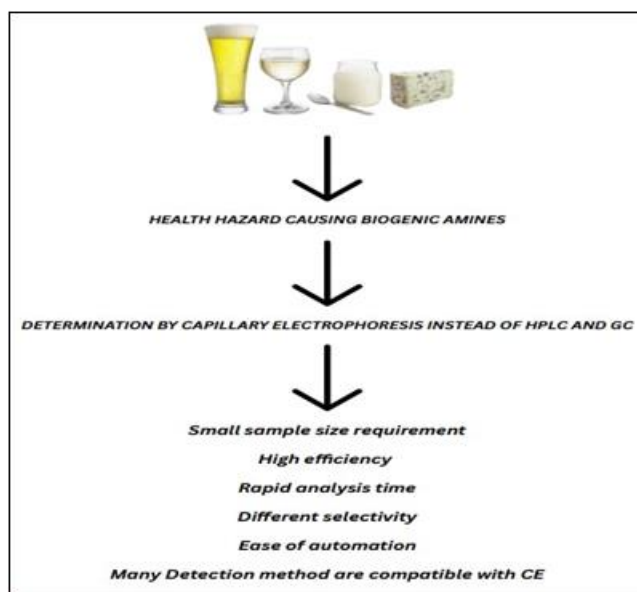


Determination of Biogenic Amines in Food by Capillary Electrophoresis

Sakshi Sinha, V K Shukla

Abstract: This review article intends to provide comprehensive analysis on biogenic amines, their source, type and classification. Biogenic amines are nitrogen containing organic compound that naturally exists in nature. They have low molecular weight and are basic in nature. They are active components that act as precursors for synthesis of alkaloids, hormones, protein, nucleotides and aromatic compounds. They occur in plants, animal and microorganism and play an essential role in metabolic and physiological function. This paper focuses on determination of biogenic amines in food by capillary electrophoresis as it causes several health problems such as histamine causes headache, digestive disorders and neurotoxicity, tyramine can cause hypertension and cadaverine can cause cytotoxicity. Biogenic amines are determined by several techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), Ion exchange chromatography. This paper further focuses on advantages of capillary electrophoresis in determination of biogenic amines in food. Capillary electrophoresis is a liquid phase separation method. The technique of capillary electrophoresis is used to identify, separate and quantify large range of components. There are several types of CE. Instrumentation of CE includes fused silica capillary which has length of 20 - 50cm. The reservoir is filled with separation buffer and the two ends of capillary tube extend between two reservoirs. CE has wide variety of application in identification, separation and quantification. In conclusion, biogenic amines cause many health hazard and is important to be identified and this paper deals with their presence and identification in food.

Graphical abstract:



Keywords: Biogenic amines, Capillary Electrophoresis, Food, HPLC, GC

1. Introduction

1.1 Biogenic Amine:

Biogenic amines are nitrogen containing organic compound that naturally exists in nature. They have low molecular weight. Biogenic amines are basic in nature. Biogenic amines occur in plants, animal and microorganism and play an essential role in metabolic and physiological function. They are active components that act as precursors for synthesis of alkaloids, hormones, protein, nucleotides and aromatic compounds. [5]

Biogenic amines are found in that food which contain protein and/or free amino acid. Due to the existence of the enzyme decarboxylase, biogenic amines are generated by the decarboxylation process of free amino acids. This reaction

leads to the removal of carbon dioxide from free amino acid to finally form amines. Enzyme decarboxylases are naturally present in plant, animal and microorganism. [1]

Biogenic amines are also widely present in food such as wine, beer, cheese and fermented food. Environmental hygiene, food storage, and food processing are all factors that contribute to the generation of substantial amounts of biogenic amines in food. [4]

The amount and type of biogenic amines formed is affected by:

- 1) Food composition
- 2) Microbial flora
- 3) Temperature of the food storage
- 4) Ripening condition and packaging of food . [1]

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1.2 Classification of biogenic amines:

On the basis of:

1) Chemical Structure:

- Aliphatic structures e. g. putrescine, spermine, spermidine
- Aromatic structures e. g. tyramine, phenylethylamine

c) Heterocyclic structures e. g. histamine and tryptamine.

