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Determination of arecoline in areca nut based on field amplification in capillary electrophoresis coupled with electrochemiluminescence detection

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ABSTRACT: A sensitive capillary electrophoresis–electrochemiluminescence (CE–ECL) assay with an ionic liquid (IL) was developed for the determination of arecoline in areca nut. The IL, 1-butyl-3-methylimidazolium tetrafluoroborate ($BMImBF_4$), was an effective additive improved not only the separation selectivity but also the detection sensitivity of the analyte. $BMImBF_4$ in the separation electrolyte made the resistance of the separation buffer much lower than that of the sample solution, which resulted in an enhanced field amplified electrokinetic injection CE. ECL intensity of arecoline is about two times higher than that of the analyte with phosphate–IL buffer system. Resolution between arecoline and other unknown compounds in real samples was improved. Under the optimized conditions (ECL detection at 1.2 V, 16 kV separation voltage, 20 mmol/L phosphate with 10 mmol/L $BMImBF_4$ buffer at pH 7.50, 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L phosphate buffer in the detection reservoir), a detection limit of 5×10^{-9} mol/L for arecoline was obtained. Relative standard deviations of the ECL intensity and the migration time were 4.51% and 0.72% for arecoline. This method was successfully applied to determination of the amount of arecoline in areca nut within 450 s. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: electrochemiluminescence; capillary electrophoresis; arecoline, ionic liquid

Introduction

The electrochemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ has been used to develop numerous analytical methods and has the potential of greatly enhancing sensitivity and selectivity for the determination of trace analytes. Its applications include immunoassays and DNA probe assays (1–3) and determination of oxalate, ascorbic acid and amines (4–10). Recently, ECL has been frequently coupled with CE for the determination of drugs and other bioanalytes (11–16). Because the CE–ECL system has the advantages of short analysis time, small sample consumption and high separation efficiency, it has become an attractive alternative to HPLC for the analysis of medicines active ingredient including Chinese traditional medicines.

Ionic liquids (ILs) are liquid at room temperature and have special physical and chemical properties. Their applications have been extensively explored, (17–20) and they have been used in various analytical separation methods. Also, an IL has been used to investigate the influences of chain lengths on CE separation (21).

Areca nut as a medicinal herb is used worldwide for its varied health benefits. Arecoline (methyl-1,2,5,6-tetrahydro-1-methylnicotinate) is the primary active ingredient in the areca nut responsible for central nervous system effects, such as sympathetic and parasympathetic effects (22). Its pharmacological activities are achieved through inducing the constriction of the bronchial smooth muscles, and stimulation of the lacrimal and intestinal glands. Due to its genotoxic, mutagenic and carcinogenic potential (23–26), arecoline is commonly associated with the development of oral leukoplakia, oral submucous fibrosis and oral cancer (27). Therefore, it is necessary to develop sensitive, rapid and effective methods for the determination of arecoline in areca nut. Several methods have been developed for the analysis of arecoline, such as HPLC (27), mass spectrometry (28) and HPLC–mass spectrometry (29,30). The types of samples tested include breast milk, meconium, urine, umbilical cord serum, human saliva and hair.

The aim of this research was to establish a sensitive and selective CE–ECL-IL assay that could be used for the sensitive analysis of arecoline in areca nut samples after its separation from other coexisting materials. An IL was used as an additive in the running buffer, since it can improve the resolution selectivity and detection sensitivity of arecoline. The CE–ECL system with IL shows the advantages of high sensitivity, good selectivity, a wide dynamic linear range, simplicity of operation and low cost for the determination of arecoline in areca nut. The conditions

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for CE separation, ECL detection and the effect of IL were systematically investigated. The proposed method was successfully applied in the analysis of arecoline in areca nut.

