

# Simultaneous Chemical Separation and Surface-Enhanced Raman Spectral Detection Using Silver-Doped Sol-Gels

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## INTRODUCTION

Over the past 30 years the combination of chemical separation and molecular analysis has proven invaluable to the analytical chemist in identifying chemicals at extremely low concentrations in complex matrices. For example, a drug and its metabolites can be effectively separated from blood plasma using gas chromatography and identified by the chemical fragments detected by mass spectrometry.<sup>1</sup> More recently, the combination of liquid chromatography or flow injection analysis with surface-enhanced Raman spectroscopy (SERS) has been investigated for such applications.<sup>2-13</sup> Advantages of this combination include minimal sample preparation, unrestricted use of water in the mobile phase, high chemical specificity through abundant molecular vibrational information, and extreme sensitivity as demonstrated by the detection of single molecules.<sup>14,15</sup> Previous research has largely employed the three most common methods of generating SERS; roughened silver electrodes, silver coated substrates, and silver colloids for detecting separated analytes.<sup>2-13</sup> The latter has gained the most attention, as colloids can be easily and inexpensively prepared, and mixing the colloids with the chromatographic column effluent using flow injection is highly reproducible.<sup>3,10</sup> However, care must be taken to control aggregation of the colloids so that the amount of Raman signal enhancement is maintained. A range of experimental variables, such as analyte concentration and pH, can strongly influence aggregation and to some extent limit applications.<sup>8</sup> Further, the choice of mobile phase is similarly limited by the need to maintain colloid integrity.

Recently, we<sup>16-20</sup> and others<sup>21-23</sup> have been developing sol-gels to trap silver or gold particles as yet another method of generating SERS. Once the sol-gel has formed the metal particle size and aggregation are stabilized. Albeit changes in pH may still result in variable Raman signal intensities, such as the case of weak acids and bases, where the relative concentrations of the ionized and unionized forms may be influenced.<sup>17</sup> In addition, we have shown that many of the common solvents, such as acetone, methanol and water can be used equally in generating SERS of analytes with these metal-

doped sol-gels.<sup>16</sup> Concurrent to our work, others have been developing sol-gels as the stationary phase in columns for both liquid- and gas-phase chromatography.<sup>24-27</sup> Advantages can be gained in both the preparation of columns and their performance. The sol-gel approach allows combining deactivation, coating, and immobilization in a single step, while the sol-gels have shown reduced tailing, improved separation, and broader application to solvents and analytes. Here we combine the following two functions of sol-gels: (1) the ability to separate chemicals; and (2) the ability to immobilize metal particles and generate SERS in order to detect chemicals in solution. This ability to perform both functions at the same time also allows performing the analysis in a new fashion. Namely, the chemicals in a suitable solvent can be forcibly separated within seconds by a pressure (push) or vacuum pulse (pull). Then a suitable optical probe, capable of excitation and collection of Raman photons, can be used to scan the length of the column detecting separated chemical species. Complete analysis can be accomplished in less than five minutes.

## EXPERIMENTAL

All chemicals were obtained at their purest commercially available grade from Aldrich (Milwaukee, WI). The columns consisted of 1-mm diameter melting point capillaries, packed with 5-mm lengths of silver-doped sol-gel. The SER-active sol-gel was obtained from our commercially available sample vials, the preparation of which has previously been reported.<sup>19</sup> In essence, the vials are prepared from two precursor solutions. The first, a silver amine complex, consists of a 5/1 v/v ratio of 1N AgNO<sub>3</sub> and 28% NH<sub>3</sub>OH. The second, the alkoxide, consists of a 2/1 v/v ratio of methanol and tetramethyl orthosilicate (TMOS). The precursors are mixed 1/8 v/v silver amine to alkoxide and 0.15 mL are transferred to a 2-mL glass vial (Wheaton, Millville, NJ), which is spun to coat the inside walls. After sol-gel formation, the incorporated silver ions are reduced with dilute sodium borohydride, which is followed by a water wash to remove residual reducing agent. Here, the sol-gel coating in the vial was removed by scraping and then made homogeneous by grinding with a mortar and pestle. A 4-cm length of capillary was cut and the sol-gel was packed into the capillary using sterile cotton to hold the powder in place. The top was fit with a 1-mL plastic disposable pipette to allow delivery of 10  $\mu$ L samples to this rudimentary liquid chromatography column. A diaphragm pump was used for vacuum assisted measurements (Gast Manufacturing, Benton Harbor, MI).

