



Recent applications of the derivatization techniques in capillary electrophoresis



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ABSTRACT

The main reasons for performing derivatization in capillary electrophoresis are largely the same as for liquid chromatography, however there are specific aspects in electrokinetic separations where derivatization plays specific roles. The review is focused on the articles published in the past 5 years with the aim to highlight this unicity. Derivatization is mainly applied to improve the inherent low sensitivity of capillary electrophoresis when optical detection is used and the introduction of originally developed derivatization approaches have been addressed mainly to the detection by laser-induced fluorescence. A further peculiarity concerns the development of automatized as well as in-capillary derivatization that can be performed using the commercially available instrumentation. The majority of the methods considered deal with the derivatization of amine group in small molecules (in particular, amino acids) as well as in proteins and peptides. Applications are also addressed to chiral analysis and for trapping unstable and reactive small molecules and inorganic ions. The analysis of proteins and saccharides in glycomics, have been covered in dedicated sections.

1. Introduction

Derivatization is the transformation of an analyte into a modified compound with a more favorable structure, the derivative, allowing for the improved determination by means of the selected analytical method [1]. The reasons for developing derivatization procedures in capillary electrophoresis (CE) are largely the same as those in liquid chromatography (LC) *i.e.*, to introduce UV-absorbing or fluorescent groups for enhancing sensitivity, to improve the stability of the analyte, and to improve the ionization when the detection system is mass spectrometry. Moreover, derivatization can be carried out for converting enantiomers into diastereomers in order to achieve enantioresolution in achiral environment. In LC, derivatization is also performed to improve the retention of very polar analytes by the most common reversed-phase columns. In capillary zone electrophoresis (CZE) the selectivity is driven by the charge-to-mass ratio under the electric field in solution, thus derivatization modifying both charge and or mass, can affect the mobility of the analytes allowing the selectivity tuning. In addition, changing the hydrophobicity of the analytes is helpful for selectivity improvement in micellar electrokinetic chromatography (MEKC) [1,2].

Important aspects of derivatization that can be considered specific for CE analysis concern *e.g.*, the conversion of some relevant neutral molecules such as majority of the saccharides, into derivatives that can

be ionized making them suitable for CZE separations [3,4], and the development of automatable derivatization strategies (in-capillary derivatization and derivatization followed by on-line preconcentration) [1,2,5]. In addition, labeling the analytes with fluorescent tags compatible with laser excitation especially in the visible spectrum, has become most common in CE with laser-induced fluorescence detection (CE-LIF) than in LC [6].

This survey reviews the main applications of derivatization in CE, published starting since 2018 with the aim at providing an overview of the trends in this area. More than one third of the considered articles dealt with the analysis of amino acids (AAs) demonstrating how this topic is always of great interest and in this regard the details of the methods have been collected in a specific table. A further aspect to be considered concerns the applications of enantioselectivity-oriented derivatization, underlying the prominent role that CE techniques have gained in the field of chiral analysis. The survey was conducted using databases as Scopus and Web of Science; along with keywords such as "derivatization", the terms "labeling" and "label" were also included, extending the overview to the derivatization for characterization and analysis of biological macromolecules such as glycans, peptides and proteins. The tables are organized by considering the analyte classes (AAs, peptides and proteins, miscellaneous) with the aim at providing an immediate readout of the applications/methods.

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2. Small molecules

2.1. Optimization of the derivatization and analysis conditions

Optimization of derivatization conditions involves the choice of the pH (and buffer type), solvent, type of reagent and its concentration, in relation to that of the analyte. Temperature and reaction time are also extremely important because it is desirable to obtain high derivatization efficiency in a short time. Despite the success of chemometric methods for optimizing multi-variable systems, the one-variable-at-a-time approach is still followed in most of the cases.

One of the most popular reagents for pre-capillary derivatization of amino group both in LC and CE applications is 9-fluorenylmethyl chloroformate (FMOC-Cl) which reacts with primary and secondary amines leading to fluorescent derivatives. FMOC-Cl fulfills the main requirements for practical and reliable methods *i.e.*, fast derivatization reaction under mild conditions (room temperature), and good stability of the derivatives that can be detected both by UV absorption and spectrofluorimetry (FL) [7–10]. Alkaline buffers as borate or tetraborate at pH 8–9 are usually selected. At higher pH values the reaction rate increases however, unfavorably also the hydrolysis of the reagent increases. A detailed investigation on the kinetics of derivatization of AAs using Pacific Blue succinimidyl ester (PBSE) as the reagent, showed that its hydrolysis is an important factor for determining the ultimate labeling efficiency and the balance of the two kinetics effects *i.e.*, PBSE hydrolysis and labeling rate, dictates the optimum pH value [11].

In optimizing the pH of the reaction mixture, also the compatibility with the matrix components of the real samples should be considered. Very fast derivatization of primary amines is achieved using *o*-phthalaldehyde (OPA) in the presence of a thiol (*e.g.*, 2-mercaptoethanol, or *N*-acetyl-L-cysteine, NAC) to give fluorescent isoindole derivatives. Although the optimum pH is established to be 9–9.5 and even higher [12], the reaction with the non-proteinogenic amino acid theanine was found to be maintained up to pH 7.5, allowing the application of the derivatization to complex samples whose matrices can be degraded at more alkaline pH values (Fig. 1) [13].

It would be desirable to carry out the labeling reaction at room temperature, however it is obvious that the derivatization kinetics are improved at higher temperatures or by using microwave [14] and ultrasonication [15]. However, in a detailed study on the optimization of derivatization of molecules of pharmaceutical and toxicological interest containing the amine group, by fluorescein 5-isothiocyanate isomer I (FITC), the temperature of 5 °C was found to be advantageous for the increased stability of the derivatives [16].

The influence of the molar ratio (reagent/analyte) on the reaction efficiency is of utmost importance. The higher the molar ratio, the higher the reaction efficiency, however, the reagent excess can disturb the subsequent separation. In analysis of aldehydes derivatized by 2,4-dinitrophenylhydrazine (DNPH), a major issue is that the large excess of the reagent can precipitate in the reaction mixture and, in addition it can interfere in the subsequent CE separation. Solid-phase extraction (SPE) procedure before injection in CE, was thus necessary [17–19]. Advantageously, using *N*-propyl-4-hydrazino-naphthalimide, (NPHNA) as the reagent, only the adducts with the aldehydes are fluorescent, whereas the reagent excess is not, thus analysis by MEKC-LIF is simplified avoiding post-derivatization sample treatments [20]. Using FMOC-Cl, the reagent excess, and the main hydrolysis product FMOC-OH are often removed by liquid-liquid extraction with *n*-pentane before the injection into the CE apparatus [8–10]. However, with respect to HPLC separations, when CE conditions are properly optimized, the reagent overloading can be less cumbersome. As an example, the analysis of glyphosate, and its microbial metabolite aminomethylphosphonic acid (AMPA), derivatized with high excess of FMOC-Cl, was carried out by field-amplified sample injection and sweeping MEKC avoiding any post-derivatization treatment allowing the determination of the herbicide residue in wheat [21]. Liu et al., observed that the derivatization

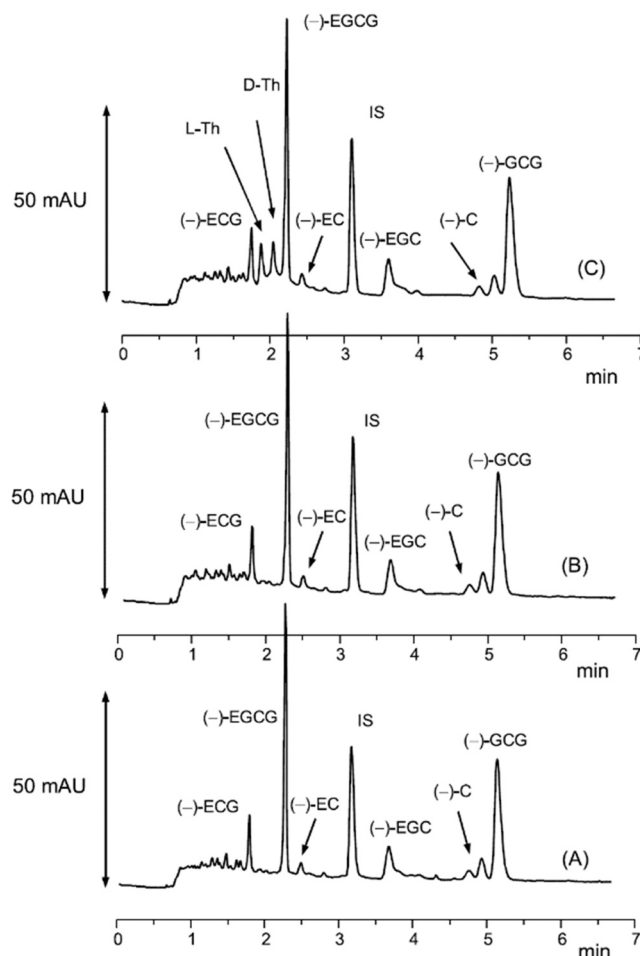


Fig. 1. Electropherograms of a green tea sample by CD-MEKC; A) analysis of the real sample before derivatization; B) analysis after derivatization using OPA/NAC; C) analysis of the real sample, spiked with D/L-theanine and derivatized using OPA/NAC. The catechins as relevant components of the samples were not deteriorated applying the derivatization conditions necessary to the detection of theanine enantiomers. BGE composed of pH 2.5 phosphate-borate buffer (25 mM) containing 65 mM SDS and 28 mM dimethyl- β -cyclodextrin. Fused-silica capillary (48.5 cm total length; 8.5 cm effective length; i.d. 50 μ m); voltage 30 kV; capillary cartridge temperature 30 °C; hydrodynamic injection at 50 mbar \times 5 s; detection at 220 nm. Symbols: (-)-catechin, (-)-C; (-)-epicatechin, (-)-EC; (-)-epicatechin gallate, (-)-EGC; (-)-epigallocatechin, (-)-EGC; (-)-epigallocatechin gallate, (-)-EGCG; (-)-gallocatechin gallate, (-)-GCG; O-phthalaldehyde, OPA; N-acetyl-L-cysteine, NAC. From [13], with permission.

yield for the different 3-hydroxyaspartate stereoisomers changes as the reagent/analyte changes, and using a ratio FMOC-Cl/analyte < 2.0 derivatization occurs preferably toward erithro isomers [8].

When more than a single reactive group is present in the analyte, the derivatization can occur either on all the reactive sites and singularly on each of them, thus producing mixtures of the derivatives. As an example, FMOC-Cl forms with His and Tyr, mono- and double labeled species [7] and the same behavior was observed in CZE analysis of His using dansyl chloride (DNS) as the reagent [22]. Favorably, when salicylaldehyde sulfonate was used for derivatization of polyamines, only the di-anionic derivatives were obtained whose electrophoretic behavior was significantly different from that of the reagent excess simplifying the subsequent CE separation also in the presence of high excess of the reagent [23]. In Table 1 and Table 2 are summarized the characteristics of the derivatization methods applied in CE analysis of AAs and other small molecules, respectively. The structures of the reagents and the reactions schemes are reported in Supplementary Material

Table 1
Derivatization of amino acids.

