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# Measurement of ascorbic acid in single rat peritoneal mast cells using capillary electrophoresis with electrochemical detection

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ABSTRACT

In this paper, the amount of ascorbic acid (AA) in single rat peritoneal mast cell was determined by the method of capillary electrophoresis (CE) with electrochemical detection (ED) at a carbon fiber microdisk bundle electrode. The CE–ED system and the single-cell injection system were rearranged to make the operation more convenient and efficient. In the experiment, a self-made holder made of foam was used to keep the capillary from swing, which kept the stability of the baseline of the electropherogram. The single cell was lysed completely within 5 s using the 0.1% sodium dodecylsulfate (SDS) as the cell lysis solution together with the lysis voltage of 2 kV. The quantitation analysis was accomplished by the use of calibration curves, and the amount of AA in single rat peritoneal mast cell was from 2.4 to 7.1 fmol.


## 1. Introduction

Ascorbic acid (AA) is one of the most important cellular antioxidants [1,2], and it improves immunological functions, such as phagocytosis, chemotaxis, and neutrophil adhesion [3]. Recent clinical studies have demonstrated that the content of AA in biological fluids can be used to assess the amount of oxidation stress in human metabolism [4], and excessive oxidative stress can result in cancer, diabetes mellitus and hepatic disease. AA also plays an important role in mast cell functions including killing malignant mast cells at low densities [5], protecting mast cell lines against peroxidative stress induced by oxidized low density lipoproteins (LDL) [6]. It is also reported that the relatively high level of AA in mast cells serves to protect the cell membrane from oxidative damage. AA may therefore contribute to the survival of mast cells after an extensive secretory response [7]. Therefore, the determination of AA in mast cells is important and significant.

Capillary electrophoresis (CE) has been widely applied to the rapid detection of biochemical and pharmaceutical analytes, and it has become a useful and powerful technique to analyze single cells [8–11] owing to its advantages such as low reagent consumption, fast analysis speed, high separation efficiency and biocompatible environments. Electrochemical detection (ED), as an inexpensive and sensitive detection method, has been successfully coupled to CE to detect electroactive compounds in single cells such as dopamine in single rat pheochromocytoma cell [12], histamine in individual rat peritoneal mast cells [13] and glutathione in single human erythrocytes [14], single mouse peritoneal lymphocytes [15] and single human hepatocarcinoma cells [16]. However, the analysis of ascorbic acid in single rat peritoneal mast cells (RPMCs) using CE with electrochemical detection has not been reported to date.

In this paper, a simple and rapid method for the analysis of ascorbic acid in single rat peritoneal mast cells with a self-made foam holder using CE with ED at a carbon fiber microdisk bundle electrode was reported.

## 2. Experimental

### 2.1. Instrumentation

The CE system used in this work was almost similar to which described in Ref. [17] in addition to some useful improvements. The CE system, the arrangement of the ED cell and the single-cell injection system were illustrated in Fig. 1 in detail. Briefly, a reversible high-voltage power supply (Model 9323-HVPS; Beijing Institute of New Technology, Beijing, China) provided a variable voltage of 0–30 kV across the capillary, with the outlet of the capillary at ground potential. Fused-silica capillaries (20 μm ID, 375 μm OD) from Yongnian Optical Conductive Fiber Plant (Yongnian, China) were cut into length of 60 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the ED cell was held at ground potential.

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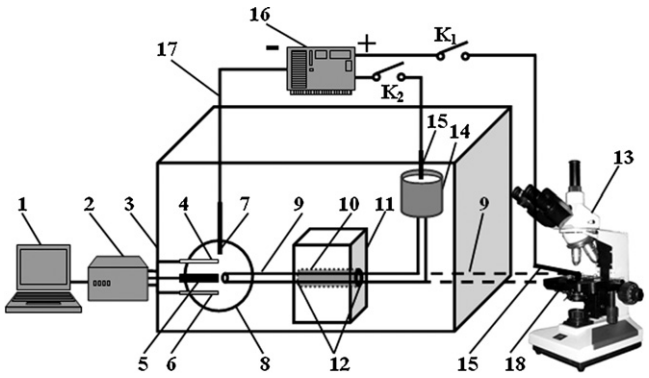


**Fig. 1.** Overview of the CE–ED detection and the single-cell analysis system. 1, computer; 2, electrochemical detector; 3, Faraday cage; 4, reference electrode; 5, working electrode; 6, auxiliary electrode; 7, high-voltage cathode; 8, CE–ED detection cell; 9, capillary; 10, rubber tube; 11, foam holder; 12, silicone glass sealant; 13, inverted microscope; 14, running buffer reservoir; 15, high-voltage anode; 16, high voltage; 17, copper wire; 18, setup of single-cell injection [19].