The column was fixed vertically just inside the focal point of a microscope object (20x0.4, Newport, Irvine, CA) that was attached to an XYZ positioning stage (Newport). The microscope objective was used to focus the beam into the sample and to collect the scattered radiation back along the same axis. A notch filter (Kaiser, Ann Arbor, MI) was used to reflect the excitation laser to the microscope objective and pass the Raman scattered radiation collected by the objective.

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Two 3-m lengths of fiber optic were used to deliver the laser energy (200 micron diameter, Spectran, Avon, CT) and collect the Raman radiation (365 micron diameter). A Nd:YAG laser provided 50 mW of 1064 nm excitation at the sample (Brimrose, Baltimore, Maryland), while a Fourier transform Raman spectrometer equipped with an InGaAs detector was used for spectra acquisition (Real-Time Analyzers, East Hartford, CT).<sup>28</sup>

## RESULTS AND DISCUSSION

The initial experiment mimicked traditional liquid chromatography. A  $8 \times 10^{-3} \text{ M}$  *p*-aminobenzoic acid (PABA) and a  $4 \times 10^{-3} \text{ M}$  phenyl acetylene (PA) solution was prepared in methanol to demonstrate separation of polar and non-polar chemicals. A 10- $\mu\text{L}$  solution was added to the top of the column and allowed to elute, driven by gravity and capillary action. The microscope objective was positioned 0.5 mm from the bottom edge of the 5-mm length of packed sol-gel. Scans were collected and averaged every 30 s to produce a spectrum. Unique bands for PA and PABA at  $1985 \text{ cm}^{-1}$  and  $850 \text{ cm}^{-1}$ , respectively were used to plot relative concentration as a function of time (Fig. 1). Chemical separation is achieved as PA and PABA reach maximum concentrations at 18 and 65 min, respectively. However, the elution profiles are far from ideal, showing considerable broadening. This can be attributed to a relatively wide distribution of pore sizes in the sol-gel stationary phase, and hence distribution of elution paths through the column. Conversely, the variety of chemical interactions for either PA or PABA with the available silver surface is limited, and therefore this contribution to the broadening is believed to be minor. Further, the smooth disappearance of PA and decrease

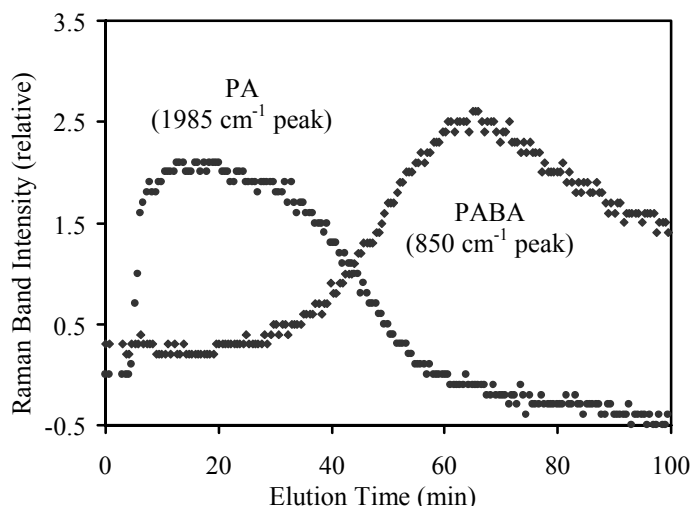


FIG. 1. Elution profile of a 1/2 M/M PA and PABA solution in methanol detected by SERS. Raman band peak heights used are indicated. Spectral conditions: 30 s per spectrum, 50 mW of 1064-nm excitation,  $8 \text{ cm}^{-1}$  resolution.

of PABA suggest that surface interactions are reversible and do not adversely affect the SER-activity of the silver particles.