Amino acid/s	Sample	Reagent	CE mode	Reaction conditions	Detection	LOD ^a	ref
3-Hydroxyspartate	Cerebrospinal fluid	FMOC-Cl ¹⁾	CD ²⁾ -CZE	-borax pH 9.2 -r.t. ³⁾ , 8 min	CE-MS, positive ionization	356 nM	8
Homocysteine	Standard	FMOC-Cl	CD-CZE Chiral separation	-borate pH 9.0 -r.t., 2 min	UV at 210 nm	n.r. ⁴⁾	9
Ala, Asp, Glu, His, Ile, Leu, Met, Phe, Thr, Trp, Val	Cerebrospinal fluid	FMOC-Cl	CD-MEKC Chiral separation	-borax pH 9.5 -r.t., 2 min	-FL ⁵⁾ ex/em 260/331 nm	10–100 nM 536 nM (Trp)	10
Biogenic amines, His	Yogurt, cheese, milk drinks	DNS ⁶⁾	CZE	-NaOH, 6 M -r.t., 5 min	UV at 214 nm	0.05 – 0.4 μM	22
3-Hydroxyaspartate	Urine	NBD-Cl ⁷⁾	CD-CZE Chiral separation	-borax pH 8.5 -microwave at 70 °C, 30 min	LIF 488 nm	4 nM	14
Arg, Asp, Glu, Gly, Leu, Lys, Met, Thr, Val	Functional food	NBD-Cl	CZE/In- capillary	-borax -waiting time 360 s	UV 475 nm	2.8–25.2 μM	25
Proteinogenic primary AAs	Cell cultures	NDA ⁸⁾	-MEKC -microchip (MCE)	-cyanide ions -borax buffer -r.t., 10 min	-MEKC UV 254 nm -MCE LEDIF ⁹⁾ 458 nm	5–10 μM	33
Ala, Arg, Asn, Asp, Gln, Glu, His, Ile, Leu, Met, Phe, taurine	Human embryos	NDA	MEKC	-borate pH 9.0 -cyanide ions 25 °C, 45 min	LIF 488 nm	12–39 nM	34
Asp, GABA, Glu, Gly, Leu	Microdialysates (brain)	NDA	CZE CZE/In- capillary MEKC/In- capillary	-borate pH 8.7 -cyanide ions -r.t., 15 min	LIF 410 nm	n.r.	64
Trp	Standard	CBQCA ¹⁰⁾	MEKC	-borate pH 9.9 -cyanide ions -25 °C, 2 h	LIF 480 nm	0.7 μM	35
Ile, Trp, Tyr, Val	Mouse plasma	CBQCA	MEKC	-borax pH 9.5 -cyanide ions -r.t., 2 h	LIF 480 nm	6 μM (Trp)	36
Asp, Glu	Cerebrospinal fluid	6-CFSE ¹¹⁾	CD-CZE Chiral separation	-no buffer -25 °C, ultrasonication 2 h	LIF 488 nm	~7 μM	15
Ala, Arg, Asp, GABA, Gly, Glu, His, Leu, Ser, Val	Samples from deep antarctic ice	PBSE ¹²⁾	-CZE -Microchip (MCE)	-borate pH 8.5 -4 °C - 1 h	LIF 405 nm	0.7–3 nM	11,32
Asn, Asp, Gln, Glu, Ser	Cerebrospinal fluid (artificial)	(+)-FLEC ¹³⁾	CZE Chiral separation	-borax pH 9.2 -r.t., 1 h	ESI-MS negative ionization	< 1 μM	58
Ala, Asp, Glu, Met, Ser, Thr, Trp, Tyr, Val	Liquid culture E. Coli	OPA ¹⁴⁾ /NAC ¹⁵⁾	CD-CZE/In- capillary Chiral separation	-borate pH 9.5 -r.t.	UV 340 nm	3–10 μM	59
Theanine	Green tea	OPA/NAC	CD-MEKC Chiral separation	-borate, pH 7.5 -r.t., 2 min	UV 220 nm	1 μM	13
Sarcosine, Ala, Cys, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Val	Urine	PA ¹⁶⁾	CZE/In- capillary	-borate buffer	UV 200 nm	0.1 μM	65
Val, Leu	Tumor cells	FITC ¹⁷⁾	CZE	-borate pH 9.2 -r.t., 15 h	LIF 480 nm	0.15 μM	28
Hydroxyproline	Hydrolyzed dairy products	FITC	CZE	-borate pH 9.2 -r.t., 16 h	LIF 474 nm	0.61 nM	30
Ala, Asp, GABA, Glu,	Rat brain sections	FITC	CAE ¹⁸⁾	-borax pH 9.2 -r.t., 12 h	LIF 488 nm	109 pM	29
Various AAs	Bovine aortic endothelial cells	-DAP ¹⁹⁾ -DEDA ²⁰⁾	CZE	-pyridine -r.t., 30 min -r.t., 70 min	CE-MS Positive ionization	76 nM	68

1) FMOC-Cl, 9-fluorenylmethyl chloroformate; 2) CD, Cyclodextrins; 3) r.t., room temperature; 4) n.r., not reported; 5) FL, Fluorescence; 6) DNS, dansyl chloride; 7) NBD-Cl, 4-Chloro-7-nitro-1, 2, 3-benzoxadiazole; 8) NDA, naphthalene-2,3-dicarboxyaldehyde; 9) LEDIF, Light-emitting diode induced fluorescence; 10) CBQCA, 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde; 11) 6-CFSE, 6-carboxyfluorescein *N*-hydroxysuccinimide ester; 12) PBSE, Pacific Blue succinimidyl ester; 13) (+)-FLEC, (+)– 1-(9-fluorenyl)ethyl chloroformate; 14) OPA, *O*-phthalaldehyde; 15) NAC, *N*-acetyl-L-cysteine; 16) PA, Phthalic anhydride; 17) FITC, Fluorescein isothiocyanate; 18) CAE, Capillary array electrophoresis; 19) DAP, 3-(Diethylamino) propionyl chloride; 20) DEDA, *N,N*-Diethylethylenediamine

^a LOD values have been reported on a unified basis (relying on data given in the papers)

Table 2
Derivatization of small molecules/miscellaneous.

Analyte	Sample	Reagent	CE mode	Reaction conditions	Detection	LOD ^a	ref
Sulfite	Seafood	Formaldehyde	CZE	-90 °C (10 min)	Indirect UV	4.4 mg/kg	44
Nitric oxide	Macrophage cells	DAMBO ¹⁾	MEKC	-pH 7.4	LIF 488 nm	0.12 nM	42, 43
		DSDMHDAB ²⁾		-37 °C, 10 min		0.43 nM	
Cyanide	Seeds from apples	FQ ³⁾	CZE	-borax pH 9.2	LIF 488 nm	26 nM	46
Acrylamide	Potato products	Cysteine	CZE	-85 °C, 60 min	C ⁴ D ⁴⁾	0.16 μM	45
				-cysteine			
				- <i>n</i> -butylamine			
Formaldehyde	HeLa cells	NPHNA ⁵⁾	MEKC	-70 °C, 10 min	LIF 488 nm	5 amol	20
				-phosphate pH 7.4			
				-37 °C, 30 min			
Formaldehyde	Urine	2-thiobarbituric acid	CZE	-HCl 1.45 M	amperometric detection	80 nM	49
				-45 °C, 1 h			
Aldehydes	Water samples	DNPH ⁶⁾	NACE ⁷⁾	-ACN/phosphoric acid	UV 360 nm	~ 0.1 μM	19
	Particulate matter		MEKC				
Aldehydes	Exhaled breath	FTZ ⁸⁾	CZE	-borate/phosphate pH 9.0	LIF 488 nm	0.16–3.4 nM	51
				-60 °C, 1.5 h			
Aldehydes	Alcoholic beverages	HBA ⁹⁾	CZE	-no buffer (pH 5)	UV 290 nm	~ 0.0035–0.01 mM	50
				-r.t., 60 min			
Carbonyl compounds	Drinking water	DNPH	MEKC	-citrate buffer (pH3)	UV 360 nm	~ 20 nM	17
				-40 °C, 1 h			
Catecholamines	Serum, urine	BODIPY-NHS ¹⁰⁾	CZE	-boric acid/borax, pH 7.5	LIF 473 nm	0.2–0.3 nM	39
				-25 °C, 20 min			
Thiols	Urine and living cells	BODIPY-IA ¹¹⁾	CZE	-borate pH 9.5	LIF 473 nm	0.5 nM	40
				-45 °C, 15 min			
Penicillamine, tiopronine	Urine and serum	TMMB-Br ¹²⁾	CZE	-phosphate pH 8.5	LIF 473 nm	0.47 nM, 0.88 nM	41
Fatty acids	Cell cultures	AMPP ¹³⁾ DEEA ¹⁴⁾ 2-PA ¹⁵⁾	CZE	-40 °C, 10 min	ESI-MS, positive ionization	25–50 nM	69
				-10–30 min		250 nM	
Drugs (amino group)	Standard sol	FITC	MEKC	-borate pH 9.7	LIF 488 nm	0.1–0.4 nM	16
				-5 °C, 32 min			
Adamantine drugs	pharmaceuticals	Fluorescamine	MEKC/In-capillary	-borate pH 10.0	UV 305 nm	6.0–8.5 μM	66
Pregabalin	pharmaceuticals	DNS	CD-CZE		UV 220 nm	~ 5 μM	60
				-bicarbonate pH 9.5			
Alendronate	Nanoparticles/ pharmaceuticals	OPA, ME ¹⁶⁾	CZE	-r.t., 45 min	UV 254 nm	~ 2.5 μM	12
				-alkaline pH (NaOH)			
				-60 °C, 15 min			
Glyphosate, AMPA, taurine	Wheat	FMOC-Cl	MEKC	-borax pH 9.2	UV 210 nm	10 nM	21
				-r.t., 10 min			
Glyphosate, AMPA	Mussels	NBD-F	CD-CZE	-borate pH 9.2	LIF 488 nm	0.17 μM (G) 0.03 μM (A)	26, 27
				-80 °C, 5 min			
Morphine and metabolite	Human urine Serum samples	Ferricyanide	CZE/In-capillary MEKC/In-capillary	-borax pH 10.5 -potassium ferricyanide (0.25 mM)	FL ex/em 340/ 450 nm	~ 2 nM	62, 63
Polyamines	Standard	Salicylaldehyde sulfonate	CZE	-HEPES ¹⁷⁾ pH 7.8	240 nm	< μM	23
				-r.t., 40 min			
Sulfonamides	Spiked urine, pharmaceuticals	DEAC-C ¹⁸⁾	MEKC	-cyanuric chloride	LIF 405 nm	0.23–0.29 nM	37
				-50 °C, 3 h			

1) **DAMBO**, 1,3,5,7-tetramethyl-8-(3',4'-diaminophenyl)-difluoroboradiaza-s-indacene; 2) **DSDMHDAB**, disodium 2,6-disulfonate-1,3-dimethyl-5-hexadecyl-8-(3,4-diaminophenyl) - 4,4'-difluoro-4-bora-3a,4a-diaza-s-indacene; 3) **FQ**, 3-(2-furoyl)quinoline-2-carboxaldehyde; 4) **C⁴D**, Capacitively coupled contactless conductivity detector; 5) **NPHNA**, *N*-propyl-4-hydrazino-naphthalimide; 6) **DNPH**, 2,4-dinitrophenylhydrazine; 7) **NACE**, Non-aqueous capillary electrophoresis; 8) **FTZ**, fluorescein 5-thiosemicarbazide; 9) **HBA**, 4-hydrazinobenzoic acid; 10) **BODIPY-NHS**, 1,3,5,7-tetramethyl-8-(*N*-hydroxysuccinimidyl propionic ester)-difluoroboradiaza-s-indacene; 11) **BODIPY-IA**, *N*-(4,4-difluoro-1,3,5,7-tetramethyl-3a,4a-diaza-s-indacene-2-yl) iodoacetamide; 12) **TMMB-Br**, 1,3,5,7-tetramethyl-8-bromomethyl-difluoroboradiaza-s-indacene; 13) **AMPP**, *N*-(4-aminomethylphenyl)pyridinium; 14) **DEEA**, *N,N*-Diethylethylenediamine; 15) **2-PA**, 2-picolylamine; 16) **ME**, 2-mercaptoethanol; 17) **HEPES**, 2-[4-(2-hydroxyethyl)piperazin-1-yl]athanesulfonic acid; 18) **DEAC-C**, 7-(diethylamino)coumarin-3-carboxylic acid.

