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Research Article

A new approach for the detection of a nonfluorescent compound by CE-resonance Raman spectroscopy based on the sweeping-MEKC mode

A CE-resonance Raman spectroscopy (CE-RRS) method based on MEKC and sweeping-MEKC modes is described. A nonfluorescent compound, malachite green (MG), and a doubled Nd:YAG laser (532 nm, 300 mW) were selected as model compound and light source, respectively. In order to carry out a quantitative analysis of MG, a monochromator (effective bandwidth, 0.4 nm) was used to collect the specific Raman line at 1616 cm^{-1} (N-φ and C–C stretch, corresponding to 582 nm when the wavelength of the exciting source was 532 nm). As a result, the LOD for MG was 10 ppm, based on the MEKC/RRS mode. This could be improved to 5 ppb when the sweeping-MEKC/RRS mode was applied. Furthermore, with the addition of nano-size silver colloids to the CE buffer the detection limits can be further improved, but the data obtained with surface-enhanced resonance Raman spectroscopy (SERRS) are less useful for quantitative purposes.

Keywords: CE / Malachite green / Raman spectroscopy / Surface enhanced-resonance Raman spectroscopy / Sweeping-MEKC   DOI 10.1002/elps.200600393

1 Introduction

The CE-LIF detection method, a highly sensitive method, is frequently used for the detection of fluorescent analytes, either native fluorescent or dye-labeled compounds. To nonfluorescent analytes, when an optical UV absorbance detection can be used, this is, in fact, the most common means of detecting a UV-absorbable analyte, due to its low costs and flexibility. 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 detection, electrochemistry, chemiluminescence, or even MS, *etc.* can be used. Raman spectroscopy (RS) is a powerful method for detection, but is seldom used for detection in CE. This is because Raman emission is based on inelastic light scattering, a very inefficient process, resulting in poor sensitiv-

ity in detection. It is acquired by irradiating a sample with a powerful laser source of visible or near-infrared monochromatic radiation, but the intensity of Raman lines is less than $\sim 1/10^5$ of the intensity of the source. The signal intensity is proportional to λ^{-4}, that is, it is strongly enhanced at short wavelengths, such as when a high power UV laser is used. In order to improve the sensitivity of RS, special modes can be exploited, such as surface-enhanced resonance Raman spectroscopy (SERRS) [1–4]. SERRS is a spectroscopic phenomenon in which an enhanced Raman intensity can be observed when an analyte is adsorbed to a roughened metal surface or a metallic substrate, usually a silver colloid. In a favorable case, typical enhancement factors are in the range of $\sim 10^5$–10^6 without a significant simultaneous increase in background: the silver particles also cause fluorescence quenching. Nevertheless, only a few reports of coupling this approach with SERRS with CE have been reported [5–8].

On-line sample concentration techniques, including the so-called stacking and sweeping modes, have recently become popular and important. Sensitivity can be improved by several orders of magnitude, when such methods are employed. Several excellent descriptions of on-line sample concentration techniques, and related applications can be found in the published literature [9–13].

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Abbreviations: MG, malachite green; **MG-Ag**, malachite green-silver colloid; **PMT**, photomultiplier tube; **RS**, Raman spectroscopy; **SERRS**, surface-enhanced resonance Raman spectroscopy

In this study, we first report on the use of a combination of a CE-surface-enhanced resonance Raman spectroscopy (CE-SERRS) detection method and the sweeping-MEKC mode. A doubled Nd:YAG laser was used as the light source and malachite green (MG) was used as a model compound, respectively. Several experimental parameters were optimized and the data for these are reported herein.