Materials and methods

Reagents

Tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate [$Ru(bpy)_3Cl_2$–$6H_2O$] was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Arecoline was purchased from the National Institute for the Control of Pharamaceutical and Biological Products, Ministry of Health (Beijing, China). The purity of arecoline standard is 99%, and it can be used directly in experiments without further purification. $BMImBF_4$ and 1-butyl-3-methylimidazolium hexafluorophosphate ($BMImPF_6$) used in this work were synthesized by the Centre for Green Chemistry and Catalysis, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, following the procedure described elsewhere (31), and the starting materials were obtained from Sigma-Aldrich (Steinheim, Germany). Chinese herb areca nut was purchased from a local pharmacy (Jilin, China). Sodium hydroxide and sodium phosphate (Na_2HPO_4 and NaH_2PO_4) were purchased from Beijing Chemical Chemicals (Beijing, China). All the reagents were of analytical grade and were used as received without further purification. Double-distilled water was prepared using a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA). All solutions were made up using double-distilled water and stored in the refrigerator at 4 °C before use.

Apparatus and equipment

CE separations and ECL detections were carried out using a computer-controlled CE–ECL system (Xi'an Remex Electronics Co. Ltd, Xi'an, China), including a high-voltage power supply for electrophoretic separation and electrokinetic injection, an electrochemical potentiostat, a multifunctional chemiluminescence detector and a multichannel data processor. All ECL signals were recorded with a photomultiplier tube installed under the ECL detection cell and processed with a data processor controlled by a computer. All experiments were carried out at room temperature.

ECL detection was employed using a three-electrode system consisting of Ag/AgCl as the reference electrode, Pt wire as the counter-electrode and Pt disk (500 μm diameter) as the working electrode. The axes of the working electrode and the separation capillary were aligned, setting the distance at 150 μm from one another with the aid of a optical microscope.

The electrophoretic separation of samples was performed in a 58 cm uncoated fused-silica capillary, 50 μm i.d and 360 μm o.d., obtained from Yongnian Optical Fabric Factory (Hebei, China). The separation capillary was filled with 0.1 mol/L NaOH overnight in order to maintain an active and reproducible inner surface, and then flushed for 10 min with 0.1 mol/L NaOH, 10 min with double-distilled water and 10 min with the running buffer prior to use. A solution consisting of 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L phosphate buffer, pH 7.50, as background electrolyte (BGE), was added to the ECL detection cell. The detection potential applied at the Pt disk working electrode was fixed at 1.2 V, and electrokinetically injected at 10 kV for 10 s. 20 mmol/L phosphate with 10 mmol/L $BMImBF_4$ buffer, pH 7.50, was chosen for the separation buffer. The samples were separated at 16 kV applied voltage.

Solutions

Arecoline was dissolved in double-distilled water to prepare a stock solution. The standard solution of the alkaloid was diluted with double-distilled water to the desired concentration just before use. Various other buffers with different concentrations were also prepared with double-distilled water.

Extraction of arecoline from areca nut

About 0.3 g areca nut powdered sample was accurately weighed, then extracted for 30 min with 2 ml double-distilled water in an ultrasonic bath. The extraction was repeated three times. The extracts were combined and filtered through a 0.45 μm membrane before use. These extracts were diluted further before analysis, as appropriate. In this experiment, a one-step extraction approach without organic solvents avoids both sample loss and environmental pollution.

Results and discussion

Selection of detection potential

Cyclic voltammetry was used to characterize the ECL behaviour of arecoline in a potential range of 0.00–1.30 V. As shown in Fig. 1, the ECL intensity began to increase at 0.8 V (Fig. 1a) and about 100 counts were displayed when the potential was close to 1.2 V. By comparison, when arecoline was added, the ECL intensity was about 3500 counts at 1.20 V (Fig. 1b). This indicated that arecoline can react with the ruthenium species in the ECL process and can enhance the emitted light intensity.