2) Number of amine groups present:

- Monoamines e. g. tyramine, phenylethylamine
- Diamines e. g. histamine, putrescine, cadaverine
- Polyamines e. g. spermine, spermidine [3] [23]

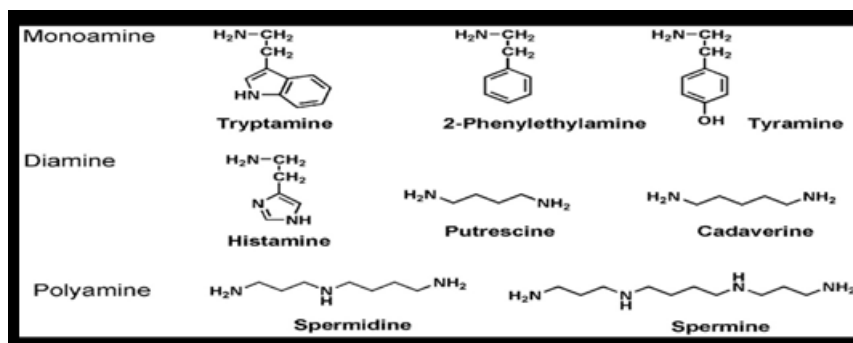


Figure 1: Different biogenic amines [23]

1.3 Functions of biogenic amine in humans:

- Histamine increases permeability of capillaries for white blood cells.
- Tyramine raises the heart rate and blood sugar concentration and also has some significant antioxidant property.
- Spermine, spermidine, putrescine and cadaverine play a vital role in synthesis of genetic material like DNA, RNA and protein. It also maintains stability of biofilm.
- Tryptamine, phenylethylamine and tyramine regulate blood pressure.
- Phenylethylamine regulate level of norepinephrine. [3]

Although Biogenic amines perform vital biological function in humans but at excessive concentration it can cause many human health problems.

1.4 Health problems caused by biogenic amines:

- Histamine can cause headache, digestive disorder and neurotoxicity.
- Tyramine can cause hypertension.
- Cadaverine and putrescine are milder in nature but cause cytotoxicity.
- Cadaverine, putrescine, Spermine and spermidine while reacting with nitrite may produce carcinogenic nitrosamine. [4] Therefore, estimation of biogenic amines is important in food to avoid such health problems.

To avoid such adverse effect of biogenic amine through excessive intake, many nations have set minimum limit on the number of biogenic amines in food product like aquatic products and wine. [10]

1.5 Determination of biogenic amines:

There are several hindrances in determining biogenic amines making it difficult to detect through conventional physiochemical method like spectrophotometry, fluorometry and electrochemical methods. [3]

Some such challenges in estimation of biogenic amines are:

- Biogenic amines have a high polar nature, which makes them more water soluble than the organic solvents that are commonly utilized.
- They are present in complex matrix sample.
- Variable concentration range which is very low.
- Presence of potentially interfering compound.
- Lack of intrinsic property of the compounds. [7]

To overcome this problem, developed analytical method are used which is based on amine extraction and derivatization followed by determination.

Estimation of biogenic amines is performed by:

- High performance liquid chromatography (HPLC)
- Gas chromatography (GC)
- Capillary electrophoresis (CE)
- Ion exchange chromatography (IEC) [7]

2. Capillary Electrophoresis

The technique of capillary electrophoresis is used to identify, separate, and quantify large range of components. This approach is based on the notion of differential rate of migration of separated components. In the presence of an electric field, separation occurs in a narrow bore capillary. [6] [8]

2.1 Advantages of CE over other electrophoresis techniques, HPLC and GC are:

- Small sample size requirement
- High efficiency
- Rapid analysis time
- Different selectivity
- Ease of automation
- Many detections method are compatible with CE [9]

Capillary electrophoresis is mainly used in determination of protein, peptide, amino acids and biogenic amines. It is an important technique for determining free amino acid concentrations as it is a good strategy of separation for polar

molecule. The main difficulty in estimation of biogenic amines by CE is their absorption on the capillary surface because of the presence of negatively charged silanol group having pH less than 2. Several strategies are used to solve this problem such as coated capillaries, background electrolyte solutions with extreme ph and addition of additives.

