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Determination of free amino acids and related compounds in amniotic fluid by capillary electrophoresis with contactless conductivity detection ☆

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Abstract

A capillary electrophoretic (CE) method with contactless conductivity detection (CCD) has been developed for the determination of free amino acids (AAs) in the amniotic fluid. Apart from 20 proteinogenic AAs, 12 other biogenic compounds have been identified including ethanolamine, choline, β-alanine, 2-aminobutyric acid, 4-aminobutyric acid, creatinine, ornithine, carnitine, citrulline, 4-hydroxyproline, 1-methylhistidine and 3-methylhistidine. The running electrolyte consisted of 1.7 M acetic acid and 0.1% hydroxyethyl-cellulose (pH 2.15). An addition of acetonitrile to the sample improved the separation of AAs significantly and permitted an increase in the amount of the sample injected. As a result, the sensitivity of the determination increased and the limit of detection (LOD) decreased by a factor of ca. 4, as compared with our previous study. The LOD values were between 1.5 μM (arginine) and 6.7 μM (aspartic acid). The CE/CCD method has then been applied to clinical analyses of the amniotic fluid collected from 20 pregnant women aged over 35 years and 24 pregnant women with whom abnormal foetus development was suspected. The latter group of women was found to exhibit systematically enhanced amniotic levels of most of the AAs studied.

Keywords: Amino acid; Contactless conductivity detection; Acetonitrile; Amniotic fluid

1. Introduction

Amniotic fluid plays an important role in pregnancy and foetal development [1]. Its volume ranges from about 50 mL in the 12th week of pregnancy, a maximum of about 800 mL is reached in the 34th week, and the amount then decreases to the final value of about 600 mL. Amniotic fluid helps to protect the foetus and plays an important role in the development of many of the foetal organs including the lungs, kidneys, and gastrointestinal tract. At the beginning of pregnancy, the composition of the fluid is qualitatively similar to that of mother plasma, except for a lower content of proteins. Since the 11th week, the fluid composition changes, because the foetus starts to swallow and inhale the fluid and to excrete urine [1,2]. Amniotic fluid is biochemically investigated if the development of the foetus seems to be abnormal, or if the pregnant woman age is over 35 years. Chromosomal and various metabolic disorders can be detected by AAs monitoring and by determining other organic acids in biological fluids such as plasma, urine, cerebrospinal or amniotic fluid. Abnormalities related to metabolism of AAs include phenylketoneuria, tyrosinemia and citrullinemia, which are characterized by enhanced levels of phenylalanine, tyrosine and citrulline, respectively, or maple syrup urine disease indicated by enhanced level of leucine, isoleucine and valine in blood [3–5].

Biochemical determinations of AAs in biological fluids have primarily used single-purpose, automatic instruments for ion-exchange chromatography, or reversed-phase liquid chromatography with post-column ninhydrine derivatization [6,7]. Since the concentrations of amino acids in body fluids are relatively high, capillary electrophoresis (CE) appears to be a suitable technique, offering a high separation efficiency, short time of analysis, low sample consumption and simpler pre-treatment of biological samples [2,4,5,8–12]. However, a suitable detection technique, generally applicable to CE analysis of proteinogenic AAs has long been lacking. Direct UV photometric or,

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sometimes, fluorimetric detection is applicable only to aromatic AAs, while other AAs have to be chemically derivatized prior to analysis using a time-consuming procedure [4,8–12]. Indirect photometric detection suffers from a low sensitivity and requires the use of rather dilute separation buffers, which are not suitable for analyses of highly conductive biological fluids [13]. Electrochemical detection utilizing the oxidation or reduction of AAs on solid electrodes (Pt, Au, C) is limited to several AAs [14]. On the other hand, all AAs should be detectable as ions by conductivity measurements, using, e.g., the contactless conductivity detector (CCD). This method has been originally developed for the detection of small inorganic ions [15–20], but its utilization for detection of free AAs in the conventional [21,22] or micro-fluidic [23–25] CE analysis has been also demonstrated. A typical construction of CCD uses two tubular or semi-tubular electrodes that are attached in series to the outer surface of the separation capillary and separated with a gap. An alternating voltage applied to one electrode is transmitted through the capillary wall, and the corresponding current changes caused by varying conductivity in the detection gap zone are sensed with the other electrode. The absence of the physical contact between the electrodes and the test solution prevents changes in their properties caused by, e.g., adsorption of solution components or interfacial electrochemical processes [26].

