

Optimization of the separation of malachite green in water by capillary electrophoresis Raman spectroscopy (CE-RS) based on the stacking and sweeping modes

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Abstract

A capillary electrophoresis Raman spectroscopy (CE-RS) method based on the stacking and sweeping modes are described. A non-fluorescent compound (malachite green, MG; crystal violet, CV) and a doubled Nd:YAG laser (532 nm, 300 mW) were selected as the model compound and light source, respectively. In order to carry out a quantitative and analysis of MG, a monochromator was used to collect the specific Raman line at 1616 cm^{-1} (the N- φ and C-C stretching, corresponding to 582 nm when the wavelength of the exciting source is 532 nm). The limit of detection (LOD) for MG was 1.6×10^{-5} and 1.1×10^{-5} M, respectively, based on the CZE and MEKC modes. This could be improved to 3.4×10^{-7} and 5.3×10^{-9} M, respectively, when the stacking and sweeping modes were applied. The method was also extended to the determination of MG in an actual sample.

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1. Introduction

Laser induced fluorescence and optical UV absorbance are the most common detection methods used in CE. However, if the analyte does not emit or absorb UV/visible radiation, alternate types of detection, such as indirect fluorescence/absorbance detection, refractive index, light scattering, chemiluminescence, electrochemistry, even mass spectrometric detection, etc. can be used. Although each method has unique advantages and disadvantages with respect to selectivity, sensitivity, precision and simplicity of use, the typical optical detections used in CE show less of selectivity, since the spectra obtained at ambient temperature are broad, and this limits the spectral characteristics needed for analyte identification. In contrast to this, Raman spectroscopy (RS) provides a unique spectrum of an analyte with a very high spectral resolution. Thus far, it is a very useful method, but is seldom used for detection in CE [1–7], although the first demonstration of Raman spectroscopic detector for CE has been reported by Morris and co-workers [8,9].

This is because Raman emission is a very inefficient process, resulting in poor sensitivity in detection, compared to fluorescence/absorbance methods. In this study, we selected malachite green (MG) oxalate and crystal violet (CV), two compounds with similar chemical structures, as model samples to determine the extent to which the selectivity can be improved when the capillary electrophoresis Raman spectroscopy (CE-RS) method is used. In order to improve the sensitivity when RS is used, we introduced on-line sample concentration techniques to the CE-RS detection method, including the stacking and sweeping modes [10–18]. The method was also extended to the determination of MG in an actual sample. Several experimental parameters were optimized and the data for these are reported herein.

2. Materials and methods

2.1. Chemicals

Malachite green oxalate and crystal violet ([4-[4,4'-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-ylidene]-dimethylammonium chloride) were obtained from Acros (NJ,

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USA). Sodium dodecyl sulfate (SDS) was obtained from Sigma (St. Louis, MO, USA). Citric acid was purchased from Yakuri Pure Chemical Co., Ltd. (Osaka, Japan), respectively. All other chemicals were of analytical grade and were commercially available.

2.2. CE apparatus

The CE set-up and data acquisition system used were similar to a previously described set-up [19], but the light source was changed to a doubled Nd:YAG laser (532 nm, 300 mW). The laser beam was focused on the CE capillary (fused silica capillary, i.d., 75 μm ; J&W Scientific, CA, USA) by means of a lens (focus length, 3 cm). The Raman emission was collected at a right angle to the light source by means of a microscope eyepiece (10 \times), passed through a long-pass filter, dispersed by a monochromator (Acton Research Corporation, Model SP-300i; detection window was set at 582 ± 0.2 nm), followed by detection using a photomultiplier tube. A commercial Raman instrument (Dilor XY800 Triple-grating spectrometer; resolution, 0.1 cm^{-1}) equipped with a charge coupled detector was also used to assist in the identification of Raman shifts.

3. Results and discussion

3.1. Improvement in selectivity between malachite green and crystal violet

Conventional Raman spectra of malachite green and crystal violet are shown in Fig. 1A and B, respectively; the numbers above the peaks indicate the Raman shifts, in wavenumbers (cm^{-1}) and are in reasonable agreement with published literature values; the strongest Raman bands at 1616 cm^{-1} of MG and 1620 cm^{-1} of CV were assigned to the ring breathing and N- ϕ stretching modes [20]. A further detailed discussion of these assignments can be found in the literature [20]. Fig. 2A shows a typical electropherogram of a mixture of MG and CV; the detection window was set at 582 ± 0.2 nm, corresponding to the strongest Raman line ($1616 \pm 7 \text{ cm}^{-1}$; indicated as arrow-marks in Fig. 1A and B). A doubled Nd:YAG laser (532 nm, 300 mW) was used for excitation. The separation mode used was the CZE mode. Two peaks were observed, corresponding to MG and CV,