Separations were carried out at an applied voltage of 20 kV. The ED at a constant potential was performed with a voltammetric analyzer (Model CHI800; Shanghai Chenhua Instrument Company, Shanghai, China). ED was carried out with a three-electrode system. It consisted of a carbon fiber microdisk bundle electrode which was the same as the one described previously [18] as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a coiled Pt wire (0.5 mm diameter, 4 cm in length) placed at the bottom of the cell as the auxiliary electrode. The Pt wire also served as the ground for the high potential drop across the capillary. The whole detection cell as well as the capillary and the running buffer reservoir were housed in a Faraday cage in order to minimize the interference from external noise sources. When AA in the cell extract was to be determined, the capillary (9) and the high-voltage anode (15) were in the position of the running buffer reservoir (14) shown in Fig. 1. When AA in a single cell was to be detected, both of the capillary (9) and the Pt wire (15) of the high-voltage anode were turned to the single-cell injection device (18) on the microscope. In the process of the single-cell analysis, it was easy for the capillary to swing because of the ionized air around or the wind. This made the baseline of the electropherogram very unstable and signal-to-noise ratio high (as shown in Fig. 5 curve 2). An easy self-made holder made of foam (11) was used to fix the capillary to solve this problem. The capillary (9) was inserted into a soft rubber tube (~350 μm ID, 600 μm OD) (10) and the rubber tube was inserted into the foam passage. Then the rubber tube was fixed to the foam tightly by filling the interfaces between the tube and the foam with silicone glass sealant (12). Compared with the CE system we previously used, the capillary was more stable after being tightly placed into the foam holder. Moreover, a good signal-to-noise ratio was obtained (as shown in Fig. 5 curves 3 and 4). After the electroosmotic current reached a constant value, the electromigration injection and lysis of the whole cell mentioned above were carried out again. Then the capillary was returned carefully to the CE buffer. Finally, a separation voltage of 20 kV was applied across the capillary and the detection potential of 0.80 V *vs.* SCE was applied at the working electrode and the electropherogram was recorded.

### 2.2. Reagents and solutions

Ficoll-400 was obtained from Pharmcia Company, Sino-American Biotec. (Beijing, China). Lymphocytes separation medium (density: 1.077 ± 0.002 g/ml) was purchased from Tianjin Haoyang Biologic Products Science and Technology Limited Company (Tianjin, China). Mast cells separation solution (density 1.085 g/ml) was prepared by mixing 1.86 ml 40% Ficoll-400 and 8.14 ml Lymphocytes separation medium. The physiological buffer saline (PBS) consisted of 0.146 mol/l NaCl and 0.006 mol/l $NaH_2PO_4$–0.014 mol/l $Na_2HPO_4$ (pH 7.4). SDS, histamine, tyrosine, serotonin and norepinephrine were purchased from Sigma (St. Louis, MO, USA). Dopamine, epinephrine and uric acid were obtained from Fluka (Buchs, Switzerland). Tryptophan was purchased from DongFang Sanitary Materials Factory (Tianjin, China). L-Cysteine was purchased from Shanghai Bio. Life Science and Technology (Shanghai, China). AA (analytical grade, content >99.7%) was purchased from BASF Chemicals Limited Company (BASF, Ludwigshafen, Germany). All chemicals and reagents were of analytical grade and used without additional purification. All solutions were prepared with deionized water. Stock buffer solutions were 0.2 mol/l $Na_2HPO_4$ and 0.2 mol/l $NaH_2PO_4$. 0.01 mol/l stock solution of AA was freshly prepared with water daily because of its sensitivity to air. The detected analytes were diluted with buffer solutions before analysis. Adult male rats (150–200 g) were purchased from Qingdao Port edicine Inspection Institute (Qingdao, China).