The present work continues in the previous studies [21,27], where the CE/CCD method was used to determine free underivatized AAs in various natural samples [21], and human plasma [27]. It will be demonstrated that all proteinogenic AAs, as well as 12 other biogenic compounds, can be separated in amniotic fluid by using the same background electrolyte (BGE) consisting of 1.7 M acetic acid and 0.1% hydroxyethylcellulose (pH 2.2) that we have previously optimized for the plasma analysis [27]. Further, it will be demonstrated that an addition of acetonitrile to the amniotic sample improves focusing of the AA zones, which allows increasing the amount of the sample injected. This leads to a considerably higher sensitivity and a lower limit of detection (LOD), as compared with our previous study [27]. As a result, the detection of 12 other biogenic components of the amniotic fluid is also possible. Finally, we shall demonstrate the application of the CE/CCD method in clinical analysis of the amniotic fluid collected from pregnant women.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade purity. Alanine (Ala, 99.5%), arginine (Arg, 99.5%), asparagine (Asp, 99%), glutamic acid (Glu, 99%), glycine (Gly, 99%), isoleucine (Ile, 99%), leucine (Leu, 99%), lysine (Lys, 98%), methionine (Met, 99%), ornithine (99%), phenylalanine (Phe, 99%), proline (Pro, 99%), serine (Ser, 99%), threonine (Thr, 99.5%), tryptophan (Trp, 99%), tyrosine (Tyr, 99%), valine (Val, 99%), β-alanine (β-Ala, 99%), 2-aminobutyric acid (99%), 4-aminobutyric acid (GABA, 99%), carnitine chloride (99%), choline chloride (99%), citrulline (99%), creatinine (99%), cystine (99%), ethanolamine (99%), 4-hydroxyproline (99%), 1-methylhistidine (1MH, 98%), acetic acid (HAc, 99%) and acetonitrile (99.9%) were purchased from Fluka. Glutamine (Gln, 99%), histidine (His, 98%), 3-methylhistidine (3MH, 98%) and hydroxyethylcellulose (HEC) were purchased from Sigma, sulfosalicylic acid (p.a.) was purchased from Lachema Brno, and acetone was purchased from Penta. Deionised Milli-Q water (Millipore, Bedford, USA) was used for preparation of BGEs and of the stock solutions of AAs (1 mg/mL), which were stored in a refrigerator at 4 °C. Cystine was dissolve in water alkalized with NaOH. Solutions of AAs for calibration were prepared by diluting the corresponding stock solutions. pH was measured using a laboratory pH meter (pMX 3000, WTW, Germany).

2.2. Instrumentation

Electrophoretic measurements were carried out using HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a built-in CCD and controlled by the ChemStation CE software. All separations took place at +20 kV and the current flow through the capillary of +20 μA. The CCD was developed and constructed in Department of Physical Chemistry, Charles University, Prague [19]. The detector was placed in the cartridge for the separation column at a distance of 13 cm from its end. A fused-silica capillary (80 cm in total length, 75 μm I.D. × 375 μm O.D., 67 cm to CCD and 72 cm to DAD, Composite Metal Services, UK) was used, covered by a protective polyimide layer at the controlled temperature of 25 °C. Before its first use, the capillary was conditioned by washing with 0.1 M NaOH (20 min), then with deionized water (20 min), and finally filled with BGE. Between each two CE runs, the capillary was washed in sequence with 0.1 M NaOH (2 min), deionized water (2 min) and BGE (4 min). BGE consisted of 1.7 M HAc + 0.1% HEC (pH 2.15). The amniotic fluid samples of the constant volume were introduced into the capillary with the help of hydrodynamic injection of 1000 mbar s.

