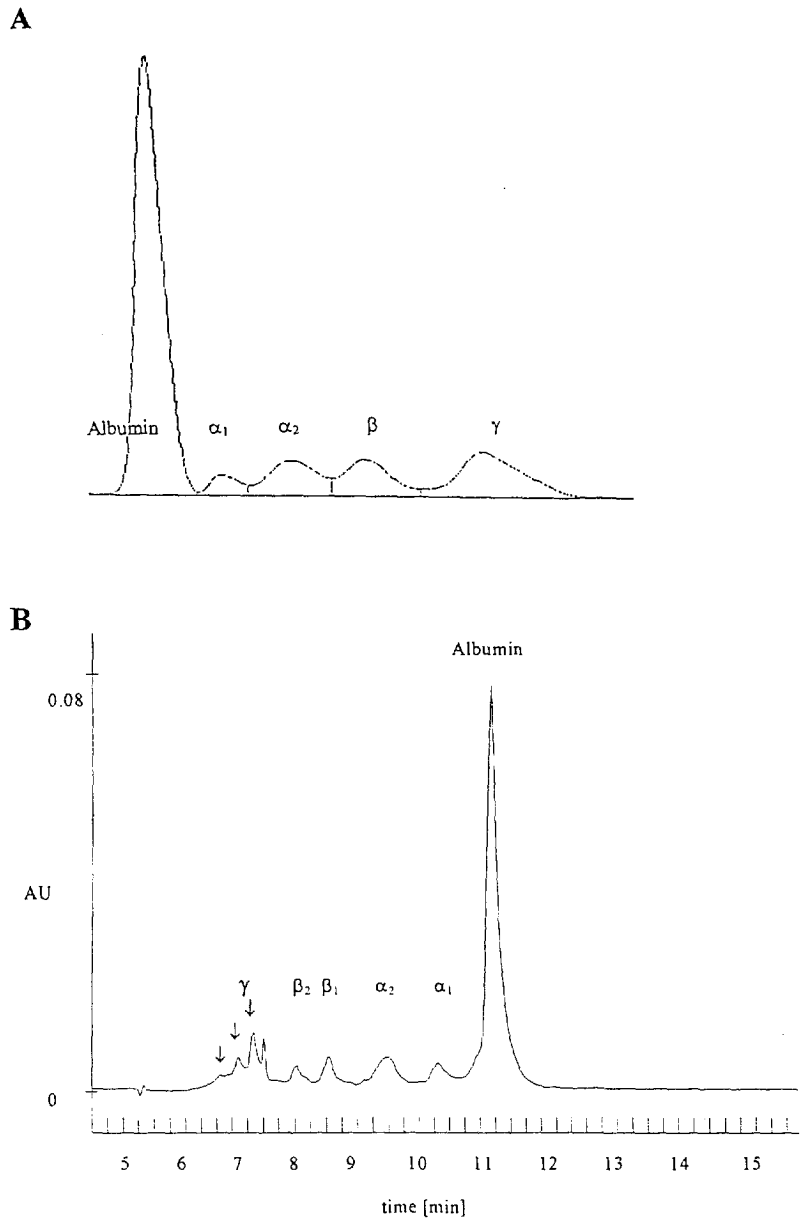

Fig. 2. (A) Electropherogram of serum proteins from an IgG λ myeloma patient run on cellulose acetate. Electrophoretic conditions, 100 V; buffer: 1.1% sodium barbital, 1.0% citric acid, pH 8.6. The cellulose acetate was stained with Ponceau red S. (B) Capillary electropherogram of the same sample. Capillary, fused-silica (55 cm×50 μm); running conditions, 35 μA; buffer, 30 m*M* borate, pH 10.0; detection, UV at λ 195 nm; equipment, Dionex capillary electrophoresis system CES I.

Immunofixation electrophoresis/subtraction is a method that allows the identification of specific peaks in a separated pattern, means in this case the classification of all kinds of gammopathies, especially the clinical most important monoclonal bands.

The instrument was designed for clinical routine

use, including seven capillaries (working in parallel) and UV detectors with a fixed wavelength at $\lambda=214$ nm. The analytical performance in SPE (42 samples per hour) is comparable to that of most agarose- or cellulose acetate membrane-based instruments. The possibility to analyze paraproteins automatically by immunosubtraction (five samples per hour) is an enormous progress as especially the traditional immunofixation procedure is very labor-intensive and time-consuming. The capillary electropherograms on the Beckman system, as well as on other systems (e.g., Ref. [69]), give comparable quantitative [61,66,67,69] and more detailed qualitative results [28,58–60,62,63,67–69] compared to classical serum protein electrophoresis. In this area, CE is not only an alternative method, but possesses the potential to replace the traditional cellulose acetate membrane and agarose gel electrophoresis, provided that the price for the analysis in total (instrumentation, analytical kits, analysis time) is comparable to or even cheaper compared to the other analytical procedures.

3.1.2. Distinct proteins, peptides and amino acids

Separation and quantification of distinct proteins from the complex serum matrix is another goal to be attacked by CE (survey, see Table 1). An example to be discussed here, are protein components, related to the risk of arteriosclerosis and coronary heart disease. CE screening for plasma apolipoprotein A-I levels (Apo A-I), the major protein constituent of human high density lipoprotein (HDL), was performed directly in serum [54]. Decreased Apo A-I levels in human serum are indicative for atherosclerotic processes [71], acute hepatitis and hepatic cirrhosis [72]. Today, the immunonephelometric (INA) and immunoturbidimetric assays (ITA) are the most common methods applied in clinical laboratories for an automated and rapid routine analysis of Apo A-I concentrations in human serum. The major drawbacks of all immunological determination methods of Apo A-I are caused by the inhomogeneity of HDL [73] and that masking lipids may prevent antigenic sites to be accessible [74,75]. Consequently, results are strongly influenced by variations in the specificity of antisera, the standardization procedure and the methodology used in a particular assay system. Furthermore, a serious problem of any INA or ITA is that hyperlipemic samples can disturb the Apo A-I determination.

Fig. 3a demonstrates that the electrophoretic conditions allow a clear-cut separation of Apo A-I from serum proteins and quantitative peak evaluation even in highly lipemic serum samples, while nephelometry may give doubtful results. The electropherogram of a patient serum sample with an elevated level of Apo A-I is seen from Fig. 3b, showing a major and an additional minor Apo A-I peak at shorter migration time. Different polymorphic Apo A-I forms can be made responsible for this separation pattern, as revealed by analytical isoelectric focusing [76] and recently also by capillary electrophoresis [28,77]. Therefore, for quantitative Apo A-I determination the area of both peaks have to be considered. The Apo A-I linearity plot, the clinical relevant detection limit and range, as well as the coefficient of variation (1.8% for the migration time and 8.7% for the relative peak area) are all acceptable for routine analysis [54]. If the sample throughput will be increased by capillaries in parallel in the future, this method will compete with traditional procedures. Until now it is used only for samples of special interest or as a confirmation test. To become a useful diagnostic tool a procedure determining the apolipoprotein B_{100} has to be supplemented.

The apolipoproteins A-I and A-II are the major protein constituents of HDL, comprising approximately 70% and 20% of the total apoliporoteins of HDL, respectively. The mixture of both apolipoproteins, as well as the other apolipoproteins, could not be separated in serum samples by CE until now, because of the high concentration of other serum proteins and therefore overlying phenomena. However, after fractionation of the individual lipoproteins via ultracentrifugation of plasma, e.g., apolipoprotein A-I and A-II from HDL [28,77–79], apolipoprotein B_{100} and B_{48} from low density lipoprotein (LDL) [78,79], apolipoprotein C-III and B_{100} from very low density lipoprotein (VLDL) [79], as well as the independent coronary heart disease marker lipoprotein (a) [80], the individual apolipoproteins can be determined by CE. However, ultracentrifugation is a very time-consuming procedure (24 to 48 h), and, therefore, apolipoprotein determinations including such a step are not suitable for clinical routine analysis.

Table 1
Survey of selected capillary electrophoretic applications with proteins and amino acids in biological matrices

Compounds	Matrix	CE mode	Detection mode	References
Proteins				
Protein fractions	serum	CZE	UV 190, 200, 214, 220, 230 nm	[2,28,58] [59–62] [63–66] [67–70] [260]
Immunoglobulin G, transferrin, albumin, prealbumin, β-trace protein, glutamine	CSF	CZE	UV 185 nm	[96]
Apolipoprotein A-I, A-II, B_{100}, B_{48}, C-III and E	serum	MECC	UV 190,200, 220 nm	[28,54,77,79]
Lipoprotein subfractions (HDL, VLDL, IDL, LDL)	serum	CITP	Vis 570 nm	[81–84]
Leucine aminopeptidase	serum, urine	CZE	LIF	[93]
Cerebrospinal fluid proteins (not specified)	CSF	CZE	UV 200, 280 nm	[97]
Myoglobin	urine, tissue	CZE	Vis 405 nm	[86]
Albumin, α-1-acid glycoprotein, transferrin, β-microglobulin, immunoglobulin light chains	urine	CZE	UV 200 nm	[90]
Monoclonal antibodies (anti-TNF, anti-CEA)	serum containing culture medium	CZE CIEF CGE	UV 200, 214, 280 nm	[261]
Imidodipeptides (prolidase deficiency)	urine	CZE	UV 200, 269 nm	[87]
Cathepsin D	breast tissue	CZE	UV 214 nm	[95]
Hemoglobin variants	plasma	CZE CIEF	UV 210, 214, 280 Vis 405, 440 nm	[32,105,106] [107–109] [110–112] [113,114]
36 low side molecular mass proteins (not specified), cut off 30 and 5 kDa	seminal and vaginal fluid, serum, saliva	CZE	UV 214 nm	[104]
Amino acids				
Hydroxyproline, sarcosine, proline	urine	MECC	fluorescence	[262]
Arginine, glutamine, valine, threonine, glycine, serine, alanine, glutamic acid, γ-aminobutyric acid, aspartic acid	CSF	MECC	LIF	[102]
Glutamate, aspartate	CSF	CZE	LIF	[99,100]
14 amino acids and 4 amines (ornithine, tyramine, citrulline, taurine)	CSF	MECC	LIF	[98]
Tryptophane, 5-hydroxytryptophane, 3-indoxyl sulfate	urine	MECC	fluorescence	[127]
Glutamine, immunglobulin G, transferrin, albumin, prealbumin, β-trace protein	CSF	CZE	UV 185 nm	[96]