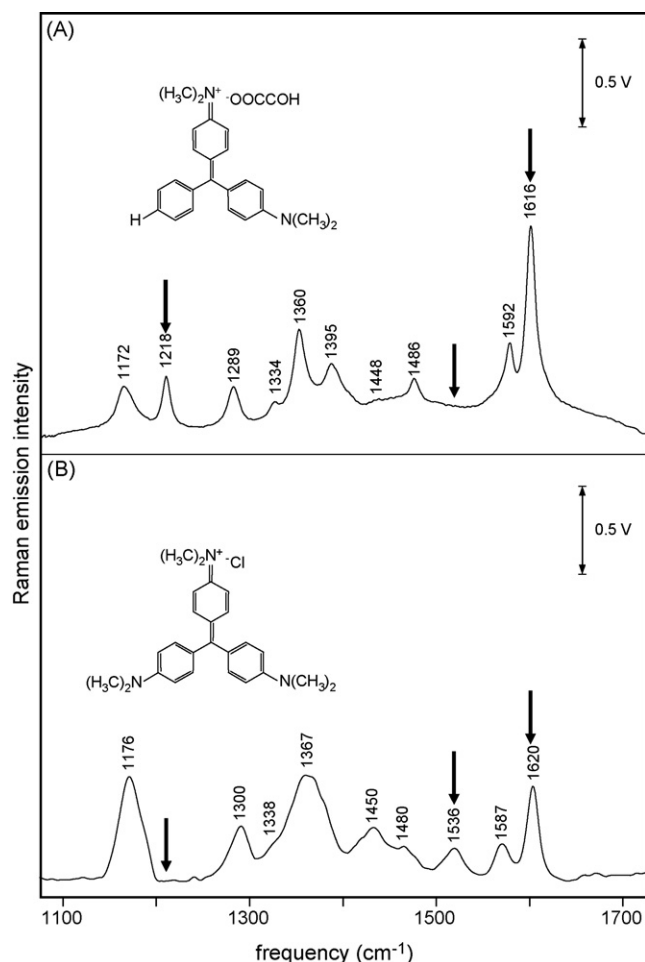


Fig. 1. Raman spectra of: (A) malachite green (1.6×10^{-4} M) and (B) crystal violet (1.2×10^{-4} M); the insets show their chemical structures.

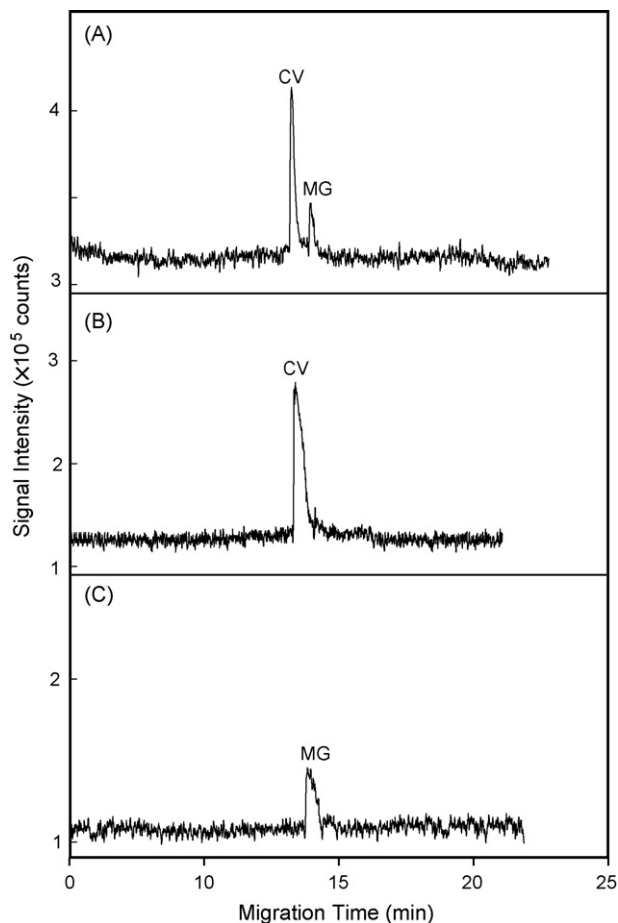


Fig. 2. Typical electropherogram of a mixture of CV and MG; the detection window was set at specific frequencies: (A) 1616 cm^{-1} ; (B) 1535 cm^{-1} ; (C) 1218 cm^{-1} (indicated as arrow-marks in Fig. 1). CE conditions used are the same as described in Fig. 3A. Sample concentrations of CV and MG used in frames (A–C): (A) 1.6×10^{-3} and 2.7×10^{-4} M; (B) 6.1×10^{-3} and 2.4×10^{-4} M; (C) 2.5×10^{-3} and 1.1×10^{-3} M, respectively. CE conditions: CZE, an aqueous citric acid (50 mM) buffer (pH 2.2; 512 $\mu\text{S}/\text{cm}$) and the applied voltage was +20 kV.