2.3. Sample preparation

Amniotic fluid was collected by amniocentesis from 20 pregnant women of age over 35, and from 24 pregnant women suspect of abnormality of the foetus. The amniotic fluid was collected between 17th and 19th week of their pregnancy, with the patients' agreement. After the collection, the samples were immediately frozen and stored at −80 °C. Before use, the amniotic fluid samples were freed of proteins by mixing 100 μL of plasma with 100 μL of acetonitrile in a microtube. The mixture was centrifuged for 5 min at 4000 × g, and the supernatant was injected directly into the separation capillary.

3. Results and discussion

3.1. Effect of deproteining of the amniotic sample on the CE separation of AAs

The precipitation of proteins in BGE and their adsorption on the capillary wall adversely affect the CE separation of the components of biological fluids and the life-time of the separation

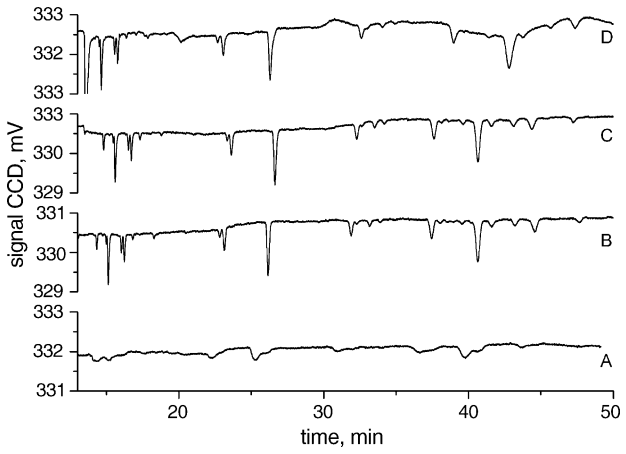

Fig. 1. CE separation of AAs in the amniotic fluid after deprotonization of the sample by using various agents: (A) sulfosalicylic acid, (B) acetonitrile, (C) acetone and (D) after dilution of the sample with water. Hydrodynamic injection 1000 mbar s.

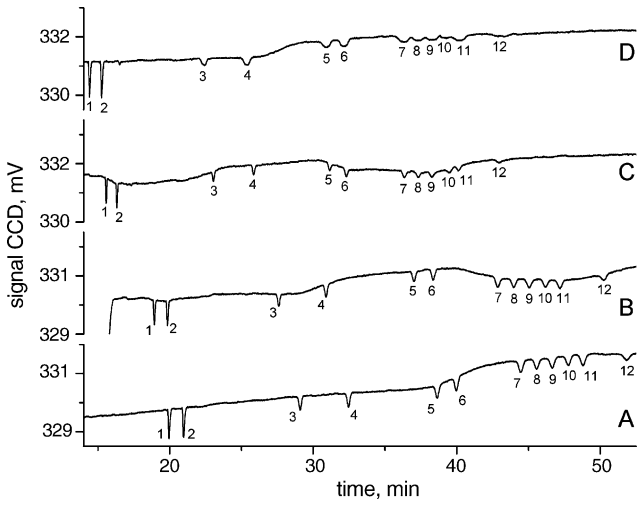

Fig. 2. CE/CCD separation of a model mixture of 12 AAs (20 μM) in various backgrounds: (A) acetonitrile–water mixture (1:1, v/v), (B) 0.15 M NaCl in acetonitrile–water mixture (1:1, v/v), (C) 0.15 M NaCl in water and (D) water. Hydrodynamic injection 1000 mbar s. Peak identification: (1) Lys, (2) Arg, (3) Gly, (4) Ala, (5) Val, (6) Leu, (7) Thr, (8) Met, (9) Gln, (10) Glu, (11) Phe and (12) Pro.

capillary. In order to avoid these complications, the biological fluids are usually freed of proteins prior to analysis by adding a strong acid (sulfosalicylic or perchloric acid), or an organic solvent (acetone, acetonitrile, isopropanol) [28–31]. Since the amount of proteins in the amniotic fluid (up to 6 g/L [32]) is still appreciable, although lower than in human plasma, we have investigated first the effect of addition of various deproteining agents including the aqueous solution of sulfosalicylic acid (40 mg/mL), acetonitrile and acetone, which all were mixed with the amniotic fluid sample in the ratio 1:1 (v/v) and centrifuged as described in Section 2.3. The effect of adding water at the same ratio was examined as a reference.