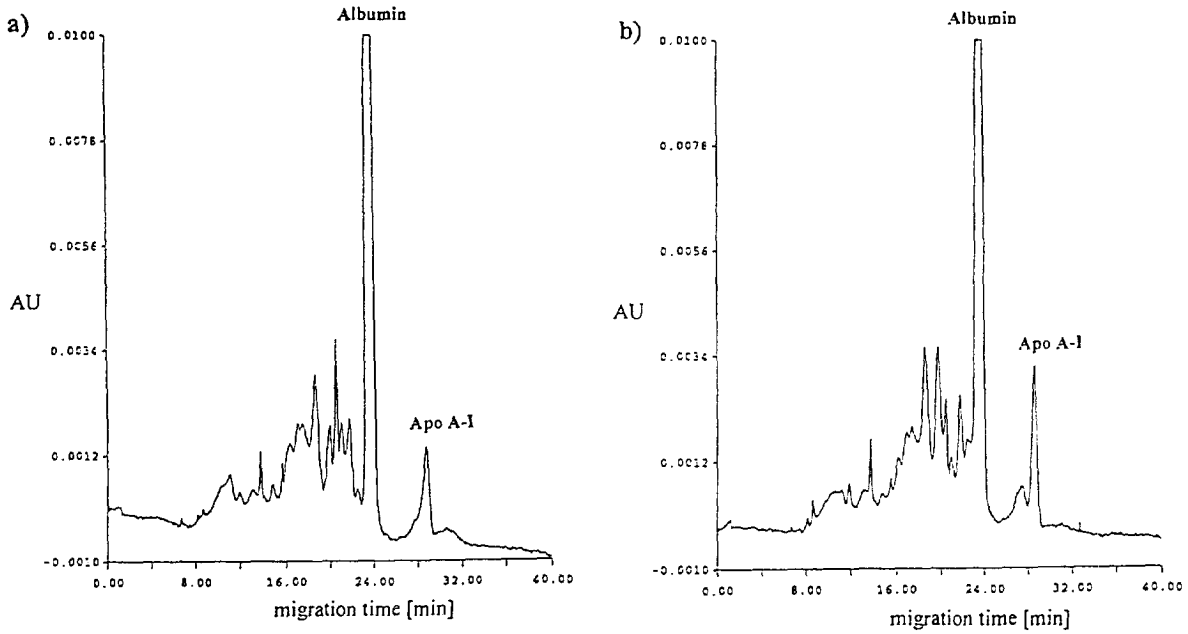

Fig. 3. (a) Capillary electropherogram of a lipemic serum sample from a patient (triglycerides: 967 mg/dl (=10.93 mmol/l); cholesterol: 298 mg/dl (=7.75 mmol/l)). Results of quantitative analysis of apolipoprotein A-I (Apo A-I): capillary electrophoresis, 167 mg/dl; nephelometry, 147 mg/dl. (b) Capillary electropherogram of a patient serum sample with an elevated level of Apo A-I. Results of quantitative analysis of Apo A-I: capillary electrophoresis, 301 mg/dl; nephelometry, 244 mg/dl. Electrophoretic conditions: Capillary, fused-silica (50 cm×50 μm); loading pressure: 27.6 kPa s; running conditions, 20 kV; buffer, Bio-Rad evaluation LLV buffer; detection, UV at λ 195 nm; equipment, BioRad BioFocus 3000 capillary electrophoresis system.

After prestaining with Sudan black or a lipophilic fluorescent dye, detection of lipoproteins directly from whole serum or plasma can be realized using the sieving effect of HPMC and an isotachophoretic separation mode [81–84]. Within 15 min, the lipoprotein particles with molecular masses ranging from 180 000 Da (HDL) up to 5 000 000–10 000 000 Da (VLDL) are separated in 14 distinct subfractions. Such an isotachophoretic analysis of serum lipoproteins in combination with determinations of total triglycerides, total cholesterol, and HDL-cholesterol levels significantly improves the diagnosis for disorders of lipid metabolism [84]. In daily routine analysis, the separation profile of this procedure includes too many lipoprotein peaks with a very high inter-individual variation of the pattern, making a diagnostic interpretation very time-consuming and complicated. But for specialized clinical departments like lipid outpatient clinics or clinical research departments, the separation patterns can give valuable information about lipid disorders, individual prognosis and treatment of the patient.

Also, CE patterns of proteins, peptides or amino acids from the urine, another clinical important human specimen, are intensively investigated, e.g., to detect or classify kidney damages [58], different forms of phenylketonuria [85], monitor myoglobinurias [86], or for the diagnosis of human metabolic disorders [68,87]. The urine contains, depending on the state of health, proteins, peptides and amino acids of clinical interest in specific amounts, but also many UV-absorbing components in high concentration. Therefore, detection via absorption often requires, as mentioned above, a sample pretreatment step, whereas the mainly used quantitative immunological techniques (e.g., nephelometry) measure the proteins directly in the patient samples. An alternative method for analysis of urinary diagnostic metabolites is described by Jellum et al. using a CE instrument, equipped with a diode-array detector

(identification of metabolites via characteristic diode-array spectra and migration time) [68].

Likewise, hemoglobin and myoglobin, due to their strong visible absorption bands, can be detected directly at 415 nm [17,86]. Myoglobin is found in urine (myoglobinuria) in patients with different disorders, such as muscular dystrophy, traumata, infections or intoxication, always reflecting acute myolysis. It is important to recognize myolysis at its earliest state to prevent acute renal failure by dialysis. CE detection and quantification of myoglobin in urine without sample pretreatment in less than 7 min is an efficient method and an alternative to immunological methods for emergency cases [86]. Monitoring myoglobin in tissue extracts, sera (as an early marker of acute myocardial infarction), or dialysate and urine samples would be a more supplementary approach to control muscular dystrophies, traumata, or acute myocardial infarction.

Electropherograms of natural or pretreated urine and urine proteins show a large number of identified and unidentified peaks [58,63,88–91]. Unequivocally, the low molecular mass components, such as uric acid, creatinine, and urea can be identified [63,90,91], useful parameters for judging the kidney function, determined in daily routine with high throughput autoanalyzers. Measuring these analytes by rapid CE methods could be useful to classify doubtful results, but so far these methods can not compete with established procedures.

For the observation of patients with malignant B-cell proliferation (multiple myeloma, chronic lymphatic leukemia, etc.), a disorder characterized by the presence of monoclonal immunglobulins in serum, there is a strong clinical demand to detect and quantify constantly urinary excretion of the renal tubular cell damaging free κ- and λ-light chains (Bence-Jones proteins) [92], which was realized by CE separations of Bence-Jones proteins [58,90]. The values are in good correlation with the semiquantitative analysis performed by high resolution agarose gel electrophoresis [90]. Until now, a correlation with the, not very specific quantification of the free κ- and λ-light chains by immunological methods is missing.

An attractive method, performing clinically significant enzyme assays in capillaries, has been recently described by Miller et al. [93] for leucine aminopeptidase (LAP). For the electrophoretically mediated microanalysis, capillary electrophoresis columns and time-resolved LIF detection was applied for the enzyme detection (down to 6×10^{-13} *M*) in human serum, urine and *Escherichia coli* supernatant. An interesting method, demonstrating the flexibility and power of CE. For practice, the enzymatic activity of LAP of several hundred of samples per day can easily be measured by a chemical autoanalyzer.

Glutathione synthetase deficiency is associated with massive urinary excretion of 5-oxoproline (pyroglutamate), severe metabolic acidosis and defective central nervous system functions, sometimes of fatal consequence [94]. The diagnosis of glutathione synthetase deficiency and adenylosuccinase deficiency presented by Jellum et al. [94] can be performed in a much faster simpler way using CZE (completed in 30 min including sample preparation) than by the routine analytical procedure (gas chromatography–mass spectrometry (GC–MS), followed by amino acid analysis; completed in 3 h). In the same paper a CZE procedure have also been elaborated for homocysteinuria and cystenuria [94]. Lately, this group published an efficient tool to further speed up the analysis and enlarge the analytical spectrum, using a CE equipped with a diode-array detector [68]. Thus, disease-specific metabolites in urine can be identified via characteristic diode array spectra and migration time. The urine was injected directly, without any pretreatment, and over 50 metabolites were separated in 15 min. The method readily diagnosed inter alia 5-oxoprolinuria, multiple carboxylase deficiency, adenylosuccinase deficiency, and propionic acidemia [68]. This method is therefore an attractive alternative to the labor-intensive (sample-pretreatment) and time-consuming (total analysis time) HPLC or GC–MS procedures to detect abnormal compounds occurring in urine due to diseases. Prolidase deficiency, another inherited disorder, is characterized by massive urinary excretion of imidodipeptides X-proline and X-hydroxyproline. The detection of these imidodipeptides by a fast and simple CZE protocol is described in Ref. [87]. The inborn disorder of phenylalanine metabolism, the so called phenylketonuria, is detectable within 12 min by CZE [85]. Caused by an enzymatic block, increased levels of phenylalanine and several aromatic

acids are secreted into serum and urine, all of them detectable by CZE [85].

A good predictor for tumor malignancy in general and of breast carcinoma in particular is the analysis of cathepsin D [95]. A CZE method is described analysing the cathepsin D activity in only 5 min. The two main advantages of this method are relative speed and low cost. Both alternative methods, the RIA and traditional enzymatic assay, require more than one day to complete the reaction, and the costs, especially for RIA, are very high.

