ANALYTICAL RAMAN SPECTROSCOPY

Constantinos A. Georgiou

Agricultural University of Athens, 75 Iera odos, Athens 118 55, Greece
Phone: +301-5294248, Fax: +301-5294265
URL: http://www.aua.gr/georgiou E-mail: cag@aua.gr

Abstract

Novel FT-Raman methods for the quantitative analysis of fenthion, diazinon, methyl-parathion, ciprofloxacin and acyclovir are presented along their evaluation in the analysis of their formulations. The proposed methods provide results that are in accordance with those obtained through standard methods. Multivariate calibration provides results that are in accordance with those obtained through univariate calibration for all methods except for methyl-parathion where the accuracy is improved by multivariate calibration. Furthermore, the proposed methods offer several advantages compared to spectroscopic and chromatographic methods

Key words: Raman spectroscopy, Pesticides, Pharmaceutics

Introduction

Raman spectroscopy has been applied in structural and qualitative analysis but quantitative analysis has not kept pace with those applications. FT-Raman spectroscopy achieves high precision in frequency and increased S/N ratio due to multiplex measurement. Excitation at 1064 nm overcomes problems of fluorescence and thermal decomposition traditionally related to dispersive techniques. In this study, Raman spectroscopy was applied in the quantitative analysis of pesticides and pharmaceutics. The ability to analyze the structure of drug substances in aqueous environments, the capability to sample through glass and the increased sensitivity to symmetric stretches and highly polarizable bands renders Raman spectroscopy a powerful analytical technique [1,2].

Experimental

Samples were analysed as received, without any treatment. An exception were the commercial formulations of ciprofloxacin that were ground in a mortar, and the resulting powder was pressed to prepare discs. Standard solutions of fenthion, diazinon and methyl-parathion were prepared during calibration, while calibration discs were prepared for ciprofloxacin and acyclovir.

FT-Raman spectra were recorded with a Nicolet 750 FT-Raman spectrometer equipped with a Nd:YAG laser source that emits at 1064 nm. A CaF_2 beamsplitter, an Indium-Galium Arsenate (InGaAs) detector and 180^0 backscattering geometry were used in the spectrometer. Liquids (standard solutions and formulations of fenthion, diazinon and methyl-parathion) were placed in NMR cells, while solids (calibration disks and formulations of ciprofloxacin and acyclovir) were placed in a laboratory made accessory. Spectra were accumulated from 100 scans collected during 3 min at a resolution of 4 cm^{-1}.

Results and discussion

Quantitative analysis of fenthion in pesticide formulations [3,4]

The Raman spectra of the organophosphorus pesticide fenthion is presented (fig. 1) and a novel FT-Raman method for the quantitative determination of fenthion in pesticide formulations is proposed. Calibration curves based on Raman intensity and band area at 2951, 1065, 661 and 604 cm^{-1} were linear (correlation coefficients: 0.991-0.998) in the concentration range of 0.4-4.36 M. Detection limits ranged between 0.14 to 0.36 M and precision from 0.4 to 6.8 %RSD (n=4). Spectra normalization against the 802 cm^{-1} cyclohexane band proved to be effective in