Fig. 1 shows electropherograms of the amniotic fluid after its deproteining. As it can be seen, the addition of sulfosalicylic acid has a rather deteriorating effect on the CE separation, which can be due to dissociation of sulfosalicylic acid leading to an increased electric conductivity of the sample, enhanced Joule heat and the peak broadening (Fig. 1, trace A). On the other hand, the sample treatment with organic solvent results in both the stabilization of the baseline and the sharpening of the separation peaks (Fig. 1, traces B and C). These effects facilitate the identification of AAs present at lower concentrations, as compared with the analysis of the sample that was only diluted with water (Fig. 1, trace D). The effect of the organic solvent can be ascribed to the effect of two factors [29–31,33]. First, an addition of the organic solvent leads to a decrease in the electric conductivity of the sample and to the zone focusing by stacking due to the high electric field strength. Second, the simultaneous presence of acetonitrile (or acetone) and inorganic ions at high concentrations in the amniotic fluid containing approx. 0.15 M NaCl gives rise to the isotachophoretic effect, i.e. to the so-called zone focusing through stacking by transient pseudo-isotachophoresis. Although the deproteining of the amniotic fluid using acetonitrile and acetone provided practically the same results, the former solvent was preferred due to its lower vapour pressure.

In order to get a deeper insight into the effect of the organic solvent, as well as of the enhanced concentration of inorganic ions in biological samples, we carried out a series of experiments with the model mixture of 12 AAs of the same concentration (20 μM) dissolved in four different backgrounds: deionized water, 1:1 (v/v) deionized water–acetonitrile mixture, 0.15 M NaCl in deionized water, and 0.15 M NaCl in the 1:1 (v/v) deionized water–acetonitrile mixture. Separation conditions were the same as for the amniotic fluid samples. The measured electropherograms (Fig. 2) were used to infer three parameters characterizing the peaks of the individual AAs: the peak height (h), the peak half-width ($w_{1/2}$), and the migration time (t_m). The latter two parameters were then used to evaluate the separation efficiency as the number of theoretical plates:

$$N = 5.54\left(\frac{t_m}{w_{1/2}}\right)^2,$$

which reflects the peak sharpening. Table 1 summarizes the values of the parameters h and N, as averaged from three independent CE separations for each of the four backgrounds above. These data indicate that the effects of the organic solvent and of the salinity depend on the nature of AA. The peaks of basic AAs (Lys, Arg) are sharp in all backgrounds, which is not the case of AAs with a longer migration time. This effect is probably associated with a degree of ionization of AAs in different backgrounds. Basic AAs are well ionized in both water and acetonitrile and move faster out of the sample zone giving rise to a sharp peak. In water, the peaks of other AAs starting with Gly are broad (Fig. 2, trace D), because of a lower ionization and a longer time of residence of AAs in the sample zone. The aqueous samples have a low electric conductivity, which gives rise to a more pronounced effect of the released Joule heat in sample zone. An addition of a salt (NaCl) to the aqueous sample leads to an increase in the electric conductivity, to a suppression of the heating of sample zone and, thereby, to the peak sharpening (Fig. 2, trace C). On the other hand, an addition of

Table 1
Peak height (*h*) and the number of theoretical plates (*N*) for various AAs as evaluated from electropherograms of the model mixture of 12 AAs (20 μM) in four different backgrounds: (A) acetonitrile–water mixture (1:1, v/v), (B) 0.15 M NaCl in acetonitrile–water mixture (1:1, v/v), (C) 0.15 M NaCl in water and (D) water