Cerebrospinal fluid (CSF) is secreted in the brain and is in steady state with the fluid surrounding brain cells. It plays a critical role in providing a constant chemical environment for sensitive neurons and glia. For the diagnosis of central nervous system disorders, the analysis of proteins, peptides and amino acids play an important role, e.g., increased levels of plasma macromolecular proteins may indicate a disturbance of the blood–CSF barrier or the levels of the amino acid glutamine are largely increased in CSF of hepatic encephalitis [96]. The CE profiles of pathophysiological CSFs performed by CZE [96], CGE [97] or MECC-LIF [98] are potentially useful for the diagnosis of various neurological disorders. To overcome concentration detection limits in CSF, e.g., for the determination of multiple neuropeptides or amino acids, LIF detection after a derivatization step was performed [98–102]. In a total analysis time of 35 min the electropherogram of a CSF sample shows approximately 50 separate peaks, ten of which were identified as arginine, glutamine, valine, threonine, glycine, serine, alanine, glutamic acid, γ-aminobutyric acid and aspartic acid [102]. CSFs of up to 74 patients with, e.g., Alzheimer's disease, epilepsy, demyelination, cerebral infarction, various other neurological disorders [96,97,102] and of acute lymphoblastic leukemic children, treated with high amounts of methotrexate [98], have been analyzed. The in vivo monitoring of amino acid neurotransmitters in brain microdialysates [99,100], whereby the separation of glutamate and aspartate is achieved within 70 s [100], demonstrates the enormous potential of CE for neurochemical research, reviewed in Refs. [101,103].

For forensic serological investigations, the CE patterns of low molecular mass proteins in seminal plasma, vaginal fluid, saliva, and serum are of interest [104].

Hemoglobins, are protein tetramers, consisting of two pairs of different globin chains (α, β, γ, δ) each of which covalently linked to four different heme groups, and more than 600 structurally different human hemoglobins are known [105]. As many congenital defects and acquired hematologic disorders exist (e.g., sickle cell disease, different thalassemia syndromes, methemoglobine, glycated hemoglobin (long-term diabetes control), etc.) quite a few test systems for their identification have been developed in the clinical laboratories. Because of the high resolution power, automatization and several other before mentioned advantages, CIEF is an alternative method to analyze and quantitate hemoglobin variants [32,105–114]. Prior to application to clinical routine analysis, additional work is necessary to investigate the dependence of resolution on the carrier ampholyte concentration, ionic strength and protein content of the sample, focusing time and voltage, the degradation of capillary coating, as well as on the composition of anolyte and catholyte, influencing the anodic and cathodic drift of the pH gradient and therefore the reproducibility [115]. These investigations may be anticipated to further improve CIEF, but already now the methods are competing with the traditional IEF analyses.

Most clinical laboratories employ at present a battery of different hemoglobin assays, like electrophoresis (alkaline and acid), ion-exchange or affinity chromatography, IEF, HPLC and immunoassays [116] for managing the multiple diagnostic needs in hemoglobin analysis [105]. It seems, that the advantages of all these analytical procedures can be integrated in one CE mode: CIEF.

3.2. Drugs

Over the last two decades, different techniques of drug monitoring in body fluids and tissues of biological specimens with clinical relevance have been developed, such as dosage adjustments, compliance control, determination of efficient drug levels or overdoses, etc. Therefore, the availability of fast, precise and reliable analytical methods for the determination of drug levels is essential in clinical chemistry.

The analysis of drug levels in body fluids is, at present, a domain of immunoassays, chromatography (thin-layer or liquid chromatography) and spectro-

photometry. All these methods suffer from several disadvantages: Developments of immunoassay kits are costly and tedious, antibodies suffer from cross-reactivities with metabolites of drugs or contaminations, HPLC procedures often require labor-intensive sample preparation, relatively long equilibration and analyses times. Thin-layer chromatography (TLC) provides mainly qualitative results (as do some of the immunoassays), though qualitative screening of plasma, urine or gastric aspirate often helps in identifying the drugs. Most of the disadvantages, mentioned for the different methods of drug analysis, can be overcome by using CE [117]: high speed, on-line sample pretreatment (if necessary) and automatic separation and quantification, will probably make it the most attractive technique for emergency and routine analysis of drugs in future times. For developing CE methods in this area, it is important to realize that the majority of these bioanalytical methods do not use just one simple analytical step, but rather include one or more sample pretreatment steps, which are often time-consuming, suffer from inaccuracy and are not very useful for emergency diagnosis. Pretreatment procedures could be necessary if, e.g., a large number of difficult to separate compounds have to be analyzed or when concentrations of drugs or metabolites are low. Thus far, mainly CZE and MECC separations were described in the literature (survey, see Table 2). Hudson et al. collected CZE relative migration times of more than 400 drugs [118]. Nevertheless, MECC is the more favorable one, as it allows to separate both neutral and charged compounds in a single run. Moreover, minimal sample pretreatment is required as contaminating proteins are well solubilized by the detergent-containing buffer. The main interest in CE method development is focused on performing single step analysis, with direct on-column injection of body-fluids. These perspectives for CE and especially MECC in drug analysis have recently been reviewed [119,120].

3.2.1. Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is a rapidly developing area in the clinical laboratory. To be effective, TDM requires the acquisition of valid specimens followed by routine, timely, and reliable determinations of drug concentrations in these specimens.

One strategy to detect one component in a complex serum matrix is to use specific antibodies. The combination of immunoassays and CE has recently attracted considerable attention [23,63,121–123]. The basic principle is an immunological reaction between antibody and analyte, followed by a CE separation of the antibody–antigen complex from the free antibody and the free antigen, thus allowing a quantification. Such assays are reported for TDM of digoxin (cardioactive digitalis glycoside) in serum [63,121], theophylline (anti-asthmatic) in serum [123], and in the field of endocrinological diagnosis for the steroid hormone cortisol [124,125]. Several advantages resulting from the combination of immunoassays and CE should be mentioned: Low costs (only a few microliters of antibody reagent, etc., are required), time-consuming procedures like multiple washing steps can be omitted, fluorescence-labeled antibodies or F_{ab}-fragments, detected by LIF, allow a drastic sensitivity increase [125] or convenient analysis of lipemic, icteric or hemolyzed sera, which are known to create problems in conventional immunoassays [121].

MECC was applied to quantitate, e.g., theophylline [29,126], quinidine (cardioactive), naproxen (antirheumatic) and salicylate [127], or ethosuximide (anti-epileptic) directly in serum or urine [30,31]. Solid-phase extraction in combination with CZE allows to determine methadone and its primary metabolites in urine [128–131]. For monitoring the levels of the local anesthetic bupivacaine (a cocaine derivative) in drain fluid and plasma, a MECC [132] as well as a CZE method [133] was developed, and the results are in good coincidence with those of gas chromatographic determinations [133]. To determine the minimal toxic, but still effective dose of the antimycotic drug flucytosine or the bactericidal antibiotic drug fosfomycin, monitoring in serum could be successfully followed by MECC respectively CZE [134,135]. After derivatization with fluorescamine, gabapentin (a new anticonvulsant drug) can be monitored in less than 11 min [136], and in one of the first communications on CE and drug monitoring in serum samples, the analysis of methotrexate (antifolate; anticancer drug) has been described by Roach et al. in 1988 [137].

Suramin, originally used for the treatment of African Sleeping Sickness, was recently discovered to be an anti-prostate-tumor drug with narrow thera-

Table 2
Survey of selected capillary electrophoretic applications of drugs in biological matrices (arranged in alphabetic order)

Compounds	Matrix	CE mode	Detection mode	References
Amiloride, metoprolol, deacetylmetipranolol, labetalol, furosemide	serum, urine	CITP	conductivity	[147]
Aminopyrine, 4-aminoantipyrine	urine	CE-ED	0.9 V	[164]
Amiodarone, desethylamiodarone	serum	CZE	UV 242 nm	[48,49]
Amphetamine, caffeine, heroin,	serum, urine	MECC	UV 200 nm	[153]
Antipyrine	saliva	MECC	UV 260 nm	[149]
Barbital, allobarbital, phenobarbital, butalbital, thiopental, amobarbital, pentobarbital	serum, urine	MECC	UV-scan	[157]
Benzodiazepines	serum	MECC	UV 200 nm	[160]
Benzodiazepines	urine	MECC	UV-scan	[159]
Bupivacaine	serum	MECC	UV 220 nm	[132]
Bupivacaine, antipyrine	drain fluid	CZE	UV 200 nm	[133]
	plasma	MECC	UV 240 nm	
Buthionine-sulfoximine	plasma	MECC	UV 190 nm	[140]
Cannabidiol metabolites (11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid)	urine	MECC	UV 195 nm	[155]
Cicletanine	plasma	MECC	UV 214 nm	[143]
Cimetidine	serum	MECC	UV 228 nm	[145]
Clenbuterol	urine	CZE	UV 210 nm	[169]
Cocaine, opiate, morphine, diphenhydramine, methaqualone, temazepam, codeine, benzodiazepines, methadone	urine	MECC, CZE	UV 195 nm	[150]
Codeine, methapyrilene, brompheniramine, amphetamine, methamphetamine, procaine, tetrahydrozoline, phenmetrazine, butacaine, medazepam, lidocaine, acepromazine, meclizine, diazepam, doxapram, benzocaine, methaqualone	plasma urine	CZE	UV 214 nm	[151]
Dextromethorphan, dextrorphan	urine	CZE	UV 200 nm	[174]
Digoxin	serum	EIA+CE	UV 260 nm	[63,121]
		MECC	LIF	[23]
Diuretics	urine, serum	CZE	UV 220 nm 215 nm	[168,170]
Ephedrine, norephedrine	urine	CZE	UV 210 nm	[167]
Ethosuximide, phenobarbital, naproxen	serum	MECC	UV 215 nm	[30]
Famotidine	urine, plasma	CGE	UV 220 nm	[144]
Flucytosine	serum	MECC	UV 210 nm	[134]
Fosfomycin	serum	CZE	indirect UV	[135]
Gabapentin	serum	MECC	UV and fluorescence	[136]
Glipizide, glyburide, acetohexamide, chlorpropamide, tolazamide, tolbutamine, acetohexamide,	urine	MECC, CZE	UV 200 nm	[45,146]
Heroin, cocaine, codeine, thiopental, 6-acetylmorphine, benzoylecgonine,	urine	MECC	UV-scan	[129]

(*Contd.*)

