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Review

Capillary electrophoresis in the evaluation of aminothiols in body fluids☆

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ABSTRACT

Thiols play a fundamental role in cell biology, biochemistry and pharmacology. Altered thiol levels in body fluids are linked to specific pathological conditions. Glutathione is the most abundant intracellular low-molecular-mass thiol, playing an essential role in protecting cells from toxic species; other relevant thiol-containing compounds are homocysteine (Hcy), cysteine (Cys), cysteinylglycine (CysGly). Plasma aminothiols can be bound to proteins but they also occur free in the disulfide (symmetrical and mixed) and in the reduced forms. The simultaneous determination of these aminothiols, their precursor and metabolites is a useful tool in studying oxidative stress, metabolic and redox regulation. Many capillary electrophoresis methods have been proposed for this purpose, the aim of the present review is to support researchers in the choice of suitable methods for the determination of thiols in body fluids evaluating the different approaches and technologies proposed from the literature.

Contents

1. Introduction

Biological thiols are compounds of main interest due to their importance in biological processes such as antioxidant defence network, methionine cycle and protein synthesis. Moreover, altered thiol levels in body fluids are linked with specific pathological conditions. Plasma/serum homocysteine concentration has been related to neural tube defects, pregnancy complications mental disorders, cancer, hyperinsulinemia [1,2] and mainly to cardiovascular diseases [3,4]. Abnormalities of glutathione metabolism have been associated with Alzheimer's and Huntington's diseases [5]; physiological decrease in glutathione plasma levels have been associated with the age in both male and female subjects [6]. Nevertheless glutathione is also a major component of the process for defence against the toxicity of xenobiotic compounds and oxidants [7].

Characteristically over 90% of plasma thiols are in the form of disulfides with a 40–50% protein bound [8]. Plasma thiols can be classified in high-molecular-mass protein mixed disulfides (i.e. protein-bound homocysteine) and low-molecular-mass (LMM) free thiols. LMM free thiols are sub-classified in the reduced and the disulfide fractions. The reduced free fraction includes homocysteine (Hcy), cysteine (Cys), cysteinylglycine (CysGly) and glutathione (GSH). The disulfide fraction is further sub-classified in symmetrical and mixed disulfides; symmetrical disulfides include glutathione disulfide (GSSG) cystine (CSSC) and homocystine (HCSSCH); between mixed disulfides, GSH–Hcy and Cys–Hcy are classified. The physical properties of the S–H bond determine the reactivity of such compounds being important determinants of protein structure and function forming disulfide linkages –S–S– between cysteinyl residues [9].

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Glutathione is the major non-protein thiol; it is responsible for the maintenance of protein thiol status in cells [10,11]. Within the cell (i.e. hepatocytes, erythrocytes, neurons, macrophages) glutathione is present mainly in a reduced form and is the main non-protein sulfhydryl. The two principal disulfide forms, glutathione disulfide (GSSG) and protein–GSH mixed disulfide (PrSSG), exhibit lower levels [5,6,12–14]. GSH acts as a free radical scavenger and also enhances the functional ability of other antioxidants, e.g., vitamin E, and ascorbic acid [15,16].

Homocysteine represents a crucial intermediate in the methionine cycle, acting as a precursor for cysteine on the trans-sulfuration pathway as well as for methionine synthesis via remethylation. Cysteine represents the most abundant thiol in plasma while cysteinylglycine, a product of glutathione enzymatic degradation, represents the runner-up. Additionally, there is increasing evidence suggesting that thiol groups located on various molecules act as redox sensitive switches thereby providing a common trigger for a variety of reactive oxygen species mediated signalling events [17].

Cysteamine is the simplest stable aminothiol and represents the degradation product of the amino acid cysteine. Cysteamine bitartrate has been used as drug to cleave the disulfide bond with cysteine to produce molecules that can escape the metabolic defect in cystinosis and cystinuria [18] and it has been also proposed as drug in the treatment of Huntington's disease and Batten Disease [19–21]. D-penicillamine (2-amino-3-mercapto-3-methylbutanoic acid, D-PEN) is a sulfur-containing amino acid obtained by hydrolytic degradation of penicillin, with no antibiotic activity; it represents another thiol drug used in the treatment of cystinuria, Wilson's disease and rheumatoid arthritis, consequently the corresponding plasma dosage is important in these disease states [22].

Captopril, 1-{3-mercapto-2-(S)-methyl-1-oxopropyl}-(S)-proline represents another thiol drug utilized as an inhibitor of the angiotensin-converting enzyme and is widely used for the treatment of hypertension and congestive heart failure [23]. The literature shows that high-performance liquid chromatography (HPLC) is a major technique used for the determination of captopril in pharmaceutical formulations [24–26], in plasma and urine for pharmacokinetic studies [27,28].

Plasma aminothiol determination has been progressively more utilized not only in clinical and translational research involving oxidative stress, but it is also used in disease states monitoring, in metabolic disorders evaluation and for therapeutic thiol drug monitoring [29]. The GSSG/GSH ratio has been shown to be an effective measure of oxidative stress [30] occurring in cardiovascular diseases and hyperhomocysteinemia, which in turn is an independent risk factor for cardiovascular diseases, which involve free radical production.

Even if numerous HPLC methods for the determination of thiols in body fluids have been proposed, capillary electrophoresis (CE) methods are increasingly affirming in this field [4,31–34].

In CE, several separation modes are available namely capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), isotachophoresis (ITP), capillary gel electrophoresis (CGE) and capillary electrochromatography (CEC). CE is a far versatile and chip separation technique, being suitable for large and small molecules separation and quantitation.

Besides the above-mentioned characteristics, CE exhibits drawbacks such as limited detection sensitivity, which is due to the restricted range of analyte loading, arising from the relatively small volumes that can be injected in the capillary (10–100 nL). Several solutions were proposed to resolve this problem, e.g., use of capillaries with extended path length (bubble- or zeta-shaped cell), more selective detectors (multiwavelength and DAD), more sensitive detectors (conductivity, laser-induced fluorescence (LIF), ECD, MS), two-dimensional CE (ITP coupled to CZE, ITP–CZE), derivatization reactions, etc. [35,36].

The first paper on the use of CE in the evaluation of thiol-containing compound came in 1991 from Jellum et al. [37], which employed monobromobimane (mBrB) as fluorescence derivatizing agent for thiols. In recent years, several CE procedures have been applied in the evaluation of Hcy and thiols in general, in body fluids. Most of them used LIF as detection but also UV and amperometric detection have been proposed with success. Despite the wide range of CE techniques mainly CZE and MECK have been applied to the evaluation of thiols in human fluids; only one chiral separation has been reported, with no application to biological samples [38]. Moreover, most of the research papers on the evaluation of thiols in body fluids by CE, mainly involve methods development, without an extensive clinical application/evaluation. The goal of the present article is to orientate researchers in the choice of a suitable thiol determination in body fluids, for clinical purpose.

2. CE detection of thiols

2.1. Photometric detection

The first report on the application of CE to thiols evaluation was by Stamler and Loscalzo in 1992 [39] analyzing underivatized thiols. They separated GSH, Cys, and Hcy from their S-nitrosated derivatives using capillary zone electrophoresis. Cys, Hcy, and GSH were separated from each other and from their corresponding symmetrical disulfides (HCSSCH, CSSC and GSSG) in 0.01 M phosphate buffer, pH 2.5, the absorbance detection was followed at 200 nm. The corresponding S-nitrosothiols were selectively detected at 320 nm. For the first time they proposed this technique for separation and identification of thiols, disulfides, and S-nitrosothiol derivatives. The first quantitation of underivatized thiols and their symmetrical disulfides in biological matrix was in 1996 by Russell and Rabenstain [29]. They used a detection wavelength of 200 nm with detection limits in the range 20–90 μM using a 50 μm-diameter capillary. As the determination exhibited a lack of sensitivity related to the molar absorbance coefficient of these compounds, to achieve lower detection limits, thiols were derivatized with the thiol-specific probe, 5,5′-dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent), improving the detection limit (LOD) to 5–50 μM.