Compound	A		B		C		D	
	h (mV)	*N*	*h* (mV)	*N*	*h* (mV)	*N*	*h* (mV)	*N*
Lys	1.03 (0.05)	151,187 (1,710)	0.85 (0.01)	189,583 (1,695)	0.87 (0.02)	226,803 (5,486)	1.23 (0.05)	106,306 (2,984)
Arg	1.10 (0.00)	145,099 (1,020)	0.85 (0.01)	178,115 (4,800)	0.84 (0.01)	207,208 (4,553)	1.27 (0.05)	97,237 (2,271)
Gly	0.45 (0.01)	122,616 (2,668)	0.40 (0.01)	132,350 (6,139)	0.32 (0.01)	147,946 (7,200)	0.23 (0.00)	22,218 (709)
Ala	0.49 (0.01)	123,668 (2,561)	0.43 (0.01)	130,235 (4,419)	0.32 (0.00)	139,405 (11,090)	0.25 (0.01)	20,615 (732)
Val	0.47 (0.00)	122,726 (1,990)	0.35 (0.01)	124,795 (2,712)	0.24 (0.00)	150,377 (14,006)	0.21 (0.00)	21,110 (741)
Leu	0.49 (0.01)	118,661 (5,026)	0.37 (0.00)	133,530 (4,147)	0.26 (0.00)	143,666 (9,071)	0.23 (0.01)	20,775 (1,001)
Thr	0.39 (0.01)	102,275 (842)	0.32 (0.00)	107,666 (10,158)	0.21 (0.01)	119,401 (9,998)	0.18 (0.00)	18,685 (1,062)
Met	0.34 (0.01)	101,156 (6,456)	0.27 (0.02)	110,282 (5,067)	0.16 (0.01)	134,287 (9,237)	0.12 (0.01)	25,778 (1,780)
Gln	0.36 (0.00)	101,046 (834)	0.28 (0.00)	102,848 (3,043)	0.16 (0.00)	116,899 (16,865)	0.12 (0.01)	26,206 (2,111)
Glu	0.34 (0.00)	110,462 (2,903)	0.26 (0.02)	107,691 (5,942)	0.14 (0.01)	101,599 (8,477)	0.10 (0.00)	20,851 (667)
Phe	0.38 (0.01)	110,622 (3,138)	0.28 (0.01)	112,612 (5,523)	0.16 (0.00)	133,424 (14,385)	0.16 (0.00)	19,970 (554)
Pro	0.24 (0.00)	83,811 (15,380)	0.19 (0.01)	94,419 (8,922)	0.09 (0.01)	134,986 (20,997)	0.10 (0.01)	15,100 (452)

Average values of the parameters and the standard deviations (in parentheses) were evaluated from three independent CE/CCD separations.

acetonitrile to the aqueous sample results in an increase of the peak height, in particular for the slowly migrating AAs (Fig. 2, traces A and B), which could be attributed to stacking due to the high electric field strength and the stacking effect by transient pseudo-isotachophoresis. Under the present experimental conditions, AAs are separated as cations, sodium ions or basic amino acids serves as the leading ions, while the organic solvent can be regarded as the pseudo-terminating ion. An addition of a salt (NaCl) to the water–acetonitrile sample does not affect the separation efficiency, but it leads to a decrease in the peak height, probably due to suppressing of stacking by the high electric field strength.

3.2. CE separation of the model aqueous samples

BGE consisting of 1.7 M HAc + 0.1% HEC (pH 2.15) has been previously optimized for the determination of AAs in the human plasma samples [27]. At pH 2.15, AAs migrate as cations. The relatively high HAc concentration ensures a high buffering capacity of BGE, as well as a high value of the Kohlrausch regulating function, while the presence of HEC helps to stabilize the electroosmotic flow and to protect the capillary inner wall against undesirable adsorption of components of the biological fluid [27]. In the present study we attempted to increase further the sensitivity of the CE/CCD method using the same BGE, but modifying the sample pretreatment. As shown in the previous section, an addition of acetonitrile or acetone to the amniotic sample improves focusing of the AA zones, which makes it possible to increase the amount of the sample injected without affecting the efficiency of the CE separation. The effect of the amount injected was studied for a model aqueous sample containing 20 proteinogenic AAs and 12 other biogenic substances that were identified in the amniotic fluid. For the purpose of evaluation of the sensitivity and LOD, these components were dissolved in the 1:1 (v/v) mixture of water and acetonitrile at a concentration of 10 μM each, except for 15 μM Asp and 4-hydroxyproline. On stepwise increasing the injection from 300 to 1500 mbar s, the peak heights increase, whereas the peak resolution deteriorates. The optimum injection was found at 1000 mbar s, which permits separation of all the 32 analytes down to the baseline in 70 min (Fig. 3); at pH 2.15 only 3MH and 1MH migrate together and can be separated at higher pH of 3.4 [34]. Owing to a high reactivity of the –SH group, cysteine is actually determined in the form of its oxidation product, cystine.