Hydrodynamic voltammograms for the arecoline standard solution were investigated to assess the influence of the detection potential on the ECL responses in a potential range of 0.80–1.40 V (Fig. 2). The results were correlated with the cyclic voltammetry experiments described above. At lower voltages, a very weak ECL response was observed. At 1.1V, the ECL signal started to become

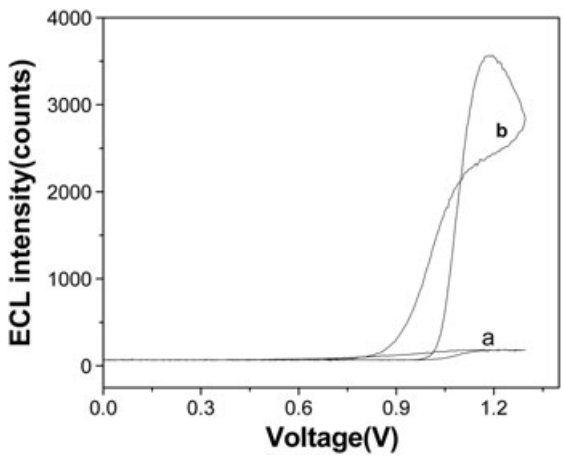

Figure 1. Dependence of the ECL intensity on applied voltage curve of 2.3 mmol/L $Ru(bpy)_3^{2+}$ without (a) and with (b) 10^{-5} mol/L arecoline; 50 mmol/L phosphate buffer, pH 7.5, in the detection cell.

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visible. Finally, the ECL intensity curve reached a plateau, with the most favourable detection potential at 1.2 V.

Effect of buffer

The running buffer pH value has a significant effect on the ECL reaction between $Ru(bpy)_3^{2+}$ and analytes, the analytes ionization and electro-osmotic flow. In this study, the influence of buffer pH on the detection sensitivity of arecoline was investigated with phosphate buffers over the pH range 3.54–9.02. Previous work (11–16) indicated that the ECL reaction between $Ru(bpy)_3^{2+}$ and amine species is a pH-dependent process, and that good ECL efficiency can be achieved under slightly basic conditions, because of the deprotonation of the amine species to form a reducing free radical intermediate. Fig. 3 shows the effect of buffer pH on the detection sensitivity of arecoline; the result agreed with the ECL mechanism reported by Richter (32). Although two ECL peaks were obtained, the maximum intensity of the arecoline with a suitable migration time was observed at pH 7.50. On the other hand, a similar effect of buffer pH in the detection cell on the detection sensitivity was obtained. Phosphate buffer at pH 7.50 was used in both the separation capillary and the detection cell in further experiments.

The effect of phosphate buffer concentration on the detection sensitivity of the alkaloid was studied in the concentration range 10–40 mmol/L. As can be seen from Fig. 4, with increasing concentration, the ECL intensity for the analyte increased gradually and showed maximum intensity with a 20 mmol/L phosphate buffer.

Effect of IL on the analysis performance

Arecoline was detected under the above optimal conditions. However, when extracts of areca nut were introduced into the CE–ECL system, arecoline was poorly separated from other unknown compounds. In order to suppress the deleterious effects of the unknown compounds and improve the resolution, further attempts were made by adding the additive to the running buffer. $BMImBF_4$ and $BMImPF_6$ as additives were investigated for the separation and the determination of arecoline.

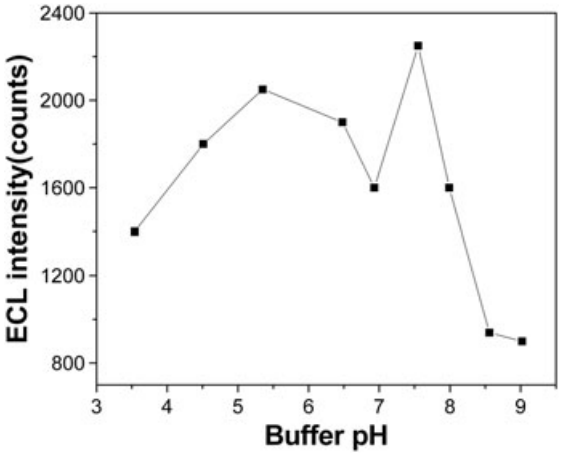

Figure 3. Effect of buffer pH value on ECL intensity of arecoline. Conditions: sample, 5×10^{-5} mol/L arecoline; detection voltage, 1.20 V; electrokinetic injection, 10 s at 10 kV; separation phosphate buffer, 20 mmol/L; separation voltage, 16 kV.