Table 2. Continued

Compounds	Matrix	CE mode	Detection mode	References
flunitrazepam, oxazepam, metamphetamine, amphetamine, methadone, methaqualone, diazepam, morphine				
7-hydroxycoumarin and 7-hydroxycoumarin-glucuronide	urine	MECC	UV 320 nm	[141,142]
Lithium	serum	CITP	conductivity	[163]
Mephenytoin, dextromethorphan, caffeine	urine	MECC	UV–Vis scan	[171]
Methadone and its primary metabolite	urine	CZE	UV-scan	[131]
Methotrexate, 7-hydroxymethotrexate	serum	CZE	LIF	[137]
Metformin	plasma	MECC	UV 230 nm	[38]
Morphine, benzoylecgonine, codeine, methaqualone, methadone, amphetamine	urine	MECC	UV-scan	[128]
Morphine, benzoylecgonine, metamphetamine, amphetamine, methadone, diazepam, morphine-3-glucoronide, carbamazepine, phenobarbitale	serum, saliva, urine	MECC	UV-scan	[130]
Morphine, heroin, codeine, dihydrocodeine, pholcodine, 6-monoacetylmorphine	urine	CZE	UV 200 nm	[154]
Morphine-3-glucoronide	urine	CZE MECC	UV 195 nm	[152]
Naproxen, quinidine, salicylate	serum, urine	MECC	UV 220 nm fluoresc. 340 nm	[127]
Phenobarbital, butalbital, amobarbital, pentobarbital, secobarbital	serum, urine, gastric cont., autopsy	MECC	UV 214 nm	[158]
Phenobarbital, phenytoin, carbamazepine, ethosuximide, primidone	serum	MECC	UV 210 nm and UV scan	[161]
Primidone, phenobarbital, ethosuximide, phenytoin	serum	MECC	UV 215 nm	[31]
Prospidin	tissue	CZE	UV 254 nm	[139]
Salicylate, acetaminophen (paracetamol), antiepileptics	serum urine	MECC CZE CITP	UV-scan	[162]
Suramin	serum	CGE	UV 254 nm	[138]
Theophylline	plasma,	CZE	UV 280 nm	[36,122]
	serum	EIA+		[29,123]
		MECC	LIF	
	urine	MECC	UV	[126]
Theophylline, caffeine, naproxen	serum	MECC	UV 240 and 275 nm	[29]
Thiopental	serum, plasma	MECC	UV 290 nm	[156]
Verapamil	serum	CITP	conductivity	[148]
Zolpidem	urine	CZE	LIF	[165]
Zopiclone	urine	MECC	LIF	[166]

peutic range (serum concentration ~200–300 mg/l) and long half-life (44–54 days), therefore frequent monitoring is required to minimize toxicity. Garcia and Shihabi [138] developed a rapid assay for suramin levels in serum in an analysis time of only 2.5 min with a linear range of 50–500 mg/l. Another widely used anticancer drug is the chemotherapeuticum prospidin (a derivative of nitrogen mustard). In the recently appearing paper [139], dealing with the detection of prospidin in human tissue, a clear quantitative determination in human papilloma species was achieved. The diastereoisomers of buthionine sulfoximine are potent blockers of glutathione biosynthesis and may therefore modulate the toxicity of cancer therapy and radiotherapy. Therefore it is now being used in clinical trials as a biochemical modulator in conjunction with radiotherapy for solid tumors. In the past, sample extraction and derivatization were necessary for the quantification of L-buthionine-(*R,S*)-sulfoximine. The MECC method developed by Sandor et al. is sensitive and adequate for determining the plasma levels of this substance in clinical trials, currently being undertaken [140]. Coumarin, used for the treatment of high-protein edemas, brucellosis, and chronic infection is currently being investigated as an anticancer drug. The direct quantitative detection of 7-hydroxycoumarin and 7-hydroxycoumarin-glucuronide in urine by MECC in comparison to HPLC is quite fast and accurate, but the sensitivity in the lower concentration range has to be improved [141,142].

A sensitive and selective CE procedure was developed few years back for the determination of the antihypertensive drug cicletanine in plasma and the values obtained were in good agreement with those found by HPLC; however, the CE method requires less than half the time compared to the chromatographic method [143]. The separation behaviour of the anti-ulcer drug famotidine was used to evaluate the optimal composition of mixed polymers for the direct determination pharmaceuticals in urine [144]. The H2-receptor antagonist, cimetidine, was quantified in serum by MECC after electrochromatographic solid-phase extraction [145].

Oral drug therapy of non-insulin-dependent diabetes mellitus (NIDDM or type II diabetes) is managed mainly by sulfonylureas or biguanides. The major acute risk of a sulfonylurea is hypoglycemia and that of biguanide is lactic acidosis, therefore fast and reliable methods to quantify the amount of these drugs are desirable. Metformin (dimethylbiguanide) is widely used in Europe and USA. Due to the life-threatening risk of lactic acidosis a precise quantification in plasma of metformin-treated NIDDM patients is indicated. In this context a MECC method to measure metformin in plasma was recently developed [38]. As metformin is not bound to protein, sample pretreatment before CE analysis (separating the drug from plasma proteins) can easily be performed by ultrafiltration. Fig. 4 demonstrates the quantitative determination of metformin in an ultrafiltrated sample of a diabetic patient. The calibration curve covers the therapeutic concentration range from 0.5 to 2.5 μg/ml [38]. Hence, this method is suitable for an exact measurement of metformin in patient plasma samples, but prior to clinical evaluation, the running time has to be shortened. The improvement of the analysis (speed-up) and the measurement of metformin in erythrocytes and urine are under way. Hypoglycemia can be caused by surreptitious abuse of sulfonyl urea drugs or an insulin-producing tumor (insulinoma), both, resulting in an abnormally low concentration of blood sugar. Insulinoma can be excluded in less than 17 min by a method, developed by Núnez et al., performing MECC analysis of seven commonly administered sulfonyl urea drugs in urine [146].

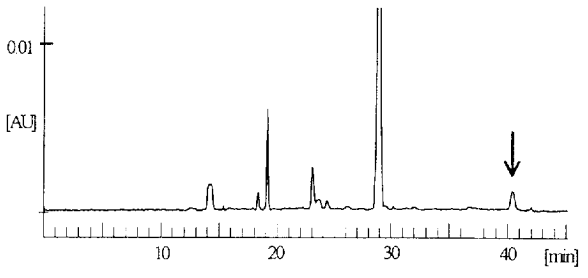

Fig. 4. Capillary electropherogram of an ultrafiltrated human plasma sample of a metformin treated diabetic patient. The metformin peak is marked by an arrow (quantitative result: 1.1 μg/ml). Electrophoretic conditions: Capillary, fused-silica (47 cm×75 μm); loading, hydrostatic: 100 mm, 45 s; running conditions, 12 kV; buffer, 20 m*M* borate, 20 m*M* Na-phosphate, 100 m*M* SDS, pH 9.2; detection, UV at λ=230 nm; equipment, Dionex capillary electrophoresis system CES I.

The separations and determinations of cardiovascular drugs (amiloride, metoprolol, deacetylmetipranolol, labetalol, furosemide) out of serum and urine samples [147], as well as verapamil [148], were recently investigated by CITP.

Checking specificities, sensitivities, recoveries and the linearities within the therapeutic range, comparative drug levels of patient samples were found to correlate well, if determined by different techniques, e.g., EMIT (enzyme multiplied immunoassay technique) versus MECC parameters of theophylline in serum [29], HPLC versus MECC levels of antipyrine in plasma, saliva and drain fluid [133,149] or CZE versus GC parameters of bupivacaine in drain fluid [133]. The large number of applications in TDM indicate the potential role of CE in the methodologies and analytical spectra in this important clinical field. A widespread use of clinical TDM by CE depends largely on the availability of commercial kits for an easy, safe and reproducible analytical procedure and the corresponding easy to handle instrument and computer software.

3.2.2. Intoxications and drug abuse

Analytical toxicology, effectively performed, is frequently an essential part of the sequence of events from diagnosis to treatment of chronic and acute poisoning. In order to fulfil its role in dealing with emergency situations, the clinical laboratory must provide a 24 h service and utilize reliable methods with short turnaround times. The compromise between service demand and resources is often achieved by a well-chosen combination of qualitative and quantitative methods representing a wide variety of analytical principles and modes. The results of the laboratory analysis may confirm a previous impression of the clinician or may raise the possibility of a toxic substance not previously suspected. In some cases, quantitative results may determine the type of treatment procedure to be instituted.

With its distinct advantages, modern CE appears to be an attractive tool for fast and quantitative monitoring of intoxications. An overall concept for efficient toxicological drug screening by capillary electrophoretic techniques is proposed in the paper of Steinmann and Thormann [150]. The time for analysis is in many cases much shorter (separation times of 1 [149] to 3 min [31,134,138]) compared to routine screening methods, the results are always quantitative and multicomponent analyses in one run can be realized. This has been demonstrated by Chee and Wan, who separated 17 basic drugs in spiked plasma and urine samples in less than 12 min [151]. The exemplary analytical spectrum of CE separations concerning intoxications and drug abuse includes opiates [128–130,152–154], cannabinoides [155], barbiturates [30,31,156–158], benzodiazepines [41,151,159,160], stimulants [151], antiepileptics [30,31,129,151,161,162], other psychopharmaceuticals [163], analgesics [30,31,131,151,162,164–166], or doping screening [167–170] within 20 min or less. Due to the lack of specificity, positive or negative results gained from routinely applied immunoassays, such as FPIA (fluorescence polarization immunoassay) and EMIT, should be considered with caution and confirmed with a more specific method, including TLC, HPLC or GC–MS [155]. From the advantages of CE over immunological methods, mentioned before, emergency and routine drug laboratories will certainly make increasing use in the future, because in analytical toxicology the sequential mode in CE is sufficient, if the total analysis time is in the range of minutes and the procedure is easy to perform, since a lot of samples sent to the laboratory at the same time is uncommon in this field.