GSH and GSSG were determined in erythrocyte from fresh blood samples [29]: after plasma removal by centrifugation, the packed erythrocytes were washed in isotonic saline solution and lysed by freeze–thawing. Protein were precipitated by trichloroacetic acid (TCA) 0.1 M containing EDTA (1.0 M), after centrifugation and filtering (0.2 μm size), samples were diluted 1:5 with 0.1 M NaH_2PO_4 (pH 2.3) running buffer. Separation conditions were: capillary 50 cm × 50 μm i.d. voltage 15 kV, buffer as already reported, injection hydrodynamic 5 psi × 3 s. The authors not only measured biological thiols but they also separated and measured D-PEN and captopril thiol drugs, although with no application to biological matrices in this case.

The thiol determination in plasma has been accomplished more recently [40,41]. Disulfides were reduced with triphenylphosphine and proteins removed by deproteinization with 5-sulfosalicylic (SSA) or TCA. Aminothiol fractions, total Hcy (tHcy), total Cys (tCys) and total GSH (tGSH) were then derivatized with mBrB or 4-fluoro-7-sulfamoylbenzofurazan (ABD-F) before the separation of conjugates by CE with photometric detection at 234 or 250 nm and 220 nm for mB-thiols and ABD-thiols, respectively (Fig. 1). The authors reported to have used an 80 mM sodium phosphate at pH 9.0 running buffer but there should be an error, since sodium phosphate is not a buffer at that pH value. The reported LOD was around

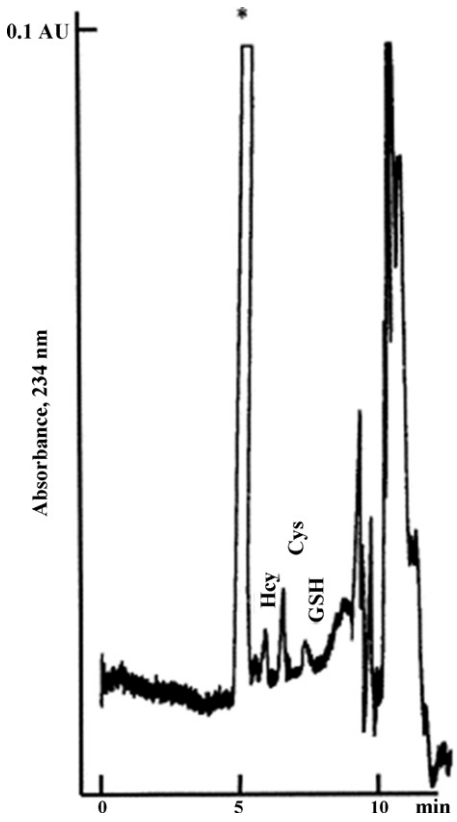

Fig. 1. CE identification of monobimane conjugates of aminothiols in human plasma with photometry detection (234 nm). The aminothiol content is 42.3 nmol/mL for tHcy (pathology) (6.19 min), 171.4 nmol/mL for tCys (6.78 min), and 10.1 nmol/mL for tGSH (7.81 min). The peak marked by the asterisk corresponds to monobimane hydrolysis by-product. Reprinted from [41] with permission.

5 μM. The concentrations of tHcy, tCys and tGSH in plasma obtained from 17 healthy overnight fasting males were: 12.21 (±4.09 SD), 211.32 (±36.44 SD) and 7.68 μM (±2.43 SD), respectively and did not differ significantly from healthy overnight fasting females. The data were in line with those obtained by chromatographic assay [42], utilizing the same derivatization procedure and others obtained by chromatographic assay with different derivatization procedure [42–44].

Kim et al. [38] performed the chiral separation of L-homocysteine and D- homocysteine by CE with UV detection at 220 nm by complexing the ABD-F derivatized Hcy forms with a γ-cyclodextrine added in 50 mM sodium phosphate buffer pH 2.25. Plasma Hcy level was increased after L-methionine oral administration [45], therefore L-Hcy could be involved in adverse reactions in atherothrombosis, and it would be desirable to determine the two stereoisomers. The authors did not report the LOD, nor did they apply their method to L-Hcy evaluation in plasma.

Several methods have been proposed for the GSH and GSSG free fraction determination in human body fluids, without pre-assay derivatization [46–49]. The measurement of GSH in the physiological concentration range is not simple since glutathione lacks appreciable chromophor and low UV region detection is necessary. Most of the buffer systems used are, however, optically transparent at, or below 200 nm and μM sensitivity is achievable [46,50].

Havel et al. [47] developed a MEKC method for determining both GSH and GSSG, utilizing direct UV detection of underivatized GSH and GSSG, using phosphate–borate buffer with SDS at pH 8.00 and minimal sample preparation (Fig. 2). 100 μL of plasma samples were mixed with 100 μL of saline and 300 μL of 20 mM H_3PO_4 in acetonitrile; after centrifugation supernatant aliquots were diluted with 800 μL of 0.1 M HCl and filtered through a 0.2-μm acrodisc filter prior to analysis. The LOD was 0.8 μM for GSH and 0.4 μM for GSSG, using high sensitivity flow cell with a 75 μm i.d. capillary. The authors applied their method to plasma samples, detecting a limit of quantitation (LOQ) of 1.6 and 0.8 μM, for GSH and GSSG, respectively. HPLC methods with UV detection utilizing 2-chloro-1-methylquinolinium tetrafluoroborate (CQMT) for pre-column glutathione derivatization gave a LOD of 0.1 and 0.3 for GSH and GSSG, respectively [29]. The same derivatization method has been recently applied to the assay of cysteamine in human plasma by CE [51]. The authors reduced cysteamine disulfides to thiol with tri-n-butylphosphine (TBP), followed by thiol derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate and deproteinization with perchloric acid. The separation of cysteamine 2-S-quinolinium derivate from those of plasma endo- and exogenous thiol derivatives was achieved by CZE, based on acetonitrile stacking in a 0.2 M Tris/HCl pH 2.1. Quantitation was obtained by UV detection at 355 nm. The LOD was 0.8 μM and LOQ 2.5 μM. The procedure was applied to the analysis of plasma samples obtained by apparently healthy volunteers, spiked with known amounts of cysteamine standard solution. The method resulted is suitable for

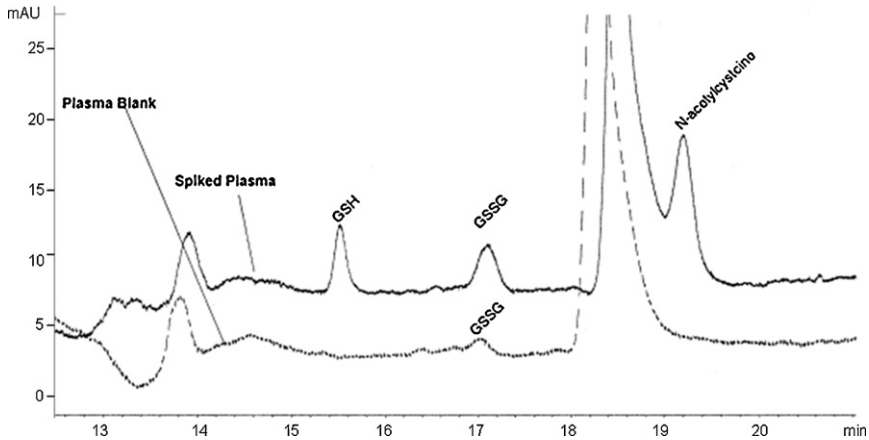

Fig. 2. Electropherograms of spiked (with GSH) and unspiked plasma samples showing that GSH and GSSG are detectable at concentrations of 1.6 μM GSH and 0.8 μM GSSG in plasma. The unspiked sample (plasma blank), is a plasma sample from a pool of plasma, blanked for GSH content by auto GSH oxidation during long period preservation (data kindly provided by the author, T. Wielgos). This account for the selectivity of the method in that no other peaks comigrate with GSH in plasma samples. Conditions: buffer, 25 mM NaH_2PO_4, 15 mM $Na_2B_4O_7$ and 50 mM SDS, pH 8.0; capillary, 80.5 cm (72 cm × 75 μm I.D. with high sensitivity flow cell, λ = 195 nm; injection: 500 mbar s. Reprinted from [47] with permission.