CE coupled with mass spectrometry is more preferred than CE coupled with optical detector. Reason being amino acids have neither absorbance or fluorescence and they must be derivatized with a chromophore or fluorophore agent, respectively, for their detection by UV detector or fluorescence detector. [9]

2.2 Types of capillary electrophoresis:

- 1) Capillary zone electrophoresis (CZE)
- 2) Non - aqueous CE (NACE)
- 3) Micellar electrokinetic chromatography (MEKC)
- 4) Capillary electrochromatography (CEC)
- 5) Capillary isotachopheresis (CITP)
- 6) Capillary isoelectric focusing (CIEF),
- 7) Chiral CE (CCE)
- 8) Capillary gel electrophoresis (CGE)
- 9) Microemulsion electrokinetic capillary chromatography (MEEKC) [22]

2.3 Electrophoretic mobility

It refers to the reaction of analyte in sample solution that develop from the application of electric filed in the capillary. i. e. Cations are drawn to the negatively charged cathode, whereas anions are drawn to the positively charged anode. [6]

$$\mu_{ep} = \frac{q}{6\pi\eta r_i}$$

2.4 Electroosmotic flow

Electroosmosis occurs when electric field is supplied to the solution present in the capillary. The fused silica capillary has fixed charges on the interior wall, it contains ionisable silanol groups having pKa between 4 to 6. At pH more than 6, silanol group ionize and give anionic form silanoate making the surface of capillary negatively charged. This pulls positive charge cations from the sample solution under investigation, resulting in the formation of a double layer with a positive charged density that decreases exponentially as distance from the wall increases. A potential difference close to the wall is created and it is called zeta potential. The difference between the flow profile is the main reason of increased efficacy of CE. [6]

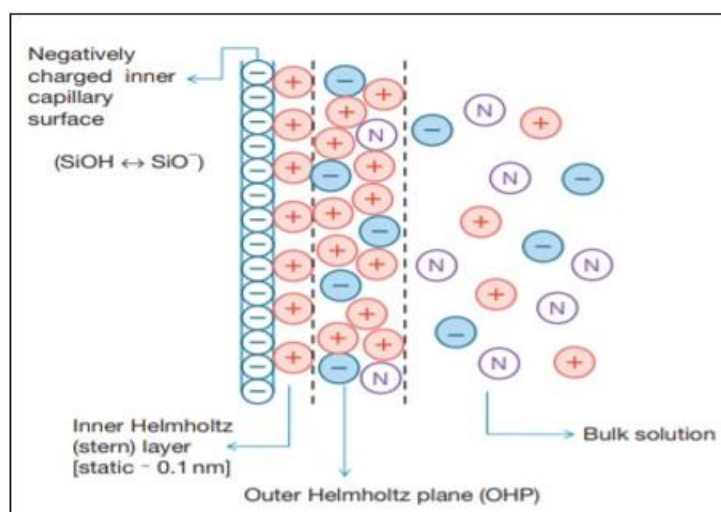


Figure 2: Electric double layer in fused silica capillary [6]

The net apparent mobility is the vector sum of the electrophoretic and electroosmotic mobility:

$$\mu_{app} = \mu_{ep} + \mu_{EOF}$$

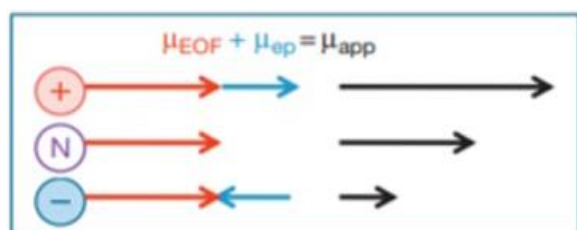


Figure 3: Migration of solute in capillary electrophoresis [6]

2.5 Instrumentation

It consists of a fused silica capillary which has length of 20 - 50cm. The two ends of the capillary stretch between two reservoirs and are filled with separation buffer.

To perform separation, the capillary's inlet is inserted into the sample to be separated for sample injection after it has been placed in buffer. The inlet is returned to buffer reservoir after sample injection.

