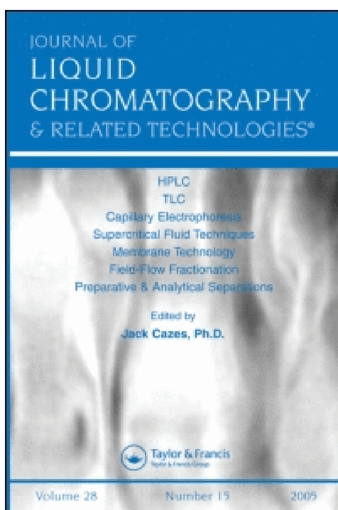
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SINGLE CELL ANALYSIS USING CAPILLARY ELECTROPHORESIS

L. M. Shaner[a]; P. R. Brown[b]
[a] Pfizer Central Research, Groton, CT, U.S.A. [b] Department of Chemistry, University of Rhode Island, Kingston, RI, U.S.A.

Online publication date: 04 October 2000

To cite this Article Shaner, L. M. and Brown, P. R.(2000) 'SINGLE CELL ANALYSIS USING CAPILLARY ELECTROPHORESIS', Journal of Liquid Chromatography & Related Technologies, 23: 7, 975 — 997
To link to this Article: DOI: 10.1081/JLC-100101502
URL: http://dx.doi.org/10.1081/JLC-100101502

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 23(7), 975–997 (2000)

SINGLE CELL ANALYSIS USING CAPILLARY ELECTROPHORESIS

L. M. Shaner,[1] P. R. Brown[2]

[1] Pfizer Central Research
Groton, CT 06249

[2] University of Rhode Island
Department of Chemistry
Kingston, RI 02881

Downloaded At: 10:00 24 January 2011

ABSTRACT

Capillary electrophoresis (CE) applied to the analysis of single cells makes possible the study of the composition of the building block of life, the cell. CE is uniquely suited for the analysis of single cells because ultrasmall samples can be injected, separated, and detected. For instance, CE is capable of detecting femtomole (10^{-15} mole) to attomole (10^{-18} mole) quantities from nanoliter (10^{-9} liter) sample sizes.[1]

Model systems of relatively large invertebrate neural cells were initially studied by CE;[1-12] however, CE has recently been used to study small cells found in mammals such as erythrocytes,[13-22] lymphocytes,[23-25] adrenomedullary cells,[26,27] pancreatic cells,[28-29] and various other mammalian cells.[30-33] It has also been used to analyze single plant cells.[34, 35] Individual differences are discernible by studying the contents of single cells. Because variations in both the types and amounts of compounds present in single cells taken from the same sample demonstrate cellular uniqueness and complexity, a better understanding of cellular function can be achieved using CE.

Copyright © 2000 by Marcel Dekker, Inc. www.dekker.com

INTRODUCTION

Electrophoresis in narrow-bore capillaries was first introduced by Jorgensen and Lukacs[36,37] in 1981. Capillaries of an inner diameter of 75 micron (μm) were used to separate amino acids, dipeptides, and amines. The application of CE to the study of samples of limited size, such as single cells, was quickly realized. Injection volumes range from nanoliters to picoliters (10^{-12} liter), which correlate well with the volumes available in single cells. Sensitive detection techniques, such as electrochemical detection and fluorescence detection, have low limits of detection, which are critical for mass and concentration samples of limited size such as single cells. CE also provides highly efficient separations with upwards of one million theoretical plates.[38] This resolving power allows separations with relatively short analysis times for cells which contain many components.

INSTRUMENTAL

Two critical instrumental parameters are modified to customize and optimize the application of CE to single cells: the sample injection and detection.

Single Cell Sampling

Direct injections of single cells into the capillary have been accomplished using both cytoplasmic (or protoplasmic for plants) sampling (CS) and whole cell sampling (WCS). CS involves the direct removal of an aliquot from the cell. In theory, CS permits repeated analyses of the same cell, which can be useful for dynamic monitoring. Whole cell sampling (WCS) consumes the entire cell sample and therefore is a static means of study. Both electrokinetic and hydrostatic injection techniques have been used to pull the cell or cell aliquot into the capillary. Because of the small dimensions involved, the injection process is typically done under a microscope with micromanipulation tools.

Modifications to the injection end of the capillary have been made for both CS and WCS. Capillaries have been etched with hydrofluoric acid (HF) to taper the tip.[5,6] The tapered tip facilitates insertion of the capillary into the cell for CS. For WCS, a tip that is comparable to the cell dimensions facilitates the selection and injection of a single cell. An alternative method for creating tapered capillary tips is hand-pulling the capillary in a flame.[39,40] Tips of 10 μm have been made from 100 μm inner diameter capillaries. The pulled tip tapers to a smooth point, which has several advantages over the uneven and jagged tip created using HF. While the jagged tip may be desirable for CS, to pierce the cell membrane, a smooth tip allows WCS of cells that are slightly larger than the tip opening to be pulled into the capillary, with less danger of cellular rup-

Downloaded At: 10:00 24 January 2011

ture. In addition, pulling the capillary into a tip results in a gradual reduction in the capillary wall thickness, thus a more rugged tip is formed. HF etched tips are thin and fragile since the etching process effects both the inside and outside of the capillary.

A schematic of a hydrostatic, whole-cell injection using a vacuum at the outlet end is shown in Figure 1. In this setup, the injection end of the capillary is moved into close proximity of the cell of interest. A slight vacuum is applied at the outlet end of the capillary, using a gas-tight syringe attached to a sealed

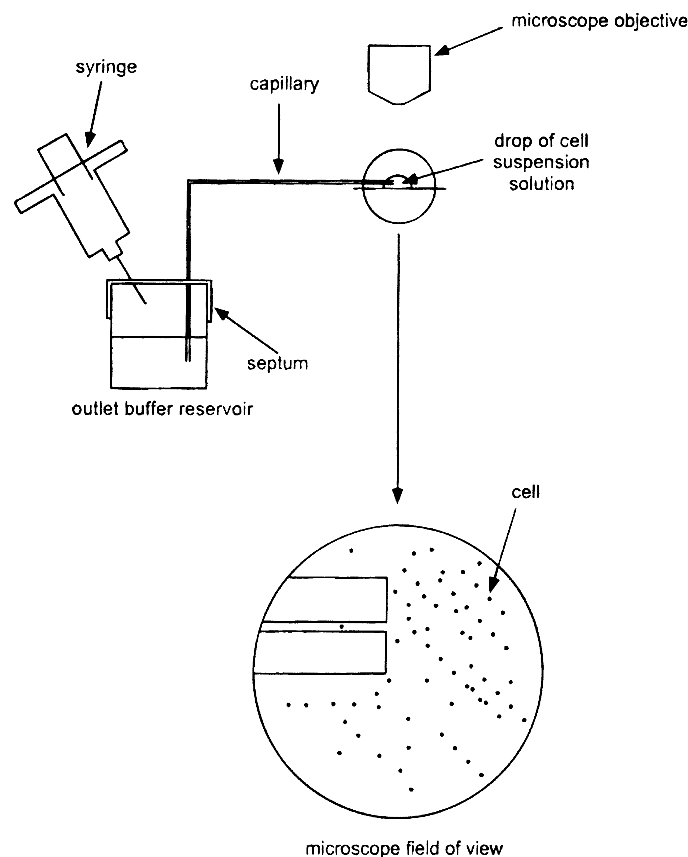


Figure 1. Schematic of single cell injection into a capillary column. (Reprinted from ref. 42, with permission).