respectively. However, when the detection frequency was set at 1536 cm^{-1} , only CV was observed (as shown in Fig. 2B). In contrast to this, when the detection frequency was set at 1218 cm^{-1} , only MG was observed (as shown in Fig. 2C). The concentrations of MG used in Figs. 1A and 2A were different, resulting in an inconsistent peak height. In principle, the separation efficiency is not dependent on the detection frequency. However, when the detection frequency is set to a specific Raman line, it can be used to select a specific analyte from a mixture, and this is achieved only with difficulty when regular optical methods are used if the analytes provide similar fluorescence/absorbance spectra.

3.2. Improvement on sensitivity of malachite green

Malachite green, a synthetic dye that is potentially dangerous to human health, is used to color fabrics and paper, and has been used illegally in the treatment of certain fish diseases, mainly, against parasites in freshwater and marine fishes. Thus far, the current detection methods for MG include HPLC [21–23], LC–MS [24–26], UV/CE-stacking [27] methods, etc. In this study, we selected MG as model compound, and investigated its separation and on-line sample concentration condi-

tions under the CZE, stacking, MEKC and sweeping-MEKC modes, respectively. The detection window was also set and matched with the strongest Raman line ($1616 \pm 7\text{ cm}^{-1}$). In the case of the CZE and stacking modes, optimal conditions involved the use of an aqueous citric acid (50 mM) buffer (pH 2.2; conductivity = $512\text{ }\mu\text{S/cm}$). When the stacking technique was introduced, the running buffer was the same as that used in the CZE mode. The sample was prepared in a matrix solution, obtained by diluting the aqueous citric acid (50 mM) buffer to 1/100, which lowered the conductivity of the solution (pH 2.6; conductivity = $4.16\text{ }\mu\text{S/cm}$), for use in the stacking mode. Fig. 3A and B show typical electropherograms of MG (sample concentrations used in the CZE mode, $2.2 \times 10^{-4}\text{ M}$; in the stacking mode, $1.1 \times 10^{-6}\text{ M}$). The buffer conditions were as described in Fig. 2A. Since the detection window was extremely narrow, the limit of detection (LOD) for MG was 1.6×10^{-5} and $3.4 \times 10^{-7}\text{ M}$, respectively, based on the CZE and stacking (sample injection length, 16 cm; effective/total length, 61/70 cm) modes. The linearity of these methods for MG was also fairly good and these data (including the calibration curve, coefficient of correlation, limit of detection values and theoretical plate numbers) are summarized in Table 1. Fig. 3C shows a typical electropherogram of MG ($5.4 \times 10^{-5}\text{ M}$) based on the