The sensitivity and LODs evaluated using the injection of 1000 mbar s are given in Table 2, together with their ratio to the values obtained previously for the injection of 300 mbar s [27]. LOD was determined from the peak height as the average concentration corresponding to S/N = 3, the background CCD noise was 0.02 mV. The data in the last two columns in Table 2 show that the change in the amount injected leads to an increase in the sensitivity by a factor of ca. 4, and to a decrease in the LOD by a factor of 2.1–4.8. Apparently, the LOD increases with the

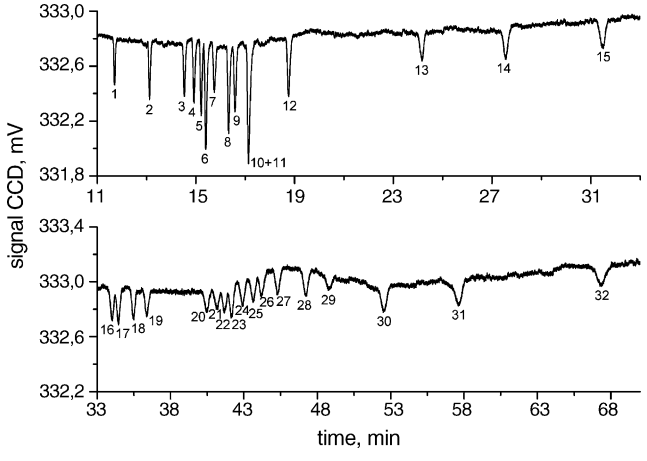

Fig. 3. CE separation of the model mixture of 20 proteinogenic AAs and 12 other biogenic substances dissolved in the water and acetonitrile 1:1 (v/v) at a concentration of 10 μM each, except for 15 μM Asp and 4-hydroxyproline. Hydrodynamic injection 1000 mbar s. Peak identification: (1) ethanolamine, (2) choline, (3) creatinine, (4) β-Ala, (5) ornithine, (6) Lys, (7) GABA, (8) Arg, (9) His, (10) 1MH, (11) 3MH, (12) carnitine, (13) Gly, (14) Ala, (15) 2-aminobutyric acid, (16) Val, (17) Ile, (18) Leu, (19) Ser, (20) Thr, (21) Asn, (22) Met, (23) Trp, (24) Gln, (25) citrulline, (26) Glu, (27) Phe, (28) Tyr, (29) Pro, (30) cystine, (31) Asp and (32) 4-hydroxyproline.

Table 2
Sensitivity of the CE/CCD method and limits of detection (LOD) for AAs and other biogenic compounds in a model aqueous sample as evaluated from measurements at four different concentrations; each measurement was repeated three times

Compound	Tested interval (μM)	Sensitivity (mV s/μM)	*R*	LOD (μM)	Sensitivity increase (times (×))	LOD decrease (times (×))
Ala	10–500	0.238	0.9999	3.2	3.6	3.3
Arg	2.5–150	0.400	0.9955	1.5	4.9	2.9
Asn	5–100	0.292	0.9993	4.3	4.3	3.6
Asp	10–100	0.314	0.9991	6.7	4.4	3.7
Carnitine	2.5–100	0.307	1.0	2.2	–	–
Citrulline	5–200	0.361	1.0	3.5	4.1	3.5
Creatinine	2.5–100	0.235	1.0	2.3	3.7	2.5
Cystine	10–200	0.408	0.9997	4.0	–	–
Ethanolamine	2.5–50	0.162	0.9997	2.3	–	–
GABA	2.5–50	0.261	0.9996	2.0	–	–
Gln	10–400	0.315	0.9992	4.0	3.7	3.2
Glu	10–500	0.323	0.9997	4.0	3.7	3.1
Gly	10–400	0.209	0.9998	3.3	3.9	3.4
His	2.5–200	0.303	1.0	2.3	4.2	2.1
Choline	2.5–50	0.202	0.9995	1.9	–	–
Ile	10–200	0.330	0.9999	2.9	4.2	3.8
Leu	10–200	0.328	0.9999	3.0	3.8	3.3
Lys	2.5–300	0.327	0.9999	1.9	4.4	2.5
Met	5–100	0.311	0.9998	3.8	3.6	3.2
Ornithine	2.5–100	0.290	0.9997	1.9	3.9	2.5
Phe	10–200	0.359	1.0	3.8	4.3	3.8
Pro	10–300	0.262	0.9998	6.0	5.1	4.8
Ser	10–200	0.269	0.9999	4.3	3.7	3.0
Thr	10–300	0.296	0.9996	4.0	3.6	3.2
Trp	5–100	0.384	0.9998	3.0	4.1	3.8
Tyr	10–200	0.381	0.9997	3.5	3.7	3.2
Val	10–300	0.298	0.9998	3.3	3.6	3.1
β-Ala	2.5–50	0.227	0.9992	1.8	–	–
2-Aminobutyric acid	5–50	0.300	0.9998	3.0	–	–
3MH	2.5–100	0.309	0.9995	1.9	4.1	2.6
4-Hydroxyproline	10–200	0.260	0.9999	8.6	–	–