Downloaded At: 10:00 24 January 2011

buffer reservoir. The cell is pulled into the capillary by suction. As smaller cells of interest are sampled, WCS becomes challenging due to both the difficulty in selectively injecting a single cell and the random movement of the cell. A recent enhancement in the sampling process has been reported by Chiu et al.[39,40] using optical trapping in conjunction with tapered capillary tips to inject cellular vesicles. Optical trapping serves to immobilize the vesicle so that the injection can be made before the cell moves away.

Once in the capillary, whole cells must be lysed to release their cellular contents for separation. Depending upon the strength of their respective cell wall, cells can be lysed in a variety of ways. The simplest method is to apply a voltage to start the separation, which causes the cell to burst due to the applied potential, or the change in ionic strength between the isotonic sample plug and the running buffer. Alternatively, injections of a non-isotonic buffer solution can also be made to induce lysing. To study cellular release dynamics, vesicular depleting agents or secretagogues can be added to the buffer to stimulate a controlled release of an analyte of interest. Following the selective release of cellular contents, complete cellular lysing is employed to measure residual cellular amounts. Finally, for more rugged cells, such as lymphocytes, a tesla coil has been used to rupture the cells under an induced electromagnetic field.[24] A diagram of this technique is shown in Figure 2.

Downloaded At: 10:00 24 January 2011

Detection

Because of the extremely small sample volumes and concentrations of analytes present in single cells, sensitive modes of detection must be employed for CE analyses. Electrochemical detection, operating in the amperometric mode, and laser induced fluorescence (LIF) are the most widely used detection techniques for single cell applications; however, UV detection[11,35] and electrospray

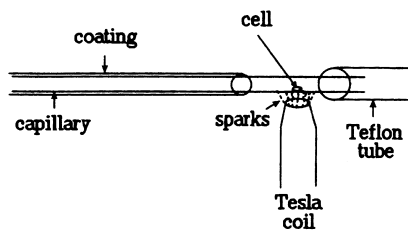


Figure 2. Method for lysing single lymphocytes with a tesla coil. (Reprinted from ref. 24, with permission from Elsevier Science).

ionization Fourier transform ion cyclotron resonance mass spectroscopy (ESI-FTICR-MS)[20] have also been used for single cell detection. To measure smaller mass ratios of cellular sub-components, even more sensitive detection is needed.

Electrochemical detection possesses low mass sensitivity and is selective to various analytes at different potentials. Detection limits are in the attomolar to zeptomolar (10^{-21} mole) range.[10] The majority of electrochemical detection applications to single cell analyses are done in the amperometric mode. In the amperometric mode, a constant potential is applied across the capillary and the resulting current is measured. Scanning electrochemical detection has been used to enhance the detection of closely eluting analytes.[10] The technique involves rapid stepping of the electrode voltage and measuring the current. As peaks elute, a characteristic voltammogram is obtained which aids in identifying analytes. Electrochemical detection is limited to analytes which can be oxidized or reduced.

LIF detection can be performed on-line by focusing the laser beam directly on the detection window of the capillary. Detection limits are in the attomolar to zeptomolar range.[41,42] While a single, or a range, of laser emission wavelengths can be selected to closely match the excitation wavelength of the analyte of interest, LIF is limited by the excitation wavelength outputs available from common lasers. Wavelength resolved laser induced fluorescence provides the ability to identify analytes with similar migration times, by simultaneously monitoring the fluorescence emission spectra of the analyte bands.[12]

LIF can be utilized where the analyte of interest possesses natural fluorescence or contains groups that can be chemically labeled with a fluorescent tag. Natively fluorescent compounds include proteins and catecholamines, which contain aromatic groups. Unfortunately, few compounds natively fluoresce. Thus, a variety of chemical tags have been developed which allow for the creation of a fluorescing species via a chemical reaction.[41,42] Fluorogenic reagents such as naphthalene 2,3 dicarboxaldehyde (NDA), o-phthaldialdehyde (OPA), 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde (CBQCA), and fluorescamine have been developed to label amino acids, proteins, peptides, and carbohydrates. These reagents possess the desirable characteristics either of being non-fluorescent prior to derivatization or producing non-fluorescing hydrolysis products.

Other tagging agents include fluorescein for amines and monobromobimane (mBBr) for sulfhydryl groups. These reagents are usually fluorescent in both reacted and unreacted states; therefore, derivatization must occur prior to separation. Other derivatization reagents include 5-dimethylaminonaphthalene-1-sulfonyl (DANSYL) chloride and fluorescein isothiocyanate (FITC).

Downloaded At: 10:00 24 January 2011

Derivatization reactions can be performed either prior to or after separation. Pre-column derivatization reactions require no special instrument modifications since the tagging reaction is performed prior to separation. Drawbacks of pre-column tagging are sample dilution with the fluorescent tag solution, reaction kinetics, and possible side reactions. In addition, derivatization can result in a multiply tagged analyte, thus giving several peaks for a single compound. Peak identification can be challenging, as the electrophoretic mobility will be that of the tagged analyte and not the original molecule. Post-column derivatization eliminates the preliminary sample preparation and migration times are of the original analyte; however, instrument modification is usually required to create a tagging reaction chamber. Finally, for analytes that do not possess native fluorescence or contain groups conducive to tagging, indirect fluorescence detection can be accomplished by using a fluorescing buffer. When the non-fluorescent analyte peak passes the detector, a drop in signal is recorded as a negative peak.

Downloaded At: 10:00 24 January 2011

APPLICATIONS

Neurons

Nerve cells from mollusks were used in the earliest demonstrations of single cell CE analysis, primarily because of their relatively large size, ease of identification during dissection, and well-characterized compositions. Neurotransmitter components, such as dopamine (DA) and epinephrine (E), could be easily oxidized allowing for amperometric detection, while the presence of amino acids provided components which are either natively fluorescent or could be tagged for fluorescence detection.

In 1989, Kennedy, Jorgenson et al.,[2] analyzed neurons from the land snail, *Helix aspersa,* using CE with LIF detection. Single, isolated neurons were placed into a microvial and homogenized. The supernatant was then transferred to another microvial and reacted with NDA for derivatization of primary amines. This solution was pneumatically injected onto the column where detection was accomplished using a helium-cadmium (He-Cd) laser as the excitation source. Several NDA-labeled amino acids were identified; however, no attempts were made at quantitation.

Direct sampling of the cytoplasm of the giant DA cell of the pond snail, *Planorbis corneus*, was initially accomplished by Wallingford and Ewing[3] in 1988 using a microinjector tip attached to the end of a 12.7 µm id capillary. The microinjector was approximately 1 mm long and tapered to a 7.5 µm tip that could be inserted into the neuron. An electrokinetic injection followed by electrochemical detection produced an electropherogram of several, unidentified peaks. In 1990, Chien et al.[4] investigated the cellular storage and release of DA from the *Planorbis corneus* giant DA neuron. CS with a microinjector followed

by electrochemical detection was used to compare the DA content of an untreated cell prepared in a ringer solution to a cell exposed to ethanol. Although the untreated neuron did not display a peak at the DA retention time, an estimated 14 femtomole (fmol) of DA was injected from the ethanol treated neuron, which corresponds to a cellular concentration of 1.4×10^{-4} to 4.7×10^{-5} molar (M). Using additional data from voltammetry to determine endogenous DA levels, 98% of the cell's DA was estimated to be bound in vesicles, with the remainder free in the cytoplasm.