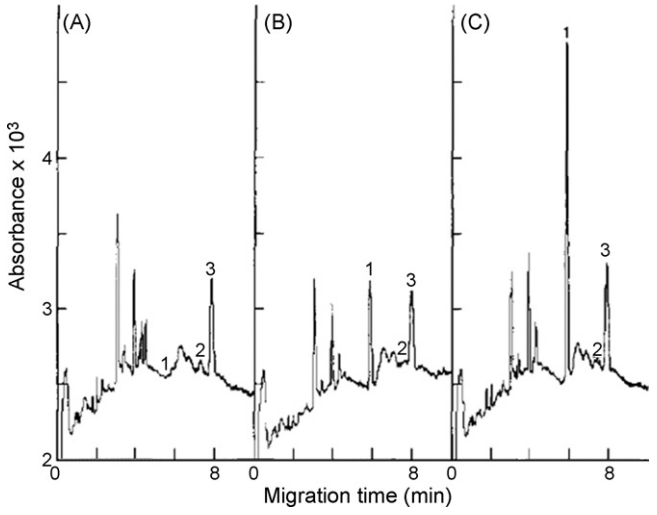

Fig. 3. Electropherogram of ABD-thiols in human plasma. (A) Sample from an healthy subject. (B) Mixture of the same plasma sample and 32.3 (M Hcy. (C) Mixture of the same plasma sample and 76.9 (M Hcy. Peaks: 1 = Hcy, 2 = GSH, 3 = Cys. Reprinted from [52] with permission.

monitoring the 12 h time-course of total cysteamine concentration in plasma of subjects under treatment.

It is evident that to obtain detection limits below μM concentration, researchers have looked to chemical derivatization. The gain in sensitivity is usually at the expense of additional sample treatment steps, employing both pre- or post-column chemical derivatization.

Derivatization with chromophores improves the analytical sensitivity, allowing also GSH stabilization during both pre-analytical and analytical phase.

Kang et al. [52] utilized ADB-F to assay thiols in human plasma, including glutathione (Fig. 3). Plasma sample (0.5 mL) was treated with 50 μL of 10% (v/v) TBP for 30 min at 4 °C for thiols reduction and decoupling them from proteins. TCA (0.5 mL at 10%) containing EDTA (1 mM) was used to precipitate proteins; after centrifugation, the supernatant was mixed with 0.2 mL of 0.1 M borate buffer (pH 8.0) containing EDTA (2 mM) and incubated for 5 min at 50 °C with 0.4 mL of ABD-F in a 0.1 M borate buffer (pH 8). After filtering (0.45 μm size), the sample was injected into CE apparatus. The separation of ABD-thiols was performed in a 50 mM sodium phosphate buffer pH 2.1, in a bare fused-silica capillary (27 cm × 50 μm i.d.) using 15 kV voltage. The authors evaluated tHcy, tGSH and tCys in less than 8 min. Diode-array detector (DAD) was employed for ABD-thiol analysis to confirm the ABD-thiol peaks. The LODs for Hcy, GSH, and Cys were 0.5, 1 and 2 μM at 220 nm, respectively.

Glatz and Maslanová [53] performed on-column reaction of GSH with 2,2′-dipyridyldisulfide (DPDS). They injected the two compounds as discrete plugs separated with a short plug of background electrolyte allowing the reaction occurring on run. During the reaction, GSH was transformed into a mixed disulfide with the formation of 2-thiopyridone, the latter was concomitantly separated and evaluated, reading at 343 nm. They measured free GSH in whole blood samples from healthy subjects. Aliquots of 0.45 mL of blood were mixed with 0.05 mL of 50% SSA solution containing 1 mM EDTA. After centrifugation, the supernatants were filtered through a 0.45-μm filter and analyzed. The concentration of free GSH was 645 μM (± 25 SD), lower compared to that obtained by other authors both by CE [54] and HPLC [55]; these last cited authors, have evidenced that the use of N-ethylmaleimide (NEM), as a quenching agent for free thiols, avoids GSH oxidation which is a common artefact, in the determination of GSH in human blood [56]. The 343 nm detection exhibited a better specificity in comparison with 200 nm UV detection. The LOD was close to 5 μM and the sensitivity comparable to that obtained using direct UV detection at very low wavelengths, 185–190 nm [46].

Žunić and Spasić [54] applied a factorial design for screening important variables (pH, concentration, temperature) in CE separation/quantitation of various thiols, allowing their measurements without derivatization procedures. The best separations were obtained in 30 min employing a 10 mM phosphate buffer, pH 2.8, 18 °C, and 15 kV voltage. Fairly good precision with a linear relationship between peak area and concentrations (r = 0.995–0.999) were obtained. The method was used to analyze human capillary blood. The method was suitable for the measurement of GSH and GSSG, as well as for aromatic amino acids (i.e. Phe, Trp and Phe + Tyr) in blood hydrolysates. Under the used experimental procedure it was not possible to measure dipeptides with disulfide bond such as HCSSCH and CSSC, due to the poor detection limits of the method. The LOD of the other compounds was, however, below 10 μM level, allowing their evaluation within run precision. The authors evaluated these analytes in capillary blood samples, where GSH and GSSG exhibited a concentration of 1078.1 (±17.0 SD) and 110.6 μM (±7.3 SD), respectively.

2.2. Amperometric electrochemical detection

Amperometric detection represents an attractive approach to thiols evaluation by CE, due to electrochemical activity of such compounds, which consents lower detection limit compared to UV detection. Unfortunately this approach is limited by unavailability of commercial instrumentation and also by difficulties in the selection of suitable potential applied to the working electrodes, in relation to different compounds.

Chen et al. [57] have proposed bioanalytical application of specially designed miniaturized CE with end-column microdisc electrode amperometric detection system for the separation and detection of Hcy, Cys, GSH, and N-acetylcysteine (NAC). The separation occurred in 130 s using a 20 mM phosphate running buffer, pH 7.8. They evidenced a LOD of 0.75, 0.8, 2.9, and 3.3 μM, respectively for the above mentioned compounds, the authors did not report applications to biological matrices.

Pasas et al. [58] described a method with amperometric detection for the determination of both tHcy and protein-bound homocysteine (pbHcy) in plasma. They evaluated both end-column and off-column detection system, the last one presenting an increased sensitivity for the determination of Hcy, suitable for tHcy and pbHcy quantification over the physiological concentration ranges in all disease states.