However, further studies are required to substantiate this point and its validity to other analytes. Methods that minimize pore distribution to reduce peak tailing, analysis of solvent effects and chemical interactions, and the ability to re-use columns is the subject of a more comprehensive study and is beyond the scope and intent of this preliminary report.

In an effort to substantially improve this separation and hence reduce analysis time, methods were explored to drive the solution through the column at greater speeds (e.g. pump the sample). Furthermore, since the entire length of the 5-mm sol-gel column is SER-active, the extent of separation could be evaluated by moving the microscope objective to different positions along the column. In effect, spectra could be collected at multiple points along the column and each analyte quickly identified. There was no need to wait for the analytes to elute past a single measurement point at the end of the column.

In addition, various methods could be used to draw the analytes into the column rapidly. To demonstrate this concept, a mild vacuum of 50 cm Hg was applied to the exit end of the column. Again a sample of  $4 \times 10^{-3} \text{ M}$  PA and  $8 \times 10^{-3} \text{ M}$  PABA was used, but this time the sample was prepared in a 50/50 v/v methanol/water solution, one of the most commonly used mobile phases. A 10- $\mu\text{L}$  sample was drawn into a 5-mm sol-gel column in 30 s. Spectra were collected at five discrete points spaced 1 mm apart with the first one at 0.5 mm from the top edge of the packed sol-gel (Fig. 2). Each spectrum consists of scans averaged for 30 s. Spectra obtained at the top and bottom of the column indicate pure PA and PABA, respectively, while the middle spectrum shows a mixture of the two analytes. This series of spectra also demonstrates the power of Raman spectroscopy in that each chemical can be easily identified either isolated or as a mixture. Admittedly previous knowledge of the sample simplifies this task, but spectral matching and deconvolution software programs could be used to handle unknowns.

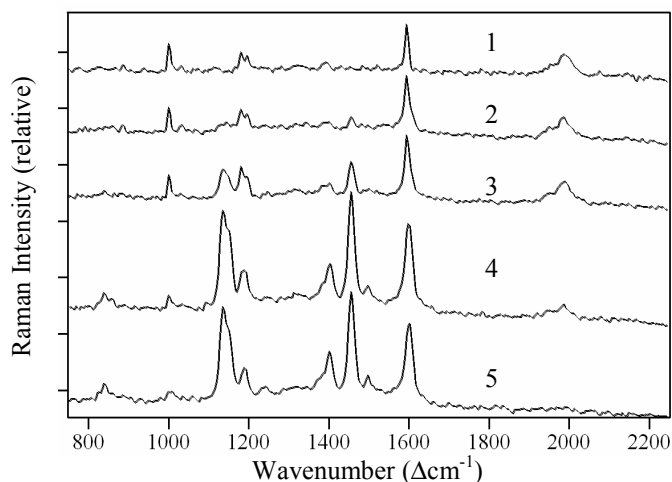


FIG. 2. SERS of 1/2 M/M PA and PABA solution in 1/1 methanol/water at five discrete points spaced 1 mm apart along a 5-mm sol-gel column, denoted as 1 through 5, where 1 is PA, 2-4 are mixtures, and 5 is PABA. Spectral conditions as in Fig. 1.

## CONCLUSION

Here we have shown that a silver-doped sol-gel can be used to both separate chemicals and produce their surface-enhanced Raman spectra. We have combined these capabilities in a liquid chromatography column and simultaneously separated and measured phenyl acetylene and *p*-aminobenzoic acid. Furthermore, since the sol-gel is SER-active along the entire column length we have profiled the separation of these chemicals by measuring spectra along the length of the column. This latter approach may prove valuable in that sample separation can be effected rapidly followed by spectral analysis. Many applications may benefit from this approach, including detection of contaminants in groundwater (e.g.  $\text{CN}^-$ ,  $\text{Cr}_2\text{O}_7^{2-}$ ), determining the efficacy of a drug by analyzing the parent and its metabolites in biological fluids, and detecting chemical agent hydrolysis products in poisoned water.