In 1990, Olefirowicz and Ewing[5,6] modified the injection end of 2 and 5 µm capillaries by etching with HF to form a tapered point of approximately 8-10 µm. The capillary could then be directly inserted into the neuron, thus constructing an on-line injector and avoiding the need for a separate microinjector tip. Injection was accomplished using an electrokinetic pulse followed by electrochemical detection, operating in the amperometric mode. A schematic of the system illustrating direct CS is shown in Figure 3. Single injections from 5 dif-

Downloaded At: 10:00 24 January 2011

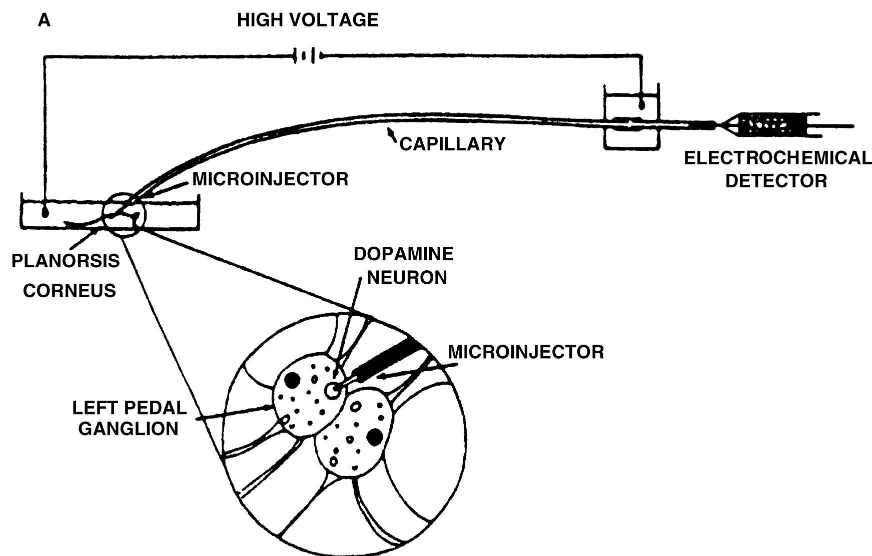


Figure 3. Schematic diagram of the capillary electrophoresis system used for the injection, separation, and detection of cytoplasmic samples. Also shown is an exploded view of the left and right pedal ganglia of *Planorbis corneus* with the microinjector inserted directly into the dopamine neuron. (Adapted from ref. 6, with permission from Elsevier Science).

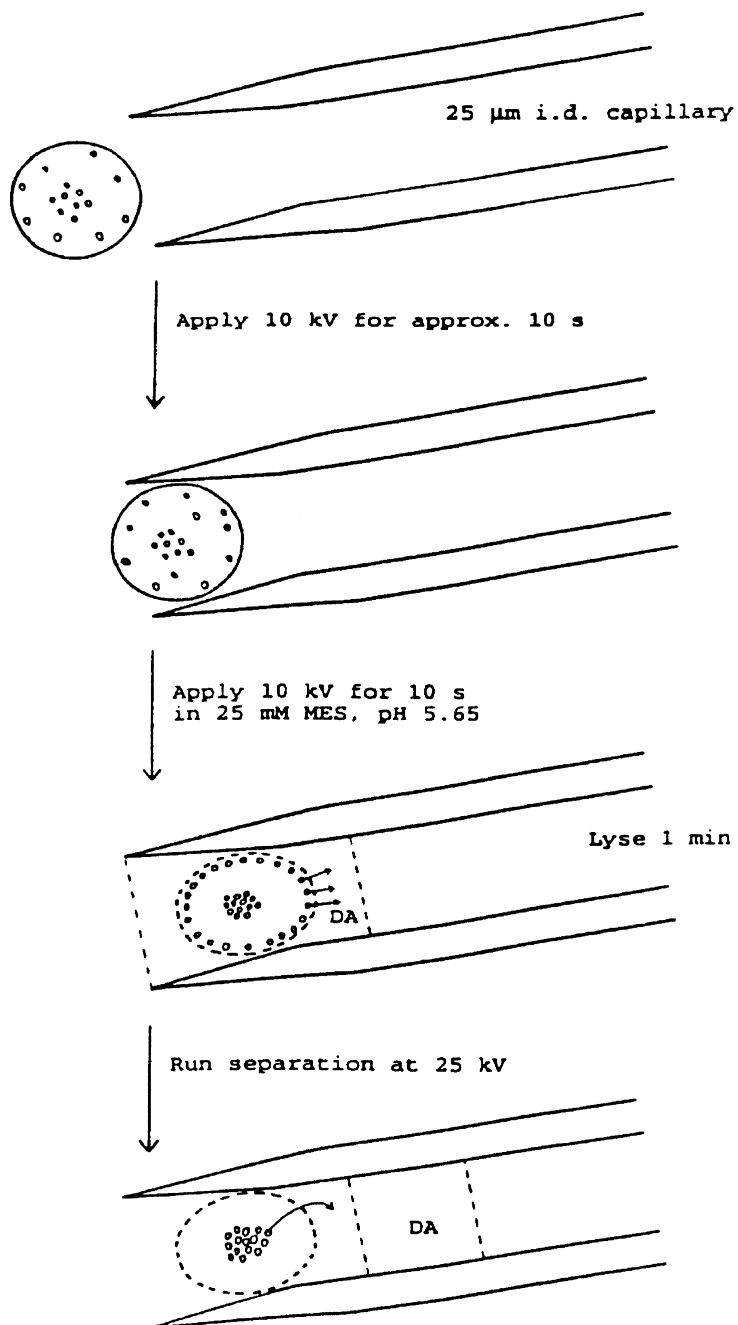
ferent snails gave DA concentrations of 2.2 ± 0.52 μM. Again in 1990, Olefirowicz and Ewing[5] used etched capillaries to study the large serotonin (5-HT) neuron of *Planorbis corneus*. Single injections from 3 different snails gave 5-HT concentrations of 3.1 ± 0.57 μM. In 1991, Olefirowicz and Ewing[7] analyzed whole DA neurons and compared the resulting electropherograms to the cytoplasmic results. Electrokinetic injection was used to draw the neuron, which was approximately 75 μm in diameter, into the 25 μm diameter capillary. The elongated neuron was lysed by a subsequent injection plug of buffer. Three main peaks were detected from the whole cell, one corresponding to DA, and two other unidentified peaks.

In 1994, Kristensen, Lau, and Ewing[8] demonstrated the presence of two vesicular compartments in the *Planorbis corneus* DA neuron. Using an electrokinetic whole cell injection with an etched tip column, three cell lysing processes were compared: lysing on-column with non-physiological buffer for 60 seconds (s), lysing on-column with non-physiological buffer for 5 minutes (min), and cell pretreatment with reserpine, a vesicle depleting agent, prior to on-column lysing for 60 s. A schematic of the injection and lysing process is shown in Figure 4. Using electrochemical detection operated in the amperometric mode, the resulting electropherogram, displayed the presence of a peak at the migration time of DA and a second, broader peak eluting just after DA. The second peak was attributed to some form of vesicular DA. The results indicated that 24% of the cell DA is cytoplasmic and 76% is vesicular.