In pbHcy determination, a 50–150 μL aliquot of fresh plasma was filtered by centrifugation; the ultrafiltrate, containing free amino acids and salts, was discarded and the pellet washed with distilled and deionized water. 50–150 μL of 100 mM tris(2-carboxyethyl)phosphine (TCEP) was added to the pellet and allowed to react for 10 min. The sample was again filtered by centrifugation; the ultrafiltrate was injected into CE apparatus and quantified by standard addition (5 μM increments ranging from 5 to 20 μM Hcy). In tHcy determination, a 45 μL aliquot of fresh plasma and a 5 μL aliquot of 1 M TCEP, pH 7.5 were mixed and incubated for 10 min; after filtration by centrifugation, the ultrafiltrate was injected into the CE apparatus. tHcy was quantified by standard addition as previously mentioned. The LOD for Hcy was 0.5 μM using off-column amperometric detection; the response was linear over the range 1–100 μM, evidencing suitable application for the tHcy quantification over the physiological concentration ranges. Plasma pbHcy and tHcy levels from a healthy individual were determined to be 2.79 (±0.31 SD) and 3.37 μM (±0.15 SD), respectively. These values are about 40–70% lower compared to other authors, which report a concentration range of 5–10 μM, assessed either by HPLC and CE [31,32,59]. The methodology was also applied to a

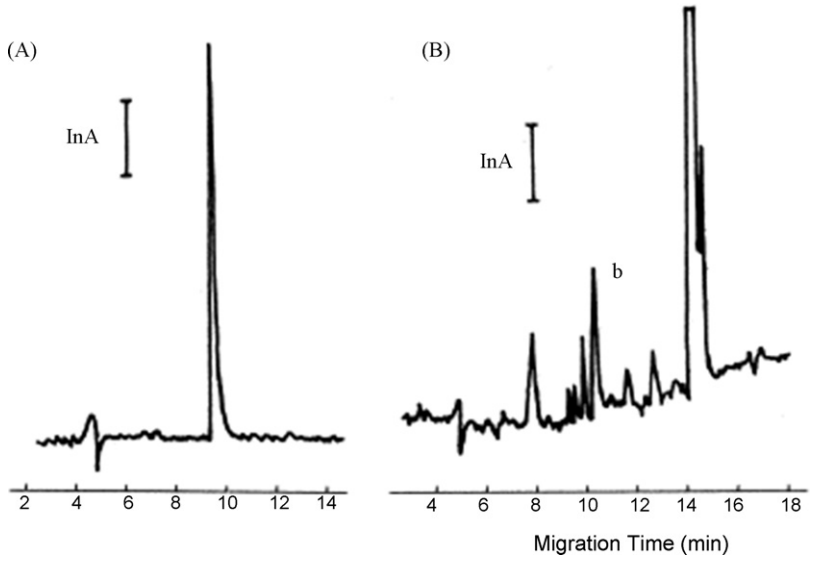

Fig. 4. Electropherograms of samples. (A) The methimazole (MMI) tablet; (B) the serum sample: the peak b is the electrophoresis peak of cysteine (Cys). Working potential: +1100 mV (vs Ag/AgCl). Fused-silica capillary, 25 μm i.d. × 70 cm; working electrode, 500 μm carbon disk electrode; separation medium, 20 mM; pH 7.4 PB solution; separation voltage, 27 kV; injection, 25 kV × 10 s. Reprinted from [60] with permission.

microchip CE format for the separation and detection of Hcy and GSH.

Amperometric detection with carbon disk electrode has been reported by Wang et al. [60] to determine the various thiol compounds, including cysteine, glutathione, 6-thiopurine (TP) and methimazole. The detection potential was chosen at +1100 mV; at this potential all analytes exhibited good response on the carbon disk electrode, utilizing 20 mM phosphate buffer, pH 7.4. The analytes were resolved in 20 min with a LOD of about 1.0 and 2.5 μM for cysteine and GSH, respectively. The method was applied to the separation and determination of free cysteine in serum sample, the serum was diluted 5-fold with running buffer and then filtered by centrifugation, prior to analysis. A 12.8 μM concentration was detected (Fig. 4).

Inoue and Kirchhoff [61] developed a chemically modified, miniaturized, electrode by entrapping the coenzyme pyrroloquinoline (PQQ) into a polypyrrole matrix, on a 245 μm graphite support. Thiol detection was achieved by their reactivity with PQQ, being thiol oxidized and PQQ reduced to $PQQH_2$. Thiols were measured monitoring the amperometric response from the reversible oxidation of $PQQH_2$ to PQQ on the electrode. An important characteristic of such electrode as detector for CE is that the detection potential is changeable as result of the pH-dependent electrochemical reaction for PQQ, being more sensitive to thiols at higher pH, because of the easy oxidation of RS^- compared to RSH. As the authors stated, higher pH values also shift the redox potential for the PQQ reaction to a more negative value, allowing a lower oxidation potential to be used and minimizing the effects of potential electroactive interferences.

The electrode has been utilized as end-column detector to measure free Hcy, Cys, GSH and NAC. The separation was performed in uncoated fused capillary (80 cm × 50 μm i.d.), utilizing 0.1 M Tris-borate buffer at pH 8.4, 30 kV voltage, +300 mV detection potential vs Ag/AgCl. The authors stated that variations in electrode response may occur from different electrodes, due to differences in PQQ entrapping into the matrix (electropolymerization step). The problem can be easily solved by calibration of each electrode which is then stable for a week. The method was applied to the separation/quantitation of mentioned thiols and to the determination of cysteine in dietary supplements and in human urine from healthy subjects. The LOD was 0.02, 0.01, 0.10, 0.13 μM for Cys, Hcy, NAC and GSH, respectively. In the application to biological matrix, urine (20 mL) was acidified (100 μL concentrated HCl) to prevent thiol oxidation; the sample was then diluted with 0.1 Tris-borate buffer containing 1 g/L EDTA and filtered (0.2 μM membrane). The free cysteine content was calculated to be 35–51 μM.

More recently Yao et al. [62] have proposed a method for the determination of free fraction of Hcy, Cys, GSH and also uric acid and ascorbic acid, in human whole blood and rat brain tissues, after protein precipitation with SSA solutions. They evaluated the effects of pH, running buffer, ionic strength, voltage and injection time, on migration time and resolution; utilizing phosphate buffer and electrokinetic injection. The detection electrode was a 500 μm platinum disk at working potential of +1.05 V. The optimal condition for compounds separation/quantitation were pH 7.8, 100 mM ionic strength, 6s injection, 18 kV voltage. The LODs were 1.29, 0.83, 2.58, respectively for Hcy, Cys, and GSH. Since, at pH of 7.8, the migration time of Hcy was close to that of the electroosmotic flow, the authors choose to not measure Hcy because the influence of some neutral electroactive species in the real biological samples.

2.3. Fluorescence detection

Fluorometric detection allows a marked improvement in LOD of about 100–1000 times compared to UV detection. Various fluorogenic reagents have been used, allowing different thiol derivatives. Fluorogenic reagents with benzofurazan structure [63,64], 5-bromomethylfluorescein [65] and fluorescein isothiocyanate (FITC) [66] have been utilized. ABD-F and ammonium 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F) were employed for specific detection of thiols by HPLC due to their good reactivity and selectivity to thiols [67,68]; in particular ABD-F exhibits milder conditions and faster reaction time with thiols compared to SBD-F. Monobromobimane has been the first derivatizing agent utilized in CE analysis of thiols [37]; it is highly reactive towards thiols but its hydrolysis products are also fluorogenic, thus necessitating complex separation procedures to obtain a satisfactory resolution of thiol adducts from interferents [69].

Since the reaction of thiols with fluorogenic reagents allows their evaluation at very low concentration, as in body fluids, most of the methods simultaneously evaluate glutathione and the other thiols.