The experimental data indicated that the migration time of the analyte was prolonged by using $BMImPF_6$, and resolution between arecoline and the unknown compound in real samples was not improved. However, by employing $BMImBF_4$, there was a significant change in separation selectivity. A possible reason is that hydrophobic $BMImPF_6$ provides a higher viscosity and a lower ionic conductivity compared with $BMImBF_4$; the latter was therefore used for the determination of arecoline. The effects of the IL, $BMImBF_4$, on the analytical performance of the CE–ECL system were investigated in the concentration range 2.0–16 mmol/L, as shown in Fig. 5. With increasing $BMImBF_4$ concentration, the resolution between arecoline and the unknown compounds was gradually improved, due to the difference of interactions between arecoline and alkyl imidazolium cations and between unknown compounds and alkyl imidazolium cations. In addition, the electrostatic attraction of alkyl imidazolium cations with the negatively charged capillary surface changes the net charge of the inner wall of the separation capillary, which results in a decrease of electroosmotic flow, and the separation system affords more opportunity for the

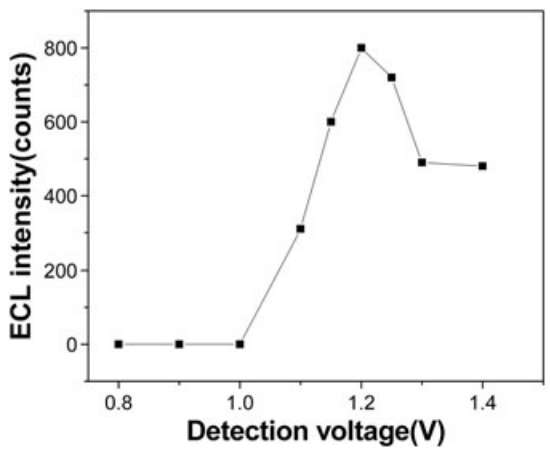

Figure 2. Dependence of the ECL intensity on the detection potential. 2×10^{-5} mol/L arecoline aqueous solution, electrokinetic injection 10 s × 10 kV, 20 mmol/L phosphate running buffer, pH 7.5; 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L buffer in the detection cell; separation potential 16 kV.

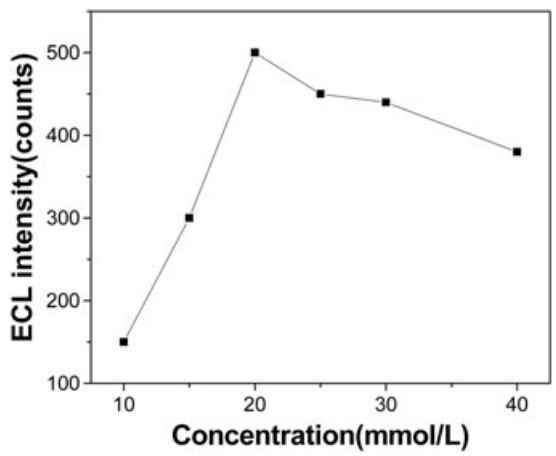

Figure 4. Effect of buffer concentration on ECL intensity of arecoline. Conditions: sample, 10^{-5} mol/L arecoline; detection voltage, 1.20 V; electrokinetic injection, 10 s at 10 kV; separation phosphate buffer, pH 7.50; separation voltage, 16 kV.

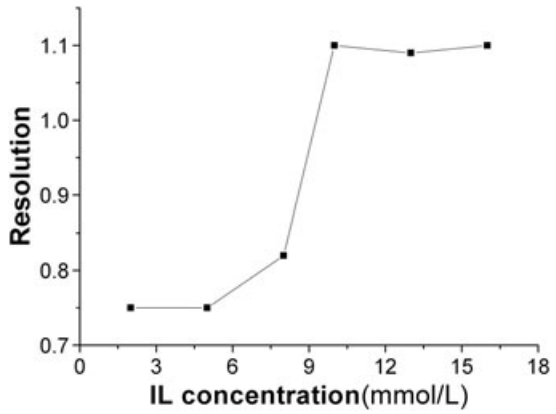

Figure 5. Effect of IL concentration on analysis performance. Conditions are the same as in Figure 4.

analyte to associate with the IL. In this study, good resolution with a suitable migration time was obtained with a running buffer composed of 20 mmol/L phosphate and 10 mmol/L $BMImBF_4$.