Other symbols and abbreviations as in Table 1.

^a LOD values have been reported on a unified basis (relying on data, given in the papers)

(Table SM1, and Table SM2).

2.2. Derivatization for laser-induced fluorescence detection (CE-LIF)

LIF is an extremely sensitive detection method thanks to the excitation by means of monochromatic, directional, and coherent sources. The coupling of LIF detector with CE instrumentation has become

commercially available and relatively affordable; light-emitting diode-induced fluorescence detector (LEDIF) is a convenient alternative to LIF, with some advantages as LEDs are less expensive than lasers, consume less energy and are stable, maintaining similar sensitivity [6,24].

4-Chloro-7-nitro-1, 2, 3-benzoxadiazole (NBD-Cl) [14,25], 4-fluoro-7-nitro-1, 2, 3-benzoxadiazole (NBD-F) [26,27] and FITC [16,28–30],

are suitable for derivatization of primary and secondary amines. The obtained derivatives can be detected using the relatively common argon ion laser (excitation at 488 nm), however the derivatization required for longer reaction time with respect to those necessary for FMOCl and OPA, and higher temperature. NBD-F was found to react 10 times faster than NBD-Cl [31] and it has been used for quantitation of glyphosate and AMPA by CE-LEDIF achieving sensitivity adequate to perform *in-vivo* studies on the bioaccumulation of these pollutants in mussels [26,27]. PBSE thanks to the favorable chemistry of the *N*-hydroxy succinimide leaving group, forms with amines stable conjugates which can be simultaneously excited at 405 nm and emit at 455 and 551 nm, respectively, facilitating two-color LIF analysis; PBSE has been used in recent applications in CE and microchip CE [11,32].

Naphthalene-2,3-dicarboxaldehyde (NDA) [33,34] and 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA) [35,36], share a similar derivatization reaction scheme (Table SM1) where the aldehyde moiety reacts in a multistep sequence with the primary amine to form an imine. The addition of cyanide ions (CN⁻) to the imine, results in intramolecular cyclization leading to cyanobenzo[*f*]-isoindole through the loss of a water molecule; the derivatization conditions should be optimized according to the pK_a of the amines (in general pH value in the range 9.0–9.3 is reported as the optimum). Excess of cyanide ions shifts the reaction toward a first-order kinetic with respect to NDA, however in the presence of high excess, cyanide induces the condensation of two aromatic aldehydes to form α -hydroxyketone. Thus, a ratio of cyanide/NDA of 1:1 is often considered as the optimum [33,34].

The derivatization of the sulfonamide nitrogen by 7-(diethylamino) coumarin-3-carboxylic acid (DEAC-C) as the reagent, has been developed as an original approach for improving detection sensitivity in CE-LIF of the drugs. Once the carboxylate of the reagent in the presence of cyanuric chloride, is converted to the cyanuric ester, the sulfonamides react yielding the *N*-acylsulfonamide (Table SM2). The derivatives were stable but only at 4 °C. The adducts with some sulfonamides of pharmaceutical and toxicological interest were detected by CE-LIF and the original derivatization approach is promising as a general procedure not only in the field of pharmaceutical analysis but also in doping control and determination of residues in environment, food etc. [37].

Among the fluorescent probes used in imaging techniques, BODIPY *i.e.*, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene dyes are very promising since the scaffold possesses high photostability, neutral total charge, high fluorescence quantum yield, and sharp absorption and emission spectra [38]. The recent literature reports some examples, where BODIPY scaffold, appropriately functionalized has been used to develop derivatization in CE-LIF applications by means of the presence of (i) the succinimidyl ester as a good leaving group in analysis of catecholamines [39], (ii) the iodoacetamido reactive moiety [40] as well as (iii) an activated methyl bromide in analysis of thiols [41], and (iv) the diaminophenyl moiety for reaction with nitric oxide (NO) [42,43] (Fig. 2A-E and Table SM2).

2.3. Derivatization for trapping unstable molecules and inorganic ions

Quantitation of reactive species in biological systems is challenging because of their limited stability, thus derivatization in this case is firstly addressed to their conversion into stable derivatives. Quantitation of sulfite ion used as a seafood additive was carried out by its conversion to the stable hydroxymethylsulfonate *via* reaction with formaldehyde. Hydroxymethylsulfonate is devoid of spectrophotometric absorption, thus the determination could be obtained by CE using indirect UV detection [44]. The reactivity of activated olefines to the sulfhydryl group, has been applied to trap the highly unstable acrylamide, a substance formed during food processing and classified as a probable human carcinogen, simply by using cysteine or other small thiols. The reaction catalyzed by butylamine leads to a derivative that even though devoid of UV absorption can be detected by CE using

capacitively coupled contactless conductivity detector [45]. Often the derivatization of unstable inorganic species must be addressed also to improve detection sensitivity with respect to the original species by introducing a chromophore or fluorophore as in the case of cyanide ions using 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) for CE-LIF determination in apples seeds [46], and using BODIPY dyes to quantitate nitric oxide (NO) in biological systems [42,43]. The latter is a gaseous signaling compound whose distribution within and outside the cells is considered as an important parameter affecting physiological processes as neurotransmission and vasodilation. To trap and stabilize intra- and extracellular nitric oxide content, two reactive BODIPY dyes were used by considering that they would have opposite behavior in term of distribution within and outside the cell *i.e.*, while the reagent addressed to trap intracellular nitric oxide must be membrane-permeable to gain the interior of the cell, the other one must be distributed only in the outside environment (Fig. 2D,E) [42,43].

Aldehydes are very reactive carbonyl compounds that can be produced in biological systems and because of their oxidative capacity as well as ability to bind to numerous biomolecules, are regarded as biomarkers for the prediction and the early diagnosis of diseases related to oxidative stress [47]. Because of their volatility and poor stability, the determination in complex matrices is challenging; a CE approach is based on derivatization aiming to trap the analytes by conversion to stable compounds with improved detectability. 2-Thiobarbituric acid reacts with saturated aldehydes giving the adduct at the 5 position of the ring (Table SM2). Under certain conditions the latter can undergo autoxidation and subsequent Michael addition [48], thus derivatization should be carried out to preserve the stability of the primary adduct, an electroactive compound detectable by CE using the amperometric detector [49].

Reagents useful to stabilize aldehydes and, at the same time, improving the detectability are DNPH [17,19], NPHNA [20], 4-hydrazinobenzoic acid [50], and fluorescein 5-thiosemicarbazide (FTZ) [51]. Using 4-hydrazinobenzoic acid, no pH adjustment of the reaction mixture was necessary for derivatization, whereas the reactivity of FTZ is optimal at pH 9, where the electronic density of the nitrogen is high enough for the nucleophilic attack to the carbon atom of the aldehyde [51].

Owing to the fast autoxidation of thiols to disulfides the derivatization of the sulfhydryl function is necessary for reliable quantitation; among the different approaches, labeling the aliphatic thiols with reactive BODIPY dyes has been recently proposed in successful applications using CE-LIF (Fig. 1B, C) [40,41].

2.4. Derivatization for chiral separations

Capillary electrophoresis is frequently used for chiral separations in pharmaceutical, food, and bio-samples [52–54]. Enantioseparation of AAs has always received considerable attention since, even though in nature only small quantities of *D*-AAs can be found, they are relevant in several functions of living organism playing physiological roles in human health [55–57]. Derivatization addressed to improve detectability is often performed using achiral reagents; to achieve the enantioseparation a suitable chiral selector must be supplemented to the BGE. Interestingly, derivatization can be useful for the indirect approach of enantioresolution where the conversion of the couple of enantiomers to the corresponding diastereoisomers, allows for chiral separation to be obtained in achiral environment. Converting enantiomers into diastereoisomers to obtain enantioseparations, is not often applied however it is a useful approach when avoiding the addition of the chiral selector to the BGE is preferable, as in CE-MS. The enantiomerically pure reagent (+)-1-(9-fluorenyl)ethyl chloroformate ((+)-FLEC), a chiral FMOCl analog, has been applied in analysis of a series of AAs for the targeted metabolomic study in cerebrospinal fluid by CE-MS [58]. The indirect chiral separation of AAs can also be obtained by derivatization using OPA and the enantiomerically