Neural cells from the abdominal, buccal, and cerebral ganglia of the gastropod mollusk, *Aplysia californica,* were isolated by Shippy et al.[9] in 1995. The cells were lysed in a derivatizing solution of fluorescamine in dimethylfor-

Downloaded At: 10:00 24 January 2011

Figure 4. Schematic of a capillary tip modeling the cell acquisition and lysing procedure for the 60-s lyse time. Starting from the top, a micromanipulator was used to contact the cell with the tip of the capillary. An injection potential of 10 kV for approximately 10 s was used to transport the whole cell into the etched capillary tip. The microinjector tip containing the cell was then placed into the buffer reservoir for capillary electrophoresis and a 10-s plug of buffer (25mM MES, pH 5.65) was drawn into the capillary to cover the cell. The voltage was stopped for 60 s to allow the cell to lyse from contact with the buffer. Separation of species released by stimulation of the cell and those species inside the cell that are washed from the cell following lysing was then carried out at 25 kV separation potential. The model shows the hypothesis that neurotransmitter vesicles near the plasma membrane (functional vesicles) release neurotransmitter into the surrounding solution upon initial lysing of the plasma membrane by the MES buffer. When the separation potential is applied, all released substances and those freely dissolved in the cytoplasm are swept past and though the cell. Buffer moving thorough the cell then lyses the internalized vesicles and their components are subsequently separated. (Reprinted from ref. 8, with permission from Elsevier Science).



Downloaded At: 10:00 24 January 2011

mamide and approximately 2 nanoliters (nL) of solution was introduced into the capillary using gravity injection. LIF detection was accomplished using a He-Cd laser at 354 nanometer (nm) excitation. Electropherograms of several identified neurons, a metacerebral, B1, and a bag cell, showed variations in the peak patterns.

In 1996, Swanek, Chen, and Ewing[10] further tested the hypothesis of the vesicular compartmental storage of DA in *Planorbis corneus* using scanning electrochemical detection to positively identify the two peaks attributed to bound and free DA. The average DA content was found to be approximately 400 fmol.

Nitrite and nitrate levels in individual buccal neurons of the mollusc, *Pleurobranchaea californica,* were measured by Cruz et al.[11] in 1997. Direct CS was performed using an electrokinetic injection. UV detection at 214 nm was used to monitor the separation of the anions. Concentrations of $2.09 \pm 0.34 \times 10^{-3}$ M nitrite and $1.22 \pm 0.24 \times 10^{-2}$ M nitrate were obtained from 5 single buccal A motoneurons taken from the same animal. For a group of 16 cells taken from 6 animals, levels were $0.3 - 4 \times 10^{-3}$ M nitrite and $2 - 30 \times 10^{-3}$ M nitrate. Good correlation was observed between NADPH-diaphorase (NADPH-d) positive staining and cells that contained measurable amounts of nitrite and nitrate, while NADPH-d negative staining correlated well with cells that contained no measurable amounts of nitrite and nitrate.

In 1998, Fuller, Sweedler et al.[12] studied neurons from *Aplysia californica* and *Pleurobranchaea californica* using wavelength-resolved laser-induced native fluorescence spectroscopy. Single cells were isolated, homogenized, and diluted to 300 nL prior to electroosmotic injection. Injection volumes were approximately 2 nL; therefore, multiple injections could be made from the same cell solution. The combination of electrophoretic migration time and fluorescence spectral information permitted positive identification of analytes with similar migration times. More than 30 compounds were identified in the single cells studied. Using neurons from *Aplysia californica* and *Pleurobranchaea californica*, Floyd, Sweedler et al.[1] in 1998 measured intracellular levels of L-arginine (Arg), L-citrulline (Cit), and other primary metabolites related to nitric oxide synthase (NOS) activity. Isolated neurons were homogenized and labeled with fluorescamine prior to electrokinetic injection. The results demonstrated that the Arg/Cit ratio correlated with the intensity of NADPH-diaphorase staining, which is an indicator of NOS activity.

Erythrocytes (Red Blood Cells)

In 1992, Hogan and Yeung[13] reported the first application of CE to separate and quantify individual components of mammalian erythrocytes, which have an

Downloaded At: 10:00 24 January 2011

estimated volume of 87 femtoliters. Under microscopic magnification, a single cell was selected and injected into the capillary using a vacuum pulse. Once in the capillary, the cell adhered to the capillary wall and was lysed upon contact with the running buffer. Both direct and indirect LIF detection were used, depending upon the inherent fluorescent properties of the analyte of interest. An argon (Ar) laser operating at 275 nm was used as the excitation source. Indirect fluorescence detection was used to analyze cellular sodium and potassium by using 6-aminoquinoline and cetyltrimethylammonium bromide as the fluorescing background.

Direct fluorescence detection was used to monitor mBBr derivatized thiols such as glutathione (GSH). Derivatization was performed using the cell as a reaction vessel, utilizing the selective permeability of the cell wall. The small nonpolar derivatization molecules were able to pass through the cell membrane into the cell; however, the larger, derivatized product was contained within the cell. Lee and Yeung[14] also studied intracellular levels of proteins, hemoglobin A_0 (HemA), methemoglobin (Met), and carbonic anhydrase (CAH) in human erythrocytes using the same injection and detection schemes. An average hemolysate electropherogram was compared to 29 individual cell electropherograms, all of which displayed a variety of differences. Because erythrocytes display rather homogeneous cell volumes, variability in HemA and CAH was attributed to cell age.

In 1994, Xue and Yeung[15] used indirect fluorescence, with fluorescein as the background fluorophore, to detect the anions lactate and pyruvate in single erythrocytes. Injection was performed by vacuum using an HF-etched capillary tip, which minimized the extraneous volume of suspension solution injected with the cell and reduced interference. An Ar laser at 330 nm was used for excitation. The average amounts of lactate and pyruvate in the 27 single erthryocytes studied were 1.3 fmol and 2.1 fmol, respectively. Xue and Yeung[16] monitored the variability in the five different forms of intracellular lactate dehydrogenase (LDH) isoenzymes present in erythrocytes by measuring the formation of nicotinamide adenine dinucleotide (NADH), a fluorescent product related to LDH enzyme activity. A vacuum pulse was used to introduce the cell into the capillary, where the cell lysed upon contact with the running buffer. Separation was performed followed by an incubation period to permit the NADH to migrate into LDH zones depending on enzymatic activity. Variations in the separation and incubation times permitted resolution of the 5 isoenzymes.

Rosenzweig and Yeung[17] used a laser based particle counting microimmunoassay to study levels of glucose-6-phosphate dehydrogenase (G6PDH) present in single erythrocytes. Single cells were injected using a vacuum pulse. The cells adhered to the capillary wall and were lysed upon contact with the running buffer, which contained an antigen specific for G6PDH. The agglutinated particles were differentiated from smaller, unreacted particles and count-

Downloaded At: 10:00 24 January 2011

ed using an Ar laser at 488 nm. Counts were converted into G6PDH amounts via a standard curve. In the 25 cells studied, G6PDH amounts varied from 4.5 to 63 zeptomole (zmol), with the 14-fold variation postulated to be related to cell age.