### 2.3. Preparation of rat peritoneal mast cells

Adult RPMCs were isolated from an adult male rat by the method almost the same as that described in Ref. [13]. An adult male rat was placed in an ether-saturated chamber for 2–3 min to be anesthetized and decapitated. This was found to be superior to other methods, which tended to cause internal bleeding into the peritoneal cavity. The abdominal fur of the rat was quickly sterilized with 75% alcohol. The abdominal wall was exposed by removal of a piece of fur 2 inches square. An injector was inserted deep into the abdomen and approximately 30 ml of phosphate-buffered saline (PBS) buffer was injected into the peritoneal cavity. After the abdomen was massaged for 2 min, the peritoneal cavity was cut open, ca. 25 ml fluid of the buffer containing cells was withdrawn and collected in three 10-ml centrifuge tubes kept in ice. In order to minimize the influence on the AA concentration either in the cells extract or in the single cells by the preparation method, all subsequent operations were carried out at 4 °C. The fluid was centrifuged for 10 min (1000 rpm, 4 °C) and the supernatants were decanted. Cell mixtures at the bottom of the three centrifuge tubes were transferred into one centrifuge tube. By the aid of pipetting, the cell mixture was resuspended in ca. 2 ml PBS buffer and was then added slowly into another centrifuge tube containing ca. 3 ml mast cells separation solution along the tube wall by pipette. It was centrifuged for 15 min at 2500 rpm and 4 °C. After the supernatant was discarded, the cells were washed twice with cold PBS buffer. The final mast cells were resuspended in ca. 2 ml PBS buffer. This was the suspension of mast cells and was stored in this medium at 4 °C for further use. The amount of the cells in the suspension was calculated with a hemocytometer (Zhejiang Yuhuan Medical Instrument Plant, Zhejiang, China) and the yield was $3.8 \times 10^5$ cell/ml. To obtain the extract of AA from the RPMCs, 1.0 ml suspension of PBS containing RPMCs was centrifuged for 10 min at 1000 rpm and 4 °C. The supernatant was discarded. After the cell solution was lysed by sonication for 15 min, it was centrifuged for 20 min at 1000 rpm. The supernatant was diluted into 1.0 ml with the running buffer ($1.83 \times 10^{-2}$ mol/l $Na_2HPO_4$–$1.70 \times 10^{-3}$ mol/l $NaH_2PO_4$ (pH 7.8)). It was the mast cells extract for the determination of AA.

### 2.4. Injection, lysis and analysis of single cells

The whole setup of single-cell injection made with microscope slide was the same as which described in Ref. [19] besides a touch switch added between the injection and the high voltage (as shown in Fig. 1 $K_1$). The single-cell injection method was almost the same as that in our previous work [18]. After the single-cell injection

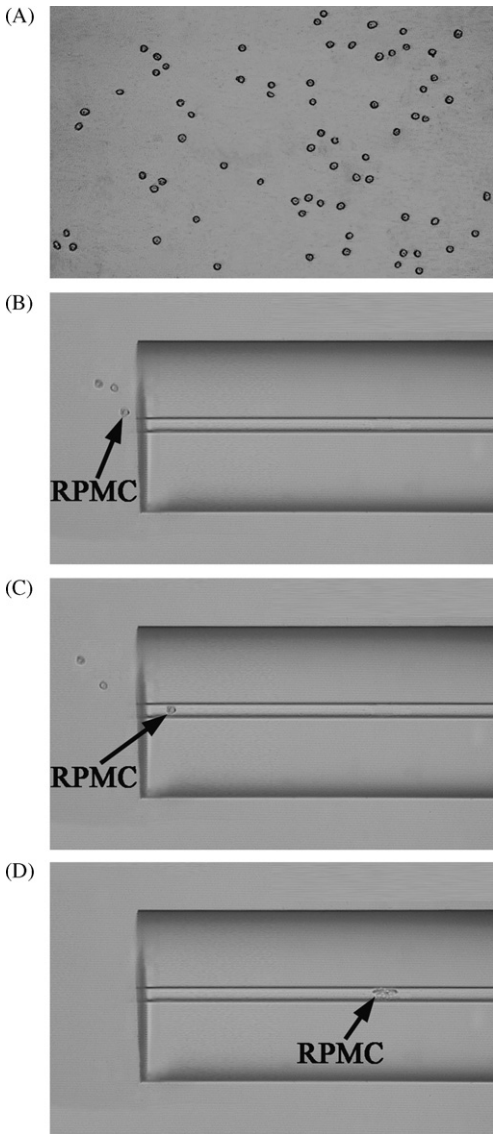


**Fig. 2.** Process of the injection and lysis of single cells. (A) The single RPMCs on the slide under the view of microscope; (B) a mast cell drifting toward the injection end; (C) the whole cell into the separation capillary tip; (D) the mast cell lysed.