2 Materials and methods

2.1 Chemicals

MG oxalate and silver nitrate were obtained from Acros (New Jersey, USA). SDS was obtained from Sigma (St. Louis, MO, USA). Hydroxylamine hydrochloride and citric acid were purchased from Fluka (Bauch, Switzerland) and Yakuri Pure Chemical Company (Osaka, Japan), respectively. Sodium hydroxide and sodium chloride were purchased from J. T. BAKER (Mallinckrodt Baker, USA). 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 setup [14], 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, id, 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 (red-color), dispersed by a monochromator (Acton Research Corporation, Model SP-300i; detection window was set at 582$\pm$0.2 nm), followed by detection using a photomultiplier tube (Hamamatsu-R928). In the off-line case, an argon ion laser (514.5 nm, 50 mW) was used as the excitation light. Various concentrations of samples were prepared, and were sealed in a glass tube (id/od, 0.8/1.1 mm) for the measurements. The laser beam was focused on the sample by a 3 cm lens. Raman emissions were collected at a right angle to the light source, and dispersed by a second monochromator (Acton Research Corporation; Model SP-500i; resolution, 0.1 nm), followed by detection using another photomultiplier tube (PMT). 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 and SERRS shifts.

2.3 Preparation of the silver colloids and sample solution

2.3.1 Silver colloids

The procedures used to prepare the silver colloids have been described previously in the literature [15]. Following the procedures, the final products were identified by UV-Visible spectroscopy.

2.3.2 Sample solution

A 0.02 g MG was dissolved in water (10 mL). A 10 μL diluted MG solution (concentration ranges: 1250–125 ppm, MEKC/SERRS; 0.5 ppm–50 ppt, sweeping-MEKC/SERRS) was simply mixed with 990 μL of silver colloid, and then to a 1.0 mL solution for use.

3 Results and discussion

3.1 Off-line conventional RS and SERRS spectra of malachite green

The conventional Raman spectrum of MG (150 ppm) is shown in Fig. 1A; the inset shows the chemical structure of MG. The numbers above the peaks indicate the Raman-shifts, in wavenumbers (cm^{-1}) and match reasonably well to published literature values [16]. Table 1 summarizes the data obtained in this study and the values

Table 1. Main peaks observed in the normal Raman and SERRS frequencies (cm^{-1}) of in a dilute aqueous solution using 514.5 nm (argon ion laser) as the exciting source

Raman[a]	SERRS[b]	Raman-SERRS shift [c]	Raman[d]	Peak assignment[d]
1172	1176	−4	1176 s	I.p. C-H bend
1218	1214	4	1221 s	N-C stretch, NR$_2$ bend
1289	1289	0	1297 s	I.p. C-C and C-C-H
1334	1330	4	1340 m	Combination
1360	1359	1	1368 s	N-φ stretch
1395	1390	5	1402 s	I.p. C-C, C-H
1448	1445	3	1449 w	NR$_2$ bend and rock
1486	1481	5	1492 m	NR$_2$ bend and rock
1592	1589	3	1596 s	I.p. ring stretch, bend
1616	1617	−1	1619 s	N-φ and C-C stretch