Compared with the phosphate separation buffer system, higher ECL intensity of the analyte can be observed with the phosphate–IL buffer system. $BMImBF_4$ in the separation electrolyte greatly enhanced the conductivity of the running buffer, which made the resistance of the separation buffer much lower than the sample solution and resulted in an enhanced field amplified effect of electrokinetic injection CE. As shown in Fig. 6, when $BMImBF_4$ was added to the running buffer, the ECL intensity of arecoline was about two times higher than that of the analyte with the phosphate buffer system. In this experiment, IL $BMImBF_4$ as an additive in the separation buffer could improve not only the separation selectivity but also the detection sensitivity.

Effect of separation voltage

The separation voltage affects the quality of the separation and the migration time of analytes. Attempts were therefore made to optimize the separation conditions by using different applied voltages in the range 12–20 kV. The effect of the separation voltage on the ECL intensity of the analyte is shown in Fig. 7. The sharp peak of analyte was observed by increasing the separation voltage. However, excess voltage ($>$ 16 kV) affected the actual detection voltage applied at the Pt working electrode and resulted in increased baseline noise. Moreover the increasing joule heat within the capillary caused peak broadening and adversely affected the separation performance. Based on these experiments, 16 kV was selected as the optimum voltage to achieve a good compromise.

Repeatability, linearity and detection limit

Under the optimum conditions, the repeatability of the analysis, including ECL intensity and migration time of analyte, was studied. The linear relationship between ECL intensity of the analyte and the corresponding concentrations was established by a series of the injections of standard solution with different concentrations. The calibration equation of the standard curve is $I = 7.58 \times 10^7 C + 92$ for arecoline, where I is ECL intensity and C is the concentration of the analyte. The limit of detection (LOD) was based on the concentration of the alkaloid that gave a signal three times greater than the baseline noise (S:N = 3). The results of detection limit, linear range, correlation coefficient and recovery are shown in Table 1.

Assay of areca nut samples

The extracts from areca nut were analysed by field-amplified sample stacking CE–ECL detection. The herbal extracts preparation procedure was adopted as described above, assuring an exhaustive extraction of the target analyte. Spiking of pure reference alkaloid standards in areca nut extracts was used for peak identification, by comparing the electropherograms of diluted herbal extracts with those of diluted herbal extracts spiked with a known standard of alkaloid, where the increase of peak height at a certain migration time was directly proportional to the amount spiked with arecoline. In the internal standard method employed, the content of arecoline found in the herb was 0.0096%.

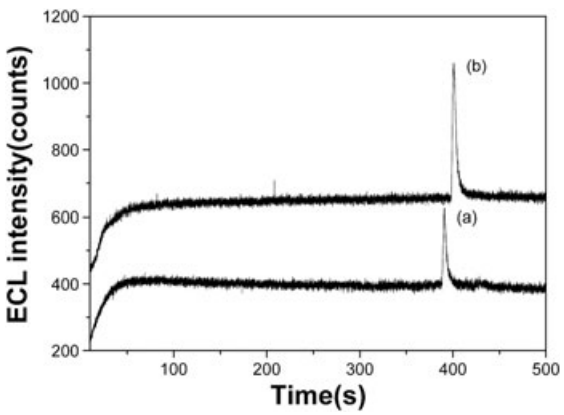

Figure 6. Effect of IL on ECL intensity of arecoline. ECL intensity of arecoline (2×10^{-6} mol/L) in 20 mmol/L phosphate running buffer with (b) 10 mmol/L $BMImBF_4$ and (a) without 10 mmol/L $BMImBF_4$. Conditions are the same as in Figure 4.