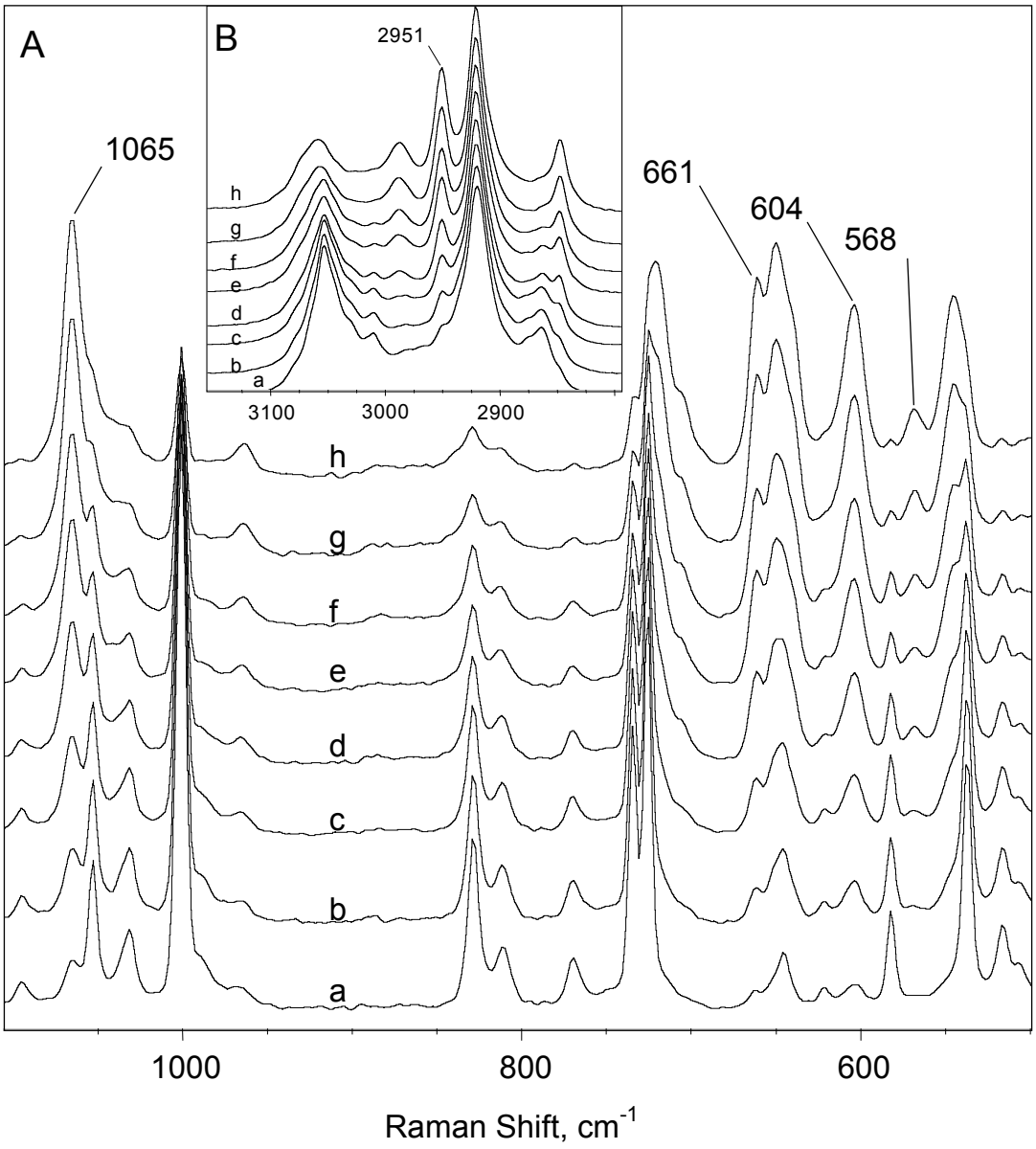
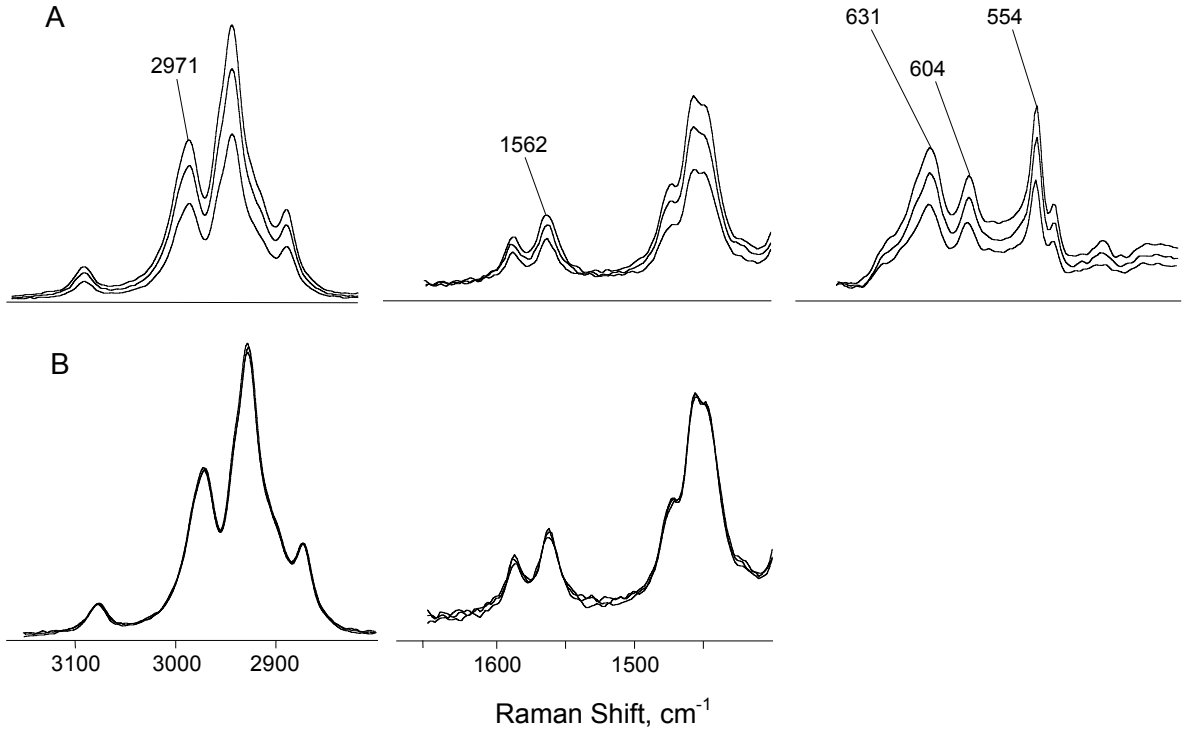