3.2.3. Function tests and phenotyping of metabolic polymorphisms

Testing the functions of organs by monitoring exogenous probes is a well established method of clinical diagnosis. For example, determination of antipyrine levels in saliva or plasma after oral administration is a non-invasive accurate way to estimate liver function (microsomal enzyme activity) and MECC yields comparable results to the HPLC method [133,149]. Phenotyping of inherited metabolic variations of human drug metabolism in serum or urine is of clinical importance, since enzymatic deficiencies can lead to unusually high plasma concentrations of certain drugs and to increased side-effects, or, in case of prodrugs, insufficient therapeutic effects [171]. As HPLC and genotyping methods are time-consuming, because of sample pretreatment (HPLC) or multiple analytical steps (genotyping), a CZE- [171] and MECC-methods [171–173] was

developed to determine drug metabolite levels in urine and thereby identifying phenotypes for hydroxylation and acetylation. Recently, also the debrisoquine-oxidation metabolic phenotype was also determined by CZE [174].

These selected examples demonstrate that automated capillary electrophoresis is best suited for therapeutic and diagnostic drug monitoring. Its superiority over chromatographic methods is based on several important facts, including the feasibility of directly injecting aqueous protein samples (such as plasma or serum), the high degree of efficiency, automation, reproducibility, speed and requiring small amounts of sample and separation buffer, mostly free of organic solvents [133]. Compared to immunoassay procedures, CE methods neither require tedious and expensive development of kits nor can cross-reactivities lead to doubtful results. The upcoming task is to compare and evaluate the various CE applications with the conventional methods under daily routine or emergency laboratory conditions, and thereby proof and underline the practical use of CE in clinical drug analysis. A second step will be the development of easy to use, low-cost analytical CE-kits, which will further increase the influence and acceptance.

3.3. Hormones, neuropeptides, biogenic amines, vitamins and ions

Endocrine glands are tissues releasing secretory products, the hormones, into extracellular and interstitial fluids. Most hormones can be grouped into three general classes: (1) steroids, (2) polypeptides or proteins, and (3) hormones derived from amino acids. Most of the hydrophobic steroid hormones circulate in plasma bound to high-affinity plasma proteins, and only a small percentage of the hormone is set free, causing biological activity [175]. For clinical diagnosis, the hormone concentrations in blood are of great relevance, and, as these are very low (steroid and thyroid hormones between 10^{-6} and 10^{-11} mol/l, proteohormones between 10^{-9} and 10^{-12} mol/l [176]), hormones are difficult to determine by conventional CE methods and until now there are only few reports dealing with human samples (survey, see Table 3). However, in other fields, like neuroscience, CE was successfully applied for, e.g., in vivo monitoring of hormone release [177,178].

Though still in an experimental stage, the successful separation and quantification of 7 corticosteroids, including 1-dehydroaldosterone, 17-isoaldosteron, cortisone, aldosterone, cortisol, 21-deoxycortisol and corticosterone by MECC is described in Ref. [179]. Steroids, relevant for the diagnosis of congenital adrenal hyperplasia (17-hydroxyprogesterone, 21-deoxycortisol, cortisol and 11-deoxycortisol) are separated with high resolution in supplemented serum, but increased sensitivity needs sample preconcentration via solid-phase extraction [180]. Estrone, estradiol, estriol in urine are well separated by MECC, but the sensitivity limits attainable with UV detection may limit its utility to the measurement of estriol and estrone [181]. Cortisol levels in serum can be determined by a new solution phase competitive immunoassay, followed by a CE separation of free and bound fluorescein-labeled tracer, thus realizing a quantitative determination of cortisol in serum [124,125]. The CE-immunoassays were performed with F_{ab}-fragments [125] and polyclonal antibodies [124]. Determinations of the serum cortisol concentrations (reference range: 83–635 nmol/l [182]) are important for the assessment of adrenocortical activity and the diagnosis of adrenal malfunctions such as Addison disease and Cushing syndrome.

One of the first applications of CE in the field of neuroscience was the detection of leucine enkephalin and methionine enkephalin in equine CSF [183]. CZE–MS and HPLC–MS have been evaluated for their suitability to determine low levels of these endogenous components with potent opiate agonist activity. Recently, a preparative and analytical way to detect and separate β-endorphin, leucine enkephalin and methionine enkephalin from bovine pituitary by CZE was reported [184]. In brain microdialysate fluid of rats nanomolar concentrations of noradrenaline and dopamine could be determined with CZE in combination with LIF after derivatization [178]. An overview of CZE–LIF determinations of neuropeptides, e.g., luteinizing hormone-releasing hormone, neuropeptide Y, β-endorphin is given by Advis et al. [101]. Urinary detection of elevated levels of catecholamine metabolites (homovanillic acid and vanillylmandelic acid) are indicative of

Table 3
Survey of selected capillary electrophoretic applications of low molecular mass substances

Compounds	Matrix	CE mode	Detection mode	References
Hormones				
17-Hydroxyprogesterone, 21-deoxycortisol, 11-deoxycortisol, cortisol	serum	MECC	UV 254 nm	[180]
Cortisol	serum	EIA+CZE	LIF	[124] [125]
Homovanillinic acid, vanillylmandelic acid, 5-hydroxyindole-3-acetic acid, tryptophane, 5-hydroxytryptophane, 3-indoxyl sulfate	urine	MECC	fluorescence	[127]
Estrone, estradiol, estriol	urine	MECC	UV 220 nm	[181]
1-Dehydroaldosterone, 17-isoaldosterone, cortisone, aldosterone, cortisol, 21-deoxycortisol, corticosterone	serum plasma	MECC	UV 260 nm	[179]
luteinizing hormone-releasing hormone, neuropeptide Y, β-endorphin	liquor, brain	CZE	UV 214 nm	[177]
β-endorphin, methionine, enkephalin, leucine enkephalin	bovine pituitary	CZE-LSIMS	UV 200 nm	[184]
Leucine enkephalin, methionine enkephalin	CSF	CZE-MS	UV 220 nm	[183]
Noradrenaline, dopamine	CSF	CZE	LIF	[178]
Vitamins				
Thiamine, nicotine amide, nicotinic acid, pyridoxine, cobalamin, ascorbic acid	experimental	MECC	UV 254 nm	[185]
Retinol	serum dried human blood	CZE	LIF	[186,187]
Ascorbic acid	plasma, serum, urine, fruitbeverages	CZE	UV 254 nm	[188]
Ions				
Lactate, pyruvate, oxalate, fumarate, inorganic phosphate, acetate, glutamate, ascorbate	CSF	CZE	UV 185 nm	[191]
Ionized calcium, total calcium	serum	CZE	indirect UV 214 nm	[198]
Orotic acid, propionate, pyroglutamate, acetoacetate, adenylosuccinate	urine	CZE	UV-scan	[68]
Pyruvate, phosphate, citrate, malate, acetoacetate, lactate	serum	CZE	indirect UV 220 nm	[190]
Oxalic, formic, malonic, fumaric, succinic, α-ketoglutaric, citric, acetic, pyruvic, lactic, isovaleric, hippuric acid	urine	CZE	UV 185 nm	[189]
Iron	serum	CZE	UV 270 nm	[199]
Potassium, calcium, sodium, magnesium	serum	CZE	indirect UV 214 nm	[192]
Nitrite, nitrate	plasma, urine	CZE	UV 214 nm	[194,195]
calcium, ammonia, potassium, sodium, magnesium, lithium, barium, creatinine	urine	CZE	indirect UV 255 nm	[193]

various diseases, including neuroblastoma and pheochromocytoma. MECC allows separation and quantification within 10 min [127].

Vitamins are organic compounds, required for reproduction, health and growth. In the Western World with its mixed diet, serious deficiencies are rarely observed, except in humans with, e.g., food fads, in patients with malabsorption or patients

treated by hemodialysis. However, excessive use of some vitamins may be found. In poorer communities, severe deficiencies can occur. In 1988, an experimental separation of six water-soluble vitamins within 22 min by MECC has been reported [185]. Vitamin A deficiency causes irreversible blindness, night blindness, xerophthalmia and keratomalacia. Serum vitamin A level determinations with CZE–LIF can be performed in less than 6 min, with a detection limit of only 10 ng/ml (reference range of vitamin A: 300–800 ng/ml [182]). Compared to HPLC [116], this method requires almost no sample preparation and is feasible for finger-prick analysis of vitamin A, of special interest for newborns and young children, due to the small sample requirement in CE [186]. Recently the same group described the CE determination of vitamin A also in dried human blood spots [187]. Due to rapid sample preparations and excellent separation characteristics, quantitative CE determination of vitamin C levels in serum, urine, and fruit beverages, are preferable to HPLC [188].

The increase of some organic acids can lead to a decrease in blood pH (i.e., acidosis), to acidotic coma and even death, well described for metabolic disturbances such as diabetic ketoacidosis or lactic acidosis. Many inherited disorders of amino acid metabolism are caused by an enzyme deficiency resulting in the accumulation of an organic acid in blood and urine (so called acidemia); e.g., propionic acidemia, methylmalonic acidemia (metabolism of propionate), isovaleric acidemia, β-methylcrotonic aciduria (leucine metabolism). Determinations of lactic, oxalic, formic, malonic, fumaric, succinic, α-ketoglutaric, citric, acetic, pyruvic, isovaleric and hippuric acid in human urine by CZE in comparison with values determined by an organic acid analyzer are described in reference [189]. However, the evaluation was performed only on urine samples of healthy volunteers. In the electropherograms of non-deproteinated sera of critically sick children, pyruvate, phosphate, citrate, malate, acetoacetate and lactate peaks could be identified within 12 min, whereby the most important lactate was quantified [190]. Also, in CSF from 40 patients with central nervous system (CNS) diseases (e.g., bacterial meningitis, acute-phase cerebral infarction, viral meningitis), the organic acids lactate, pyruvate, oxalate, fumarate, acetate, glutamate, ascorbate were analyzed by CZE, and the results show that CZE can become a powerful tool in the biochemical diagnosis and differentiation of CNS diseases [191].

The determination of inorganic cations and low molecular mass anions in body fluids is of considerable importance for diagnostic purposes, and part of the routine tasks in clinical laboratories. In serum samples, the cations potassium, calcium, sodium, magnesium can be quantitatively separated in one run with a minimal effort of sample preparation [192]. Also in urine, calcium, ammonia, potassium, sodium, magnesium, lithium, barium and creatinine are simultaneously detectable [193]. In daily clinical routine, the cations, mentioned before, are determined by sophisticated clinical chemistry autoanalyzers. CE has the advantage of analyzing several components in one run, but cannot be an alternative with regard to sample throughput.