Jellum et al. [37] have employed CE for the diagnosis and the study of metabolic disorders such as homocystinuria, cystinuria and GSH sythetase deficiency. They evaluated thiol compounds in red blood cells (RBC) and urine after mBrB derivatization, comparing the characteristics of CE assay to those of HPLC. In the case of urine samples, thiols were reduced with DTT (2.5 mM final concentration) and diluted with equal part or running buffer, adjusted to pH 7.5 and filtered through a 0.22 μm filter. Aliquots were incubated 10 min with thiolyte mBrB in acetonitrile (50 mM). In the case of RBC, cells were lysed by freeze–thawing and protein precipitated by perchloric acid; after centrifugation supernatant was neutralized and thiols reduced with DTT, filtered and labelled with mBrB as for urine samples. An uncoated fused-silica capillary 40 cm × 75 μm i.d. (100 μm i.d. in the case of GSH evaluation in RBC) was used for the CE analysis; the electrolyte was 50 mM sodium phosphate buffer at pH 7.5; the applied voltage was 24 kV. The excitation wavelength was 375 and 480 nm for emission. In this early stage of CE applications to thiols evaluation in biological matrices the authors stated that when dealing with cells and body fluids, the concentration of metabolites is often low for CE sensitivity, and the better way to improve the CE sensitivity is to employ LIF detector.

Kang et al. reported a CE method with UV absorbance to detect the Hcy derivatized with ABD-F by pre-column derivatization, in human plasma (see Section 2.1) [52]. The method presented adequate sensitivity for the detection of tHcy in the blood and urine of patients with diseases such as renal failure and malignant states (10–100 μM levels). In healthy subjects, however, the concentration of tHcy is close to the lower limit of the mentioned levels and the concentration of reduced Hcy is even below μM level. The same author [70] has, in fact, developed a fully automated CE assay with on-column derivatization with ABD-F to detect various thiols with LIF detection (Fig. 5). Homocysteine and ABD-F were injected for 30, 10–15, and 20 s, respectively at a 15 cm height. The running buffer was injected for 3 min to transfer the reaction sample into the heating zone. +1, −1, and +1 kV of voltage were applied to achieve the mixing of Hcy and ABD-F for 1 min, respectively. Then the sample was incubated for 10 min at 50 °C. The LOD of ABD-Hcy, -GSH, and -Cys by pre-column derivatization with UV detection was in the range of 0.5–2.0 μM and 2.5–5.0 nM with LIF detection. The result of LIF detection showed almost 1000 times increased sensitivity compared to UV detection, allowing the thiols to be determined in clinical samples. The LOD of Hcy on-column derivatization with LIF detection was slightly poorer than that of pre-column derivatization, but sufficient for the determination of homocysteine in human plasma. 5.0 nM vs 2.5 nM, respectively.

Caussé et al. [71] utilized the 6-iodoacetamidofluorescein (6-IAF) as derivatization agent for the determination of tHcy in plasma after thiols reduction with TCEP, followed by protein precipitation with SSA. The method allows to quantify tHcy, tCys, and tGSH simultaneously in a single analysis, with a 0.25 μM LOD for Hcy. The same authors have previously utilized FITC to quantify Hcy and all amino acids through its reaction with amine groups, giving fluorescein thiocarbamyl (FTC) thiol-amino acids. In this case they obtained a LOD of diluted standard FTC–Hcy and FTC–Cys of 0.8 nM, working with 50 mM boric acid, 10 mM SDS buffer, pH 9.8. Plasma samples were diluted 1000× in water prior to analysis. The authors stated that the FITC-buffer by-products produced, however, numerous interfering peaks of unreacted FITC, making difficult its utilization [66]. Besides, the IAF-based procedure allows a more selective detection of thiol-containing amino acids. In addition, in the IAF method, the migration times are shorter, the peak resolution is better and co-injection of a calibrator is not required. The authors also evaluated pre-analytical conditions for optimal quantitation of thiols in plasma by CE with LIF detection mode, utilizing 50 mM boric acid and 20 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer, pH 10 [72]. They investigated

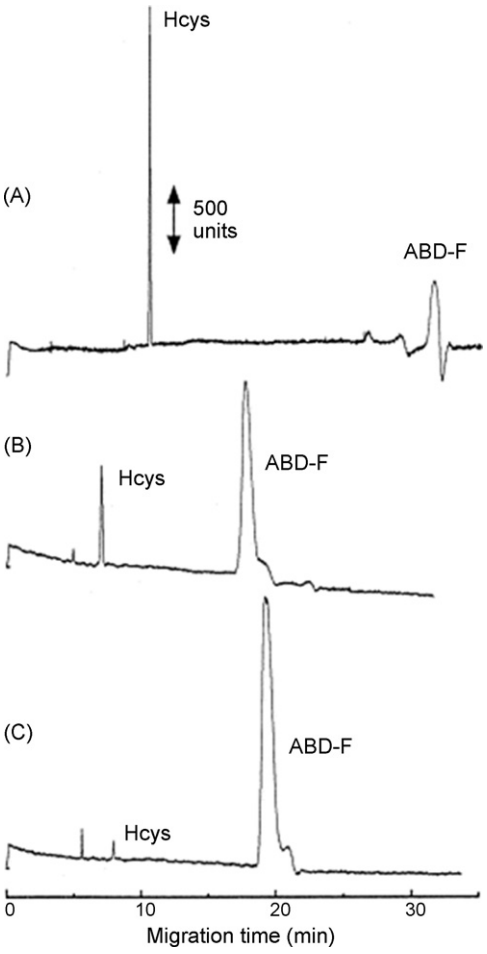

Fig. 5. Electropherograms of homocysteine obtained by various derivatization methods with UV detection. (A) Hydrodynamic injection for 5 s at 15 cm height with pre-column derivatized ABD-Hcy (167 μM) for 5 min at 50 °C. (B) Hydrodynamic injection of a mixture of 1 mM ABD-F and 1 mM Hcy (1:1, v/v) for 5 s followed by the running buffer for 3 s at 15 cm height. Voltage was applied (+1 kV) for 30 s, and the sample was incubated for 60 min at 38 °C for on-column derivatization. (C) Hydrodynamic injection of 1 mM ABD-F, Hcy, and ABD-F for 5, 5 and 3 s respectively at 15 cm height. Voltage was applied (+1, −1, and +1 kV) for 60, 60, and 30 s, respectively, and the sample was incubated for 20 min at 38 °C for on-column derivatization. CE conditions: running buffer, 20 mM Trizma® Phosphate (pH 2.1 with 0.1 M H_3PO_4); column, fused-silica capillary (60 cm × 50 μm I.D., 35 cm effective length); applied voltage, +25 kV, 220 nm detection. Reprinted from [70] with permission.

the influence of anticoagulants and protein precipitation agents on the thiol determination in serum and plasma. The authors evidenced that serum and EDTA plasma gave the same results; serum protein precipitations by acetonitrile, acetone, SSA, perchloric acid and TCA, evidenced that SSA and acetonitrile precipitations are both well adapted, whereas acetone cannot be used because no Hcy peak was detected in the expected range of concentration.

Vecchione et al. [65] evaluated sulfur-containing amino acids (methionine, Cys, Hcy) in plasma utilizing 5-bromomethyl fluorescein (5-BMF). The dye labels both thiols and carboxylic groups; besides the free carboxyl group of aminothiols can react with the CH_2Br moiety of 5-BMF to form a fluorescent conjugate which can be monitored by an argon ion laser fluorometer [73].