Then, electric potential of 10 - 30 Kv is applied between the two reservoir containing buffers. Based on the electrophoretic mobility and electroosmotic flow, analyte migrates through the column. Analytes are determined depending upon the type of detector used. [6]

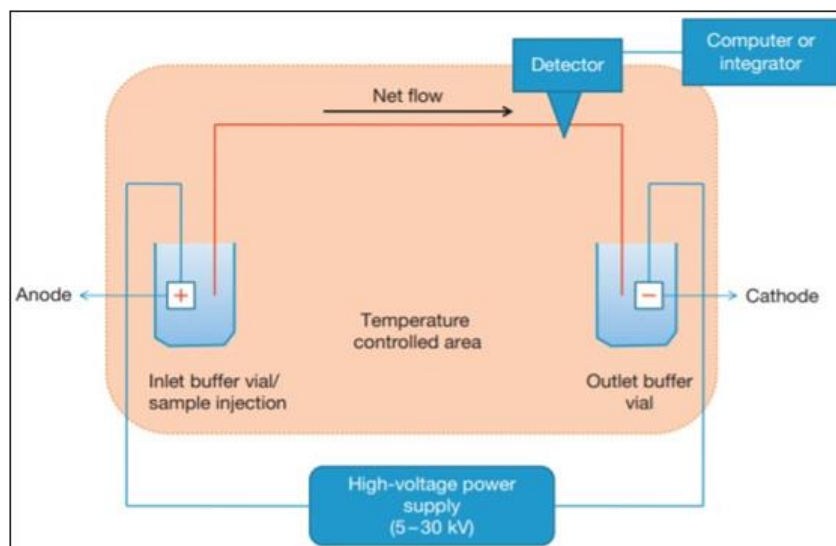


Figure 4: Basic capillary electrophoresis instrumentation [6]

3. Application of CE

1) Determination of drug related impurities:

Capillary electrophoresis is high resolution technique which makes it suitable for analyzing related substance in drugs. Apart from assessing the purity of prescription pharmaceuticals, CE is also utilized in forensic science to profile illegal compounds and determine their chiral purity.

2) Determination of counter ions:

The counter ions of acidic and basic medicines are inorganic or organic ions and as constituents in parenteral solutions they can be analyzed by CE.

3) Chiral analysis:

In chiral analysis, capillary electrophoresis is employed because of its great resolution.

4) Bioanalysis:

Capillary electrophoresis has been used to screen for licit and illicit drugs in body fluids and hair in clinical and forensic samples.

5) Biopharmaceuticals:

Capillary electrophoresis techniques are used to segregate proteins based on their charge - to - hydrodynamic size ratio, allowing for the investigation of various size and charge variations.

6) Miscellaneous application:

- Reaction monitoring
- Drug stability studies
- Drug evaluation in cell culture - based transport studies
- Calculation of content release testing from tablet [8]

4. Determination of biogenic amines in food by capillary zone electrophoresis with conductometric detection: [2]

4.1 Materials

- 1) Biogenic amines like Cadaverine dihydrochloride, putrescine dihydrochloride, agmatine sulphate, phenylethylamine hydrochloride, histamine

dihydrochloride, tryptamine, and tyramine hydrochloride

- 2) 1 M HCl, 0.5N sulphuric acid, hydroxyethylcellulose, L-histidine, adipic acid, ethylenediaminetetraacetic acid (EDTA - II) and methanol (MeOH).
- 3) The electrolyte, standard solution, and sample preparation were all done with deionized water.
- 4) Food samples

4.2 Instrumentation

- 1) The electrophoretic analyser EA 100 was employed, which was fitted with a fluorinated ethylene-propylene copolymer (FEP) analytical capillary (90 mm 0.3 mm I. D.).
- 2) A contact conductivity detector was used to detect zones.
- 3) A PC software package that came with the analyzer was used to analyse the electropherograms.

4.3 Condition of analysis

- 1) Under the following conditions, biogenic amines were identified as cations.
- 2) 15 mM histidine + 5 mM adipic acid + 0.1 mM EDTAII + 1.5 mM sulphuric acid + 50 percent MeOH + 0.1 percent HEC (pH 5.8) was the optimum background electrolyte.
- 3) The capillary was driven at a constant current of 30 μ A (about 4.5 kV).
- 4) Using a sample valve, samples and standards were pumped into a fixed internal loop (200 nL).
- 5) It took 10 minutes to complete one of the analyses.

4.4 Calibration

- 1) The technique was based on an external standard.
- 2) Biogenic amines stock solutions of 10 mM were made by dissolving biogenic amines in 20% methanol and keeping them in the refrigerator at 8°C.
- 3) From this solution, five calibration solutions (5, 10, 20, 50, and 100 nmol/ml) were produced and injected (in duplicate).