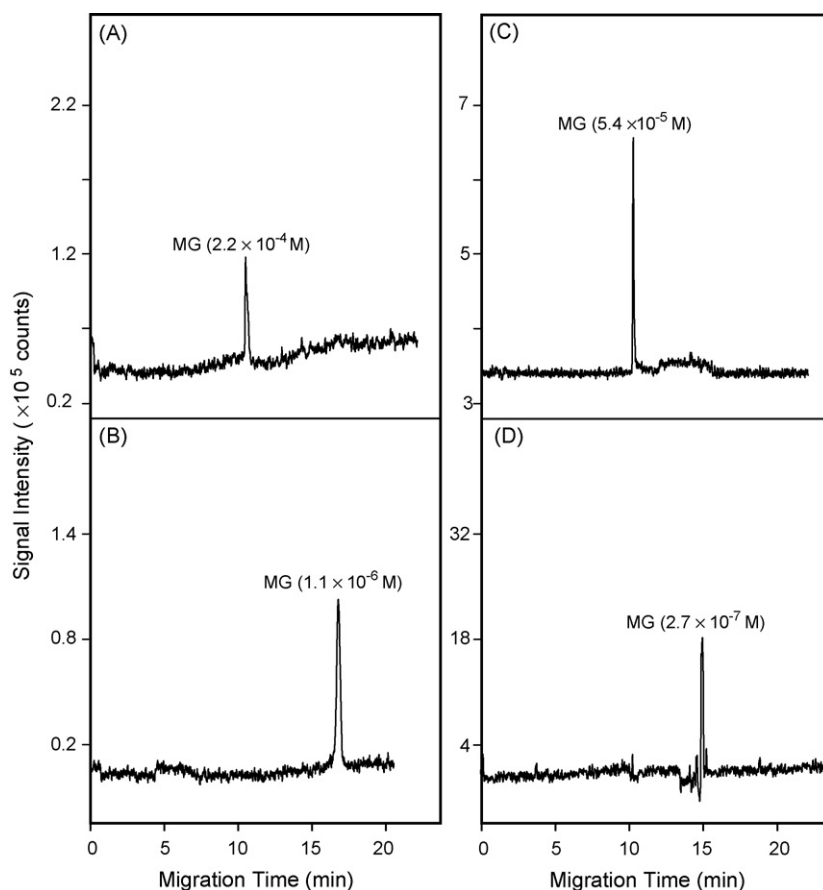


Fig. 3. Typical electropherograms of MG obtained by the CZE (A), stacking (B), MEKC (C) and sweeping-MEKC (D) modes, respectively. CE conditions are described as in Fig. 2A; stacking, buffer was the same as that used in the CZE mode but the samples were prepared in the matrix solution (diluted aqueous citric acid buffer, with pH and conductivity values of 2.56 and $4.16\text{ }\mu\text{S/cm}$, respectively). MEKC, an aqueous citric acid (50 mM) containing SDS (50 mM) buffer (pH, 2.1); sweeping-MEKC, the same running buffer system as described for the MEKC mode, but the sample was dissolved in the matrix (50 mM citric acid aqueous buffer without SDS). The applied voltages used were +20 kV in the CZE and stacking modes; -16 and -8 kV in the MEKC and sweeping-MEKC modes, respectively.

MEKC mode. The detection limit for MG was determined to be 1.1×10^{-5} M ($S/N=3$). Fig. 3D shows a typical electropherogram of MG (2.7×10^{-7} M) obtained by the sweeping-MEKC mode. The CE buffer system was basically identical to that used for the MEKC mode. The sample was dissolved in the matrix (50 mM citric acid aqueous buffer without SDS) and an injection length of 22 cm was used (effective/total length, 61/70 cm). When the sweeping-MEKC mode was applied, a dramatic improvement in detection sensitivity was obtained, and the limit of detection was improved to 5.3×10^{-9} M (at $S/N=3$). Basically the signal intensity increased with increasing injection length. However, an optimal sample injection length should be determined because if a longer capillary column is used for sample concentration strategies, the subsequent CE separation becomes insufficient. The linearity of these methods for MG was also fairly good and these data are also summarized in Table 1.

3.3. Application to a real-life sample

The sample, water in which marketed fish are kept, was obtained from a supermarket near the campus in Taipei. The sample was filtered, and a 20 μ L aliquot was placed in a vacuum chamber for drying. The residue was acidified by the addition of 20 μ L of citric acid (50 mM) and was used in the subsequent CE

Table 1

Calibration curve, coefficient of correlation, limit of detection values ($S/N=3$) and theoretical plate numbers (N) for malachite green by CZE/Raman, stacking-CZE/Raman, MEKC/Raman, sweeping-MEKC/Raman methods, respectively, by using a green diode laser

(A) CZE/Raman	
Equation of the line	$y = 5.27 \times 10^7 x + 9.08 \times 10^2$
Coefficient of correlation	$R^2 = 0.9978$
Detection range (M)	5.4×10^{-4} to 5.4×10^{-5}
Sample injection length (cm)	0.4
LOD (M)	1.6×10^{-5}
Theoretical plate number	8.4×10^3 to 3.5×10^4
(B) Stacking-CZE/Raman	
Equation of the line	$y = 1.08 \times 10^4 x + 1.53 \times 10^4$
Coefficient of correlation	$R^2 = 0.9917$
Detection range (M)	2.2×10^{-6} to 5.4×10^{-7}
Sample injection length (cm)	16
LOD (M)	3.4×10^{-7}
Theoretical plate number	6.6×10^3 to 1.8×10^4
(C) MEKC/Raman	
Equation of the line	$y = 3.6 \times 10^5 + 1.8 \times 10^6$
Coefficient of correlation	$R^2 = 0.9911$
Detection range (M)	1.1×10^{-4} to 1.3×10^{-5}
Sample injection length (cm)	0.4
LOD (M)	1.1×10^{-5}
Theoretical plate number	2.2×10^4 to 4.2×10^4
(D) Sweeping-MEKC/Raman	
Equation of the line	$y = 1.5 \times 10^7 + 1.9 \times 10^5$
Coefficient of correlation	$R^2 = 0.9905$
Detection range (M)	5.4×10^{-6} to 5.4×10^{-9}
Sample injection length (cm)	22
LOD (M)	5.3×10^{-9}
Theoretical plate number	2.3×10^5 to 3.0×10^5

Light source: doubled Nd:YAG laser (532 nm, 300 mW); detection frequency was set at 1616 ± 7 cm^{-1} .