In 1995, Lillard, Yeung et al.,[18] separated hemoglobin (Hb) variants present in the erythrocytes of a normal adult, a diabetic adult, and fetal cord blood. A vacuum pulse injection was used to introduce the cell into the capillary. The cell was lysed upon contact with the running buffer, and the Hb molecules denatured to four constituent polypeptide chains. Native LIF was measured using an Ar laser at 275 nm excitation. Normal adult lymphocytes contained 9.6% glycated β chains and 4.8% glycated α chains, while diabetic adult lymphocytes contained 30% glycated β chains and 12% glycated α chains. The fetal Hb displayed different variants from the adult.

Downloaded At: 10:00 24 January 2011

Dual channel fluorescence monitoring was used by Wong and Yeung[19] in 1995 to measure intracellular amounts of GSH, HemA, and CAH. Thiol groups in GSH and HemA were tagged with mBBr. Various labeling reaction times and temperatures were used to compare the fluorescence yield, which was optimized at room temperature for 5 min. Single cells were hydrodynamically injected onto the column using a vacuum pulse. Using an excitation wavelength of 275 nm from an Ar laser, the derivatized compounds were observed at 495 nm; while native fluorescence of CAH and HemA was monitored at 350 nm. HemA was detected at both emission wavelengths due to its thiol tagged groups and natively fluorescent aromatic amino acid residues. Variations in the 22 cells studied were observed, with ranges of 84-293 amol of GSH, 6-39 amol of CAH, 214-802 amol of HemA (native fluorescence), and 110-631 amol of HemA (mBBr tagged).

In 1996, Hofstadler, Ewing, et al.[20] performed the first analysis of single erythrocytes using CE with mass spectrometric detection. Using an HF etched column tip, single whole cells were electroosmotically injected onto the column and lysed upon contact with the running buffer. A 7 tesla ESI-FTICR-MS was used for detection. Zhang and Yeung[21] demonstrated the use of a postcolumn reactor for the LIF detection of derivatized proteins, amino acids, and amines contained in a single erythrocyte. Single cells taken from the blood of a healthy adult, a diabetic adult, and a newborn were compared. Cells were injected onto the capillary using a vacuum. Electropherograms are shown in Figure 5, with Peak 1 identified as carbonic anhydrase I and Peak 2 Hb. Hb variants contained within a single erythrocyte were analyzed by Lillard and Yeung.[22] Injection based capillary isoelectric focusing was used with native LIF detection. The anolyte, catholyte, and carrier ampholytes (with methylcellulose) were diluted to minimize baseline instability. Adult, sickle, and fetal erythrocytes were compared with variants differing by as little as 0.025 pI units resolved.

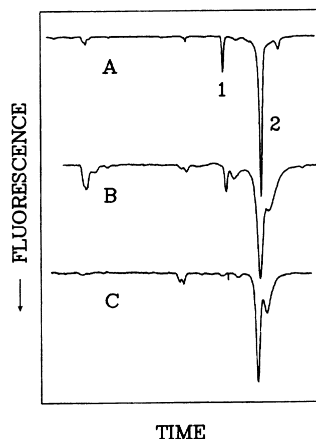


Figure 5. Electropherograms of single human erythrocytes from (A) normal adult, (B) diabetic adult, and (C) newborn. Separation capillary: 15 µm I.D., 70 cm long; reaction capillary: 30 µm I.D., 12 cm long. Electric field strengths: 300 V/cm and 150 V/cm for separation and reaction capillaries, respectively. [Peak 1 is carbonic anhydrase I, Peak 2 is hemoglobin.] (Adapted from ref. 21, with permission from Elsevier Science).

Downloaded At: 10:00 24 January 2011

Lymphocytes

In 1994, Bergquist, Ewing, et al.[23] analyzed lymphocytes taken from human cerebrospinal fluid (CSF) by CE with electrochemical detection, operating in the amperometric mode. Single cell injections were accomplished using an HF-etched capillary tip in conjunction with electroosmosis. A peak identified as DA was detected; however, because of its wider than usual width, the peak was suspected to contain other amines with similar electrophoretic mobilities, such as norepinephrine (NE), E, or 5-HT. The identity of the peak as DA was determined based on the detection of another peak with a similar retention time as the major metabolite of DA, dihydroxyphenylacetic acid (DOPAC). For the 3 CSF lymphocytes assayed, an amount of 2.3 ± 1.7 amol of catecholamine was quantified. This corresponded to 1.3×10^{-5} M, if a lymphocyte volume of 180 femtoliters (fL) was approximated.

Xue and Yeung[24] in 1996 applied CE with laser induced native fluorescence detection to the study of lymphocytes. LDH isoenzymes were measured from normal and leukemia cells. A tesla coil was used to lyse the cell (Figure 2). Although the running buffer could not lyse the more rugged lymphocytes, the tesla coil lysed the cell via an induced electric field, which did not affect the

LDH activity. The tesla coil method for cell rupture is not as successful as lysing the cell with the hypertonic process, which is a more complete and gentle method. Electropherograms from single cell analyses from three different cell types are shown in Figure 6. The five peaks correspond to the five LDH isoenzymes. Difficulties in the lysing process and adsorption resulted in only 40% of successful single cell analyses. Variations in LDH activities were attributed to variations in cell size (6-14 μm) and cell age. Because of variability observed in the cells, it was emphasized to use a population of cells to diagnose disease rather than the testing a single cell.

Human peripheral blood mononuclear cells were studied by Bergquist, Ewing, et al.[25] in 1997 using CE with electrochemical detection. Basal levels of DA and NE were measured by single cell analysis of 3 cells, 1.61 ± 0.72 amol DA and 0.96 ± 0.86 amol NE were measured. Cell populations were then incubated with DA, DA and nomifensine, or DA and tetrabenzine. Lysate samples showed that the DA incubated samples increased DA and NE levels by 48

Downloaded At: 10:00 24 January 2011

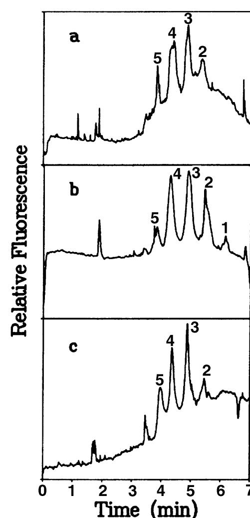


Figure 6. Electropherograms of single-cell analysis: (a) normal lymphocyte; (b) T-type lymphoblastic cell; and (C) B-type lymphoblastic cell. [Peaks 1-5 correspond to LDH-1, LDH-2, LDH-3, LDH-4, and LDH-5.] (Adapted from ref. 24, with permission from Elsevier Science).

and 8 times, respectively, while both nomifensine and tetrabenzine combined with DA showed lower levels of both DA and NE. The results indicate that lymphocytes regulate DA and NE concentrations.

