行政院國家科學委員會補助專題研究計畫成果報告

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行政院國家科學委員會專題研究計畫成果報告
毛細管電泳在臨床上的應用 (III)
The application of capillary electrophoresis in the clinical settings (III)

一、中文摘要

尼古丁是煙草中的成分之一，而且它有害人體. 這篇報告利用毛細管電泳來分析血液中尼古丁的含量. 我們發現介面活性劑與有機修飾劑的使用，可以很容易的定量出人類血清中尼古丁的含量.

關鍵詞：尼古丁、毛細管電泳

Abstract

Nicotine is one of the elements in the tobacco and it is toxic to human health. This report provide an alternative method to determine the concentration of nicotine in the human blood. We found that surfactants and organic modifiers do improve the separation of nicotine in the serum.

Keywords: Nicotine, Capillary electrophoresis

二、Introduction

Nicotine is one of the major elements in cigarettes and it has great influence on human's health. With the increase of smoking population and the age tendency of being younger, we must pay attention to this increasingly serious problem.

The prepulse inhibition (PPI) will reduce due to increase the concentration of nicotine in the human blood. The nicotine concentration increase will lead to decrease the PPI. Also, the heartbeat will be faster while inhaling high concentration of nicotine from the cigarette [1]. Nicotine in the body will affect the timing response process and its accuracy. If we inhale 8mg nicotine, the range of the concentration of nicotine in plasma will be between 6.9 ~ 11.5 ng/ml and

it will make the motor coordination of sport descend [2]. Smoking and other ways of inhaling nicotine will lead to heart diseases and there is a closely relationship between nicotine and cancers [3].

Gas chromatography, High-performance liquid chromatography and radioimmunoassay way are the methods to determine its concentration in the sample [4-10]. It takes time to meet the requirements and the waste materials are the problems to be carefully handled. This report provides an alternative method - micellar electrokinetic capillary chromatography - to detect the concentration of nicotine in the serum and this will be one of the best way to run the routine samples in the clinical laboratory.

三、Result and Discussion

Figure 1 is the molecular sturture of nicotine. Figure 2 shows that high concentrations (0.05M and 0.1M) of sodium dodecyl sulfate (SDS) has better separation than the lower one (0.01M) in 0.038M phosphate buffer with 0.06M sodium borate. We choose 0.05M SDS to run the following sample because it is the optimum condition in this study.

The resolution, separation proficiency, selectivity and the elution time of the analytes can be optimized by the capacity factor, k' (eg. Assorted surfactants, surfactant concentrations, organic modifiers, temperature, voltages, etc.) during the separation in capillary electrophoresis. [12-15]

MECC with SDS and organic modifier

Figure 3 shows the chromatograms

with two different organic modifiers as acetonitrile and methanol with 0.05 M SDS in phosphate buffer. The retention time of nicotine peak in acetonitrile does appear earlier than it in methanol solution. The peak in methanol solution also shows the tailing effect. Figure 4 is the chromatogram of 0.038M phosphate buffer, acteonitrile, 25C, 20KV with 0.05M SDS.

Variation of voltage

Variation of voltage to change the electrical field can have the several effects (e.g. variation in the migration time of samples, EOF, peak area and joule heating). The joule heating that results from an increase in voltage might lead to changes in EOF, ion mobility, analyte diffusion and band broadening. Figure 5 shows the electropherogram of the nicotine running in 0.038M phosphate/0.06M borate buffer solution with 0.05M SDS and acetonitrile at different applied voltages. The migration time increases when the applies voltages decrease. For example, the migration time of nicotine increased from 3.04 minutes to 10.98 minutes when the applied voltage was from 30 to 15 KV. The effect in the retention time is due to the change in the mobility of analyte and EOF.

Temperature effect

Viscosity is a function of time. As the temperature increases, the viscosity decreases. The electrophoretic mobility and EOF increase, too. Some analytes may not be stable at higher temperatures and the variation of temperature may lead to conformational change. Figure 6 shows the electropherograms of nicotine running in 0.038M phosphate/0.06M borate buffer solutions with 0.05M SDS and acetonitrile at different temperatures. An increase in EOF and electrphoretic mobility with increasing temperature leads to a shorter retention time for nicotine sample.

Limit Of Detection and Linear Range

Figure 7 shows the linear range is 50~300 ng/ml in the serum and the LOD is 50 ng/ml. The relative standard deviation in the plasma samples is 3.75%

Conclusion

In conclusion, nicotine can be separated by MECC in 0.038 phosphate buffer,0.06 m sodium cholate with 0.05M SDS and acetonitrile at 20C and 20 KV. This MECC approach can be applied in the clinical settings.