Fig. 1. Raman spectra acquired during calibration: (a) 0.400, (b) 1.00, (c) 1.50, (d) 2.00, (e) 2.50, (f) 3.00, (g) 3.50 and (h) 4.00 M fenthion standards measured in the range of: (A) 1100-500 and (B) 3150-2800 cm^{-1}

minimizing the effect of excitation intensity fluctuations and sample positioning changes in calibration data, resulting in improved long term stability of calibration curves. Results obtained during the analysis of the Lebaycid formulation compare well with those obtained by the GC reference method. The proposed FT-Raman method is fast, simple, safe, avoids sample pre-treatment and minimizes handling of toxic samples.

Quantitative analysis of diazinon in pesticide formulations [5]

FT-Raman spectroscopy based on band intensity and band area measurements, was used for the quantitative determination of diazinon in pesticide formulations. Bands at 554, 604, 631, 1562 and 2971 cm^{-1} were used for calibration. Calibration curves were linear (correlation coefficients: 0.992-0.9992 and 0.99-0.999 for band intensity and band area measurements, respectively) in the range of 0.2-3.5 M for the 554 and 2971 cm^{-1}, 0.3-3.5 M for the 604 cm^{-1}, 0.6-3.5 M for the 1562 cm^{-1} and 1.0-3.5 M for the 631 cm^{-1} band. Normalization of calibration curves against the 802 cm^{-1} cyclohexane band improved their long term stability and minimized the effect of laser beam



power fluctuations (fig. 2). No interference was found by commonly used surfactants and the proposed method was applied to the analysis of diazinon formulations. Results obtained compare well as indicated by the *t*-test, with those obtained by the HPLC reference method. Precision ranged between 0.2-7.8 and 0.1-7.2 %RSD, (n=4) for band intensity and band area measurements, respectively. The proposed method is rapid, simple and safe, as toxic samples are analyzed "as received" without sample pre-treatment, permiting the routine analysis of pesticides formulations.

Quantitative analysis of methyl-parathion in pesticide formulations [6]
An FT-Raman method for the quantitative determination of methyl-parathion in pesticide formulations is described. The proposed method was applied to the analysis of formulations of methyl-parathion. Univariate and multivariate calibration were used and compared for quantitative analysis. Bands observed at 634, 661, 1113, 1348 and 1527 cm^{-1} were used for univariate calibration. Calibration curves were linear (correlation coefficients: 0.996-0.998 and 0.994-0.999 for band intensity and band area measurements, respectively) in the concentration range of 0.6-3.75 M for the 634, 661 and 1527 cm^{-1} bands, 0.05-3.75 M for the 1113 cm^{-1} band and 0.1-3.75 M for the 1348 cm^{-1} band. Precision ranged from 0.5-5.2% and 0.1-6.8 %RSD (n = 4) for band intensity and band area measurements, respectively. Spectra normalization against the 802 cm^{-1} cyclohexane band improved the long-term stability of calibration allowing the use of calibration data acquired up to 30 days prior to analysis. The applicability of the method was extended through multivariate calibration by multiple linear regression using the 858 and 2952 cm^{-1} bands (fig. 3). Results obtained compare well with those obtained by the HPLC reference method. The FT-Raman method developed is rapid, simple and safe, as toxic samples are analyzed "as received" without sample pre-treatment.