a) This work; concentration, 150 ppm.
b) This work; concentration, 150 ppb.
c) Uncertainty, $\sim \pm 1$ cm^{-1}
d) Data and assignment taken from [16]; O.o.p = out-of-plane; I.p. = in-plane
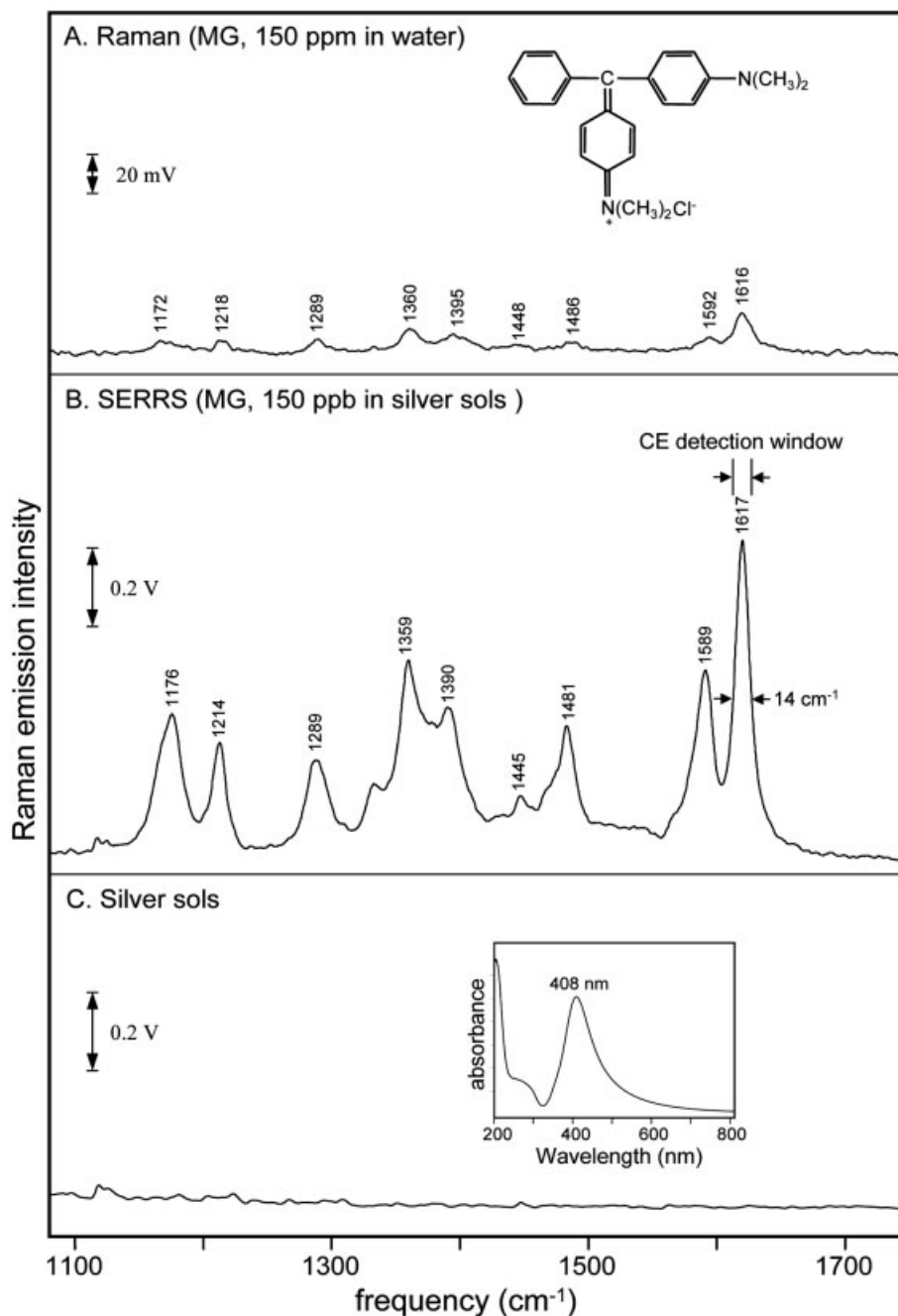
Figure 1. Conventional Raman and SERRS spectra of MG (A, 150 ppm; B, 150 ppb; C, 0 ppm) are shown in (A)–(C), respectively. Table 1 summarizes the data obtained in this study and the values found in the literature for comparison.

found in the literature for comparison. The strongest Raman band at 1616 cm^{-1} was assigned to the ring breathing and N-φ stretching modes. A detailed discussion of these assignments can be found in the literature [17]. Compared to a conventional Raman spectrum, some minor shifts were found in the SERRS spectrum (Fig. 1B) in this study, but a detailed discussion of this is omitted in this paper. As can be seen, the intensity of the conventional Raman emission is weak; the intensity of the maximum peak (1616 cm^{-1}) is only 21 mV, in this case. Such a

weak emission would be difficult to use in a CE separation. Figure 1B shows the SERRS spectrum of MG (150 ppb in a silver colloidal solution). In the cases of SERRS experiments, the sample solutions were prepared by simply mixing the MG solution and the silver colloidal solution (MG-Ag). It can be seen that the intensity of the Raman emission was dramatically enhanced to 892 mV. That is, a 42 000-fold enhancement was realized. These data for Raman/SERRS frequencies (cm^{-1}) and the relative intensity ratios are all important and are useful in a

