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Optimization of the separation of benzoic acid and sulphanilamide by capillary zone electrophoresis (CZE)

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Highlights

- **Development of analytical techniques is a challengeable subject nowadays, which requires more scientific considerations in order to increase the abilities of several techniques to be used in various function with high-value outcome.**
- **Capillary zone electrophoresis is one of the analytical methods that still need to be enhanced to achieve the separation of components with high efficiency.**
- **In this work, different strategies were applied to develop the separation of Sulfonamide and Benzoic acid, in which various parameters were adjusted and separation efficiency was exanimated to reach the optimum condition for the mentioned separation process.**

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ABSTRACT

Sulfonamide and Benzoic acid play a significant role regarding the treatment and inhibition of bacteria and its side effect on human health. Due to this, different analytical techniques with various adjustments to their parameters have been highlighted as an important challenge that should be tested in order to achieve the most efficient separation process, and thus to enable both qualitative and quantitative measurements of these substances. Therefore, the separation of the above-named compounds had been conducted by capillary zone electrophoresis (CZE) technique. Moreover, the comparison between both compounds in terms of organic, buffer and voltage at 25kV, 20kV had was achieved. Hence, this work was performed to determine the optimum experimental conditions of capillary voltage, with keeping ionic strength of buffer and percentage of organic modifier constant due to experimental time constraints.

1. Introduction

Capillary electrophoresis (CE) was found in 1981 as a new technique of separation and analysis for chemicals (Volpi and Maccari, 2013). Capillary electrophoresis (CE) is one of the electrokinetic separation techniques, which is conducted in submillimeter capillaries, in micro- as well as nanofluidic channels (Camilleri, 1993 (Fig.1) & Brittain, 2016). It is usually referred to capillary zone electrophoresis (CZE). In fact, the basis of this technique is to migrate analytes through electrolyte solutions under an electric field impact (Tian *et al*., 2013 & Zhao *et al*., 2016). In which, analytes may be separated by ionic mobility. In addition to that, they can be concentrated by both means of gradients in conductivity and pH (Tian *et al*., 2013).

In the last few years, The CZE has been provided as an alternative of HPLC techniques. This is due to the unlimited advantages of CZE, which are over the benefits of HPLC method (Patrick and Spencer, 2009). An example of that, the CZE has the ability to provide higher efficiency, shorter time for analysis, lower reagent and lower sample consumption more than HPLC technique (Patrick and Spencer, 2009 & Zheng *et al*., 2004).

The principal aim of this work is to use factorial design, which is to examine the optimal conditions of capillary voltage, ionic strength of buffer and the percentage of organic modifier for the separation of sulphanilamide and benzoic acid by using capillary electrophoresis (CE) technique.

2. Experimental Method

2.1. Chemical

Three various samples, which were Benzoic acid, Sulphanilamide and a mixed standard solution of both compounds, were prepared into 10ml volumetric flasks using deionized water. The benzoic acid solution (200 ppm) was prepared by the dilution of the stock solution of benzoic acid (1000 ppm). In addition, the sulphanilamide solution (100 ppm) was prepared by the same procedure of benzoic acid, in which the stock solution of sulphanilamide (1000 ppm) was used instead of with considering the difference in the sample volume. A mixture (200 ppm of benzoic acid and 100 ppm of sulphanilamide) was obtained by diluting 2 ml of stock solution benzoic acid and 1 ml of stock solution sulphanilamide into 10 ml volumetric flasks and then risen the mixture with water to the flask sign.

2.2. Instrumental

The basic instrumental conditions were used is that, the Beckman P/ACE MDQ capillary electrophoresis system with the software of 32 KARAT. The length of the capillary was set at 57 cm, and the effective length was at 50 cm. the chosen diameter of capillary was 75 µm. The pressure of sample injection was at 0.5 psi/10 second for 20 min. as well as, 215 nm wavelength for the UV detector. The cathode was placed at the outer buffer and the anode at inlet buffer. Then the influence of capillary voltage was determined with keeping other two factors constant owing to the need for a long time for the experiment. Thus, electrophoresis for the three solutions was determined at 20 kV as well as 25 kV.

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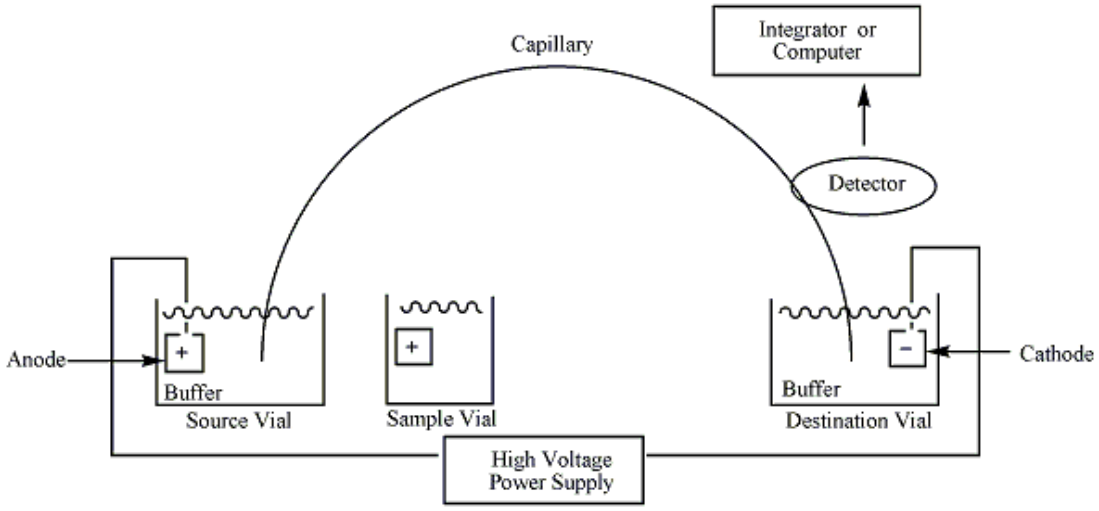

Fig. 1. Diagram of capillary electrophoresis system (Camilleri, 1993).