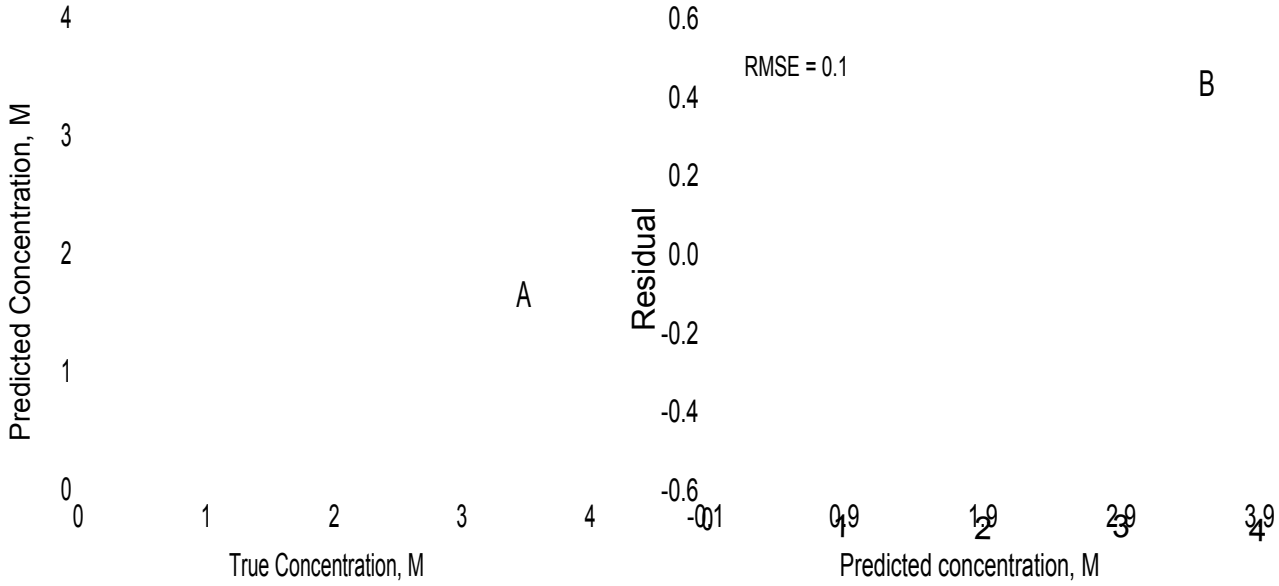


Fig. 3. Predicted methyl-parathion *vs*. true concentrations (A) and residuals as a function of concentration (B) for the multivariate regression model using band intensity measurements

Quantitative analysis of ciprofloxacin in pharmaceutics [7]
FT-Raman spectroscopy based on band intensity or band area measurements was used for the quantitative determination of ciprofloxacin in pharmaceutical solid dosage forms. Univariate calibration was used for quantitative analysis. Bands observed at 1708, 1624, 1548, 1493, 1273, 1253, 1238, 1024, 805, 787, 752, 718, 665 and 638 cm^{-1} were used (fig. 4). Calibration curves were linear in the concentration range of 3-100 % w/w with correlation coefficients: 0.99-0.996 and 0.991-0.9993 for band intensity and band area measurements, respectively. Precision ranged from 0-11 and 0.4-12 %RSD (n = 3) for band intensity and band area measurements, respectively and results were in good agreement to those acquired through the current United States Pharmacopeia (USP 24) and National Formulary (NF 19) method. Multivariate calibration was also used for quantitative analysis. Multiple linear regression using the intensities of the 1545 and 1272 cm^{-1} bands gave results in accordance to those obtained by the current United States Pharmacopeia (USP 24) and National Formulary (NF 19) method. The developed solid-state FT-Raman method does not require dissolution of solid dosage forms and can be used to replace more tedious and time-consuming methods.