In shock and organ dysfunction, observed in critically sick patients, nitric oxide is one of the main pathogenic factors, and therefore it is important to monitor its biosynthesis in these patients [194]. In blood, nitric oxide is rapidly oxidized to nitrite and nitrate, the markers of nitric oxide generation, which are normally determined spectrophotometrically after sample pretreatment [194]. In the literature two CZE methods are described to determine nitrate and nitrite levels within 16 and 7 min, respectively, without sample pretreatment in plasma [194] and urine samples [195]. Recently, a MECC method for anion separations in plasma and milk was introduced analyzing thiocyanate, iodide, nitrate and nitrite in about 12 min [196].

Calcium is the most abundant cation in the human body (1.0–1.4 kg in the adult; over 98% in the bones and teeth as crystalline hydroxyapatite). The small percentage of body calcium present in plasma and other extracellular fluids is, however, very important, e.g., for normal neuromuscular transmission, glandular secretion, and enzymatic systems (especially blood coagulation) [197]. Calcium exists in plasma in three physicochemical states: protein-bound (~135 mg), complexed with small diffusible ligands such as citrate, lactate, phosphate and bicarbonate (~30 mg), and ionized (~135 mg). Only the ionized fraction is the biologically active form, and its concentration is affected by pH and plasma protein content [197]. In

routine analysis, only the total plasma calcium concentration is determined, and therefore no information about the biologically active calcium concentration can be obtained. Direct determination of free-ionized calcium is, for technical reasons, confined to special cases. Both ionized and total calcium was determined quantitatively in human serum by CZE via indirect photometric detection in a single run and compared with the reference method (atomic absorption) for total calcium of the National Institute of Standards and Technology (Baltimore, MD, USA); for ionized calcium a special ion-selective electrode method was applied [198]. Besides ionized and total calcium, other cations like Na^+, K^+ and Mg^{2+} can also be quantitatively determined by this CZE method. These first results are very promising with regard to clinical applications, especially because only one instrument is required to determine both the ionized and total calcium levels, instead of two different techniques conventionally used [198].

Quantitation of trace amounts of iron in human serum by CZE was recently reported [199]. The method is sensitive, accurate and requires only 10 μl of serum, including sample pretreatment, eliminating interferences from copper and other serum components. The method seems to be suitable to detect iron deficiency or disorders of iron metabolism, especially in paediatric patients, where iron deficiency is common and available sample amounts are low.

3.4. Molecular biological applications

PCR, one of the standard tools in molecular biology, opened an easy access to in vitro syntheses of millions of copies of DNA segments of genetic target sequences using highly specific primers as starting materials. As has been demonstrated by several communications, CE is far superior compared to HPLC [200–202] checking the purity and identity of the synthetic oligonucleotides, prerequisite for specific DNA amplification. Its fundamental role in basic research is indisputable, and at present time major activities concentrate on its application to three broad areas of routine diagnosis: infectious diseases, neoplastic and inherited disorders.

The standard screening method for the analysis of PCR products is the time-consuming, labor-intensive, difficult to quantify, and hardly standardizable slab gel electrophoresis (agarose or polyacrylamide). Therefore a progressive impulse towards clinical routine analysis of PCR products is the combination of PCR and CE (survey, see Table 4). CE point mutation screening using different molecular biological techniques was recently reviewed by Mitchelson and Cheng [203]. The high degree of automatization, rapid performance of electropherograms, direct evaluation of mutations, detection of virus-DNA, quantitative analysis of PCR products, etc., make this combination an attractive tool for clinical molecular diagnosis. Quantitation of amplified PCR products is of key importance for the determination of the concentration of target DNA or the gene expression of the protein of special interest. CE hold promise for the precise quantitation of PCR amplicons in biological samples [204–208]. Moreover, sequencing of DNA is an analytical task for which CE in combination with laser-induced fluorescence is predestinated [209–215]. Direct DNA sequencing is a real alternative with the possibility to replace the traditional polyacrylamide slab gel techniques, documented by promising publications using capillaries in parallel or microfabricated capillary array electrophoresis chips [216–219]. All DNA molecules, single- or double-stranded, have an almost chain length-independent charge density, and consequently a sieving medium is needed for their CE separation (i.e., by CGE).

3.4.1. Infectious diseases

One of the first PCR product analyses using CGE was performed in 1991, detecting HIV-1 in blood [220]. Another application in this field is the quantification of polio virus RNA after PCR [206]. Recently, the detection of single-point mutations within *Mycobacterium tuberculosis* strains was reported [221], using both, fixed (crosslinked) gels and polymeric networks. The significance of these analyses is to rapidly identify those organisms harboring a mutation that may be associated with resistance to the drug rifampicin. Another paper describes the post amplification detection of hepatitis C virus-specific DNA product in human serum within 8 min [222]. Using LIF the sensitivity is comparable to or better

Table 4
Survey of selected capillary gel electrophoretic applications of clinical relevant diagnostic DNA

Disease	Mode of DNA amplification	Sieving mode	Detection mode	References
human genetics				
Cystic fibrosis	allele specific PCR and restriction digest of PCR products (deletion)	6% linear PAA	UV 254 nm	[224]
Coronary heart disease (ACE gene)	allele specific amplification	0.6% HEC	UV 260 nm	[235]
Duchenne muscular dystrophy, Becker muscular dystrophy	PCR multiplex reaction	6–10% linear PPA	UV 254 nm	[225]
Coronary heart disease (familial defective apolipoprotein B_{100} gene)	allele specific amplification	0.7% HEC	UV 260 nm	[234]
Coronary heart disease (apolipoprotein B_{100} gene)	amplification of VNTR alleles	0.7% HMC	UV 260 nm	[236]
Follicular lymphomas, non-Hodgkin's lymphoma (bcl-2 gene)	quantification by competitive PCR	4% linear PAA	UV 260 nm	[207]
Fetal DNA detection	PCR	Beckman dsDNA 1000 gel buffer	LIF	[238]
Congenital adrenal hyperplasia (P450c21B functional gen)	PCR (deletion)	6% linear PAA	UV 254 nm	[229]
von Willebrand factor gene	amplification of VNTR alleles	1% HEC	LIF	[230]
Cancer (ERBB2 oncogene)	RFLP	0.5% HPMC	UV 260 nm	[231]
Cancer (Tx gene)	RFLP	3% linear PAA or 1% HPMC	LIF	[263]
Medium-chain acyl-coenzyme A dehydrogenase deficiency	allele specific amplification	fixed PAA gel	LIF	[227]
Dystrophin gene	RFLP	0.5% HPMC	UV 260 nm	[226]
Cancer (p53 gene)	SSCP	4% linear PAA 2% linear PAA +5% glycerol	UV 260 nm fluorescence	[232] [239]
Coronary heart disease, M. Alzheimer (apolipoproteinE gene)	RFLP	fixed PAA gel (3%T; 0.5%C)	UV 260 nm	[237]
Cancer (DT-diaphorase expression)	RT-PCR (quantitative)	Beckman dsDNA 1000 gel buffer	LIF	[204]
Morris disease (complete androgen insensitivity syndrome)	nested-PCR	6% linear PAA	UV 254 nm	[228]
Cancer (microsatellite instability)	PCR	PCR product analysis buffer (Bio-Rad)	UV 260 nm	[223]
Cystic fibrosis	RFLP (temperature gradient capillary, gel electrophoresis)	8% linear *N*-acryloylamino-ethoxyethanol	UV 254 nm	[223]
Microbiology/virology				
Polio virus (detection and quantification)	RT-PCR	linear PAA	UV 254 nm or LIF	[206]
Mycobacterium tuberculosis (detection of single point mutations)	SSCP and ddF	Dionex sieving buffer and fixed PAA gel (3%T; 0.5%C)	LIF	[221]
HIV-1 (detection and quantification)	restriction fragments	0.1–1% HPMC and 5% PEG	UV 260 nm	[220]
Hepatitis C infection	RT-PCR	1% HEC	LIF	[222]
Therapy				
Antisense oligonucleotides in plasma		PAA gel (12% T; 0% C)	UV 260 nm	[208]
Antisense oligonucleotides		PAA gel (10%T; 0%C)	UV 260 nm and MALDI–MS or LIF	[247] [242]

than Southern blot analysis with chemiluminescence detection showing a 100% correlation for the detection of the specific PCR-fragment analyzing 39 patient samples.

3.4.2. Inherited and neoplastic disorders

In the past years, a permanent increasing number of communications dealing with PCR/CGE applications in connection with inherited diseases [223–

230], neoplastic disorders [207,231–233] or special risk factors (e.g., arteriosclerosis risk [234–237]), and others appeared in the literature. The paper of Mitchelson and Cheng examines the use of techniques such as PCR-restriction fragment length polymorphism (PCR-RFLP), heteroduplex polymorphism, single-stranded conformation polymorphism (SSCP), and denaturing gradient gel electrophoresis (DGGE) for mutation detection using CE [203].