Bovine Adrenal Medullary and Chromaffin Cells

In 1995, Chang and Yeung[26] studied chromaffin cells from the bovine adrenal medulla and measured the catecholamines, NE and E. A hydrodynamic injection using an HF-tapered capillary tip was used to facilitate selection and injection of a single cell from a droplet of cells on a microscope slide. A quick injection minimized cell adherence to the slide and the secretion of catecholamines. Upon contact with the running buffer in the capillary, the cells lysed within seconds. Native fluorescence detection using an Ar laser at 275 nm was used for excitation. Several cells taken from glands from two different cows were analyzed, with different amounts of catecholamines observed in the cells from each cow. For each of the two glands, the ratio of E/NE concentration was relatively constant, 2.7 and 5.6, which correlated with reported values. The cell to cell variations observed from each gland were attributed to differences in cell size.

Expanding on this work in 1997, Tong and Yeung[27] simultaneously measured NE and E amounts both secreted from and remaining in the cells using the secretagogue acetylcholine. Similar single cell hydrodynamic injection and native LIF detection techniques were used. To stimulate release of catecholamines from the intact cell, acetylcholine was hydrodynamically introduced into the capillary and permitted to contact the cell. Electrophoresis was conducted for 5 min using a buffer without bovine serum albumin (BSA), which prevented lysing of the cell while moving the secreted zone away. Finally, a solution containing sodium dodecylsulfate (SDS) was hydro-dynamically introduced, which caused the cell to lyse and release residual NE and E. These steps are illustrated in Figure 7. The resulting electropherogram, with two sets of doublets corresponding to the released and residual NE and E, is shown in Figure 8. Seventeen glands from six different cows were tested and showed variations in the absolute amount, with the average percentages of NE and E being 34 ± 21% and 25 ± 23%, respectively. The ratios of NE/E released and total NE/E content showed no correlation. In addition, the timing of the release process was estimated from the peak widths and shapes; however, the time resolution of 1 second was not fast enough to temporally monitor exocytosis.

Pancreatic Cells

In 1996, Tong and Yeung[28] measured the insulin content in single pancreatic β-cells using CE with hydrodynamic injection and laser-induced native fluorescence detection. Insulin producing, induced tumor cells taken from rat

Downloaded At: 10:00 24 January 2011

Downloaded At: 10:00 24 January 2011

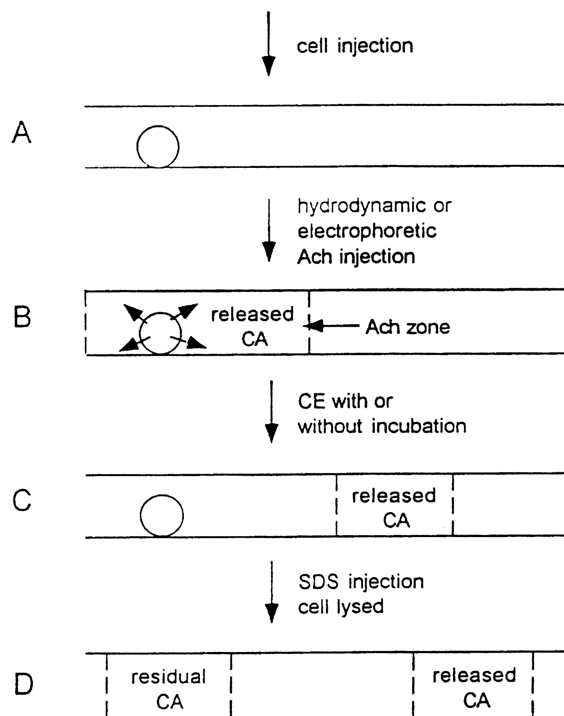


Figure 7. Schematic diagram of on-column release and lysis processes for a single cell. (Reprinted from ref. 27, with permission from Elsevier Science).

RINm5F and mouse βTC3 lines were used as models. Separations using a bare and a non-bonded poly-(ethylene oxide) (PEO)-coated capillary were compared. Standard insulin levels of 73 amol could be detected using a PEO coated capillary; however, a bare capillary had to be used for the single cell analyses. The tumor cells, which were resistant to lysing in the buffer, adhered to the bare capillary but not to the coated capillary. Adsorption to the capillary wall was critical to immobilize the cell for the subsequent SDS injection to lyse the cell. Two batches of βTC3 cells were studied, with the first batch of thirteen cells averaging an insulin content of 1.55 ± 0.74 fmol and the second batch of nine cells averaging 1.89 ± 1.03 fmol.

In later work, Tong and Yeung[29] in 1997 studied the pharmacokinetics of insulin release by βTC3 cells. After hydrodynamically injecting a single cell, a subsequent injection of digitonin was used to cause release of residual insulin,

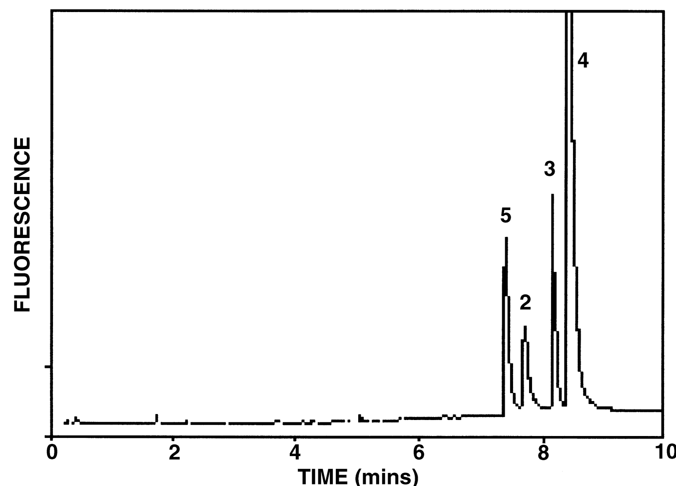


Figure 8. Electropherogram from on-column static release from a single adrenal chromaffin cell. Peaks 1 and 2 correspond to the released NE and E, respectively. Peaks 3 and 4 represent the residual NE and E inside the cell after secretion. The ordinate has been expanded to highlight peaks 1-3. (Reprinted from ref. 27, with permission from Elsevier Science).

Downloaded At: 10:00 24 January 2011

i.e., digitonin dissolves cholesterol in the cell wall resulting in permeable pores. This release of insulin was followed by a lysing of the cell to release the remaining insulin, allowing simultaneous measurement of both the amounts of released and residual insulin. A hydrodynamic flow experiment was conducted to confirm the validity of peak identification and temporal separation of 0.57 ± 0.04 min.

Various Mammalian Rodent Cells

On-column derivatization for LIF detection of single rat pheo-chromocytoma (PC12) cells was performed by Gilman and Ewing[30] in 1995. They determined the amounts of DA and five amino acids (aspartic acid, taurine, glycine, glutamic acid, and alanine) in the cells. An electrokinetic injection using HF etched capillaries was used to introduce the cell into the capillary, where it adhered to the capillary wall. The cell injection was followed by a second electrokinetic injection of a buffer containing derivatizing agents (NDA and NaCN) and lysing agent (digitonin). After a 10 min reaction period, electrophoretic separation was performed with peaks being identified by comparison of elution

times with derivatized standards. DA content was quantified at 2.9 ± 1.1 fmol and amino acid concentrations ranged from 0.18 ± 0.11 fmol (aspartic acid) to 5.1 ± 1.5 fmol (taurine).