qualitative analysis when a CCD detector is used. In order to investigate the stability of MG-Ag binding as a function of time, various periods were tested, after preparation of the solutions. The findings show that the MG-Ag solution provides the strongest emission 15 min after its preparation; a half-intensity was obtained, even when the solutions were stored for 4 h. This indicates that the MG-Ag solution can be used during the entire CE separation process. Figure 1C shows the spectrum of a silver colloidal solution and none of the characteristic peaks are present. The inset in Fig. 1C shows the UV absorbance spectrum of the silver colloidal solution. The maximum absorbance wavelength was 408 nm, indicating that the size of the silver colloid is about 23 nm, detailed information can be found in the published literature [15].

3.2 Comparison of MEKC/Raman and sweeping-MEKC/Raman methods

MG, a compound that is potentially dangerous to human health, is a synthetic dye used to color fabrics and paper, and has been used illegally in the treatment of certain fish diseases, mainly, against parasites for freshwater and marine fishes. Thus far, the current detection methods for MG include HPLC [17–19], LC-MS [20, 21] and UV/CE-stacking [22] methods, *etc.* Each method has unique advantages and disadvantages with respect to sensitivity, precision and simplicity of use. In this study, we found that MG can be easily detected and quantified using the MEKC and sweeping-MEKC modes [9]. In the case of the MEKC mode, optimal conditions were achieved using an aqueous citric acid (50 mM) buffer (pH 2.1) containing SDS (50 mM). Figure 2A shows a typical electropherogram of MG (100 ppm); the detection window was set to 582 ± 0.2 nm, corresponding to the strongest Raman line (1616 ± 7 cm^{-1}) when a 532 nm laser is used for excitation. The dye compound MG has a strong absorption in the red, but also absorbs at 532 nm. Therefore, resonance Raman spectroscopy (RRS) could be carried out, leading to low detection limits. In this case, the detection window was extremely narrow, the detection limit for MG was determined to be 10 ppm (S/N = 3). In order to perform a quantitative analysis, it would be desirable to collect the entire range of Raman emissions. This could be accomplished by removing the monochromator and using a PMT directly, and as a result, the LOD could be improved to 0.4 ppm (data not shown). Figure 2B shows a typical electropherogram of MG (25 ppb) obtained by the sweeping-MEKC mode (emission observation window, 582 ± 0.2 nm). The CE buffer system BGE 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 its

injection length was 22 cm (effective/total length, 61/70 cm). The pH of these solutions should be kept at a low value to suppress the EOF, since this method is independent of EOF. When the injection of BGE and the sample solution are complete, a negative polarity is applied to power the CE separation. Meanwhile, the cations move toward the inlet; anions move in the reverse direction. As a result, anionic SDS micelles enter the capillary and the analytes (Ag-MG) are concentrated by the sweeping-MEKC mode. Once the analytes are completely swept by SDS, the subsequent separation occurs by the MEKC mode. As a result, a dramatic improvement in detection sensitivity could be obtained, and the LOD was improved to 5 ppb (at S/N = 3). Again, by removing the monochromator to collect the entire range of emissions, the LOD was further improved to 0.2 ppb (data not shown). The linearity of these methods for MG was also fairly good and these data (including the calibration curve, coefficient of correlation, LOD values, and theoretical plate number) are summarized in the Table 2.