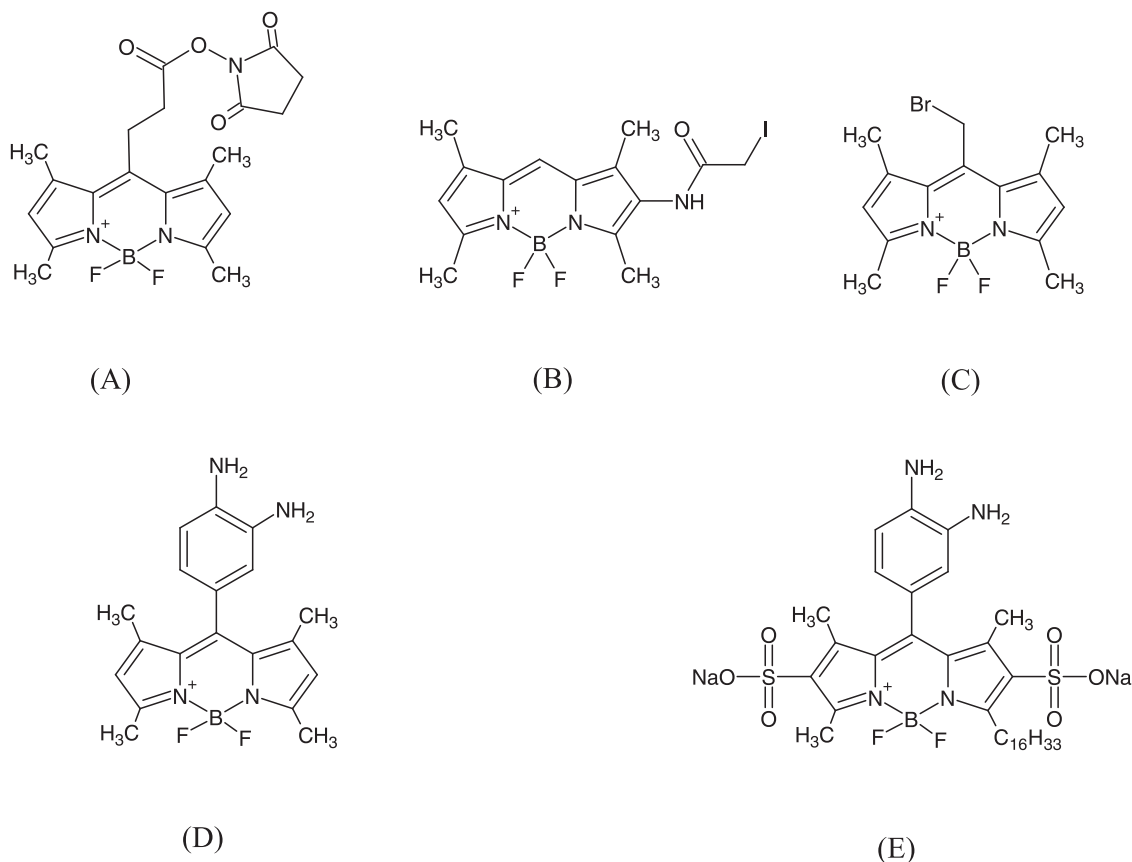


Fig. 2. BODIPY dyes used for derivatization of catecholamines (A), thiols (B, C) and nitric oxide (D, E). (A) 1,3,5,7-Tetramethyl-8-(*N*-hydroxysuccinimidyl propionic ester)-difluoroboradiaza-*s*-indacene. (B) *N*-(4,4-Difluoro-1,3,5,7-tetramethyl-3a,4a-diaza-*s*-indacene-2-yl) iodoacetamide. (C) 1,3,5,7-Tetramethyl-8-bromomethyl-difluoroboradiaza-*s*-indacene. (D) 1,3,5,7-Tetramethyl-8-(3',4'-diaminophenyl)-difluoroboradiaza-*s*-indacene. (E) Disodium 2,6-disulfonate-1,3-dimethyl-5-hexadecyl-8-(3,4-diaminophenyl) - 4,4'-difluoro-4-bora-3a,4a-diaza-*s*-indacene.

pure thiol NAC. Improved selectivity can be obtained by supplementing the running buffer with cyclodextrins (CDs) allowing chiral analysis of proteinogenic [59] and non-proteinogenic amino acids (*i.e.*, theanine) in complex mixtures with simultaneous enantioseparation of chiral catechins in green tea samples (Fig. 1) [13]. Interestingly, in some specific applications addressed to the chiral separation of AAs, it was found that small amounts of CDs as the chiral selector supplemented to the BGE, are compatible with CE-MS. To improve the interaction of small and highly polar analytes with the CD cavity for enhancing enantioselectivity, the derivatization was found to be beneficial. As observed by NMR studies, the FMOC-derivatives of 3-hydroxyaspartate isomers, establish improved enantiodiscriminating interactions with the β -CD hydrophobic cavity, with respect to those of the underivatized enantiomers [8]. FMOC-AAs enantiomeric derivatives could also be resolved in CE by supplementing the BGE with γ -CD [9,15] chiral ionic liquids [9], and β -CD in CD-MEKC conditions [10]; heptakis(2,3,6-tri-*O*-methyl)- β -CD (TM- β -CD) allowed the chiral resolution of pregabalin (an anticonvulsant/analgesic drug) derivatized with DNS [60].

2.5. In-capillary derivatization

One of the most attractive features of CE is the operational flexibility that allows to perform electrokinetic or hydrodynamic sample injections, carry out polarity switching, apply pressure during the electrophoretic runs (up to 12–13 bar), *etc.* Using the commercially available instrumentation and software it is possible to develop tailored strategies to perform on-line preconcentration [61], and on-line enzymatic reactions [5]. Similarly, derivatization can potentially be performed in-capillary by electrophoretically mixing the reagent/s and the sample/s once sequentially injected. The major advantages of in-capillary

derivatization are that only a small amount of reagent is necessary, the sample dilution is limited to a minimum and, as in the case of unstable derivatives (*e.g.*, the isoindole obtained by derivatizing AAs with OPA/NAC [59]), the time from product formation to detection is very short, allowing minimum loss from degradation. Fast derivatization reactions are necessary for the in-capillary approach and some recent examples in analysis of different matrices (food, biological samples) have been shown. Potassium ferricyanide was supplemented to an alkaline BGE as the oxidizing reagent for the in-capillary conversion of morphine and its metabolite to the highly fluorescent dimer products [62,63]. The combination of NDA/cyanide [64], the use of phthalic anhydride (PA) [65], and NBD-Cl [25], allowed determination of AAs and biogenic amines whereas fluorescamine [66] was applied for in-capillary derivatization of adamantane drugs by MEKC (Table 1 and Table 2).

2.6. Derivatization for detection by mass spectrometry

In many cases the derivatization is not necessary when MS is used for detection since an adequate ionization is directly achieved for many of the analytes [67]. Derivatization may still be necessary if enantioseparation must be obtained [8,58], and when introduction of an ionizable moiety in the analyte is beneficial for selectivity and/or sensitivity [1]. To this regard it is worth mentioning a strategy applied in the analysis of various types of metabolites by multifunctional derivatization involving two steps using: (i) 3-(diethylamino) propionyl chloride, followed by (ii) *N,N*-diethylethylenediamine. The procedure allows the introduction of the tertiary amine tag in molecules with hydroxy or carboxyl groups, establishing very general CE-MS conditions (5 mM ammonium formate/methanol, pH 2.5) using positive ionization, for the determination of the major metabolites in mammalian

cells [68]. Positive ionization in CE-MS can be also favorable to circumvent corona discharge reactions, using acidic BGE; under these conditions the analysis of fatty acids is possible only by derivatization introducing positively charged moieties [69].

The separation of neutral compounds in CE is approached by MEKC, which by employing non-volatile BGE is not fully compatible with MS detection. Therefore, in the case of separating neutral compounds in CE-MS, derivatization is employed to introduce electrical charge, such as in the case of carbohydrate analysis. This topic will be addressed in a later section.

3. Proteins and peptides

The UV absorption of peptide bond (up to 220 nm) and the presence of aromatic AAs, allow for the direct UV detection of most proteins, but the achieved sensitivity is often not suitable for CE applications such as in biomarkers quantification which requires to reach concentration levels in the nM range. Higher response can be obtained by profit of the presence in many proteins/peptides of Trp, Tyr and Phe residues thanks to their laser-induced fluorescence emission. However, the excitation wavelength is in the deep-UV, requiring lasers whose availability and affordability can be a problem [70,71]. Derivatization of peptides and proteins addressed to CE analysis is usually performed to introduce fluorescent tags to the *N*-terminus. A further approach is based on using fluorophores establishing noncovalent interactions with the target proteins [72].

Some specific considerations regarding the derivatization of proteins and peptides are the following. In proteins and peptides because of the presence of more than two reactive functions per molecule, multiple labeling could result in differently tagged compounds. As an example, trypsin inhibitors (from soybean) possess from three to six amine groups and when derivatized with Alexa Fluor 488, multi-tagged species are produced according to the different reactivity of the primary amine groups providing peak broadening in CZE analysis [73]. An intensive research is addressed to the evaluation of fibrinogenic proteins as amyloid β (A β 1–42) and other A β peptides [73–75] and alpha-synuclein [76] in cerebrospinal fluid focusing on the replacement of antibody-based methods. The lack or the limited availability of isotopically labeled peptides, makes MS-based approaches poorly affordable, prompting the applications toward the CE-LIF upon suitable derivatization. Fluorescent labels as FluoProbe 488 NHS (a succinimidyl ester) [74], FITC, CBQCA, fluorescein-NHS and others, have been successfully used; among them, NBD-F provided the best performance in terms of sensitivity, easiness of derivatization process, and peak shape under capillary gel electrophoresis (CGE) separation conditions in analysis of alpha-synuclein [76]. To achieve the sensitivity necessary for applications on cerebrospinal fluid, pre-concentration strategies based on proper selection of organic BGE at high concentration [73], large-volume sample stacking with an electroosmotic flow pump [74], magnetic immuno-capture [75], sample pre-concentration based on C8 reversed-phase monolith integrated in microchip devices [77], were adopted.

Large biological assemblies like bacteria, extracellular vesicles (EVs), and viruses, all playing important roles in physiological and physio-pathological conditions, possess electrophoretic mobility and can be subjected to CZE experiments for characterization/separation. Detection-oriented derivatization of these species is carried out by labeling the amine groups of the proteins/peptides on the particles surface (bacteria or released lipopolysaccharides) [78,79], or by using reagents able to passively diffuse inside the assembly to covalently bound the intracellular proteins (EVs analysis) [80]. In the purity assessment of adeno-associated virus (AAV) used for gene therapy, the capsid proteins (primary amine groups) were derivatized with a pyrylium dye (Chromoem P503) [81], or 3-(2-furoyl) quinoline-2-carboxaldehyde (FQ) [82]. The developed methods allowed the quantitation of proteins subunits (VP1–3) in the presence of impurities and

degradation products, achieving sensitivity from 100- to 1000-fold better than that of UV detection [81,82]. The main characteristics of the CE method addressed to proteins/peptides analysis are summarized in Table 3.

4. Carbohydrates

CE as a high-resolution analytical-scale separation technique has gained a prominent position in glycoscience for mapping glycopeptides as well as in carbohydrate characterization for achieving information of biomedical significance and in quality control of biopharmaceutical manufacturing [3]. The main applications of CE in glycomics deal with *N*-glycosylation assessment by means of the analysis of the intact proteins, their fragments, glycans and saccharides. *N*-glycans are conventionally released by the enzymatic cleavage using Peptide-*N*-Glycosidase F (PNGase F) of the glycoproteins; the subsequent analysis of the carbohydrates is challenging because of the lack of significant chromophores/fluorophores and for the poor ionization efficiency, making both optical and MS detection not suitable for the direct sensitive determination. In addition, except for acidic sugars, most saccharides are neutral molecules and their separation by CZE requires the application of specific strategies [3,4,83–86]. Derivatization, as in the case of permethylation, allows the stabilization of the compounds forming methyl ethers. Focusing on the CE analysis, derivatization would label the released carbohydrates introducing tags enhancing optical responses and providing the charge resulting in favorable electrophoretic behavior together with better ionization efficiency when MS detection is applied [3,84].