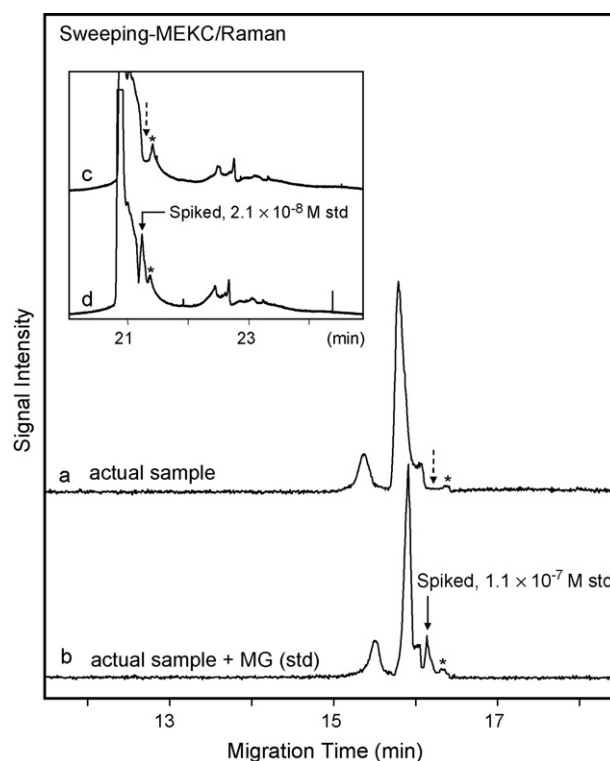


Fig. 4. Typical CE electropherograms obtained from an actual water sample, in which fish were kept in a commercial supermarket, by applying the sweeping-MEKC mode (conditions, as described in Fig. 3D). Electropherograms a and b show the results obtained before and after spiking with an MG standard (1.1×10^{-7} M), respectively. Electropherograms c and d in the inset show results obtained before and after spiking with an MG standard (2.1×10^{-8} M) by recording all emissions.

separation. Fig. 4 shows typical CE electropherograms obtained for an actual sample, obtained from a fish market, by applying the sweeping-MEKC/Raman mode. The CE conditions are as described above in Fig. 3D. Electropherograms a and b show the results obtained before and after spiking with an MG standard (1.1×10^{-7} M), respectively. Herein, the sample was directly used without any pretreatment procedures. All of these peaks were detected at an emission of 1616 ± 7 cm^{-1} , either native fluorescence or Raman lines. When a single-wavelength set-up was performed upon Raman emission, it can exclude specific emissions from a number of unknown compounds. Electropherograms a and b in the inset also show the results obtained before and after spiking with an MG standard (1.1×10^{-7} M). The entire range of emissions was detected, and, as a result, a broad background was detected in electropherograms c and d. For a comparison of the separation efficiency, an indicated peak (marked as “*” in electropherograms a–d) was selected for evaluation. In electropherograms a and c, the broken arrow indicates the expected migration time for MG, but it did not show up in the sample. This indicates that, if it exists, its concentration is below a certain LOD. Using the standard addition method, the results were compared and the findings show that the peaks (marked spiking peaks in electropherograms b and d) clearly appeared. If it were possible to arrange a CCD detector to record the entire range of emission, further accuracy in identification could be achieved, since characteristic Raman shifts (spectral

fingerprinting) can be used for the unambiguous identification of a compound.

4. Conclusions

This work successfully demonstrates a new approach for detecting a non-fluorescent compound by a capillary electrophoresis Raman spectroscopy method combined with an on-line sample concentration technique. This proposed method may solve problems that are frequently encountered for non-fluorescent analytes, even when they are present at low levels. Thus, a combination of a compact high power laser, interference filters, a PMT detector based on either a CE or microchip system, would be useful as a rapid-screening tool, in which only a miniaturized system would be needed. Further applications of this technique are currently under investigation.

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