In 1995, Orwar, Zare, et al.[31] analyzed NG 108-15 cells (mouse neuroblastoma x rat glioma) to measure cellular GSH using NDA labeling for LIF detection. Droplets of cell suspension and labeling reagent were mixed on a microscope slide over the capillary inlet. A reaction time of 10 minutes elapsed to allow the tagging reagent to permeate the cell and to form a fluorescent product. A vacuum injection was used to introduce a single cell into the capillary. The cellular GSH content was measured to be 1.28 ± 1.15 fmol based on 10 injections, 6 of which were single cells and 4 being multiple cells.

Rat peritoneal mast cells were studied by Lillard, Yeung, and McCloskey[32] in 1996 using native LIF to measure simultaneously the amount of 5-HT in single cells which are subsequently degranulated and then fully lysed. The process provides temporal information regarding the 5-HT release. Individual mast cells were injected into the capillary using an outlet vacuum pulse. The cell adhered to the capillary wall. A plug of polymyxin B sulfate, a secretogogue which induces degranulation and release of 5-HT, was introduced into the capillary electophoretically for 0.5 min to cover the cell. This process was followed by separation in the running buffer for 1 min to migrate the released zone of 5-HT away. Finally, SDS was introduced into the capillary to fully lyse the cell and release residual 5-HT. In each electropherogram, the main peak, attributed to exocytosis, was broader than the lysis peak, indicating that the initial release occurs slower than cell lysis. The average amount of 5-HT contained per cell was 1.6 ± 0.6 fmol, which was calculated as the sum of release and lysis peaks. Two batches of cells were studied on different days to calculate release. Batch one used 8 cells with an average of 28 ± 14% released per cell. Batch two used 3 cells with an average of 38 ± 15%. A detection limit of 1.7 amol of 5-HT was achieved.

Wang and Yeung[33] in 1997 used a dual enzyme on-column reaction leading to a fluorescent product to assay glutamate in single baby rat brain neurons and erythrocytes. Detection was accomplished by monitoring the fluorescent reaction product, NADH, which was related to the glutamate concentration in each cell. The detection limit was reported at 10^{-8} M, with a mass detection limit to a few attomoles. The results agreed well with reported values.

Plant Cells

In 1997, Honda et al.[34] analyzed parenchymatous cells from the citrus fruit, *Citrus unshuu*, for ascorbic acid concentration by CE using single cell samplingwith UV detection. Direct sampling of the cell fluid was achieved using grav-

Downloaded At: 10:00 24 January 2011

Downloaded At: 10:00 24 January 2011

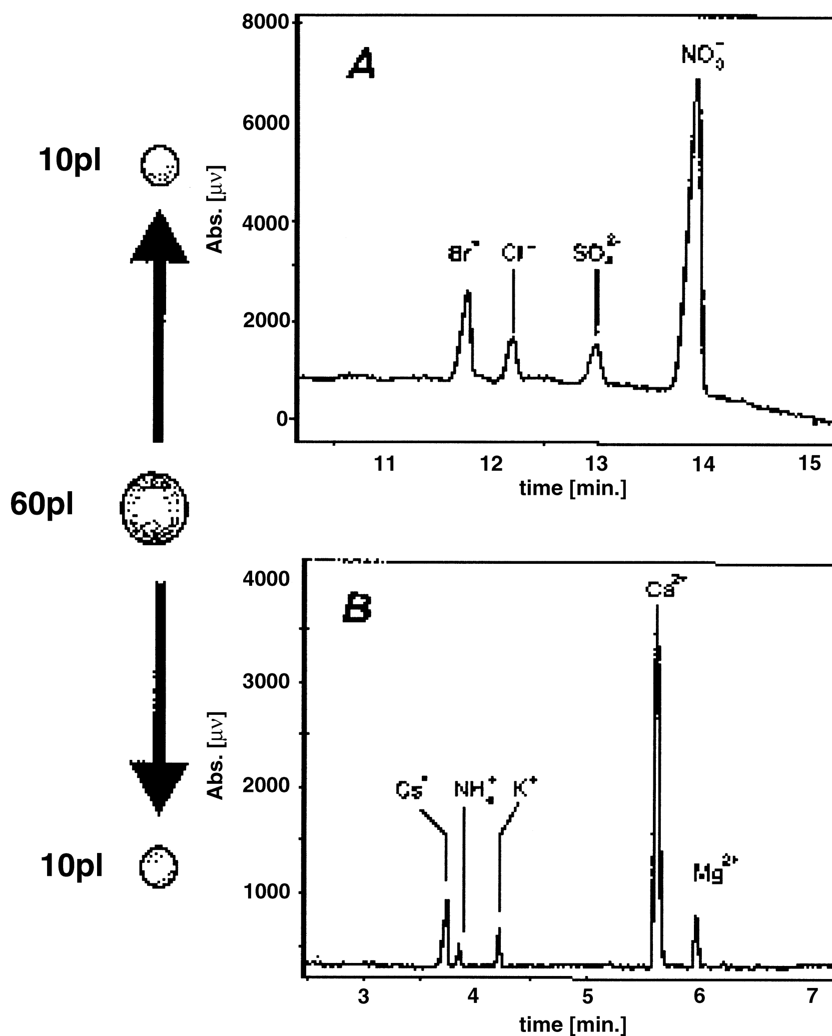


Figure 9. Determination of (A) inorganic anions and (B) cations in subsamples from the same vacuolar sample. Electrolytes: (A) 2.5 mM pyromellitic acid, 15 mM Tris, 1 mM DoTAOH, pH 8.1; (B) 5 mM imidazolesulphate, 2 mM 18-crown-6, pH 4.5. Capillaries: (A) length 99.5 cm (80 cm to detector) x 75-µm I.D. x 360-µm O.D., 150 µm O.D. at the capillary inlet; (B) length 92 cm (70 cm to detector) x 75-µm I.D. x 360-µm O.D., 150µm at the capillary inlet. Conditions: (A) voltage -25 kV, current 9 µA; (B) voltage 30 kV, current 8 µA. Detection: indirect UV, (A) 254 nm; (B) 214 nm. (Reprinted from ref. 35, with permission from Elsevier Science).

ity induced hydrodynamic injection. The injection end of the capillary was sharpened with sandpaper to create a pointed tip to pierce the rigid cell wall. The volume of a cell was estimated at 17 µL. Identification and quantitation of typical cell constituents was achieved using co-injections of an authentic standard solution and the plant cell aliquot. The use of borate complexes and SDS allows for the separation of neutral components. The concentration of ascorbic acid was estimated from introduced volumes and peak areas. For five different citrus fruits, ascorbic acid concentrations ranging from 2.7 - 7.9 mM were determined. Vacuoles from single wheat epidermal cells were sampled by Bazzanella et al.[35] using CE with indirect UV detection to measure the concentrations of inorganic cations and anions. Vacuolar sap was sampled from the cell using a microcapillary with a 1 µm tip that was quickly inserted into the plant cell and removed. A sap aliquot of 20 to 60 picoliters (pL) was forced into the capillary by the cell's turgor pressure. The aliquot was combined with an internal standard of CsBr using precise dilution techniques. Because of the relatively large cell aliquot, repeated analyses from the same vacuole were possible. Electropherograms of anions and cations from a single vacuole are shown in Figure 9.