Quantitative analysis of acyclovir in pharmaceutical solid dosage forms through their PVC-blister package [8]
FT-Raman spectroscopy based on band intensity or band area measurements was used for the quantitative determination of acyclovir in pharmaceutical solid dosage forms through their PVC-blister package (fig. 5). Univariate calibration using the bands observed at 1690, 1630, 1574, 1482, 1181, 578, and 508 cm^{-1} was found to be sufficient for the analysis. Calibration curves were linear, the correlation coefficients being 0.997-0.9993 and 0.996-0.9991 for band intensity and band area measurements, respectively. Results obtained compare well as indicated by the *t*-test, with those obtained by the current United States Pharmacopoeia (USP 24) and National Formulary (NF 19) method. Precision ranged from 0.7-5.6 and 0.4-4.5 %RSD (n = 3) for band intensity and band area measurements, respectively. The developed non-destructive FT-Raman method is rapid, simple and can be used for the on-line, real-time monitoring of acyclovir formulations production lines.

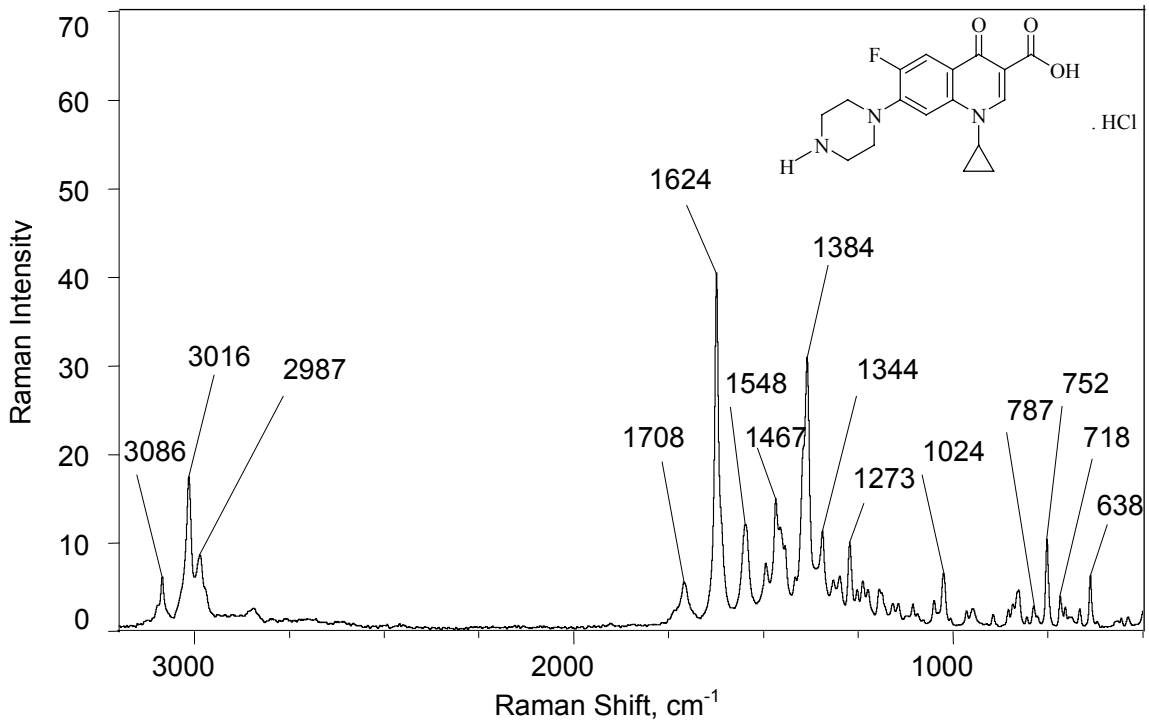


Fig. 4. Ciprofloxacin hydrochloride FT-Raman spectrum acquired at 0.51 w excitation intensity in the range of 3200 – 500 cm^{-1}

Conclusions

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- **Low analysis time:** 30 s are sufficient for acquiring a spectrum region convenient for quantitative analysis
- **Simplicity:** Minimally trained personnel can perform the analyses
- **Increased safety:** Samples were analyzed "as received" avoiding pre-treatment of toxic samples
- **Long term calibration stability:** By using external standards
- **Non-Destructive analysis:** Solid dosage forms are analyzed without dissolution
- **Non-Invasive analysis:** Solid dosage forms are analyzed through their PVC-blister package. The developed methods can be applied to on-line, real-time monitoring of production lines