Total Hcy, Cys and methionine fractions were determined in plasma after reduction with TBP and protein precipitation with TCA. For tHcy the authors determined a LOQ of 10 nM, similar sensitivity was evidenced for cysteine, an even lower one for methionine, which may be labelled on its carboxylic group. The authors also performed a comparative analysis of HPCE and HPLC quantitation of Hcy in 61 blood samples. Plasma concentrations measured by

HPCE were in good agreement with those obtained employing a HPLC-based method, with a satisfactory correlation ($r = 0.9972$).

Lochman et al. [33] reported the determination of total concentrations of Hcy, Cys, GSH, CysGly and γ-glutamylcysteine (Glu-Cys) in plasma and urine. They simultaneously evaluated other three compounds used to treat disorders of aminothiol metabolism: penicillamine, mercaptopropionylglycine and cysteamine. Samples were reduced with TCEP and labelled with 5-BMF. The electrophoretic separation was performed in an uncoated fused-silica capillary (25 μm i.d. × 27 cm) using 60 mM borate – 15 mM sodium dodecyl sulfate – 2-amino-2-methyl-1-propanol at pH 10.0, with laser-induced fluorescence detection; total analysis time was less than 2 min. The assay was linear up to 500 μM. The LOD was lower than 0.19 μM for all analyzed compounds, with the lowest ascribed to GSH (0.06 μM). Results agreed well with a standard HPLC method [74]. The authors applied the method to plasma and urine samples of healthy subjects. tHcy mean concentration was 10.6 μM (±3.9 SD) in plasma and 1.8 mM/mol creatinine (±0.9 SD) in urine.

Plasma D-PEN evaluation, a thiol drug used in the treatment of cystinuria and Wilson's disease, has been proposed by Zinellu et al. [22]. Many methods have been proposed for plasma determination of such compound: colorimetric [75,76] radioimmunoassay [77], HPLC with various detection mode [78–80], gas chromatography [81] and CE [82], however these methods are not suitable to discriminate different drug forms, which is a fundamental aspect in the evaluation of disulfide interchange reactions and in accurate pharmacokinetic studies.

Zinellu et al. [22] discriminated the various form of plasma thiols, included D-PEN, by different sample treatments with a final common derivatization step with 5-IAF (Fig. 6). Total plasma thiols were evaluated after TBP (10% v/v) disulfide reduction; proteins were precipitated with SSA (6%) and the supernatant was than derivatized (procedure 1). Protein-bound thiols were evaluated after sample SSA (15%) treatment. In this case the protein pellet was resuspended in Tris buffer and thiols released by adding TBP and protein reprecipitated adding SSA (15%); the supernatant was then derivatized (procedure 2). In the case of free plasma thiol determination, plasma was deproteinized with SSA (15%); after protein precipitation the supernatant was treated with NaOH (0.5 mM) and TPB (10%), afterward samples were derivatized (procedure 3). In the determination of reduced plasma thiols, samples were deproteinized with SSA (15%); after centrifugation NaOH (1 mM) was added to the supernatant which was then derivatized (procedure 4). Oxidized D-PEN (disulfide) was calculated by difference (free minus reduced form). The separation conditions were: uncoated fused capillary 57 cm × 75 μm i.d.; 14.4 mM boric acid as electrolyte solution with 75 mM N-methyl-D-glucamine at pH 11.4; 22 kV voltage; 0.5 psi × 2s hydrodynamic injection. The LOD was not specified by authors while the LOQ in plasma was about 30 mM for all thiol forms. The method was applied to the determination of the redox status of plasma D-PEN in a patient affected by Wilson's disease treated with oral dose of 1.2 g/day of the drug. The authors [22] observed that treated patient exhibited lower levels of total Hcy and Cys as for their protein-bound fractions, compared to healthy subjects. As they suggested, these findings point out that D-PEN treatment removes notable amount of Hcy and Cys, especially from the protein-bound fraction.

CE is regarded as really interesting attractive in drug analysis as mentioned below. In the 90's some authors have proposed CE methods for the analysis of captopril and its degradation products but with no applications to biological matrices. This thiol drug and its by-products, have been separated by CE in 100 mM sodium phosphate buffer (pH 5.5), containing Cetyl Trimethyl Ammonium Bromide (CTAB) to reverse electroosmotic flow; the authors utilized UV detection at 214 nm [83]. Derivatization has been also proposed; Lin Ling et al. utilized the fluorogenic reagent SBD-F using photometric or fluorometric detection [84]. The conditions of these existing methods however showed that the lowest LOD was higher than 0.5 μg/mL and not suitable for therapeutic drug monitoring.

Pérez-Ruiz et al. [85] developed a sensitive method for the analysis of the anti-hypertensive thiol drug captopril using LIF detection. The method is based on the derivatization of captopril with the fluorescent label 5-IAF at pH 12.5 which permitted the highest value of peak area. Separations were performed in uncoated fused-silica capillary 57 cm × 75 μm i.d. at 10 kV voltage, utilizing hydrodynamic injection 0.5 psi for 5 s. The electrolyte was 20 mM phosphate

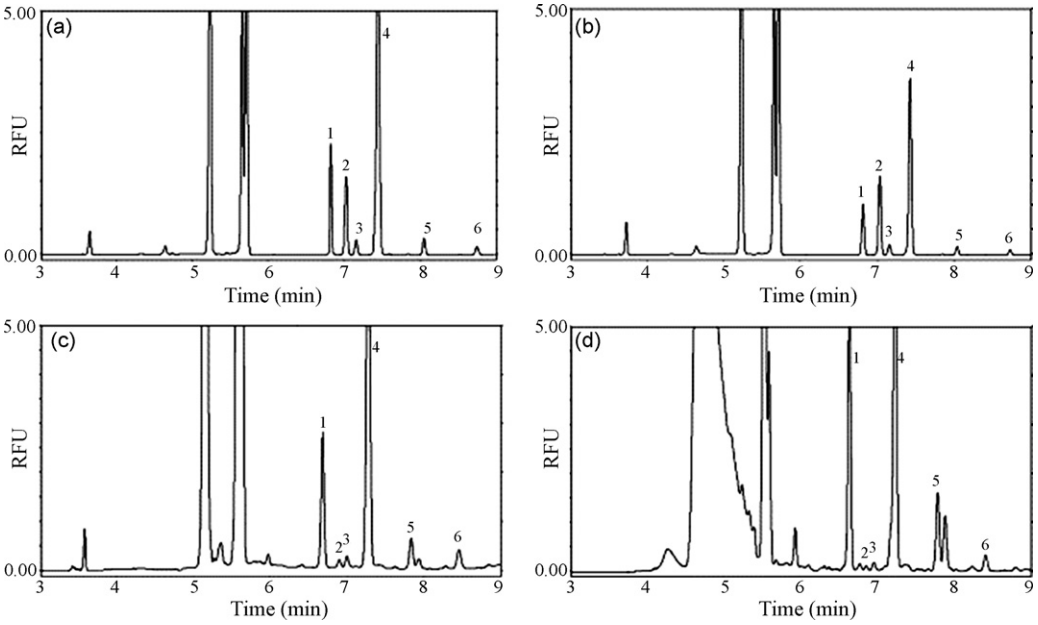

Fig. 6. Plasma D-Pen redox status in a Wilson's disease subject treated with an oral dose of 1.2 g/day of drug: procedure 1 (a): CysGly (20.9 μM), D-Pen (15.69 μM), Hcy (3.84 μM), Cys (145.3 μM), GSH (7.27 μM), Glu-Cys (3.74 μM); procedure 2 (b): CysGly (8.85 μM), D-Pen (14.98 μM), Hcy (2.63 μM), Cys (57.1 μM), GSH (2.06 μM), Glu-Cys (1.44 μM); procedure 3 (c): CysGly (12.13 μM), D-Pen (0.66 μM), Hcy (1.20 μM), Cys (88.3 μM), GSH (5.20 μM), Glu-Cys (2.30 μM); procedure 4 (d): CysGly (7.14 μM), D-Pen (0.13 μM), Hcy (0.33 μM), Cys (17.5 μM), GSH (2.87 μM), and Glu-Cys (0.70 μM). Reprinted from [22] with permission.