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Fig. 1   nicotine
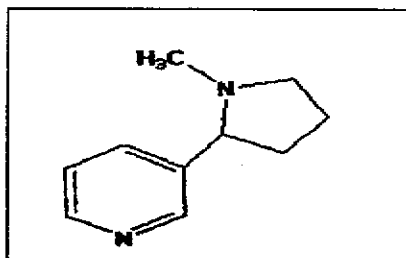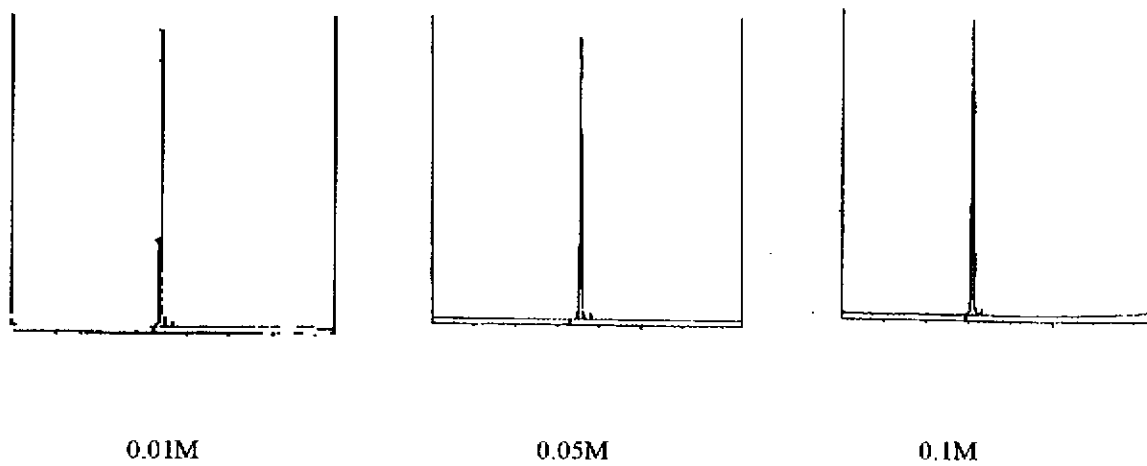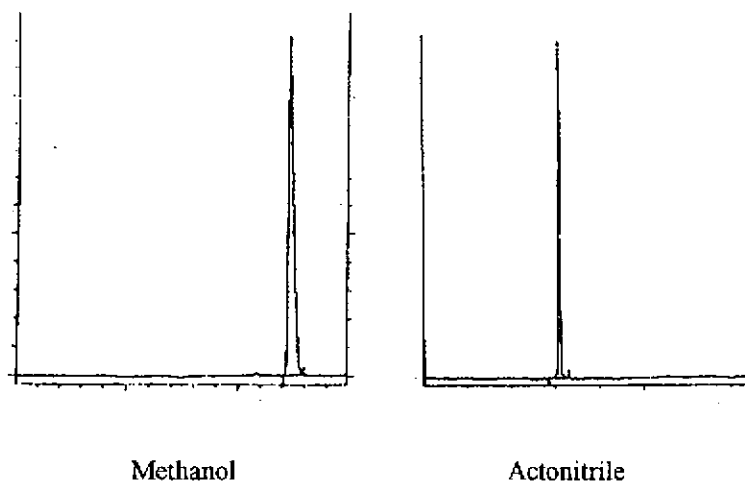

Fig. 2. MECC with SDS

0.01M   0.05M   0.1M

Fig. 3. MECC with SDS and organic modifier

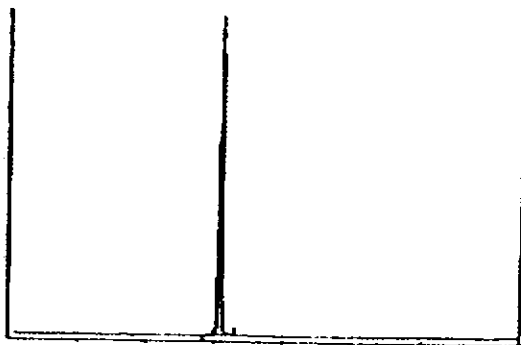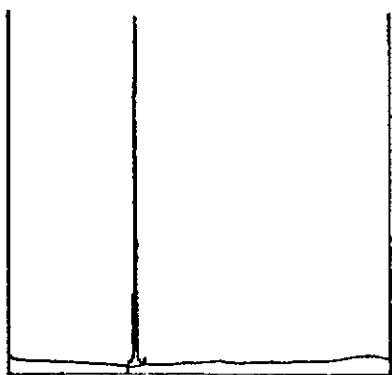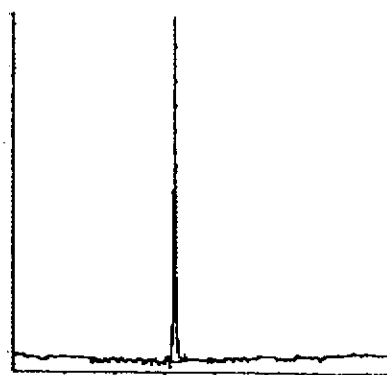Methanol   Actonitrile

Fig 4   electropherogram of nicotine in the condition of 25C, voltage,20 KV., 0.05MSDS, 0.038M phosphate butter of 120% acetonitrile, and 0.06M sodium borate buffer solution