Table 2. Calibration curve, coefficient of correlation, LOD values (S/N = 3), and theoretical plate number (*N*) for MG by MEKC/Raman, sweeping-MEKC/ Raman, MEKC/SERRS, and sweeping-MEKC/ SERRS methods, respectively, by using a doubled Nd:YAG laser[a]

A.	MEKC/Raman	[b]observed: 582 ± 0.2 nm
	Equation of the line	$y = 3.6 \times 10^5 x + 1.8 \times 10^6$
	Coefficient of correlation	$R^2 = 0.9911$
	Detection range	$1.1 \times 10^{-4} \sim 1.3 \times 10^{-5}$ M
	LOD	1.1×10^{-5} M
	Theoretical plate number	$2.2 \times 10^4 \sim 4.2 \times 10^4$
B.	Sweeping-MEKC/Raman[c]	
	Equation of the line	$y = 1.5 \times 10^7 x + 1.9 \times 10^5$
	Coefficient of correlation	$R^2 = 0.9905$
	Detection range	$5.4 \times 10^{-6} - 5.4 \times 10^{-9}$ M
	LOD	5.3×10^{-9} M
	Theoretical plate number	$2.3 \times 10^5 - 3.0 \times 10^5$
C.	MEKC/SERRS	
	Equation of the line	—
	Coefficient of correlation	—
	Detection range	$1.3 \times 10^{-5} - 1.3 \times 10^{-6}$ M
	Theoretical plate number	$4.0 \times 10^3 - 2.7 \times 10^4$
D.	Sweeping-MEKC/SERRS[c]	
	Equation of the line	—
	Coefficient of correlation	—
	Detection range	$1.1 \times 10^{-5} - 1.1 \times 10^{-9}$ M
	Theoretical plate number	$8.7 \times 10^4 - 4.8 \times 10^5$

a) Light source: doubled Nd:YAG laser (532 nm, 300 mW)
b) The wavelength of monochromator was set at 582 ± 0.2 nm.
c) Capillary: total length/effective length, 70/61 cm; sample injection length, 22 cm.
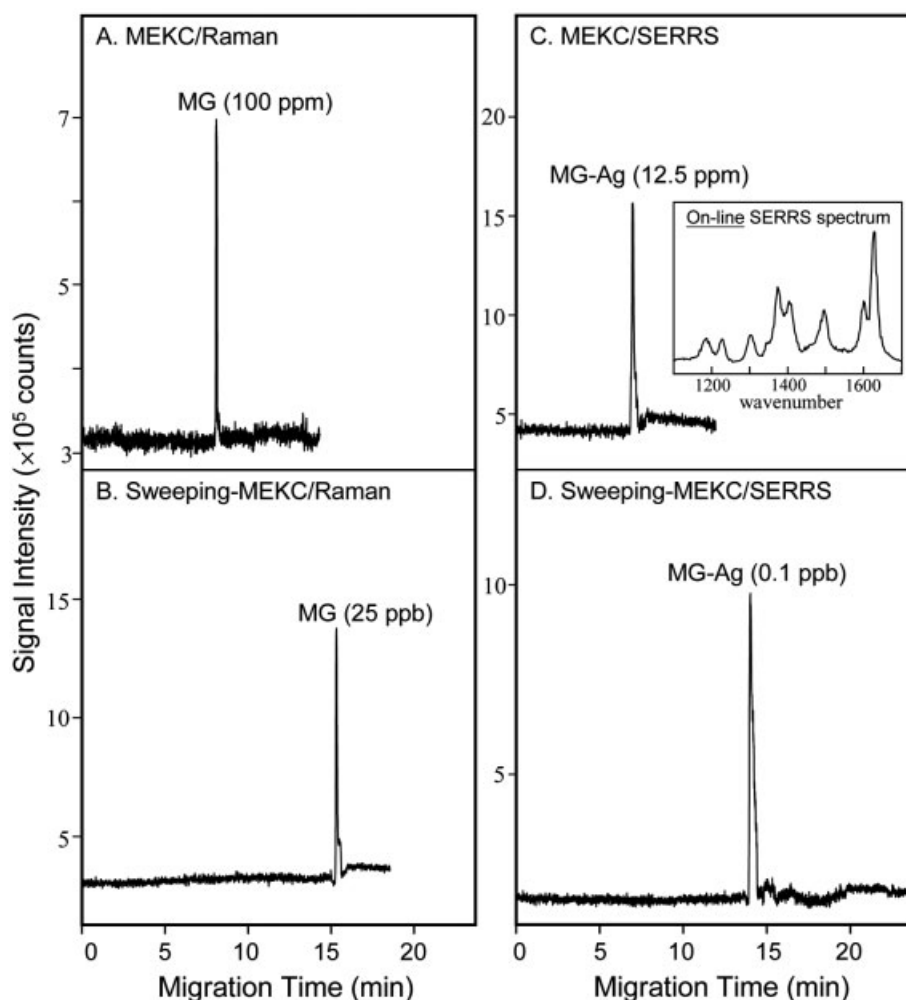
Figure 2. Typical electropherograms of MG obtained by MEKC/RS (A, 100 ppm) and sweeping-MEKC/RS (B, 25 ppb), MEKC/SERRS (C, 12.5 ppm) and sweeping-MEKC/SERRS (D, 0.1 ppb), respectively. The detection window (582 ± 0.2 nm) was set and matched with the strongest Raman line, corresponding to the specific shift at 1616 cm^{-1}. CE buffer: MEKC (A and C), an aqueous citric acid (50 mM) containing SDS (50 mM) buffer (pH 2.1); sweeping-MEKC (B and D), the same running buffer system as described as the MEKC mode, but the sample was dissolved in the matrix (50 mM citric acid aqueous buffer without SDS). The applied voltages were −16 kV (A and C) and −8 kV (B and D).