Table 1
Characteristics of CE methods for the evaluation of aminothiols in biofluids.

Analytes	Matrix	Derivatization	Capillary	kV	BGE	CE mode	Detection	Run time	LOD μM	Content	Ref.
GSH Cys Hcy	Plasma	ABD-F SBD-F	57 cm ⌀ 50 μm	25	Borate 20 mM pH 10.5	CZE	UV 200 nm	<10	1	–	[52]
GSH Cys Hcy	Plasma	BrB ABD-F	65 cm ⌀ 50 μm	16	Phosphate 80 mM pH 9.0	CZE	UV 234 nm UV 220 nm	<8	5	7.6 ± 2.4 μM 211.3 ± 36.4 μM 12.2 ± 4.1 μM	[41]
GSH GSSG NAC	Plasma	No	64.5 cm ⌀ 50 μm	15	NH_2PO_4 5 mM N_2HPO_4 15 mM SDS 50 mM pH 8	MEKC	UV 195 nm	<15	0.8	1.6 μM	[47]
Cysteamine	Plasma	No	64.5 cm ⌀ 50 μm	30	Tris–HCl 200 mM pH 2.1	CZE-ITP	UV 355 nm	<12	0.8		[51]
GSH Cys Hcy	Whole blood	DPDS On column	64 cm ⌀ 50 μm	28	Phosphate 50 mM SDS 50 mM pH 7.5	MEKC	UV 200 nm UV 343 nm	<4	5	645 ± 25 μM	[53]
GSH Cys TP	Serum		70 cm ⌀ 25 μm	27	Phosphate 20 mM pH 7.4	CZE	Amperometric +1100 mV	20	0.002 0.001 0.001	0.06 μM	[60]
GSH Cys Hcy NAC	Urine		80 cm ⌀ 50 μm	30	Tris-borate 100 mM pH 8.4	CZE	Amperometric +300 mV	15		–	[61]
GSH Cys Hcy	Whole blood		50 cm ⌀ 25 μm	18	Phosphate 100 mM pH 7.8	CZE	Amperometric +1050 mV	12	2.58 0.83 1.29	–	[62]
GSH Cys Hcy	Authentic compounds	ABD-F On-column reaction	50 cm ⌀ 50 μm	15	Trizma Phosphate 80 mM pH 2.1	CZE	Argon ion LIF Ex 354 nm Em 447–651 nm	15	0.004	–	[70]
GSH Cys Hcy NAC	Blood Plasma	FM	57 cm ⌀ 75 μm	15	Phosphate 10 mM SDS 50 mM, ACN pH 7.0	MECK	Argon ion LIF Ex 488 nm Em 520 nm	<8	0.01	0.64 μM 7.4 ± 1.8 μM	[43]
GSH Cys Hcy CysGly	Serum Plasma	6-IAF	85 cm ⌀ 50 μm	15	Borate 50 mM CAPS 20 mM, ACN pH 10.0	CZE	Argon ion LIF Ex 488 nm Em 515 nm	<14	 0.25	–	[71] [72]
GSH Cys Hcy CysGly	Plasma Urine	5-BMF	27 cm ⌀ 25 μm	8	Borate 50 mM SDS 15 mM, Amino-methyl-propanole pH 10.0	CZE	Argon ion LIF Ex 488 nm Em 522 nm	<2	0.061 0.156 0.076 0.062	11.7 ± 2.2 152.9 ± 55.4 10.6 ± 3.9 40.2 ± 10.8	[33]
Cys Hcy Met	Plasma	5-BMF	67 cm ⌀ 75 μm	30	Phosphate 0.25 mM pH 6.4	CZE	Argon ion LIF Ex 488 nm Em 520 nm	13	0.01 0.01 >0.01	–	[65]
GSH Cys Hcy	Urine RBC	mBrB	40 cm ⌀ 75 μm	25	Phosphate 50 mM pH 7.5	CZE	Fluorescence Ex 375 nm Em 480 nm	10	–	–	[37]
D-PEN	Plasma	5-IAF	57 cm ⌀ 75 μm	22	Borate 14.4 mM N-methyl-D-glucamine 75 mM pH 11.4	CZE	Argon ion LIF Ex 488 nm Em 520 nm	9	30 (LOQ)	–	[22]
Captopril	Urine	5-IAF	57 cm ⌀ 75 μm	10	Phosphate 20 mM pH 12.0	CZE	Argon ion LIF	15	0.5		[85]
GSH Hcy Gluc-Cys	Urine	OPA	40 cm ⌀ 75 μm	10	Phosphate 10 mM SDS 10 mM (only for Hcy) pH 12.0	CZE	Argon ion LIF Ex 355 nm Em 480 nm	6	0.022 0.042 0.008	1.76 0.52 0.81	[90]
GSH Hcy Cys	Plasma	5-IAF	57 cm ⌀ 75 μm	28	Borate 4 mM Phosphate 5 mM N-methyl-D-glucamine 75 mM		Argon ion LIF	5	– – –	2.5 10.7 255.5	[86]

Table 1 (*Continued*)

Analytes	Matrix	Derivatization	Capillary	kV	BGE	CE mode	Detection	Run time	LOD μM	Content	Ref.
CysGly					pH 11.0				–	21.3	
GSH Hcy Cys	Plasma	5-IAF	67 cm ⌀ 75 μm	28	Borate 16 mM Phosphate 20 mM N-methyl-D-glucamine 75 mM	CZE	Argon ion LIF	10	– – –	1.73 14.1 200.0	[89]
Gluc-Cys					pH 11.4				–	1.69	
CysGly									–	23.2	
NAC									0.0002	4.1 (after first day therapy)	

buffer at pH 12.0. Captopril was determined in pharmaceutical formulation and in human urine. The urine samples were spiked with different amounts of captopril to obtain a final concentration within the range 10–100 mg/mL; after urine clarification by centrifugation, the supernatant was diluted 1:100 with deionized water and 200 μL of the solution was used for the derivatization with 5-IAF and subsequent CE analysis. The LOD was stated to be 0.5 ng/mL. The authors evaluated the matrix effect of the methods determining captopril in the presence of endogenous thiols Cys and GSH. At the derivatization conditions selected for captopril, Cys and GSH did not react with 5-IAF. The endogenous levels of these compounds in urine and the 100-fold dilution utilized, meant that no peak was observed for these compounds. Authors also stated that the potential formation of these endogenous thiol derivatives migrated after those of captopril.

Chassaing et al. [34] developed a specific CE-LIF method to measure reduced and total Hcy, GSH and NAC in human plasma. The reduced thiols were derivatized at 25 °C, pH 7.5 with fluorescein-5-maleimide (FM). The total plasma concentration of thiols, including the fraction coupled to proteins, was drawn with dithiothreitol followed by perchloric acid deproteinization. The FM-thiol separation was performed in an acetonitrile/10 mM sodium phosphate, 50 mM SDS buffer [25:75 (v/v)], pH 7.0 in an uncoated fused-silica capillary 57 cm × 75 μm i.d., at 45 °C. The electric field was 530 V/cm and the time needed for the separation of FM-Hcy, FM-GSH and FM-N-acetylcysteine was less than 8 min. The LOD was 3 μM for the total thiols and 10 nM for the reduced thiols. The method was applied to the determination of Hcy levels in plasma from healthy subjects and patients with end-stage renal disease. Among the group of healthy subjects, a mean tHcys concentration of 7.41 μM (±1.82 SD) was observed; the patient group exhibited a mean concentration of 21.58 μM (±5.83 SD). The sensitivity of the assay allowed the monitoring of tHcy level in healthy subjects as well as in patients with end-stage renal disease.