Last two columns give the factors of the increase in the sensitivity and the decrease in LOD as compared with the previous CE/CCD analysis made using a lower amount of the sample injected [27].

migration time; the lowest values of LOD were found for basic AAs, which exhibit the highest mobility and shortest retention times. The increased sensitivity also made it possible to extend the analysis of the amniotic fluid to other biogenic compounds.

3.3. Analysis of the amniotic fluid

Fig. 4 shows an electropherogram of the amniotic fluid, together with the identification of its components, which was carried out by spiking the samples with the corresponding standard. Apart from all 20 proteinogenic AAs, the method allows determining a number of other low molecular biogenic nitrogen compounds migrating at pH 2.15 as cations. These compounds are mainly the derivatives of the proteinogenic AAs, and the metabolically related compounds, including ethanolamine, choline, creatinine, β-Ala, ornithine, GABA, 1MH, 3MH, carnitine, 2-aminobutyric acid, citrulline and 4-hydroxyproline.

The clinical study was then focused on the whole profile of AAs in the amniotic fluid collected from 20 pregnant women of age over 35, and 24 pregnant women suspect of abnormal development of the foetus. The results of this study are summarized in Table 3. AA levels in both groups were found to fall within the

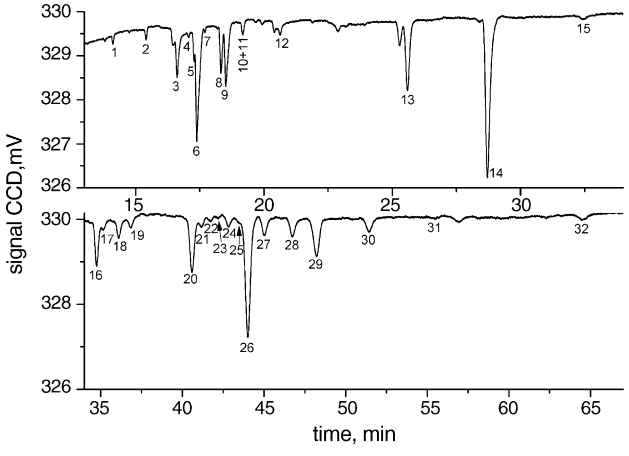

Fig. 4. CE separation of AAs and other biogenic substances in the amniotic fluid sample after its deproteinization with acetonitrile 1:1 (v/v). Hydrodynamic injection 1000 mbar s. Peak identification: (1) ethanolamine, (2) choline, (3) creatinine, (4) β-Ala, (5) ornithine, (6) Lys, (7) GABA, (8) Arg, (9) His, (10) 1MH, (11) 3MH, (12) carnitine, (13) Gly, (14) Ala, (15) 2-aminobutyric acid, (16) Val, (17) Ile, (18) Leu, (19) Ser, (20) Thr, (21) Asn, (22) Met, (23) Trp, (24) Gln, (25) citrulline, (26) Glu, (27) Phe, (28) Tyr, (29) Pro, (30) cystine, (31) Asp and (32) 4-hydroxyproline.