The two major approaches in carbohydrate labeling are (i) reductive amination and (ii) hydrazone formation (Fig. 3). 8-Aminopyrene-1,3,6-trisulfonic acid (APTS) is one of the first dyes used (Fig. 4A), suitable for CE-LIF and compatible with the argon ion laser. The hydrolytically unstable Schiff bases produced by the reaction of APTS with the saccharides need to be reduced by sodium cyanoborohydride [87,88] or 2-picoline borane complex [89,90], to give stable derivatives. APTS followed by reductive amination has been applied in several applications such as in CGE-LIF for *N*-glycan characterization of monoclonal antibody therapeutics [87], milk oligosaccharides [88] and markers of stem cells [89]. In multicapillary modality, the throughput of the separation system was significantly increased allowing the simultaneous analysis of 12 samples (human milk oligosaccharides) to be performed [88]. The labeling procedure using APTS has shown to be automatable in an original microfluidic droplet platform [91] and by using a commercially available CE instrument. In the latter application, the CE capillary works as a microreactor for all the derivatization steps (APTS tagging and reduction with sodium cyanoborohydride in acetic acid) performed by hydrodynamic introduction of the reagents. Mixing of the plugs is done by transverse diffusion of laminar flow profile, then the separation of the labelled *N*-glycans is performed by the application of the electric field through the same fused-silica capillary. The method was optimized for the analysis of glycans of IgG and rituximab released offline; the promising results sets the conditions for developing the digestion online of the glycoproteins, prior to in-capillary derivatization [92]. With the aim at improving the performances of APTS as a fluorescent tag, facilitating the progresses in glycomics, original fluorescent dyes suitable for CE-LIF having sulfonamides functions, have been developed with bright fluorescence and multiple negative charge [93,94].

As an alternative to reductive amination, the derivatization of carbohydrate by hydrazine reagents is often applied (Fig. 3B) with some interesting advantages such as the stability of the obtained hydrazones, and the higher reaction yield achieved avoiding the reduction step that could produce conversion of the saccharides to the corresponding alcohols [89,95]. Commercially available reagents as Turquoise™ and Cascade Blue (CBH) (8-(2-hydrazino-2-oxoethoxy)pyrene-1,3,6-trisulfonic acid) (Fig. 4B), are suitable for derivatization followed by CE-LIF with argon ion laser. CBH has shown a significantly higher yield in

Table 3
Derivatization of proteins and peptides.

Protein/peptide	Sample	Reagent	CF mode	Reaction Conditions	Detection	LOD	ref
HSA ¹⁾ , β -lactoglobulin, transferrin	Standard	Squarylium boronics	-Pre-capillary -In-capillary	-Water solution -Ammonium phosphate buffer pH 9.8 (online)	LIF 635 nm	~0.1 μ M	72
Ferritin, corticotropin-releasing factor	standard	Alexa Fluor 488 TFP ester	MicrochipCE In-channel CZE	-bicarbonate pH 9.9 -r.t., 30 min -phosphate pH 7.4 -r.t., 2 h	LIF 488 nm	n.r.	77
Amyloid A β 1–42 Trypsin Ovalbumin	standard	-FluoProbe 488 NHS -Alexa Fluor 488 -Fluorescein 488, Alexa-488, FP-488, CF-488 Fluoroprobe 488			LIF 488 nm	n.r.	73
Amyloid peptides	Cerebrospinal fluid		CZE In-capillary preconcentration CGE ²⁾	-borate pH 10.5 -r.t., 2 min	LIF 488 nm	0.2–0.4 nM	74, 75
Alfa-sinuclein	standard	NBD-F		-borate pH 8.0 -30 °C, 1.5 h -PBS ³⁾	LIF 488 nm	< 0.5 μ M	76
Bacteria: <i>E. coli</i> , <i>B. licheniformis</i> , <i>B. subtilis</i>	Bacteria cultures	FTTC	MEKC	-r.t., 12 h	LIF 473 nm	1.6 – 2.8 (x10 ⁶) CFU ⁴⁾ /mL	78
Viral proteins	Adeno-associated virus	Chromoem TM P503	CE-SDS	-bicarbonate pH8.3 -DTT ⁵⁾ /SDS	LIF 488 nm	0.8 ng/mL	81
Viral proteins	Adeno-associated virus	FQ	CE-SDS	-93 °C, 8 min -denaturation -cyanide ions	LIF 488 nm	3.9 \times 10 ⁹ TP ⁶⁾ /mL	82
Extracellular vesicle (EVs)	Bovine milk	6-CFSE	CZE	-70 °C, 10 min -PBS	LIF 488 nm	8 \times 10 ⁹ EV/mL	80
Lipopolysaccharides	<i>Klebsiella pneumoniae</i>	FTTC	CZE	-37 °C, 2 h -borax -50 °C, 1.5 h	LIF 488 nm	1.32 ng/mL	79

1) HSA, Human serum albumin; 2) CGE, Capillary gel electrophoresis; 3) PBS, phosphate borate saline buffer; 4) CFU, colony-forming unit; 5) DTT, 1,4-Dithiothreitol; 6) TP, Total proteins. Other symbols and abbreviations as in Table 1 and Table 2.

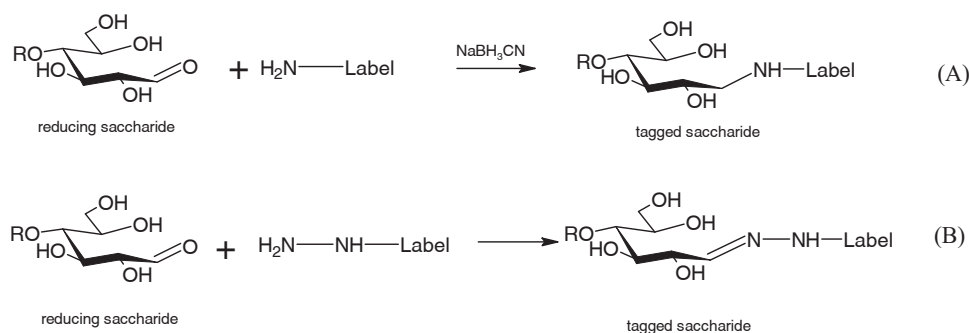


Fig. 3. Scheme of labeling reactions: (A) reductive amination and (B) hydrazone formation. Modified from [98], with permission.

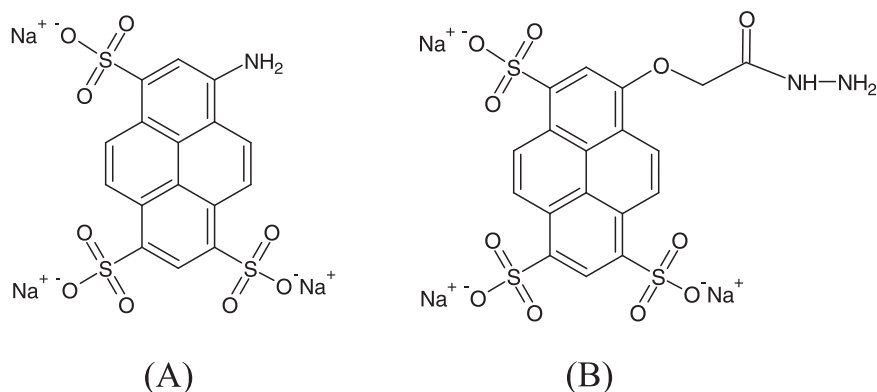


Fig. 4. Trisulfonate derivatives for saccharides labeling: (A) APTS, (B) 8-(2-hydrazino-2-oxoethoxy)pyrene-1,3,6-trisulfonic acid (CBH). Modified from [95], with permission.

labeling the *N*-glycans released from polyclonal human immunoglobulin (hIgG) and bovine ribonuclease B (RNase B) compared to APTS [95]. APTS-labeling oriented to CE-MS allows to achieve good sensitivity in carbohydrate analysis, and the negative ion mode applied is beneficial for structural identification thanks to the simpler spectra produced with respect to those obtained in positive mode [96]. Nevertheless, negative ionization is inherently less sensitive than the positive one [84]. Thus, cationic tags can be a useful alternative for derivatization of *N*-glycans and standard oligosaccharides via reductive amination. Mono-cationic reagents as (2-aminoethyl)trimethylammonium chloride gave multiple products when employed for *N*-glycans labeling. The hydrazone analog *i.e.*, (hydrazinocarbonylmethyl)-trimethylammonium chloride did not require the reduction step, and the obtained derivatives resulted to be more stable; however, the single positive charge of the obtained derivatives makes it not suitable for labeling acidic glycans [97], thus as an alternative, a hydrazine peptide rich in histidine residues has been proposed [98]. Multi-cationic reagents [99,100] have shown to be promising for analysis of oligosaccharides by CE-MS whereas they are less suitable in CE-LIF, because of a limited labeling efficiency due to the lower reactivity compared to APTS. Combining the permanent multi-cationic charge and hydrazide moiety, non-reductive amination for CE-MS analysis of glycans has been recently proposed using phosphonium salts such as (4-hydrazidebutyl) triphenylphosphonium bromide (P₄HZD), as an originally developed dye showing to provide higher sensitivity in comparison to that achieved using the commercially available Girard's reagent P [101].

5. Conclusion

The derivatization in CE is a current and active analytical approach with specific uniqueness among the separation techniques in liquid phase, as in the field of automated methods and in-capillary labeling

reactions. A further focus is also addressed to the LIF-oriented derivatization *e.g.*, by employing commercially available reagents developed for fluorescence imaging techniques, which can contribute to improve the limited sensitivity of the conventional CE detection.

The optimization of a derivatization procedure is a critical step in the whole analytical method affecting all the key validation parameters. Reaction yield mainly influences the sensitivity; precision and accuracy are highly dependent on the stability of the derivatives, thus the balance between reactivity and hydrolysis/degradation of the derivatization reagent and products should be carefully assessed. The development of a derivatization procedure requires adequate chemical knowledge on the reaction mechanisms to carry out a careful investigation on the optimization parameters (reactant/analyte ratio, temperature, reaction time, pH of the reaction mixture and its composition, *etc.*). In this regard, chemometric approaches based on design of experiments and quality by design, that appear to be underutilized in this field, could be a valuable contribution.

CRediT authorship contribution statement

Roberto Gotti: Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Benedetta Pasquini:** Data curation, Writing – original draft. **Serena Orlandini:** Writing – original draft. **Sandra Furlanetto:** Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jpba.2023.100003](https://doi.org/10.1016/j.jpba.2023.100003).