Downloaded At: 10:00 24 January 2011

Sub-Cellular Components - Secretory Vesicles

In 1997, Chiu et al.[54,55] injected vesicles obtained from the atrial gland *Aplysia californica* using capillaries with tapered tips in conjunction with optical trapping and LIF. The vesicles range in size from 30 to 2000 nm with volumes of zeptoliters to low femtoliters. Labeling was accomplished on-line with NDA prior to separation.

CONCLUSIONS

CE provides a unique analytical tool for the study of individual cells. Modifications to injection techniques have been made to customize CE for single cell studies. Enhancements in sensitive detection schemes permit the identification of constituents present in zeptomolar quantities. These innovations have helped the progress of single cell analysis by CE from initial studies using relatively large invertebrate neuronal cells to more recent work on small mammalian cells, such as erythrocytes and lymphocytes. Information on cellular composition is critical to develop an understanding of cellular function, communication, and speciation. Single cell analysis is key to measure such critical individual cellular variations. The knowledge of cellular constituents could have an impact on the early prognosis of disease or in the development of customized drugs.

Downloaded At: 10:00 24 January 2011

REFERENCES

1. P. D. Floyd, L. L. Moroz, R. Gillette, J. V. Sweedler, Anal. Chem., **70**, 2243-2247 (1998).

2. R. T. Kennedy, M. D. Oates, B. R. Cooper, B. Nickerson, J. W. Jorgenson, Science, **246**, 57-63 (1989).

3. R. A. Wallingford, A. G. Ewing, Anal. Chem., **60**, 1972-1975 (1988).

4. J. B. Chien, R. A. Wallingford, A. G. Ewing, J. Neurochem., **54**, 633-638 (1990).

5. T. M. Olefirowicz, A. G. Ewing, Anal. Chem., **62**, 1872-1876 (1990).

6. T. M. Olefirowicz, A. G. Ewing, J. Neurosci. Methods, **34**, 11-15 (1990).

7. T. M. Olefirowicz, A. G. Ewing, Chimia, **45**, 106-108 (1991).

8. H. K. Kristensen, Y. Y. Lau, A. G. Ewing, J. Neurosci. Methods, **51**, 183-188 (1994).

9. S. A. Shippy, J. A. Jankowski, J. V. Sweedler, Anal. Chim. Acta, **307**, 163-171 (1995).

10. F. D. Swanek, G. Chen, A. G. Ewing, Anal. Chem., **68**, 3912-3916 (1996).

11. L. Cruz, L. L. Moroz, R. Gillette, J. V. Sweedler, J. Neurochem., **69**, 110-115 (1997).

12. R. R. Fuller, L. L. Moroz, R. Gillette, J. V. Sweedler, Neuron, **20**, 173-181 (1998).

13. B. L. Hogan, E. S. Yeung, Anal. Chem., **64**, 2841-2845 (1992).

14. T. T. Lee, E. S. Yeung, Anal. Chem., **64**, 3045-3051 (1992).

15. Q. Xue, E. S. Yeung, J. Chromatogr. A, **661**, 287-295 (1994).

16. Q. Xue, E. S. Yeung, Anal. Chem., **66**, 1175-1178 (1994).

17. Z. Rosenzweig, E. S. Yeung, Anal. Chem., **66**, 1771-1776 (1994).

18. S. J. Lillard, E. S. Yeung, R. M. A. Lautamo, D. T. Mao, J. Chromatogr. A, **718**, 397-404 (1995).

19. K.-S. Wong, E. S. Yeung, Mikrochim. Acta, **120**, 321-327 (1995).

20. S. A. Hofstadler, J. C. Severs, R. D. Smith, F. D. Swanek, A. G. Ewing, Rapid Commun. Mass Spectrom., **10**, 919-922 (1996).

21. L. Zhang, E. S. Yeung, J. Chromatogr. A, **734**, 331-337 (1996).

22. S. J. Lillard, E. S. Yeung, J. Chromatogr. B: Biomed. Appl., **687**, 363-369 (1996).

23. J. Bergquist, A. Tarkowski, R. Ekman, A. G. Ewing, Proc. Natl. Acad. Sci. U.S.A., **91**, 12912-12916 (1994).

24. Q. Xue, E. S. Yeung, J. Chromatogr. B: Biomed. Appl., **677**, 233-240 (1996).

25. J. Bergquist, E. Josefsson, A. Tarkowski, R. Ekman, A. Ewing, Electrophoresis, **18**, 1760-1766 (1997).

26. H.-T. Chang, E. S. Yeung, Anal. Chem., **67**, 1079-1083, (1995).

27. W. Tong, E. S. Yeung, J. Neurosci. Methods, **76**, 193-201 (1997).

28. W. Tong, E. S. Yeung, J. Chromatogr. B: Biomed. Appl., **685**, 35-40 (1996).

29. W. Tong, E. S. Yeung, J. Chromatogr. B: Biomed. Appl., **689**, 321-325 (1997).

30. S. D. Gilman, A. G. Ewing, Anal. Chem., **67**, 58-64 (1995).

31. O. Orwar, H. A. Fishman, N. E. Ziv, R. H. Scheller, R. N. Zare, Anal. Chem., **67**, 4261-4268 (1995).

32. S. J. Lillard, E. S. Yeung, M. A. McCloskey, Anal. Chem., **68**, 2897-2904 (1996).

33. Z. Wang, E. S. Yeung, J. Chromatogr. B: Biomed. Appl., **695**, 59-65 (1997).

34. S. Honda, Y. Naitoh-Ohta, K. Togashi, A. Taga, Anal. Sci., **13**, 557-564 (1997).

35. A. Bazzanella, H. Lochmann, A. D. Tomos, K. J. Bachmann, J. Chromatogr. A, **809**, 231-239 (1998).

Downloaded At: 10:00 24 January 2011

36. W. Jorgenson, K. D. Lukacs, J. Chromatogr., **218**, 209-216 (1981).

37. J. W. Jorgenson, K. D. Lukacs, Anal. Chem., **53**, 1298-1302 (1981).

38. F. D. Swanek, S. S. Ferris, A. G. Ewing, "Capillary Electrophoresis for the Analysis of Single Cells: Electrochemical, Mass Spectrometric, and Radiochemical Detection," in **Handbook of Capillary Electrophoresis**, 2nd ed., J. P. Landers, ed., CRC Press, Boca Raton, FL, 1997, pp. 495-521.

39. D. T. Chiu, A. Hsiao, A. Gaggar, R. A. Garza-Lopez, O. Orwar, R. N. Zare, Anal. Chem., **69**, 1801-1807 (1997).

40. D. T. Chiu, S. J. Lillard, R. H. Scheller, R. N. Zare, S. E. Rodriguez-Cruz, E. R. Williams, O. Orwar, M. Sandberg, J. A. Lundqvist, Science, **279**, 1190-1193 (1998).

41. P. B. Hietpas, A. G. Ewing, J. Liq. Chromatogr., **18(18&19)**, 3557-3576 (1995).

42. S. J. Lillard, E. S. Yeung, "Capillary Electrophoresis For the Analysis of Single Cells: Laser-Induced Fluorescence Detection," in **Handbook of Capillary Electrophoresis**, 2nd ed., J. P. Landers, ed., CRC Press, Boca Raton, FL, 1997, pp. 523-544.

Received March 21, 1999
Author's Revisions December 6, 1999
Accepted October 7, 1999
Manuscript 5020

Downloaded At: 10:00 24 January 2011