Table 3
CE/CCD analysis of the amniotic fluid collected from 20 pregnant women of age over 35 (A), and 24 pregnant women suspected for the development abnormality of the foetus (B), the results of the analysis are compared with normal physiological levels determined by LC (C) [35]

Compound	A		B		C
	Mean (μM)	Concentration range (μM)	Mean (μM)	Concentration range (μM)	Concentration range (μM)
Ala	393.3	295.0–523.1	465.6	360.5–623.9	280–840
Arg	35.0	18.0–63.0	48.0	31.5–90.0	30–140
Asn	26.0	14.4–40.1	34.0	18.5–52.4	5–50
Asp	11.3	*x*–22.0	10.3	*x*–25.8	0–80
Carnitine	12.7	6.8–19.5	18.2	9.8–26.4	Not reported
Citrulline	12.3	5.8–18.3	13.0	6.6–21.6	Not reported
Creatinine	43.6	34.5–53.6	55.3	42.1–80.4	Not reported
Cystine	27.3	19.9–36.0	35.7	19.1–44.9	Not reported
Ethanolamine	13.7	7.4–18.5	14.3	5.6–22.2	Not reported
Gln	15.4	*x*–184.8	27.6	*x*–148.6	35–370
Glu	356.0	215.5–507.1	435.2	300.0–545.2	40–610
Gly	167.2	100.5–262.7	199.0	91.9–277.0	95–390
His	96.5	72.3–135.6	121.1	74.3–166.3	70–170
Choline	11.2	5.9–22.3	11.6	7.4–14.9	Not reported
Ile	29.0	14.5–50.9	34.7	16.4–57.3	10–90
Leu	67.7	42.1–105.2	84.0	50.3–125.3	25–200
Lys	202.2	134.9–297.2	260.5	162.4–375.2	110–500
Met	14.1	6.8–25.1	17.5	10.6–30.9	10–60
Ornithine	24.6	14.5–35.2	29.0	13.4–49.7	20–100
Phe	52.6	37.6–75.2	63.6	43.5–91.1	30–140
Pro	180.8	139.7–253.1	205.7	140.8–282.8	50–230
Ser	21.0	13.4–37.9	25.8	12.3–44.6	25–90
Thr	155.9	90.2–219.9	197.3	116.6–318.2	140–340
Trp	10.4	4.7–18.0	11.2	3.9–19.5	0–20
Tyr	50.1	39.4–74.8	61.2	42.5–96.9	25–135
Val	159.2	111.7–224.5	201.9	114.8–303.0	80–350
β-Ala	4.7	2.6–7.9	5.1	2.6–7.9	Not reported
MH	8.3	1.9–20.4	9.9	1.9–33.0	Not reported
2-Aminobutyric acid	10.7	5.0–17.0	13.3	7.0–33.0	Not reported
4-Hydroxyproline	34.9	12.7–48.5	32.0	21.9–40.4	20–50

Values "*x*" is below LOD.

interval of normal physiological values that were determined by an LC method [35,36], except for Gln. In both groups of pregnant women, the concentration of Gln was very low, and in 80% of samples Gln was found to be below LOD (3.7 μM). Only two of 44 samples contained Gln at a concentration that was several times higher, i.e. 185 and 149 μM. The values for GABA are not given in Table 3, because its concentration was below LOD in all samples. The most remarkable difference between the two groups of pregnant women was systematically higher mean concentration of majority of AAs for women suspect of abnormal foetus development.

4. Conclusions

Capillary electrophoretic method with the contactless conductivity detection allowed the sensitive determination of 20 proteinogenic AAs and 12 other biogenic compounds in the amniotic fluid. Addition of acetonitrile to the sample improved significantly the separation of AAs, due to the AA zone focusing by way of stacking caused by high electric field strength and transient pseudo-isotachophoresis. This made it possible to increase the amount of the sample injected, and to increase the sensitivity of determination, and to decrease the limit of detection (LOD) by a factor of ca. 4, as compared with our previous study. The application of the CE/CCD method to analysis of the amniotic fluid collected from 20 pregnant women of age over 35, and 24 pregnant women suspect of abnormal development of the foetus, revealed systematically enhanced amniotic levels of major AAs in the latter group.

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