References

- S. Görög, Derivatization of Analytes, in: P.J. Worsfold, A. Townshend, C.F. Poole (Eds.), *Encyclopedia of Analytical Science*, 2nd edition., Elsevier, Oxford, 2019, pp. 263–272, <https://doi.org/10.1016/B978-0-12-409547-2.14229-5>
- A. Wuethrich, J.P. Quirino, Derivatization for separation and detection in capillary electrophoresis (2015–2017), *Electrophoresis* 39 (2018) 82–96, <https://doi.org/10.1002/elps.201700252>
- G. Lu, C.L. Crihfield, S. Gattu, L.M. Veltri, L.A. Holland, Capillary electrophoresis separations of glycans, *Chem. Rev.* 118 (2018) 7867–7885, <https://doi.org/10.1021/acs.chemrev.7b00669>
- V. Mantovani, F. Galeotti, F. Maccari, N. Volpi, Recent advances in capillary electrophoresis separation of monosaccharides, oligosaccharides, and polysaccharides, *Electrophoresis* 39 (2018) 179–189, <https://doi.org/10.1002/elps.201700290>
- S. Huang, P. Paul, P. Ramana, E. Adams, P. Augustijns, A. Van Schepdael, Advances in capillary electrophoretically mediated microanalysis for on-line enzymatic and derivatization reactions, *Electrophoresis* 39 (2018) 97–110, <https://doi.org/10.1002/elps.201700262>
- T. Kaneta, Laser-induced fluorimetry for capillary electrophoresis, *Chem. Rec.* 19 (2019) 452–461, <https://doi.org/10.1002/tcr.201800051>
- A. Jámor, I. Molnár-Perl, Amino acid analysis by high-performance liquid chromatography after derivatization with 9-fluorenylmethoxycarbonyl chloride. Literature overview and further study, *J. Chromatogr. A* 1216 (2009) 3064–3077, <https://doi.org/10.1016/j.chroma.2009.01.068>
- M. Liu, H. Zhao, Z. Zhang, X. Li, L. Mao, R. Zhang, Y. Wang, Derivatization reagent-assisted enantioseparation of 3-hydroxyaspartate with two chiral centers in rat cerebrospinal fluid by capillary electrophoresis-mass spectrometry, *Anal. Chim. Acta* 1047 (2019) 257–266, <https://doi.org/10.1016/j.aca.2018.09.070>
- M. Greño, M.L. Marina, M. Castro-Puyana, Effect of the combined use of γ -cyclodextrin and a chiral ionic liquid on the enantiomeric separation of homocysteine by capillary electrophoresis, *J. Chromatogr. A* 1568 (2018) 222–228, <https://doi.org/10.1016/j.chroma.2018.07.023>
- A. Prior, G. Coliva, G.J. de Jong, G.W. Somsen, Chiral capillary electrophoresis with UV-excited fluorescence detection for the enantioselective analysis of 9-fluorenylmethoxycarbonyl-derivatized amino acids, *Anal. Bional. Chem.* 410 (2018) 4979–4990, <https://doi.org/10.1007/s00216-018-1148-x>
- L.D. Casto-Bogges, M. Golzar, A.L. Butterworth, R.A. Mathies, Optimization of fluorescence labeling of trace analytes: application to amino acid biosignature detection with pacific blue, *Anal. Chem.* 94 (2022) 1240–1247, <https://doi.org/10.1021/acs.analchem.1c04465>
- J.P. Cattalini, V.S. Mourinho, S.E. Lucangioli, Development and validation of a novel sensitive UV-direct capillary electrophoresis method for quantification of alendronate in release studies from biomaterials, *Electrophoresis* 39 (2018) 616–619, <https://doi.org/10.1002/elps.201700362>
- J. Fiori, B. Pasquini, C. Caprini, S. Orlandini, S. Furlanetto, R. Gotti, Chiral analysis of theanine and catechin in characterization of green tea by cyclodextrin-modified micellar electrokinetic chromatography and high performance liquid chromatography, *J. Chromatogr. A* 1562 (2018) 115–122, <https://doi.org/10.1016/j.chroma.2018.05.063>
- M. Liu, L. Chen, X. Li, J. Meng, Y. Bai, H. Liu, Separation and determination of 3-hydroxyaspartate by online concentration capillary electrophoresis/laser induced fluorescence with microwave-assisted derivatization, *J. Sep. Sci.* 44 (2021) 3646–3653, <https://doi.org/10.1002/jssc.202100398>
- Y.-H. Hsieh, F.-Y. Liao, Y.-H. Yang, J.-R. Weng, S.-H. Chen, C.-H. Feng, Enantioselective determination of aspartate and glutamate in biological samples by ultrasonic-assisted derivatization coupled with capillary electrophoresis and linked to Alzheimer's disease progression, *J. Chromatogr. A* 1550 (2018) 68–74, <https://doi.org/10.1016/j.chroma.2018.03.041>
- P. Emonts, H.T. Avohou, P. Hubert, E. Ziemons, M. Fillet, A. Dispas, Optimization of a robust and reliable FITC labeling process for CE-LIF analysis of pharmaceutical compounds using design of experiments strategy, *J. Pharm. Biomed. Anal.* 205 (2021) 114304, <https://doi.org/10.1016/j.jpba.2021.114304>
- J. He, J. Liu, Y. Liu, Z. Liyin, X. Wu, G. Song, Y. Hou, R. Wang, W. Zhao, H. Sun, Trace carbonyl analysis in water samples by integrating magnetic molecular imprinting and capillary electrophoresis, *RSC Adv.* 11 (2021) 32841, <https://doi.org/10.1039/d1ra05084b>
- S. Fang, Y. Liu, J. He, L. Zhang, Z. Liyin, X. Wu, H. Sun, J. Lai, Determination of aldehydes in water samples by coupling magnetism-reinforced molecular imprinting monolith microextraction and non-aqueous capillary electrophoresis, *J. Chromatogr. A* 1632 (2020) 461602, <https://doi.org/10.1016/j.chroma.2020.461602>
- Y. Li, H. Sun, J. Lai, X. Chang, P. Zhang, S. Chen, Determination of carbonyl pollutants adsorbed on ambient particulate matter of type PM_{2.5} by using magnetic molecularly imprinted microspheres for sample pretreatment and capillary electrophoresis for separation and quantitation, *Microchim. Acta* 185 (122) (2018), <https://doi.org/10.1007/s00604-017-2650-0>
- Y.-J. Fu, L. Chen, X.-F. Guo, H. Wang, Determination of formaldehyde in single cell by capillary electrophoresis with LIF detection, *Electrophoresis* 40 (2019) 1027–1033, <https://doi.org/10.1002/elps.201800399>
- R. Gotti, J. Fiori, S. Bosi, G. Dinelli, Field-amplified sample injection and sweeping micellar electrokinetic chromatography in analysis of glyphosate and aminomethylphosphonic acid in wheat, *J. Chromatogr. A* 1601 (2019) 357–364, <https://doi.org/10.1016/j.chroma.2019.05.013>
- J. Oliveira Fernandes Mantoanelli, L. Moreira Gonçalves, E. Alves Pereira, Dansyl chloride as a derivatizing agent for the analysis of biogenic amines by CZE-UV, *Chromatographia* 83 (2020) 767–778, <https://doi.org/10.1007/s10337-020-03896-x>
- T. Kaneta, Determination of polyamines by capillary electrophoresis using salicylaldehyde-5-sulfonate as a derivatizing reagent, in: R. Alcázar, A.F. Tiburcio (Eds.), *Methods in Mol. Biol. Polyamines: Methods and protocols*, vol. 1694, Springer Science and Business Media LLC, 2018, pp. 61–68, https://doi.org/10.1007/978-1-4939-7398-9_5
- A. Rodat-Boutonnet, P. Naccache, A. Morin, J. Fabre, B. Feurer, F. Couderc, A comparative study of LED-induced fluorescence and laser-induced fluorescence in SDS-CGE: application to the analysis of antibodies, *Electrophoresis* 33 (2012) 1709–1714, <https://doi.org/10.1002/elps.201200132>
- Y.-L. Yu, M.-Z. Shi, S.-C. Zhu, J. Cao, Rapid stacking of amino acids in soybean and *Dendrobium officinale* by on-capillary sandwich derivatization in capillary electrophoresis, *Food Res. Int.* 162 (2022) 112071, <https://doi.org/10.1016/j.foodres.2022.112071>
- R. Gotti, J. Fiori, S. Furlanetto, S. Orlandini, M. Candela, S. Franzellitti, Assessment of bioaccumulation of glyphosate and aminomethylphosphonic acid in marine mussels using capillary electrophoresis with light-emitting diode-induced fluorescence detection, *J. Chromatogr. A* 1681 (2022) 463452, <https://doi.org/10.1016/j.chroma.2022.463452>
- R.H.G. Wathsala, E.C. Folgueras, L. Iuffrida, M. Candela, R. Gotti, J. Fiori, S. Franzellitti, Glyphosate and its breakdown product AMPA elicit cytoprotective responses in haemocytes of the Mediterranean mussel (*Mytilus galloprovincialis*), *Environ. Toxicol. Pharmacol.* 96 (2022) 103997, <https://doi.org/10.1016/j.etap.2022.103997>
- F. Huo, T. Wan, Y. Wang, Y. Liu, P.G. Karmaker, X. Yang, Enhanced light-emitting diode induced fluorescence detection system with capillary electrophoresis, *J. Chromatogr. A* 1619 (2020) 460935, <https://doi.org/10.1016/j.chroma.2020.460935>
- Q. Zheng, Z. Guo, Y. Chen, Capillary array electrophoresis imaging of biochemicals in tissue sections, *Talanta* 240 (2022) 123183, <https://doi.org/10.1016/j.talanta.2021.123183>
- H. Ji, X. Zhang, F. Yang, J. Wang, H. Yuan, D. Xiao, Sensitive determination of L-tryptophan in dairy products by capillary electrophoresis with in-capillary optical fiber light emitting diode-induced fluorescence detection, *Anal. Methods* 10 (2018) 2211–2216, <https://doi.org/10.1039/C7AY02356A>
- T. Toyooka, Y. Watanabe, K. Imai, Reaction of amines of biological importance with 4-Fluoro-7-nitrobenzo-2-oxa-1,3-diazole, *Anal. Chim. Acta* 149 (1983) 305–312, [https://doi.org/10.1016/S0003-2670\(00\)83187-6](https://doi.org/10.1016/S0003-2670(00)83187-6)
- M.F. Mora, F. Kehl, E. Tavares da Costa, N. Bramall, P.A. Willis, Fully automated microchip electrophoresis analyzer for potential life detection missions, *Anal. Chem.* 92 (2020) 12959–12966, <https://doi.org/10.1021/acs.analchem.0c01628>
- S. Mikkonen, L. Josefsson, M.E.L. Mäkinen, V. Chotteau, Å. Emmer, Capillary and microchip electrophoresis method development for amino acid monitoring during biopharmaceutical cultivation, *Biotechnol. J.* 17 (2022) 2100325, <https://doi.org/10.1002/biot.202100325>
- A. Celá, A. Mádr, M. Ješeta, J. Žáková, I. Crha, Z. Glatz, Study of metabolic activity of human embryos focused on amino acids by capillary electrophoresis with light-emitting diode-induced fluorescence detection, *Electrophoresis* 39 (2018) 3040–3048, <https://doi.org/10.1002/elps.201800265>
- L. Perquis, H.Y. Ta, V. Ong-Meang, A. Poinso, F. Collin, V. Poinso, F. Couderc, Capillary electrophoresis/visible-LED induced fluorescence of tryptophan: what's new? *Electrophoresis* 40 (2019) 2342–2348, <https://doi.org/10.1002/elps.201900058>
- H.Y. Ta, L. Perquis, C. Sarazin, B. Guiard, V. Ong-Meang, F. Collin, F. Couderc, 3-(4-Carboxybenzoyl)quinoline-2-carboxaldehyde labeling for direct analysis of amino acids in plasma is not suitable for simultaneous quantification of tryptophan, tyrosine, valine, and isoleucine by CE/fluorescence, *Electrophoresis* 42 (2021) 1108–1114, <https://doi.org/10.1002/elps.202000263>
- C. Wu, Y. Sun, Y. Wang, W. Duan, J. Hu, L. Zhou, Q. Pu, 7-(Diethylamino)coumarin-3-carboxylic acid as derivatization reagent for 405 nm laser-induced fluorescence detection: A case study for the analysis of sulfonamides by capillary electrophoresis, *Talanta* 201 (2019) 16–22, <https://doi.org/10.1016/j.talanta.2019.03.093>
- T. Kowada, H. Maeda, K. Kikuchi, BODIPY-based probes for the fluorescence imaging of biomolecules in living cells, *Chem. Soc. Rev.* 44 (2015) 4953–4972, <https://doi.org/10.1039/C5CS00030K>
- L. Cao, L. Wu, H. Zhong, H. Wu, S. Zhang, J. Meng, F. Li, Analysis of neurotransmitter catecholamines and related amines in human urine and serum by chromatography and capillary electrophoresis with 1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl propionic ester)-difluoro-boradiazas-indacene, *Acta Chromatogr.* 34 (2022) 276–286, <https://doi.org/10.1556/1326.2021.00924>
- L. Cao, Q. Liang, T. Wei, Y. Shi, T. Deng, J. Meng, Chromatographic determination and in-situ cell imaging of thiol compounds based on a fluorogenic probe, *J. Chromatogr. A* 1577 (2018) 47–58, <https://doi.org/10.1016/j.chroma.2018.09.045>
- L. Cao, T. Wei, Y. Shi, X. Tan, J. Meng, Determination of D-penicillamine and tiopronin in human urine and serum by HPLC-FLD and CE-LIF with 1,3,5,7-tetramethyl-8-bromomethyl-difluoroboradiazas-indacene, *J. Liq. Chromatogr.* 41 (2018) 58–65, <https://doi.org/10.1080/10826076.2017.1348953>