microscope slide was placed on the inverted microscope (Nanjing Jiangnan Optical Instrument Limited Company, Nanjing, China) with a magnification of 160×, a drop of ~10 μl aliquots of the RPMCs suspension was deposited in the cell suspension reservoir on the microscope slide. Fig. 2A shows the single RPMCs on the slide under the microscope. Then the bare injection end of the capillary filled with electrophoresis running buffer was gently immersed in the RPMCs suspension under the guidance of a three-dimensional micromanipulator. A platinum wire was placed in the cell suspension to serve as the electrophoresis anode. As soon as a mast cell was drifting toward the injection end under the field of vision of the microscope vision (as shown in Fig. 2B), the touch switch ($K_1$) was on and an injection voltage of 2 kV was applied to transport the whole cell into the separation capillary tip (as shown in Fig. 2C). The touch switch ($K_1$) was off after the single-cell injection finished. The anode (15) no longer needed to be manipulated up out of the buffer reservoir. This made the single-cell operation very convenient, quick and safe. The entire process of cell injection typically took less than 1 min. The mast cell lysed rapidly with the cooperation of the 0.1% sodium dodecylsulfate as the cell lysis solution and the lysis voltage of 2 kV. After the cell was lysed, the separation capillary was carefully immersed in a reservoir containing electrophoresis buffer with an anode for electrophoretic separation. Finally, the separation voltage of 20 kV was applied through the capillary and the detection potential was applied at the working electrode.

### 2.5. Data treatment

Both AA in RPMCs extract and in single RPMCs were identified on the basis of the migration time in comparison with standard AA in the electropherograms. Peak currents and the concentration standard calibration were used to quantify AA in RPMCs extract. Due to the difference in volume for each cell and low analytic level, peak areas and the mass external standardization were used for the quantification of AA in single cells.

## 3. Results and discussion

### 3.1. Lysis and analysis of the RPMCs

To achieve rapid lysis, the cell has to be close to the boundary between the running buffer and cell medium, so that SDS surfactant can rapidly reach the cell through diffusion. In our laboratory, lysis of rat peritoneal mast cells and rat hepatocytes has been investigated. Rat peritoneal mast cells and rat hepatocytes could not be lysed in 0.1% SDS within 5 min and could be lysed in NaOH easily. Jin et al. reported similar observations [13]. In this study, however, we found that the pH value of the running buffer had a great influence on the detection of AA and the buffer pH could be changed by the introduction of NaOH. The experimental results showed that the background noises greatly increased and the signal-to-noise ratio decreased when the pH was over 9.0. The reason might be that at alkaline pH conditions AA was unstable and the negative-charged AA would be absorbed to the capillary wall [20,21]. It was further found that 0.1% SDS together with the lysis voltage of 2 kV made the mast cells lysed quickly. This method was selected to lyse mast cells. When the whole cell was injected into the separation capillary tip (as shown in Fig. 2C), a drop of ~10 μl aliquots of the 0.1% SDS was deposited in the cell suspension reservoir on the microscope slide to lyse and an injection voltage of 2 kV was applied for 5 s to inject SDS into the capillary tip. After the plug of the lysis solution covered the cell for about 10 s in the injection end of the separation capillary, a voltage of 2 kV was reapplied and lysis of the mast cell was observed with the aid of the microscope. We found that the cells lysed completely within 5 s after the use of the lysis voltage (as shown in Fig. 2D). We confirmed that, cell lysis occurred within 20 s of injection by this method. When separation voltage was applied, the lysate migrated to the detector and the electropherogram was recorded.