4.5 Sample treatment

- 1) Liquid samples were diluted (2–10 times) in demineralized water before being analysed directly.
- 2) Solid samples (5 g) were extracted in an ultrasonic

bath for 30 minutes with 100 ml of 0.1 M HCl, then filtered and Before analysis, the sample was diluted (two to five times) with demineralized water.

Table 1: Determination of biogenic amine in food and beverages

Food and beverages analysed	Biogenic amines present	Technique used	Buffer	Detector	Reference
Dark beer	Tyramine Trptophan	Microchip capillary electrophoresis	20 mM phosphate (pH 2.5)	Electrochemical (Ruthenium - containing films coated glassycarbon electrodes)	[12]
Beer and wine	Histamine Tyramine Putrescine Cadaverine Phenilethylamine Tryptophan Spermine spermidine	Capillary isotachophoresis	Leading electrolyte: 5 mM Ba (OH) 2 þ 15 mM valine þ 1% Hydroxyethylcellulose (pH 8.5), Terminating electrolyte: 0.02 M TRIS 0.1 M HCl (pH 8.3).	Conductometric detector	[13]
Wine	Histamine Tyramine Putrescine Cadaverine Phenilethylamine Tryptophan Spermine spermidine	Non - Ionic Micellar Electrokinetic Chromatography	Brij 35, borate buffer (pH 9.6)	Laser induced fluorescence (LIF) lex/em: 488/520 nm	[14]
Wine	Histamine Tyramine Phenilethylamine	Capillary Isotachophoresis (CITP) and Capillary zone electrophoresis (CZE)	CITP: Leading electrolyte: potassium (pH 6), terminating electrolyte: EACA (aminocaproic acid) (pH 4.3). CZE: Background electrolyte: GABA (PH 4.1)	UV: 280 nm	[15]
Beer and wine	Histamine Tyramine Putrescine Cadaverine Tryptophan Spermine Spermidine Phenilethylamine Tryptophan	Capillary electrophoresis	0.5 M acetic acid (pH 2.5)	MS/MS (ESI)	[1]
Meat	Putrescine Cadaverine Histamine Tyramine Spermine Spermidine	Capillary electrophoresis	CE - SDS	-	[13]
Sea food	Tyramine Putrescine Cadaverine Spermidine Tryptophan Histamine	Micellar Electrokinetic Chromatography	BGE: ethanol	LIF	[16]
Fish	Spermine Spermidine Putrescine Cadaverine	Micellar Electrokinetic Chromatography	25 mM pH 9.5 boric acid, 25 mM SDS and 27% ACN	LIF	[17]
Fish	Putrescine Cadaverine Spermine Tyramine tryptophan	capillary zone electrophoresis	6mM copper sulfate + 6mM - crown - 6 - ether + 4mM formic acid - pH 2 in water	photometric detection	[18]
Soy sauce	Spermine Spermidine Putrescine Cadaverine	Micellar Electrokinetic Chromatography	25 mM pH 9.5 boric acid, 25 mM SDS and 27% ACN	LIF	[17]
Oyster	Tyramine Histamine Putrescine Spermine	Capillary electrophoresis	Phosphate buffer at Ph7 in the detection cell, 5 mmol/ ru (bpy) 32 + and 50mmol/l phosphate buffer at pH 7 as the separation buffer.	electrochem iluminescence	[19]
Sausage	Putrescine Cadaverine Spermine Tyramine Tryptophan Spermidine	capillary zone electrophoresis	6mM copper sulfate + 6mM - crown - 6 - ether + 4mM formic acid - pH 2 in water	photometric detection	[18]
Cheese	histamine	Capillary electrophoresis	Sodium phosphate buffer	Diode array detection	[20]
rice spirit	tryptamine tyramine tyrosine tryptophan	Micellar Electrokinetic Chromatography	Sodium hydroxide buffer	Electrochemical detector	[21]

5. Conclusion

Biogenic amines are significantly present in good containing protein, amino acid, wine, beer and cheese which causes adverse effects on humans. Therefore, it is important to determine the presence of biogenic amines in food for their proper elimination and promotion of health. This paper concludes that capillary electrophoresis facilitates better determination of biogenic amines in food as compare to HPLC and GC in terms of small sample size requirement, High efficiency, Rapid analysis, time Different selectivity and Ease of automation.

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Human and Animal Rights: Nil

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