- [42] H.-W. Yao, X.-F. Guo, H. Wang, Simultaneous determination of NO released inside and outside cells at the single-cell level using CE-LIF, *Anal. Sci.* 38 (2022) 913–916, <https://doi.org/10.1007/s44211-022-00105-7>
- [43] H.-W. Yao, X.-F. Guo, H. Wang, Simultaneous quantitation of intra- and extracellular nitric oxide in single macrophage RAW 264.7 cells by capillary electrophoresis with laser-induced fluorescence detection, *Anal. Chem.* 92 (2020) 11904–11911, <https://doi.org/10.1021/acs.analchem.0c02283>
- [44] S. Gonçalves, L. Molognoni, H. Dagher, R. Pimenta, R.B. Hoff, G.A. Mücke, L. Vitali, Simultaneous extraction/derivatization for the analysis of sulfite by capillary electrophoresis: A high-throughput reference method to meet the demand of seafood inspection, *Food Res. Int.* 161 (2022) 111780, <https://doi.org/10.1016/j.foodres.2022.111780>
- [45] S. Yang, Y. Li, F. Li, Z. Yang, F. Quan, L. Zhou, Q. Pu, Thiol-ene click derivatization for the determination of acrylamide in potato products by capillary electrophoresis with capacitively coupled contactless conductivity detection, *J. Agric. Food Chem.* 67 (2019) 8053–8060, <https://doi.org/10.1021/acs.jafc.9b01525>
- [46] D.B. Craig, M.S. Guimond, Analysis of cyanide using fluorogenic derivatization and capillary electrophoresis, *Food Chem.* 370 (2022) 131377, <https://doi.org/10.1016/j.foodchem.2021.131377>
- [47] N. Kishikawa, M.H. El-Maghraby, N. Kuroda, Chromatographic methods and sample pretreatment techniques for aldehydes determination in biological, food, and environmental samples, *J. Pharm. Biomed. Anal.* 175 (2019) 112782, <https://doi.org/10.1016/j.jpba.2019.112782>
- [48] H. Kosugi, K. Kikugawa, Reaction of thiobarbituric acid with saturated aldehydes, *Lipids* 21 (1986) 537–542, <https://doi.org/10.1007/BF02534048>
- [49] F. Yi, L.-Z. Liu, M.-J. Zhang, T.-T. Wang, J.-N. Ye, Q.-C. Chu, D.-P. Huang, Electrophoretic determination of formaldehyde in human urine: application to Alzheimer's disease, *Anal. Lett.* 51 (2018) 1358–1372, <https://doi.org/10.1080/00032719.2017.1378661>
- [50] L.F. de Lima, P.F. Brandão, T.A. Donegatti, R.M. Ramos, L. Moreira Gonçalves, A.A. Cardoso, E.A. Pereira, J.A. Rodrigues, 4-Hydrazinobenzoic acid as a derivatizing agent for aldehyde analysis by HPLC-UV and CE-DAD, *Talanta* 187 (2018) 113–119, <https://doi.org/10.1016/j.talanta.2018.04.091>
- [51] T. Wang, D. Luo, Z. Chen, Y. Qu, X. Ma, J. Ye, Q. Chu, D. Huang, Sensitive determination of aldehyde metabolites in exhaled breath condensate using capillary electrophoresis with laser-induced fluorescence detection, *Anal. Bioanal. Chem.* 410 (2018) 7203–7210, <https://doi.org/10.1007/s00216-018-1327-9>
- [52] B. Chankvetadze, Application of enantioselective separation techniques to bioanalysis of chiral drugs and their metabolites, *TrAC, Trends Anal. Chem.* 143 (2021) 116332, <https://doi.org/10.1016/j.trac.2021.116332>
- [53] S. Orlandini, G. Hancu, S. Zoltán-Istvánc, A. Modroiu, L.-A. Papp, R. Gotti, S. Furlanetto, New trends in the quality control of enantiomeric drugs: quality by design-compliant development of chiral capillary electrophoresis methods, *Molecules* 27 (2022) 7058, <https://doi.org/10.3390/molecules27207058>
- [54] S. Bernardo-Bermejo, E. Sánchez-López, M. Castro-Puyana, M.L. Marina, Chiral capillary electrophoresis, *TrAC, Trends Anal. Chem.* 124 (2020) 115807, <https://doi.org/10.1016/j.trac.2020.115807>
- [55] Y. Song, C. Xu, H. Kuroki, Y. Liao, M. Tsunoda, Recent trends in analytical methods for the determination of amino acids in biological samples, *J. Pharm. Biomed. Anal.* 147 (2018) 35–49, <https://doi.org/10.1016/j.jpba.2017.08.050>
- [56] G. Carenzi, S. Sacchi, M. Abbondi, L. Pollegioni, Direct chromatographic methods for enantioresolution of amino acids: recent developments, *Amino Acids* 52 (2020) 849–862, <https://doi.org/10.1007/s00726-020-02873-w>
- [57] C. Furman, M. Howsam, E. Lipka, Recent developments in separation methods for enantiomeric ratio determination of amino acids specifically involved in cataract and Alzheimer's disease, *TrAC, Trends Anal. Chem.* 141 (2021) 116287, <https://doi.org/10.1016/j.trac.2021.116287>
- [58] R.-C. Moldovan, E. Bodoki, A.-C. Servais, B. Chankvetadze, J. Crommen, R. Oprean, M. Fillet, Capillary electrophoresis-mass spectrometry of derivatized amino acids for targeted neurometabolomics-pH mediated reversal of diastereomer migration order, *J. Chromatogr. A* 1564 (2018) 199–206, <https://doi.org/10.1016/j.chroma.2018.06.030>
- [59] L.A. Kartsova, D.O. Moskvichev, In-capillary chiral derivatization of amino acids, *J. Anal. Chem.* 77 (2022) 618–624, <https://doi.org/10.1134/S1061934822050057>
- [60] H. Harnisch, Y.-H. Chien, G.K.E. Scriba, Capillary electrophoresis method for the chiral purity determination of pregabalin derivatized with dansyl chloride, *Chromatographia* 81 (2018) 719–725, <https://doi.org/10.1007/s10337-018-3495-3>
- [61] M.C. Breadmore, W. Grochoccki, U. Kalsoom, M.N. Alves, S.C. Phung, M.T. Rokh, J.M. Cabot, A. Ghiasvand, F. Li, A.I. Shalhan, A.S. Abdul Keyon, A.A. Alhusban, H.H. See, A. Wuethrich, M. Dawod, J.P. Quirino, Recent advances in enhancing the sensitivity of electrophoresis and electrochromatography in capillaries and microchips (2016–2018), *Electrophoresis* 40 (2019) 17–39, <https://doi.org/10.1002/elps.201800384>
- [62] S. Emara, W. Zarad, M. Kamal, A. Ali, Y. Aboulella, Sensitivity enhancement for direct injection capillary electrophoresis to determine morphine in human serum via in-capillary derivatization, *J. Chromatogr. Sci.* 57 (2019) 177–185, <https://doi.org/10.1093/chromsci/bmy092>
- [63] W. Zarad, A. Shawky, A. Ali, Y. Aboulella, M. Kamal, T. Masujima, S. Emara, H. El-Gendy, Field amplified sample stacking and in-capillary derivatization for forensic analysis of morphine and morphine-6-glucuronide in human urine by capillary electrophoresis, *Talanta Open* 3 (2021) 100041, <https://doi.org/10.1016/j.talo.2021.100041>
- [64] L. Denoroy, S. Parrot, Brain Glutamate Monitoring by Microdialysis and Separation Methods with a Special Focus on Capillary Electrophoresis with Laser-Induced Fluorescence Detection, in: S. Parrot, L. Denoroy (Eds.), *Biochemical Approaches for Glutamatergic Neurotransmission*. *Neuroinformatics*, vol 130, Humana Press, New York, 2018, pp. 395–429, https://doi.org/10.1007/978-1-4939-7228-9_13
- [65] Z. Ramezani, M. Safdarian, A.A. Ghadiri, Metal-coded hydrogel magnetic molecularly imprinted polymer for preconcentration and cleanup of sarcosine: Determination in urine coupled to on-column capillary electrophoresis, *Talanta* 230 (2021) 122309, <https://doi.org/10.1016/j.talanta.2021.122309>
- [66] P. Prapatpong, N. Nuchtavorn, M. Macka, L. Suntornasuk, In-capillary derivatization with fluorescamine for the rapid determination of adamantane drugs by capillary electrophoresis with UV detection, *J. Sep. Sci.* 41 (2018) 3764–3771, <https://doi.org/10.1002/jssc.201800591>
- [67] S. Della Posta, C. Fanali, V. Gallo, S. Fanali, Recent advances in the hyphenation of electromigration techniques with mass spectrometry, *TrAC, Trends Anal. Chem.* 157 (2022) 116800, <https://doi.org/10.1016/j.trac.2022.116800>
- [68] T. Huang, M. Armbruster, R. Lee, D.S. Hui, J.L. Edwards, Metabolomic analysis of mammalian cells and human tissue through one-pot two stage derivatizations using sheathless capillary electrophoresis-electrospray ionization-mass spectrometry, *J. Chromatogr. A* 1567 (2018) 219–225, <https://doi.org/10.1016/j.chroma.2018.07.007>
- [69] M.G.M. Kok, M.F. Mora, A.C. Noell, C.W. Parker, P.A. Willis, A novel and sensitive method for the analysis of fatty acid biosignatures by capillary electrophoresis-mass spectrometry, *Anal. Chem.* 94 (2022) 12807–12814, <https://doi.org/10.1021/acs.analchem.2c02716>
- [70] V. Kašička, Recent developments in capillary and microchip electroseparations of peptides (2019–mid 2021), *Electrophoresis* 43 (2022) 82–108, <https://doi.org/10.1002/elps.202100243>
- [71] F. Couderc, V. Ong-Meang, V. Poinso, Capillary electrophoresis hyphenated with UV-native-laser induced fluorescence detection (CE/UV-native-LIF), *Electrophoresis* 38 (2017) 135–149, <https://doi.org/10.1002/elps.201600248>
- [72] M.M. Sebaili, A.A. El-Shanawany, M.M. Baraka, L.M. Abdel-Aziz, Novel mono-functional and bifunctional boronic acid functionalized squarilium dyes as pre-column and on-column labels for protein analysis by capillary electrophoresis with laser induced fluorescence, *J. Chin. Chem. Soc.* 66 (2019) 179–187, <https://doi.org/10.1002/jccs.201800211>
- [73] M. Morani, M. Taverna, T.D. Mai, A fresh look into background electrolyte selection for capillary electrophoresis-laser induced fluorescence of peptides and proteins, *Electrophoresis* 40 (2019) 2618–2624, <https://doi.org/10.1002/elps.201900084>
- [74] C. Crosnier de Lassichère, T.D. Mai, M. Otto, M. Taverna, Online preconcentration in capillaries by multiple large-volume sample stacking: an alternative to immunoassays for quantification of amyloid beta peptides biomarkers in cerebrospinal fluid, *Anal. Chem.* 90 (2018) 2555–2563, <https://doi.org/10.1021/acs.analchem.7b03843>
- [75] T.D. Mai, P.C. Hauser, S. Descroix, C. Crosnier de Lassichère, M. Taverna, C. Smađija, In-capillary immuno-preconcentration with circulating bio-functionalized magnetic beads for capillary electrophoresis, *Anal. Chim. Acta* 1062 (2019) 156–164, <https://doi.org/10.1016/j.aca.2019.02.006>
- [76] A. Napp, V. Houbart, A. Demelene, M.P. Merville, J. Crommen, M. Dumoulin, G. Garraux, A.C. Servais, M. Fillet, Separation and determination of alpha-synuclein monomeric and oligomeric species using two electrophoretic approaches, *Electrophoresis* 39 (2018) 3022–3031, <https://doi.org/10.1002/elps.201800224>
- [77] V. Sahore, M. Sonker, A.V. Nielsen, R. Knob, S. Kumar, A.T. Woolley, Automated microfluidic devices integrating solid-phase extraction, fluorescent labeling, and microchip electrophoresis for preterm birth biomarker analysis, *Anal. Bioanal. Chem.* 410 (2018) 933–941, <https://doi.org/10.1007/s00216-017-0548-7>
- [78] W. Wang, H. Zhang, X. Yu, S. Zhang, Study of antagonism between some intestinal bacteria with high-speed micellar electrokinetic chromatography, *Electrophoresis* 42 (2021) 1196–1201, <https://doi.org/10.1002/elps.202000372>
- [79] R.R.X. Lim, F.M. Fung, H.-T. Feng, S.F.Y. Li, Analysis of lipopolysaccharides by coupling microscale solid-phase extraction with capillary electrophoresis-laser induced fluorescence, *Microchem. J.* 161 (2021) 105771, <https://doi.org/10.1016/j.microc.2020.105771>
- [80] M. Morani, T.D. Mai, Z. Krupova, P. Defrenaix, E. Multia, M.L. Riekkola, M. Taverna, Electrokinetic characterization of extracellular vesicles with capillary electrophoresis: A new tool for their identification and quantification, *Anal. Chim. Acta* 1128 (2020) 42–51, <https://doi.org/10.1016/j.aca.2020.06.073>
- [81] Z. Zhang, J. Park, H. Barrett, S. Dooley, C. Davies, M.F. Verhagen, Capillary electrophoresis-sodium dodecyl sulfate with laser-induced fluorescence detection as a highly sensitive and quality control-friendly method for monitoring adeno-associated virus capsid protein purity, *Hum. Gene Ther.* 32 (2021) 628–637, <https://doi.org/10.1089/hum.2020.233>
- [82] R.P. Fernandes, J.M. Escandell, A.C.L. Guerreiro, F. Moura, T.Q. Faria, S.B. Carvalho, R.J.S. Silva, P. Gomes-Alves, C. Peixoto, Assessing multi-attribute characterization of enveloped and non-enveloped viral particles by capillary electrophoresis, *Viruses* 14 (2022) 2539, <https://doi.org/10.3390/v14112539>
- [83] S. Fekete, D. Guillaume, P. Sandra, K. Sandra, Chromatographic, electrophoretic, and mass spectrometric methods for the analytical characterization of protein biopharmaceuticals, *Anal. Chem.* 88 (2016) 480–507, <https://doi.org/10.1021/acs.analchem.5b04561>
- [84] S. Gaunitz, G. Nagy, N.L.B. Pohl, M.V. Novotny, Recent advances in the analysis of complex glycoproteins, *Anal. Chem.* 89 (2017) 389–413, <https://doi.org/10.1021/acs.analchem.6b04343>
- [85] H. Kaur, J. Beckman, Y. Zhang, Z. Jian Li, M. Szigeti, A. Guttman, Capillary electrophoresis and the biopharmaceutical industry: Therapeutic protein analysis and characterization, *TrAC, Trends Anal. Chem.* 144 (2021) 116407, <https://doi.org/10.1016/j.trac.2021.116407>
- [86] C.D. Gutierrez-Reyes, P. Jiang, M. Atashi, A. Bennett, A. Yu, W. Peng, J. Zhong, Y. Mechref, Advances in mass spectrometry-based glycoproteomics: An update covering the period 2017–2021, *Electrophoresis* 43 (2022) 370–387, <https://doi.org/10.1002/elps.202100188>
- [87] C. Filep, M. Szigeti, R. Farsang, M. Habberger, D. Reusch, A. Guttman, Multilevel capillary gel electrophoresis characterization of new antibody modalities, *Anal. Chim. Acta* 1166 (2021) 338492, <https://doi.org/10.1016/j.aca.2021.338492>