Genetic markers are important tools in the study of human disease risk factors, such as arteriosclerosis. Familial defective apolipoprotein B_{100} (FDB) is a disorder associated with a strikingly high incidence of arteriosclerosis and tends to cause disease and premature death. We recently described preliminary results on CGE separations of PCR products and screening of apoB_{100} arginine$_{3500}$→glutamine mutations of codon 3500 in the south-west German population [234]. In a pilot study, investigating the DNA of 43 selected patients, the efficiency of CGE for large cohort analyses in a fully automated way in comparison with the conventional slab gel electrophoresis technique is demonstrated. In Fig. 5a,b), the CGE patterns for the different genotypes are shown: the normal allele exhibits one 227 bp peak with a mean migration time of 10.91 min (Fig. 5a), while the mutation pattern shows two peaks, one with a mean migration time of 10.0 min (113 bp) and a second one with a coincident migration time, as observed for the non-FDB patient, indicating a heterozygous mutation (Fig. 5b), thus allowing to differentiate unequivocally between FDB and non-FDB types. To reduce the total analysis time even more, the PCR products (diluted 1:5 (v/v) with water) have been directly injected without sample cleanup. Fig. 5c illustrates the silver-stained, time-consuming and labor-intensive routine polyacrylamide gel electrophoretic separation of apoB_{100}

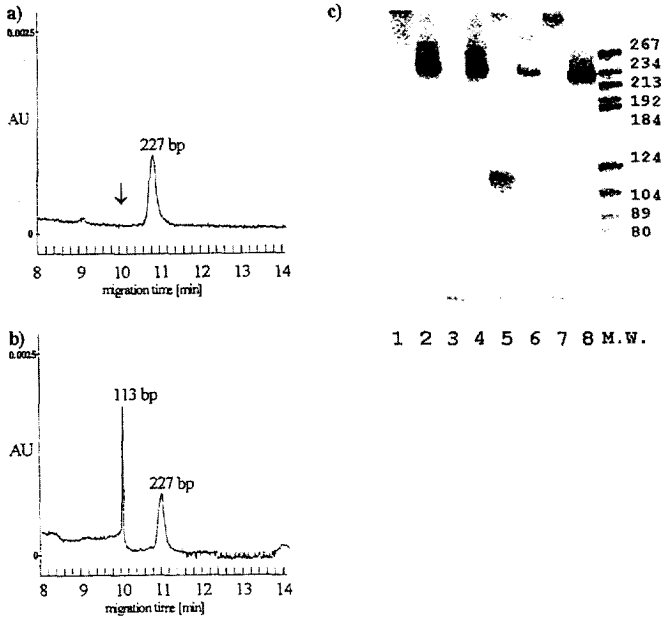

Fig. 5. Capillary electropherograms of PCR products from patients with different apolipoprotein B_{100} genotypes. (a) Non familial defective apolipoprotein B_{100}. (b) familial defective apolipoprotein B_{100} (FDB). Electrophoretic conditions: Capillary, polyacrylamide coated (47 cm×50 μm); loading, electrokinetic: 60 s at 4 kV; running conditions, 12 kV; buffer, 100 m*M* Tris–borate–EDTA, pH 8.3, plus 10 μ*M* ethidium bromide and 0.7% hydroxyethyl cellulose; detection, UV at λ 260 nm; equipment, Dionex capillary electrophoresis system CES I. (c) Horizontal polyacrylamide gel of PCR fragments from normal individuals and a FDB heterozygote on a 5% gel (0.5×260×125 mm), stained with silver. Running conditions: 100 V for 1 h followed by 200 V for 1 h; buffer, 20 m*M* MOPS, pH 8.0. Lane 1 and 2=non-FDB patient; lane 3 and 4=non-FDB patient; lane 5 and 6=FDB patient (lane 5 shows a 113 bp fragment, indicating the heterozygous status of this subject; lane 6 with the 227 bp fragment corresponds to the normal allele); lane 7 and 8=non-FDB patient; lane 9=molecular mass marker (DNA fragments length in base pairs).

PCR fragments from various individuals showing an $apoB_{100}$ Arg_{3500}→Gln mutation in lane 5 (two lanes per individual). Four of 43 hypercholesteremic patients were found to have the predominant apoB 3500 codon mutation. These results demonstrate that CGE has several advantages over slab gel electrophoresis: (1) high voltage in the kilovolt range is applicable, reducing separation times to minutes, (2) no gel casting and staining is needed, (3) samples can be loaded automatically from a carousel, (4) a minute amount of sample volume (in the nanoliter range) is required, as detection through the capillary is performed and (5) fully automated analyses of DNA-mixtures over a vast range of molecular sizes including data reporting and storage can be performed [234]. Other capillary electrophoretic analyzed markers for estimating the risk of coronary heart disease (CHD) are, e.g., the insertion/deletion polymorphism of the angiotensin I-converting enzyme gene, which is thought to be associated with CHD [235], or the variable number of tandem repeats (VNTR) in the apoB100 gene, analyzable within 17 min in a range between 600 and 1000 bp [236] and the apolipoprotein E gene by RFLP, where CGE resolves fragments differing in only 6 bp with high reproducibility [237].

CGE proved to be successful for the genetic typing and diagnosis of inherited diseases like Duchenne and Becker muscular dystrophies [225,226], cystic fibrosis [223,224], medium-chain acyl-coenzyme A dehydrogenase deficiency [227], congenital adrenal hyperplasia [229], androgen insensitive syndrome [228] or von Willebrand factor gene [230]. Detection of fetal DNA in maternal circulation and genetic diagnosis from a single cell is also reported [238].

In cancer research and diagnosis, point mutations in the p53 gene (a molecular marker for (pre-) malignant cells) were investigated by a combination of SSCP and CGE [232,239]. RFLP analysis of ERBB 2 oncogene [231], quantitative analysis of DNA abbreviations of follicular lymphomas [207] and DT-diaphorase gene expression [204] or identification of microsatellite instability demonstrated by the hereditary nonpolyposis colorectal cancer [233] could all be successfully investigated by CGE separations of corresponding PCR products.

CGE DNA-sequencing, first reported in 1991 [209] and followed by numerous communications [210–212,216–218,240–243], is the most advanced method of DNA analysis, also with impacts to clinical research and diagnosis.

Besides DNA sequencing, nearly all kinds of DNA analyses can easily be performed on the first fully automatic CGE-based DNA autoanalyzer, the ABI PRISM™ 310 (Perkin Elmer, Weiterstadt, Germany) [241]. It is a sequentially working, multicolor-fluorescent CE instrument, designed as a walk-away system. Four different fluorescent dyes in the kit and LIF detection allows this system to do DNA sequencing directly. Also, an automated PCR product sample preparation unit for CE analysis equipped with a robotic workstation was recently described [244].

Future trends are ultra-high-speed DNA sequencers, equipped with microfabricated capillary electrophoresis chips allowing a sequencing extension to ~150 bp in only 540 s and using a four color system [219], with a simultaneous DNA fragment monitoring unit and a multiplex array of capillaries [217,245], and ultra-high speed DNA fragment separations on chips (effective length of the channels 3.5 cm) analyzing DNA fragments from ~70 to 1000 bp in only 120 s [218].

3.4.3. Antisense therapeutics

The idea behind the antisense or anti-gene agents is that they inhibit a specific gene expression, either in the nucleus by binding to double-stranded DNA and pre-mRNA or in the cytoplasm by binding to mRNA. In this way the production of a specific, harmful protein is prevented. A growing number of chemical modified antisense oligonucleotides are in preclinical development or clinical trials for use as human therapeutics. Oligonucleotides as a new class of therapeutic compounds lack established bioanalytical methods for studying metabolism and pharmacokinetics, and therefore pose unique analytical challenge. The first goal of the CGE analysis is to determine the purity of the antisense-DNA after their chemical synthesis. Conversely, a quantitative method capable to differentiate between a full-length intact antisense oligonucleotide, as well as putative metabolic products truncated by the loss of one or two nucleotides is essentially necessary. CGE is far superior to all other analytical procedures, because it

is fully automatic with high speed and is the only method capable of separating oligonucleotides that differ in length by one nucleotide [246].

In the context of inhibiting a specific gene expression by antisense oligonucleotides with the consequences of preventing harmful protein production, the stability of antisense oligonucleotides in their cellular environment has been successfully monitored by CGE in combination with MALDI-MS [247]. Moreover, a method for extraction of therapeutic DNA analogs from human plasma to permit quantitation by CGE was recently successfully introduced [208]. Human plasma samples from Phase I clinical trails were analyzed, demonstrating that this method may be applicable to the detection and quantitation of the numerous therapeutic phosphorothioate oligonucleotides being studied.

The advantages of CGE over other techniques for the analysis of certain DNA analogs lies in its superior resolving power (single nucleotide resolution) and sensitive detection without use of radioactively labeled compounds, which was lately summarized in the review of DeDionisio and Lloyd [246].

As this chapter demonstrates, the CGE method is successfully applied to RFLP, VNTR, SSCP and polymorphism analysis, antisense monitoring and DNA sequencing, major areas of DNA analysis, and in contrast to many conventional methods, in a fully automated way. Especially in the field of genetic diagnosis, automatic evaluation is, besides the high accuracy and speed, an important progress in quality assurance, as conventional, visual interpretations of stained slab gels suffer from misinterpretations with all its consequences. In our opinion, the combination of PCR and CGE will open a new era for routine genetically-based diagnosis in the majority of clinical laboratories. It seems that it will be only a matter of time until automatic capillary electrophoresis will be the dominant technique in this analytical field.

3.5. Miscellaneous analyses

A common clinical problem is the evaluation of the anoxic period during the birth period and the oxygen supply to organs of newborns and preterm babies during the first weeks of life. Two principal markers of asphyxia and/or ischemia in human body fluids are hypoxanthine and uric acid [248]. An optimized separation of purine bases and nucleosides in human cord plasma of 27 neonates by CZE, studying the concentration of adenine, guanosine and hypoxanthine, appeared recently in the literature [249]. The concentrations of all compounds were significantly increased in serum of babies with asphyxia and acidic pH in comparison to those of controls, what may be a consequence of increased purine degradation during the birth and asphyxia and/or acidosis [249]. With this method, the quantitative analysis of adenine, guanosine and hypoxanthine, also in minimal sample volumes, can be performed. In addition, the determination of xanthine in 53 serum, 17 urine and 12 CSF samples by CZE after deproteinization in less than 10 min has been described [37] (Table 5). The serum concentration of xanthine, also a precursor of uric acid, is indicatively increased, e.g., with Lesch–Nyhan syndrome, hereditary xanthinuria patients, or patients after administration of allopurinol, as well as prenatal asphyxia and hydrocephalus [37]. The lack of adenylosuccinate lyase activity, an enzyme catalyzing two reactions in purine metabolism, causes secondary autism and severe psychomotoric retardation in early childhood [250]. These defects lead to an increase of two substrates, succinyladenosine and succinylaminoimidazole carboxamide riboside, in all body fluids of these patients. Comparing the electropherograms of two serum samples of patients, suffering from the enzyme deficiency, with 50 healthy control subjects, the two succinyladenosine and succinylaminoimidazole carboxamide riboside peaks were only observed in those of the patients [250]. Determination of the adenylosuccinate lyase activity by following the conversion of adenylsuccinate into AMP in vitro, of purified lysed erythrocytes or lymphocytes from the patients, is an alternative method to diagnose this disease [251]. At different periods of time, volumes of the incubation mixture were analyzed by CZE and the ratio of AMP to adenylosuccinate allowed a clear differentiation between a patient and normal controls [251].