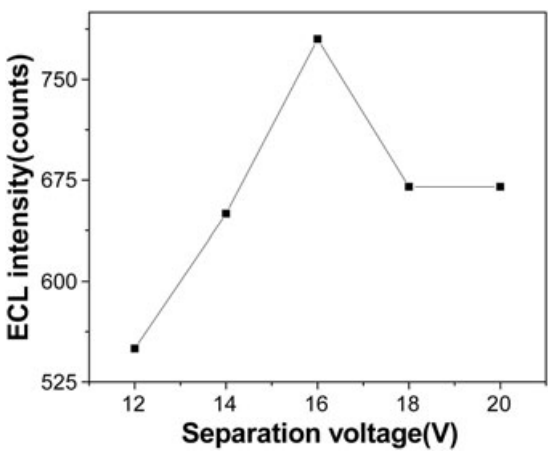

Figure 7. Effect of the separation voltage on the ECL intensity of arecoline. Conditions: sample, 10^{-5} mol/L arecoline; detection voltage, 1.20 V; electrokinetic injection, 10 s at 10 kV; separation phosphate buffer, 20 mmol/L.

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Table 1. Linearity, detection limit, recovery and reproducibility of the determination of arecoline by the proposed method

Linear range (mol/L)	Correlation coefficient	Detection limit (mol/L)	Recovery (%)	RSD (%, $n = 7$)	
				For ECL intensity	For migration time
1×10^{-7}–1×10^{-5}	0.996	5×10^{-9}	96.3	4.51	0.72

Conditions: 1.20 V detection voltage, 20 mmol/L phosphate with 10 mmol/L $BMImBF_4$ buffer at pH 7.50, 16 kV separation voltage, 5 mmol/L $Ru(bpy)_3^{2+}$ and 50 mmol/L phosphate buffer, pH 7.50, in the detection reservoir.

The phosphate–$BMImBF_4$ running buffer system can improve not only the separation selectivity but also the detection sensitivity of the analyte. The results showed that the method can be used for the sensitive and selective determination of arecoline in real samples. A typical electropherogram for a real sample solution is depicted in Fig. 8. A baseline separation was accomplished with a running buffer composed of 20 mmol/L phosphate and 10 mmol/L $BMImBF_4$. To further evaluate the accuracy of the method, recovery experiments under the optimum conditions were also conducted with real samples. The recoveries of arecoline obtained are listed in Table 1. The reproducibility of this method was tested by six identical injections of 10^{-5} mol/L arecoline standard solution. Relative standard deviations of the ECL intensity and the migration time were 4.51% and 0.72%, respectively, for arecoline.

Concluding remarks

In conclusion, a sensitive field-amplified sample stacking CE–ECL technique was established for the determination of arecoline in areca nut with one-step real sample pretreatment. $BMImBF_4$ as a high conductivity additive in the running buffer can improve both the separation selectivity and the detection sensitivity of the analyte. The newly proposed CE–ECL with IL method enables a much faster, yet improved, separation and detection of arecoline. The developed method can surely be considered as an equivalent alternative to HPLC for the determination of active components in the complex extracts of medicinal plants.

Acknowledgements

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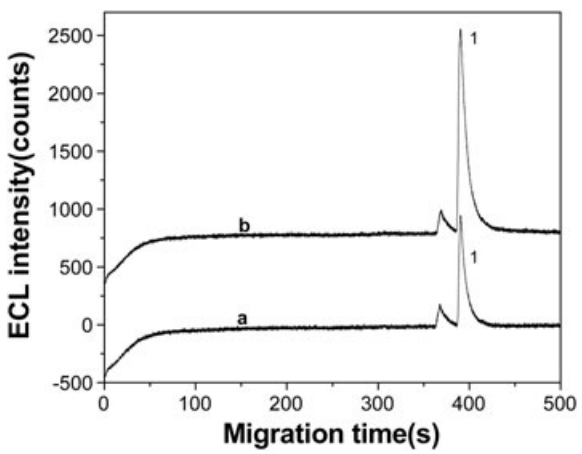

Figure 8. CE–ECL detection profile of alkaloid in real sample spiked with (b) arecoline (5×10^{-5} mol/L) and (a) without spiking. Peak 1, arecoline. Conditions are the same as in Figure 6.

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