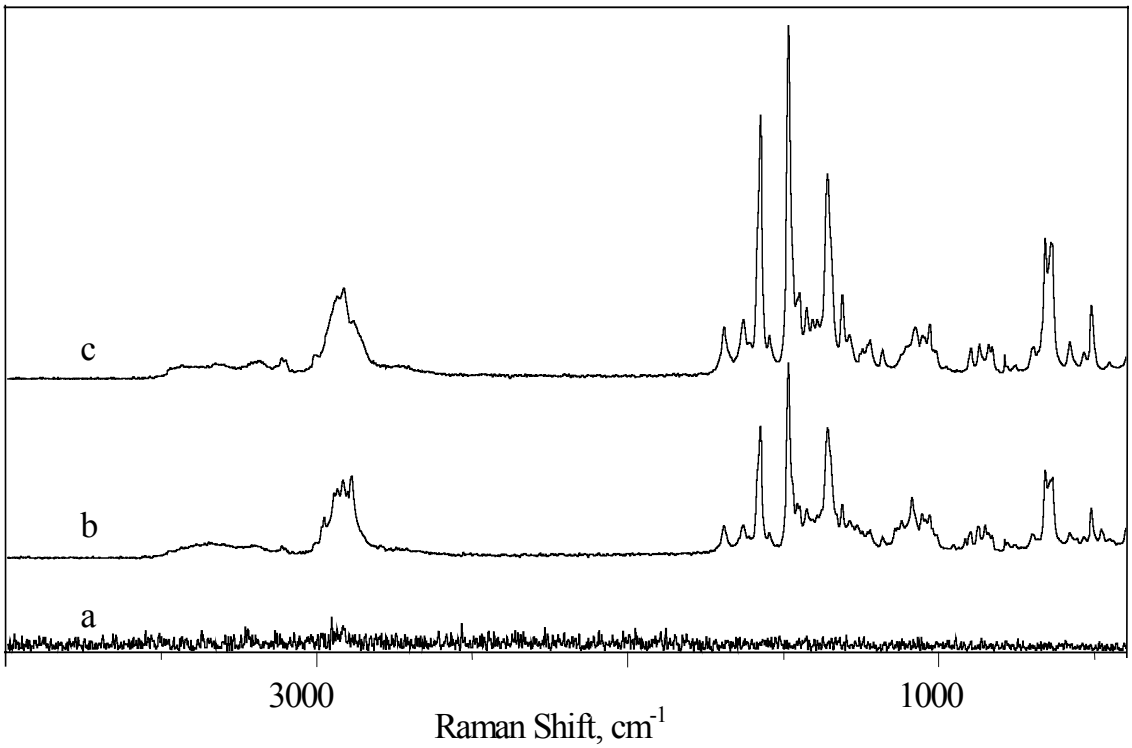

Fig. 5. FT-Raman spectra acquired from (a) the PVC-blister, and packaged tablets of the (b) Zovirax 200mg and (c) Herzkur 400mg formulations. The scale of spectrum (a) is 10 times the scale of spectra (b) and (c)

References

1. R.L. McCreery, Raman Spectroscopy for Chemical Analysis, John Wiley & Sons, UK, 2000.
2. M.J. Pelletier (Ed.), Analytical Applications of Raman Spectroscopy, Blackwell Science, UK, 1999.
3. S.G. Skoulika, C.A. Georgiou and M.G. Polissiou, Appl. Spectrosc. 53 (1999) 1470.
   Simplified presentation: http://www.aua.gr/georgiou/page23.html
4. S.G. Skoulika, C.A. Georgiou and M.G. Polissiou, *Internet Journal of Vibrational Spectroscopy* [www.ijvs.com] **4**, edition 3, (2000).
5. S.G. Skoulika, C.A. Georgiou and M.G. Polissiou, Talanta 51 (2000) 599.
   Simplified presentation: http://www.aua.gr/georgiou/page21.html
6. S.G. Skoulika and C.A. Georgiou, Appl. Spectrosc. 54 (2000) 747.
   Simplified presentation: http://www.aua.gr/georgiou/page22.html
7. S.G. Skoulika and C.A. Georgiou, Appl. Spectrosc. *in press* (September 2001).
   Simplified presentation: http://www.aua.gr/georgiou/page82.html
8. S.G. Skoulika and C.A. Georgiou, *in preparation*.
   Simplified presentation: http://www.aua.gr/georgiou/page83.html