### 3.2. Determination of AA in RPMCs extract

The separation and detection conditions for standard AA at a carbon fiber disk bundle electrode with CE–ECD were: $1.83 \times 10^{-2}$ mol/l $Na_2HPO_4$–$1.70 \times 10^{-3}$ mol/l $NaH_2PO_4$ (pH 7.8) for the running buffer solution, 20 kV for the separation voltage, 0.80 V for the detection potential, 5 kV and 10 s for the injection voltage and the injection time. The electropherogram of $5.0 \times 10^{-5}$ mol/l AA was shown in Fig. 3 (curve 1). A peak with the migration time, $t_m$, of 7.0 min, could be identified as the peak of AA. Under optimized experimental conditions, good linear relationship held between the logarithm of the concentration of ascorbic acid and the logarithm of the peak current in the range of $5.0 \times 10^{-6}$ mol/l to $5.0 \times 10^{-4}$ mol/l, the regression equation was $Y = 0.8498X - 5.078$ with a correlation coefficient of 0.9962. The R.S.D. of the method for 10 consecutive injections of

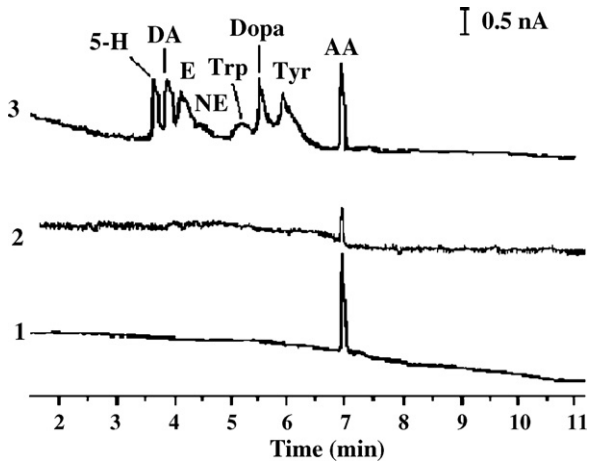


**Fig. 3.** Electropherograms of 1, $5.0 \times 10^{-5}$ mol/l AA; 2, the RPMCs extract; 3, a solution containing $5.0 \times 10^{-5}$ mol/l dopamine (DA), epinephrine (E), tryptophan (Trp), uric acid (UA), AA, tyrosine (Tyr), L-cysteine (Cys), L-glutathione (GSH), norepinephrine (NE), serotonin (5-HT), dopa (Dopa), histidine (HA). Conditions: $1.83 \times 10^{-2}$ mol/l $Na_2HPO_4$–$1.70 \times 10^{-3}$ mol/l $NaH_2PO_4$ (pH 7.8); capillary, 60 cm in length, 25 μm ID, 375 μm OD; injection, 5 kV for 10 s; separation voltage, 20 kV; detection potential, 0.80 V (*vs.* SCE).

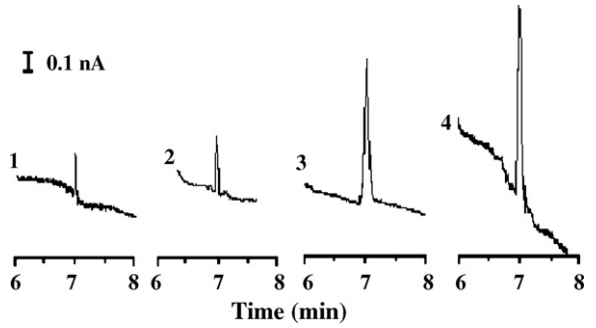


**Fig. 4.** Electropherograms of mast cell extract without and with the standard solution of AA. The added concentration of AA: 1, 0 mol/l; 2, $6.94 \times 10^{-6}$ mol/l; 3, $2.50 \times 10^{-5}$ mol/l; 4, $4.31 \times 10^{-5}$ mol/l. Other conditions as in Fig. 3.

$5.0 \times 10^{-5}$ mol/l AA ($n = 10$) were 0.79% for the migration time and 1.6% for the peak current, respectively. The concentration limit of detection ($LOD_c$) of AA was $1.7 \times 10^{-6}$ mol/l, when the signal-to-noise ratio was 3. Using the calculated injection volume of 1.75 nl, the mass LOD was 3.0 fmol. The electropherogram of the RPMCs extract was shown in Fig. 3 (curve 2), in which a peak eluting at 7.0 min should be the peak of AA in the RPMCs extract by comparing the migration time with that in curve 1. In order to determine whether the other electroactive compounds, such as dopamine, epinephrine, tryptophan, uric acid, tyrosine, L-cysteine, L-glutathione, norepinephrine, serotonin, dopa, histidine which can be oxidized at the working electrode directly, interfered with the determination of AA, their electrophoretic behavior was investigated, and the electropherogram was shown in Fig. 3 (curve 3). It