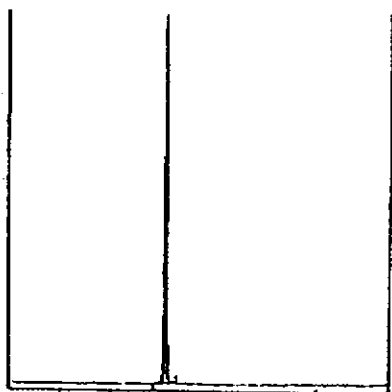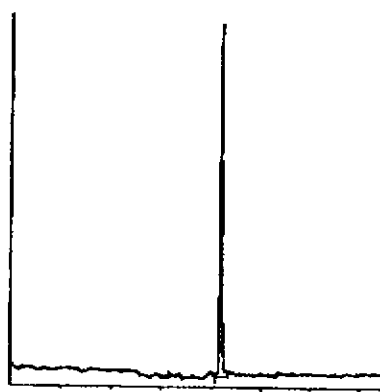Fig.5   a. 30 KV   b. 25 KV   c. 20 KV   d. 15 KV

a.

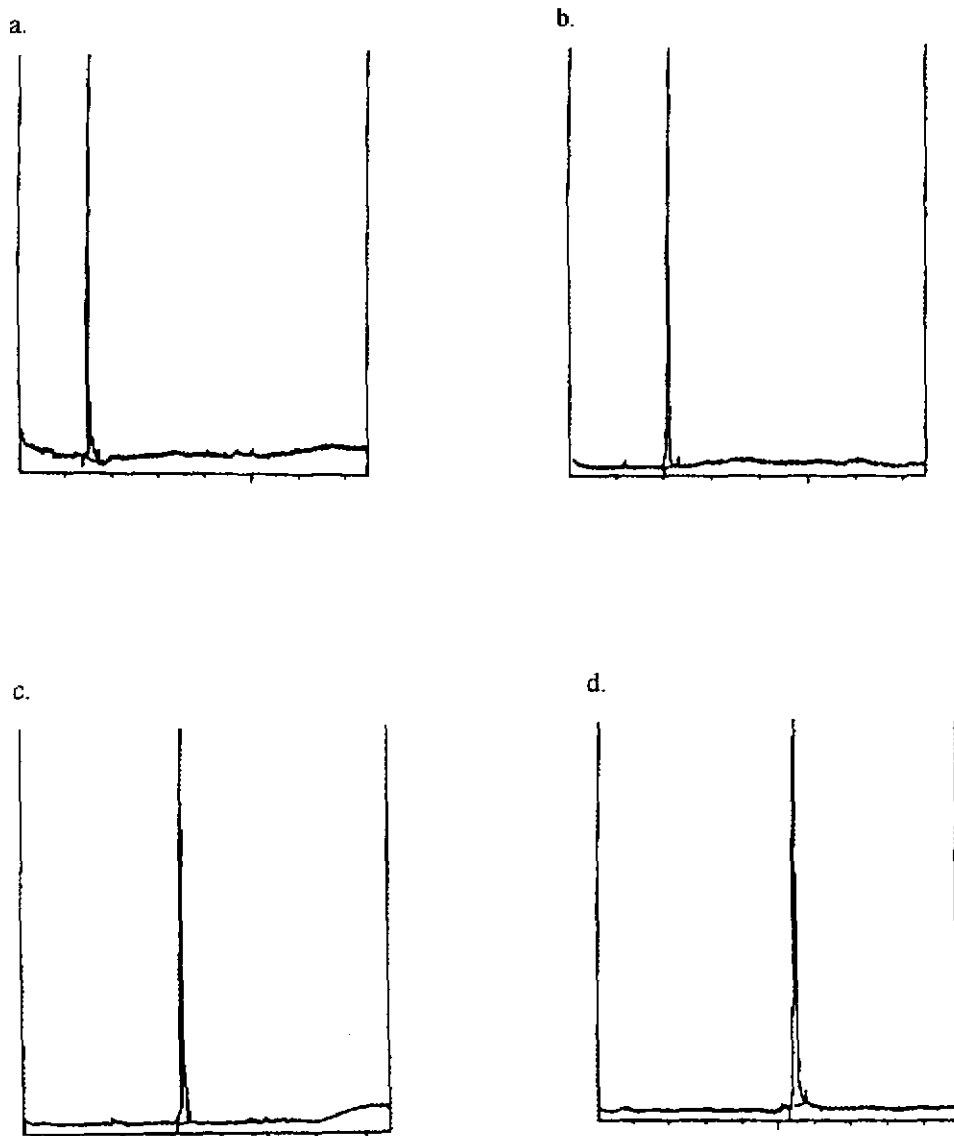b.

c.

d.

Fig.6   a. 50 ℃ b. 35 ℃ c. 25 ℃ d. 15 ℃

a.

b.

c.

d.

Fig. 7

concentration