- [88] D. Sarkozy, B. Borza, A. Domokos, E. Varadi, M. Szigeti, A. Meszaros-Matwiejuk, D. Molnar-Gabor, A. Guttman, Ultrafast high-resolution analysis of human milk oligosaccharides by multicapillary gel electrophoresis, *Food Chem.* 341 (2021) 128200, <https://doi.org/10.1016/j.foodchem.2020.128200>
- [89] P. Li, L. Wang, R. Guo, H. Feng, Y. Ji, S.Y. Lim, B.H. Ng, A.K. Carrasco Laserna, S. Khan, S.-M. Chen, S.F.Y. Li, Cross-identification of N-Glycans by CE-LIF using two capillary coatings and three labeling dyes, *Talanta* 239 (2022) 123061, <https://doi.org/10.1016/j.talanta.2021.123061>
- [90] C. Rossdam, S.A. Konze, A. Oberbeck, E. Rapp, R. Gerardy-Schahn, M. von Itzstein, F.F.R. Buettner, Approach for profiling of glycosphingolipid glycosylation by multiplexed capillary gel electrophoresis coupled to laser-induced fluorescence detection to identify cell-surface markers of human pluripotent stem cells and derived cardiomyocytes, *Anal. Chem.* 91 (2019) 6413–6418, <https://doi.org/10.1021/acs.analchem.9b01114>
- [91] T. Liénard-Mayor, C. Bricteux, A. Bendali, N.-T. Tran, A. Bruneel, M. Taverna, T.D. Mai, Lab-in-droplet: from glycan sample treatment toward diagnostic screening of congenital disorders of glycosylation, *Anal. Chim. Acta* 1221 (2022) 340150, <https://doi.org/10.1016/j.aca.2022.340150>
- [92] B. Yang, T.D. Mai, N.T. Tran, M. Taverna, In capillary labeling and online electrophoretic separation of N-glycans from glycoproteins, *J. Sep. Sci.* 45 (2022) 3594–3603, <https://doi.org/10.1002/jssc.202200340>
- [93] E.A. Savicheva, J. Seikowski, J.I. Kast, C.R. Grünig, V.N. Belov, S.W. Hell, Fluorescence assisted capillary electrophoresis of glycans enabled by the negatively charged auxochromes in 1-aminopyrenes, *Angew. Chem. Int. Ed.* 60 (2021) 3720–3726, <https://doi.org/10.1002/anie.202013187>
- [94] E.A. Savicheva, G.Y. Mitronova, L. Thomas, M.J. Böhm, J. Seikowski, V.N. Belov, S.W. Hell, Negatively charged yellow-emitting 1-aminopyrene dyes for reductive amination and fluorescence detection of glycans, *Angew. Chem. Int. Ed.* 59 (2020) 5505–5509, <https://doi.org/10.1002/anie.201908063>
- [95] J. Krenkova, F. Dusa, R. Cmelik, Comparison of oligosaccharide labeling employing reductive amination and hydrazone formation chemistries, *Electrophoresis* 41 (2020) 684–690, <https://doi.org/10.1002/elps.201900475>
- [96] C.M. Snyder, X. Zhou, J.A. Karty, B.R. Fonslow, M.V. Novotny, S.C. Jacobson, Capillary electrophoresis–mass spectrometry for direct structural identification of serum N-glycans, *J. Chromatogr. A* 1523 (2017) 127–139, <https://doi.org/10.1016/j.chroma.2017.09.009>
- [97] J. Krenkova, P. Bobal, J. Partyka, R. Cmelik, F. Foret, Investigation of a side reaction occurring during N-linked glycan labeling by cationic tags, *J. Chromatogr. A* 1570 (2018) 67–74, <https://doi.org/10.1016/j.chroma.2018.07.066>
- [98] J. Partyka, J. Krenkova, R. Cmelik, F. Foret, Multi-charged labeling of oligosaccharides and N-linked glycans by hexahistidine-based tags for capillary electrophoresis-mass spectrometry analysis, *J. Chromatogr. A* 1560 (2018) 91–96, <https://doi.org/10.1016/j.chroma.2018.05.030>
- [99] J. Krenkova, F. Dusa, R. Cmelik, Characterization of multi-cationic aminopyrene-based tag for oligosaccharide labeling by capillary electrophoresis with laser-induced fluorescence detection, *Electrophoresis* 42 (2021) 1333–1339, <https://doi.org/10.1002/elps.202100012>
- [100] J. Krenkova, M. Liskova, R. Cmelik, G. Vigh, F. Foret, Multi-cationic aminopyrene-based labeling tags for oligosaccharide analysis by capillary electrophoresis-mass spectrometry, *Anal. Chim. Acta* 1095 (2020) 226–232, <https://doi.org/10.1016/j.aca.2019.10.032>
- [101] Q. Ma, W. Wang, X. Yang, Y. Chen, Y. Liu, H. Chen, Y. Zhao, Development and application of a sensitive phosphonium-hydrazide oligosaccharide labelling reagent in capillary electrophoresis-electrospray ionization-mass spectrometry, *J. Chromatogr. A* 1680 (2022) 463409, <https://doi.org/10.1016/j.chroma.2022.463409>