3. Results and Discussion

3. 1 single – factor ANOVA of experimental results

Table 1

The time of benzoic acid alone and mixture at a different value of voltage

Analyte	Benzoic acid (minute)	Benzoic acid in mix (minute)
20 kV	6.504	———
20 kV	6.500	6.463
25 kV	5.117	5.112

Table 2

One- way ANAOVA analysis of benzoic acid migration time alone and mixture at 20 KV & 25 KV

Groups	count	sum	Average	variance
20 kV	3	19.467	6.489	0.000511
25 kV	2	10.229	5.1145	1.25E-05

Table 3

The migration time of sulphanilamide alone and mixture at a different value of voltage

Analyte	Sulphanilamide in (minute)	Sulphanilamide in mix (minute)
20 kV	3.671	3.679
25 kV	2.908	2.913

Table 4

The One –way ANAOVA analysis of sulphanilamide migration time alone and mixture at 20kV & 25 kV

Groups	count	sum	Average	variance
20 kV	2	7.350	3.675	3.2E-05
25 kV	2	5.821	2.9105	1.25E-05

As it is clear from the ANOVA, there is a significant difference between the values , with P value = 4.13467E-06 (benzoic acid) and 3.81E-05 (Sulfanilamide) in the experiment, which means there is an effect of increasing the voltage that leads to decrease the migration time and make the analytes to spend less time (Miller and Miller, 2005).

3. 2 *factorial design of model data:*

Table 5

The 3^2 full factorial design and response obtained

Voltage (kV)	Buffer Ionic Strength (Mm)	Organic modifier (%)	ΔMigration time (mins)
20	10	0	4.03
25	10	0	2.95
20	20	0	4.69
25	20	0	3.20
20	10	30	7.67
25	10	30	5.65
20	20	30	11.40
25	20	30	7.25

Table 6

Collection of responses for the Effect of voltage

% O	mM B	20 kV	25 kV	Differences
0	10	4.03	2.95	-1.08
0	20	4.69	3.20	-1.49
30	10	7.67	5.65	-2.02
30	20	11.40	7.25	-4.15
Average				**-2.19**

Table 7

The Collection of responses for the effect of organic modifier

kV V	mM B	0%	30%	Difference
20	10	4.03	7.67	3.64
20	20	4.69	11.40	6.71
25	10	2.95	5.65	2.70
25	20	3.20	7.25	4.05
Average				**4.28**

Table 8

The Collection of responses for the effect of buffer concentration

kV V	% O	10 mM	20 mM	Difference
20	0	4.03	4.69	0.66
20	30	7.07	4.40	0.25
25	0	2.95	3.20	0.25
25	30	5.65	7.25	1.60
Average				**1.56**

For single-factor interaction, the migration time was increased with 4.28 minutes with regarding the organic modifier effects, while the concentration of buffer has affected the migration time by an increase of 1.56 minutes, whereas the migration time was only decreased in terms of Voltage effect with a value of -2.19 minutes. For values of two-factor interaction indicate that the interactions between (V, B), (O, V), and (B, O) were not additive, also a random error was existing (Schmitt-Kopplin, 2016).

The combined effect of the three factors was very small compared to that of the percentage of organic modifier alone. The three-factor interaction has shown migration time with 0.43 minutes, which demonstrates that the interactions of the individual factors were not addictive because If they were additive, the combine influence would have been higher than the individual influence (Schmitt-Kopplin, 2016).

4. Calculation

According to (Miller and Miller, 2005) the Calculation of the interactions are as the following:

4. 1 Voltage effect and buffer concentration (V, B)

Average effect when the buffer = 10 mM

= (-1.08 + (-2.02)) ÷ 2 = - 1.55 mins

Average effect when the buffer = 20 mM

= (-4.15 + (-1.49)) ÷ 2 = -2.82 mins

To get the effect of voltage and buffer concentration interaction

= (-2.82 - (-1.55))/ 2 = - 0.64 mins

4.2 % Organic modifier and voltage (O, V)

Average effect when the voltage = 20 kV

= (-3.64 + 6.71))/ 2 = 5.18 mins

Average effect when the voltage = 25 kV

= (4.05+2.70))/ 2 = 3.38 mins

To get the effect of organic modifier and voltage concentration

= (3.375- 5.1750) / 2 = - 0.90 mins

4.3 Buffer concentration and the percentage of organic modifier (B, O)

Average of effect when the percentage of organic modifier = 0%

= (0.25+0.66)/ 2 = - 0.46 mins

Average of effect when the percentage of organic modifier = 30 %

= (3.73 + 1.60)/ 2 = 2.67 mins

To get the effect of buffer concentration and the percentage of organic modifier

= (2.67 – 0.46) / 2 = 1.11 mins

5. Conclusion

Capillary zone electrophoresis (CZE) has been named as the simplest and most popular mode of high-performance capillary electrophoresis. The separation in this technique was depending on the different electrophoretic mobilities of the analysts in free solution (Benzoic acid and Sulphonamide) under the impact of an electric field. This difference can be caused by various charged in addition to the masses and/or the structure of the analyte on the mobility of charge compounds. It is a technique that is expanding and evolving at an enormous rate and this must, therefore, reflect both its versatility and its use in the modern biochemistry laboratories. Different variants of the technique have been discussed with their intrinsic suitability for different types of molecular separation.

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