Current research involves manufacturing sol-gel packed columns, developing coatings in glass and plastic microchannels, and incorporating both in microchip analyzers. In addition, gold-doping and various sol-gel alkoxide precursors are being explored to broaden separation and SER-detection capabilities.

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1. J. Chamberlain, *The Analysis of Drugs in Biological Fluids*, (CRC Press, Boca Raton, 1995) 2nd ed., chap. 6 and 7.
2. J-M. L. Sequaris and E. Koglin, *Anal. Chem.*, **59**, 525 (1987)
3. R. D. Freeman, R. M. Hanmaker, C.E. Meloan, and W. G. Fateley, *Appl. Spectrosc.*, **42**, 456-460 (1988)
4. F. Ni, R. Sheng and T. M. Cotton, *Anal. Chem.*, **62**, 1958 (1990)
5. G. T. Taylor, S. K. Sharma, and K. Mohanan, *Appl. Spectrosc.*, **44**, 635 (1990)
6. R. Sheng, F. Ni and T. M. Cotton, *Anal. Chem.*, **63**, 437 (1991)
7. N. J. Pothier and R. K. Force, *Appl. Spectrosc.*, **46**, 147 (1992)
8. L. M. Cabalin, A. Ruperez, and J. J. Laserna, *Talanta*, **40**, 1741 (1993)
9. K. T. Carron and B. J. Kennedy, *Anal. Chem.*, **67**, 3353 (1995)
10. L. M. Cabalin, A. Ruperez, and J. J. Laserna, *Anal. Chim. Acta*, **318**, 203 (1996)
11. N. J. Szabo and J. D. Winefordner, *Appl. Spectrosc.*, **51**, 965 (1997)
12. B. J. Kennedy, R. Milofsky, and K. T. Carron, *Anal. Chem.*, **69**, 4708 (1997)
13. W. F. Nirode, G. L. Devault, M. J. Sepaniak, and R. O. Cole, *Anal. Chem.*, **72**, 1866 (2000)
14. K. Kneipp, Y. Wang, R. R. Dasari, and M. S. Feld, *Appl. Spectrosc.*, **49**, 780 (1995)
15. S. Nie and S. R. Emory, *Science*, **275**, 1102 (1997)
16. Y. H. Lee, W. Smith, S. Farquharson, H. C. Kwon, M. R. Shahriari, and P. M. Rainey, *SPIE*, **3537**, 252 (1998)
17. S. Farquharson and Y. H. Lee, *SPIE*, **4200**, 89 (2000)
18. S. Farquharson, W. Smith, and Y. H. Lee, *IFPAC*, **7**, 85 (2001).
19. Y. H. Lee and S. Farquharson, *SPIE*, **4206**, 140 (2001)
20. S. Farquharson, P. Maksymiuk, K. Ong and S. D. Christesen, *SPIE*, **4577**, 166 (2002).
21. F. Akbarian, B. S. Dunn, and J. I. Zink, *J. Chem. Phys.*, **99**, 3892 (1995)
22. T. Murphy, H. Schmidt, H.-D. Kronfeldt, *SPIE*, **3105**, 40 (1997)
23. Y. Lee, S. Dai, and J. Young, *J. Raman Spectrosc.*, **28**, 635 (1997)
24. Y. Guo and L. A. Colón, *Anal. Chem.*, **67**, 2511 (1995).
25. S. A. Rodríguez and L. A. Colón, *Appl. Spectrosc.*, **55**, 472 (2001).
26. D. Wang, S.L. Chong, and A. Malik, *Anal. Chem.*, **69**, 4566 (1997)
27. S. Bigham, J. Medlar, A. Kabir, C. Shende, A. Alli, A. Malik, *Anal. Chem.*, **74**, 752(2002)
28. S. Farquharson, W. Smith, R. C. Carangelo, and C. Brouillette, *SPIE*, **3859**, 14 (1999)