**Table 1**
Migration time, $t_m$, peak current, $i_p$, peak area, $q$ and amount of AA, in single RPMCs. Other conditions as in Fig. 3.

| Run no. | $t_m$ (min) | $i_p$ (nA) | $q$ (nC) | Amount (fmol) |
|---|---|---|---|---|
| 1 | 7.08 | 0.634 | 1.141 | 2.5 |
| 2 | 7.02 | 1.360 | 2.856 | 7.1 |
| 3 | 7.07 | 0.609 | 1.096 | 2.4 |
| 4 | 7.02 | 1.365 | 2.730 | 7.1 |
| 5 | 7.05 | 1.106 | 2.101 | 5.3 |
| 6 | 7.05 | 0.798 | 1.357 | 3.5 |
| 7 | 7.03 | 0.830 | 1.328 | 3.5 |

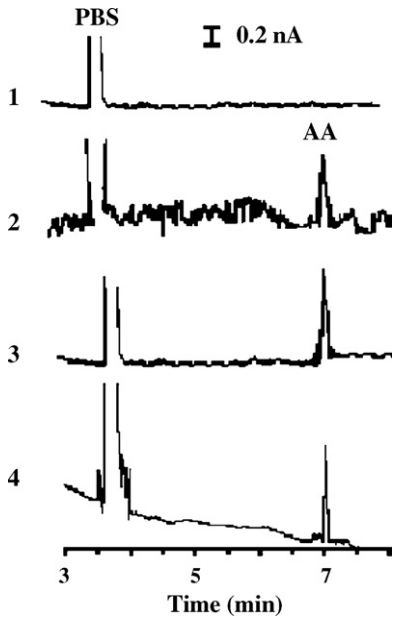


**Fig. 5.** Electropherograms of 1, PBS; 2, the single RPMC (without a holder); 3 and 4, the single RPMCs (with a holder). Other conditions as in Fig. 3.

was found that the peaks of these compounds could be well separated from AA, so they did not interfere with the determination of AA. The concentration of AA in the RPMCs extract obtained by the concentration standard calibration method was $2.1 \times 10^{-6}$ mol/l. Fig. 4 shows the typical electropherograms of the RPMCs extract without and with the standard solution of AA. The recovery rate was between 93% and 97%. Since the cell concentration in the RPMCs extract was $3.8 \times 10^5$ cell/ml, the mean mass of AA in a single RPMC could be calculated to be 5.5 fmol.

### 3.3. Identification and quantification of AA in individual RPMCs

The electropherograms of the contents of individual RPMCs were shown in Fig. 5 (curves 2–4). Curves 3–4 were the electropherograms of two individual mast cells with a self-made holder. Compared with the electropherogram of an individual mast cells without a holder (as shown in Fig. 5 curve 2), the baseline of the electropherogram was stable and the good signal-to-noise ratio was obtained.

There were two peaks appearing in the electropherograms. Comparing curves 2–4 with curve 1 in the electropherogram of the PBS solution used to suspend the RPMCs, it could be found that the peak eluting at 3.70 min came from PBS solution, the peak eluting at 7.00 min should be corresponding to AA based on the migration time. The quantization results of analysis for seven single RPMCs were listed in Table 1. The peak area together with the large linear dynamic range (from 3.0 to 300 fmol) for standard AA made it suitable to use external standardization for the quantification of AA in single RPMCs. Table 1 shows that amounts of AA in single RPMCs differed from cell to cell. The amounts of AA determined in seven cells were 2.4–7.1 fmol. The value in the RPMCs extract 5.5 fmol/cell was in the range of the values.

## 4. Conclusions

The results of this study show that capillary electrophoresis with electrochemical detection at a carbon fiber microdisk bundle electrode can be used to determine ascorbic acid in individual rat peritoneal mast cells. In this method, The CE–ED system and the single-cell injection system are rearranged as the mode described in

this paper and it is more convenient and efficient for the operation. A self-made holder made of foam was used to keep the capillary from swing so as to keep the stability of the baseline of the electropherogram. The cooperation of the 0.1% sodium dodecylsulfate as the cell lysis solution and the lysis voltage of 2 kV make the mast cell lysed rapidly. The method is a simple, sensitive and reliable technique for the analysis of chemical species in single cells.

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