Zinellu et al. [86] reported a CE-LIF method using 5-IAF and adding N-methyl-D-glucamine to the buffer to blank sylanols and slow down the electroosmotic flow. The separation of total plasma Hcy, Cys, Cys-Gly, and GSH was achieved in less than 5 min. The thiols were reduced by 10% TBP and deproteinized by 10% TCA; derivatization was carried out at room temperature for 10 min. The method was compared to HPLC with fluorescence detection using Bland–Altman test and Passing–Bablok regression demonstrating that the results are highly comparable. The same method has been applied to determine plasma tHcy and tCys levels in healthy subjects and in patients with retinal vein occlusion (RVO) [87].

The method has then been improved and applied to clinical samples to assay the various fractions of plasma thiols [88]. Hcy, Cys, Cys-Gly, GSH and Glu-Cys, were measured by varying the order of disulfide reduction with TBP and protein precipitation with SSA. After derivatization with 5-iodoacetamidofluorescein, samples were analyzed by CE-LIF using a phosphate/borate buffer in the presence of 75 mM N-methyl-D-glucamine, pH 11.0. Separating conditions were: 22–28 kV utilizing a 57 cm × 75 μm i.d. uncoated fused-silica capillary. Total, free (reduced plus disulfide forms), free reduced, disulfide forms (symetrical and mixed) and protein-bound thiols were determined. Disulfide (free minus reduced thiols) and protein-bound (total minis free thiols) fractions were calculated by difference.

The same research group has recently developed a CE-LIF method for the simultaneous evaluation of a cysteine derivate, namely NAC [89]. NAC is a therapeutic drug widely used as mucolytic agent in the treatment of respiratory diseases. Since it has been proposed that NAC administration may modify the plasma LMM thiol levels, the method has been utilized to measure the drug and the physiological LMM thiols, in NAC administered chronic obstructive broncho-pneumopathy (COPB) disease patients. After derivatization step using 5-IAF as selective thiol reagent, analytes were separated with 20 mM sodium phosphate, 16 mM boric acid with 75 mM N-methyl-D-glucamine, run buffer at pH 11.4 with a run time shorter than 10 min. The LOD of NAC was calculated to be 0.2 nM. The authors also evidenced that NAC treatment influences thiol plasma levels, after the first drug administration.

Shen et al. [90] reported an interesting method for the selective extraction of thiols, using gold nanoparticles (AuNPs), as a result of the formation of Au–S bonds. The AuNPs were coupled with non-ionic surfactant Tween 20, providing high selectivity for aminothiols with respect to previously reported use of AuNPs capped with citrate [91]. After the extraction procedure, thiols were derivatized using *o*-phthanaldehyde (OPA) and analyzed by CE-LIF, to measure ultra low concentration of free Hcy, GSH and Glu-Cys in urine samples. The authors stated that derivatization procedure with OPA was optimal at pH 12.0, the same pH value was optimal for the background electrolyte. The best separation conditions were 10 mM phosphate buffer pH 12.0 (plus 10 mM SDS only for Hcy determination), 10 kV, excitation wavelength 355 nm, utilizing a fused-silica capillary (75 μm i.d. 40 cm effective length). The LODs were 42.3, 22.5 and 8.1 nM, respectively for Hcy, GSH and Glu-Cys. The concentration of Hcy, GSH and Glu-Cys in urine samples were calculated to be 1.76 (±0.09 SD), 0.52 (±0.02 SD) and 0.81 μM (±0.04 SD) respectively, in agreement with previously reported values [92] (Table 1).

3. Conclusions

Numerous articles have been published in the last decade focusing on aminothiol evaluation in biological matrices and accounting for the clinical relevance of these biomolecules. Capillary electrophoresis really represents a valuable tool in this field of research, allowing a simple and fast analysis. While GSH and GSSG can be evaluated without any derivatization process, the other thiols such as Hcy and Cys need laborious sample treatment and suitable detectors. Plasma, like most biological samples, contains considerable amounts of sodium chloride, which causes band spreading in CE due to the low field strength, especially when the sample volume significantly goes above 1% of the capillary volume. Because of this and due

Table 2
CE and HPLC method comparison and correlation of thiol concentrations in biological matrices.

Method characteristics	HPLC	CE	HPLC	CE	HPLC	CE
n samples evaluated	51		57		61	
Matrix	Plasma		Plasma		Plasma	
Analytes	Hcy		GSH, Hcy, Cys, Cys-Gly		Hcy	
Statistical analysis	Bland-Altman (CE-HPLC)		Bland–Altman (CE-HPLC) and Passing–Bablok		–	
Reducing agent	TCEP	TCEP	TBP	TBP	$NaBH_4$	TBP
Precipitation	PCA	PCA	TCA	TCA	PCA	TCA
Derivatizing agent	5-BMF	5-BMF	ABDF	5-IAF	OPA	5-BMF
Detection	Fluorescence	LIF	Fluorescence	LIF	Fluorescence	LIF
Column	Hypersil ODS (5 μm, 125 × 4.0 mm)	Uncoated 27 cm × 25 μm	Hypersil ODS-2 (5 μm, 250 × 4.6 mm)	Uncoated 57 cm × 75 μm	Ultrasphere ODS (5 μm, 250 × 4.6 mm)	Uncoated 67 cm × 75 μm
Buffer	Acetic acid-acetate 100 mM, pH 5.5 – MeOH 3%	Borate 50 mM, SDS 15 mM, amino-methyl-propanole, pH 10.0	K-phosphate 0.1 mM 88% – EDTA 0.12 mM – ACN 12%, pH 2.1	Borate 4 mM, Phosphate 5 mM, N-methyl-D-glucamine 75 mM, pH 11.0	Sodium propionate – ACN, pH 6.5	Phosphate 0.25 mM, pH 6.4
Run time (min)	8	<2	<10	5	35	13
LOD μM	0.16	0.07	–	–	0.05	0.01
Bland-Altman mean CE-HPLC	0.5 μmol/L		−0.08 μmol/L (GSH)	–		
Bland-Altman 2SD	4.5, −3.5 μmol/L		0.42, −0.59 μmol/L (GSH)	–		
Passing-Bablok slope	–		1.017 (GSH)		–	
R^2	–		0.983 (GSH)		0.994	
References	[74]	[33]	[93]	[86]	[94]	[63]

MeOH: methanol; CAN: acetonitrile; PCA: perchloric acid; TCA: trichoroacetic acid; TCEP: tri(2-carboxyethyl)phosphine; TBP: tri-n-butylphosphine.

to the short light path of the capillary, the LOD in CE, when we refer to concentration, is worse compared to HPLC and not sufficient for the evaluation of many aminothiols. In these cases improved sensitivity is possible primarily by the use of electrochemical detectors, by laser-induced fluorescence detection after derivatization and by sample stacking. When these types of detector are employed, CE method performances are comparable to that of HPLC [74,93,94], with a good correlation of values in biological matrices [33,65,86] (see Table 2).

Besides these detection methods, microchip CE techniques are rapidly coming forward in this field. During the past decade, microfluidic analytical systems have undergone an explosive growth, due to their advantages of high performance, design flexibility, reagent economy, high throughput, miniaturization, and automation [95,96]. The microchip analysis is largely promising for biomedical analysis and clinical diagnostics; it has been already applied to the evaluation of aminothiols [55,97] and it could really represent the future of CE techniques.

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