3.3 Comparison of MEKC/SERRS and sweeping-MEKC/SERRS methods

Although SERRS has also been successfully coupled with a number of separation techniques [23–27], there are few reports on the coupling SERRS with CE [5–8]. This is probably due to the technical difficulties, which involve placing and stabilizing a microsized SERRS-active substrate inside or outside of the capillary for on-line or post-column detection. In this study, the silver colloidal solution was deposited directly in the CE running buffer, and the detection window was set at the specific Raman line for the 1616 cm^{-1} shift, the same as described above. Figure 2C shows a typical MEKC/SERRS electropherogram of MG-Ag (12.5 ppm) under the same CE conditions that were described in Fig. 2A. Herein, a plug of sample solution was injected first, and a portion of silver colloidal solution (in length 1.3 cm; pH 8.2) was then injected. When a negative polarity was used to power the CE separation, the anionic SDS micelles from the inlet vial

enter the capillary, carry the analytes to mix with the Ag particles, and the separation of MG-Ag occurs by the MEKC mode. With the assistance of nano-Ag particles, the LOD can be improved to ~10-fold compared to the normal MEKC/Raman method. The inset, as shown in Fig. 2C, is an on-line spectrum of the separated peak obtained by stopping the EOF and measured by a scanning monochromator, which was equipped with a PMT. The spectrum is identified with the standard as shown in Fig. 1B. However, signal enhancement depends on the degree of completion of the MG-Ag reaction during the CE separation process. Since this is difficult to control, the data obtained for MEKC/SERRS were less useful for quantitative purposes. Figure 2D shows a typical sweeping-MEKC/SERRS electropherogram of MG-Ag (0.1 ppb) under the same CE conditions, as described in Fig. 2B. Herein, the length of the injected sample solution and the silver colloidal solution were 22 and 1.3 cm, respectively. The findings show that the enhancement in SERRS is nonlinear; the greatest improvement is achieved for a

sample concentration of $\sim 10^{-7}$ M. Table 2 summarizes the detection ranges of the two modes. We found that the linearity of Raman methods is fairly good, but data obtained by SERRS methods is not good, and is also less useful for quantitative purposes. Thus, the sweeping-MEKC/SERRS mode is possibly more useful for detecting MG at very low levels, but it is difficult to use as a routine tool in a quantitative analysis.

4 Concluding remarks

This work successfully demonstrates a new approach for detecting a non-fluorescent compound by a CE-RRS method combined with an on-line sample concentration technique, the sweeping-MEKC mode. When the CE buffer was doped with silver colloids, the LOD could be further improved. 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, where only a miniaturized system would be needed. Further applications of this technique are currently under investigation.

5 References

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