Uremic serum was analyzed for the first time in 1990 by CZE using a 200 μm I.D. non-coated Teflon capillary and compared to a HPLC gradient elution method [252]. In the separation pattern, achieved in 8 min, hippuric acid, *p*-hydroxyhippuric acid and

Table 5
Survey of selected clinical capillary electrophoretic applications of different components

Compounds	Matrix	CE mode	Detection mode	References
Miscellaneous				
Hippuric acid, *p*-hydroxyhippuric acid, uric acid	uremic serum	CZE	UV 254 nm	[252]
Creatinine	serum	CZE	UV 215 nm	[264]
Xanthine	serum, urine, CSF	CZE	UV 280 nm	[37]
Adenine, guanosine, hypoxanthine, uric acid	cord plasma	CZE	UV 260 nm	[249]
Bilirubin species	serum	MECC	LIF	[258]
Succinyladenosine, succinylaminoimidazole, carboxamide riboside	urine	CZE	UV 254 nm	[250]
Adenosine monophosphate (bioassay: adenylosuccinate to adenosine monophosphate)	peripheral blood cells	CZE	UV 254 nm	[251]
Porphyrins	urine	MECC	fluorescence	[257]
Interleukine-1 (IL-1), IL-6, IL-8, (-interferon, tumor necrosis factor α, monocyte chemotactic protein-1, regulated on activation normal T-cell expressed and secreted (RANTES)	biopsies	CZE	UV 200 nm	[259]
Hypoxanthine, pseudouridine, hippuric acid, uric acid	serum, hemodialysate	CZE	UV-scan	[253]
Uric acid, hyaluronan, chymopapain, papain	synovial fluid	CZE	UV 200 nm 280 nm	[265]
Phenylpyruvate, 2-hydroxyphenylacetate, phenyllactate	urine	CZE	UV 260 nm	[85]
Nicotinic acid, nicotinamide, nicotinamide *N*-oxide, *N'*-methylnicotinamide, 6-hydroxynicotinic acid, nicotinuric acid	plasma	CZE	UV 254 nm	[266]
Oxalate, citrate	urine	CZE	indirect UV	[254]

uric acid peaks were identified. In a recently published communication, quantitative CE determinations of pseudouridine, hypoxanthine, hippuric acid and uric acid in 20 serum samples from healthy individuals and renal-failure patients and hemodialysate samples are described [253]. These studies demonstrate that the physiology and pathology of medical disorders, such as renal failure, can be studied by CZE in a single screening run.

CZE analysis of urinary oxalate and citrate allows also to evaluate calcium oxalate nephrolithiasis [254]. Advantageously, CZE allows the determination of, both oxalate and citrate in a single run with high precision, short analysis time (less than 5 min), and free of interferences from urinary components affecting the results obtained with enzymatic methods [254].

Porphyrias are uncommon hereditary or toxic disorders of the porphyrin-heme pathway. Heme, which is a tetrapyrrol protoporphyrin-chelated iron, is the end product of this pathway [255]. Fifteen different types of porphyrias have been described, most of them are inherited as Mendelian autosomal dominant traits, but some are recessive and others acquired. The acute porphyrias are of highest importance, because attacks of these may be life-threatening. Patients suffering from porphyria, accumulate permanently low or in an acute attack a massive excess of specific neurotoxic porphyrin precursors or porphyrins [256]. Typical urinary separation profiles, routinely analyzed by HPLC [116], can be also obtained by CZE or MECC in combination with fluorescence detection [257]. Compared with HPLC, the CE methods are also suitable for the determination of porphyrins in clinical urine specimens.

Bilirubin, a metabolic breakdown product of blood heme, is a highly significant marker playing an important role in the understanding, diagnosis, and treatment of a variety of diseases associated with liver dysfunction, like insufficient hepatic uptake, neonatal jaundice, etc., [258]. In serum, bilirubin exist under normal conditions almost completely in its unconjugated form (α-fraction), excess amounts of the conjugated species (bilirubin monoester (β-fraction), diester (γ-fraction)) are indicative for liver diseases, e.g., biliary obstruction. Covalently bound

bilirubin to serum albumin is the so called δ-fraction. Using the MECC method with LIF detection, all bilirubin species in serum can be separated within 15 min [258]. In contrast to the currently used methods, based on unselective coupling of bilirubin with a diazonium salt, the CE determination yields the most sensitive, selective and reliable results.

At present, citokine determinations, associated with immunological regulations and inflammatory processes are gaining increasing interest. To study the pathobiology of renal disease in AIDS patients, inflammatory citokines in frozen microsections of renal biopsy material were determined by CZE [259]. To verify the validity of quantitative CZE data of interleukine-1 (IL-1), IL-6, IL-8, γ-interferon, tumor necrosis factor α, monocyte chemotactic protein-1 and RANTES (regulated on activation normal T-cell expressed and secreted), the data were compared with those of a chemiluminescence-enhanced immunoassay. This preliminary work opens an attractive path for sensitive and fast determination of citokines in human biopsies.

4. Conclusions

As it becomes obvious from the different chapters of clinical applications, CE with its various modes, has a whole series of advantageous features compared to the well-established procedures, installed for diagnostic routine analyses in the clinical laboratories, such as extreme analytical flexibility separating a wide spectrum of biological molecules, requirement of minute quantities of low-cost separation buffer for multiple analyses (mostly free of organic solvents), use of almost any kind of detection mode, high speed, efficiency and reproducibility, and the potential to install fully automated procedures. CE allows furthermore a rapid change from one buffer system to another (i.e., from one analytical procedure to another) and direct injections of minute sample volumes (nanoliter) of complex body fluids like serum, plasma, urine, saliva, cerebrospinal and drain fluid, hemodialysate, sweat, and lysates of single cells. Compared to immunoassay procedures, CE techniques require neither tedious and costly developments of kits nor can cross-reactivities of sample contaminations lead to doubtful results and multiple solutes can be determined in one run. Running in the sequential mode, CE can be an advantageous alternative to procedures like HPLC, GC, TLC, etc. and in the parallel mode with on-line sample pretreatment it could well compete with automated special analyses like drug monitoring, DNA analysis, immunoassays, etc.

For a broader acceptance of clinical CE in the near future two aspects should be provided soon: (1) The development of chemical reagent kits, instruments and software suitable for an easy to use introduction of CE in daily routine, and (2) to compare and evaluate the various CE applications with conventional laboratory methods under daily routine or emergency laboratory conditions, directing the attention to: accuracy, reproducibility ($n \geq 50$), sample throughput, speed and susceptibility to disturbances (methods, capillaries, CE instruments). Other important criteria are easy to perform routine work (automation), costs per test, cheap and long-lasting of reagents, wide accessible clinically relevant concentration range, etc.

As demonstrated by this review, the advantages of CE attracted many research groups analyzing clinically relevant substances in biological fluids. However, the number of reports evaluating these applications under clinical routine conditions in comparison to the traditional procedures is very low. Few obvious examples in that direction would certainly cause clinical chemists to replace time-consuming and costly conventional methods by simple and rapid CE procedures. Until now, the only routinely performed CE analyses in the clinical laboratories are the quantification of serum protein fractions and the immunofixation/subtraction procedure. Different opinions exist about the applicability of CE in clinical chemistry, but we think that the most promising ones for routine use are, e.g., the quantification of drugs in biological samples or the analyses of all kinds of molecular biological products (e.g., PCR products, antisense oligonucleotides, etc.), more and more needed in clinical routine and research laboratories.

However, for the general adoption of capillary electrophoretic techniques in the clinical laboratories, further developments are needed, some of which to be mentioned here: increase of sensitivity and sample throughput, reliable automated long-time operations

or establishment of fully automated chains, including sample identification (via bar code), dilution, injection, recognition of unexpected interferences, online data evaluation and registration. Nevertheless, in a time with exploding costs of medical care the price per analytical value, calculated on the basis of costs of the equipment, and its service, reagent kits, personnel, etc., will make the decision to introduce or replace an analytical method in the clinical laboratory, and CE has a good chance of becoming an economical alternative.

About 20 years ago the microliter-age started in clinical chemistry, and it seems that CE now opens the door to the nanoliter-era. In our opinion, it is to imagine that fully automatic analyzers, based on CE separation techniques, will become irreplaceable instruments in clinical laboratories within the next decade.

5. List of abbreviations

ACE	Angiotensin I-converting enzyme
Apo A-I	Apolipoprotein A-I
Apo B_{100}	Apolipoprotein B_{100}
CE-ED	Capillary electrophoresis–electrochemical detection
CGE	Capillary gel electrophoresis
CHD	Coronary heart disease
CIEF	Capillary isoelectric focusing
CITP	Capillary isotachophoresis
CSF	Cerebrospinal fluid
CZE	Capillary zone electrophoresis
EIA	Enzyme-immunoassay
ddF	Dideoxy fingerprinting
EMIT	Enzyme multiplied immunoassay technique
FDB	Familial defective apolipoprotein B_{100}
FPIA	Fluorescence polarization immunoassay
GC	Gas chromatography
HDL	High density lipoprotein
HEC	Hydroxyethyl cellulose
HPLC	High-performance liquid chromatography
HMC	Hydroxymethyl cellulose
HPMC	Hydroxypropylmethyl cellulose
I.D.	Inner diameter
INA	Immunonephelometric assay
ITP	Isotachophoresis
LDL	Low density lipoprotein
LIF	Laser-induced fluorescence
MALDI-MS	Matrix-assisted laser desorption and ionization mass spectrometry
MECC	Micellar electrokinetic capillary chromatography
mRNA	Messenger RNA
NIDDM	Non-insulin-dependent diabetes mellitus
PAA	Polyacrylamide
PEG	Polyethylene glycol
RFLP	Restriction fragment-length polymorphism
RIA	Radio-immunoassy
RT-PCR	Reverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulfate
SPE	Serum protein electrophoresis
SSCP	Single-stranded conformation polymorphism
TDM	Therapeutic drug monitoring
TLC	Thin-layer chromatography
VLDL	Very low density lipoprotein
VNTR	Variable number of tandem repeats

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