

Identification of some of biologically active polyamines using *Identity* Raman Plate Reader.

INTRODUCTION



Figure 1. *Identity* Raman Plate Reader

Polyamines are well known to play a significant role in cell functioning [1-6]. All known cellular organisms possess polyamines such as spermine, putrescine, and others, often in significant (up to 10mM), but always tightly regulated, concentrations [7]. Although the precise role of polyamines in cellular processes is not always understood, some important areas of polyamines' involvement are known. These include regulation of levels of *c-fos*, *c-jun* and other transcriptional factors [1] and participation in early signal transduction and cell proliferation [2], control of growth arrest and apoptosis [3], sequence-specific binding to DNA and regulation of chromatin acetylation [4, 5]. The

latter makes polyamines important in epigenetics and specifically, in cancer development and prevention [6]. Bioactive polyamines do not possess any chromophore moieties, thus making their non-invasive label-free *in situ* monitoring difficult. On the other hand, polyamines possess distinct Raman characteristics in the 500-3500 cm^{-1} spectral region [8], which makes detecting them feasible by this technique. The use of Surface Enhanced Raman Spectroscopy (SERS) may additionally increase the sensitivity of polyamine detection below the level of physiologically relevant concentrations. Digilab, Inc. has recently introduced *Identity* (Figure 1), an innovative microplate reader based upon the power of Raman spectroscopy, which is suitable for many applications, including polyamine analysis [9,10]. This Application Note addresses the issues of identification and quantitation of different polyamines in solutions by traditional Raman, and SERS techniques using Digilab's *Identity* Raman Plate Reader.

PROCEDURE

1. All measurements were performed with a Digilab *Identity* Raman plate reader (Figure 1). The system is configurable with either a 532 nm (Model #RMI-53200-1) or a 785 nm (Model #RMI-78500-1) laser. Raman scattering is collected in an 180° backscatter configuration by a spectrometer with a Peltier-cooled CCD array detector capable of <10 cm^{-1} spectral resolution. The *Identity* supports standard clear, flat bottom 96 and 384 well microtiter plates, as well as custom plate formats. The laser is focused through the bottom of the plate into the well for the analysis of liquids. Sensitivity of the reader is below 1ppm in regular mode (with iso-propanol as a standard) and below 0.2ppb for silver colloid-based SERS method (with melamine as a standard).

APPLICATION NOTE

- Silver colloids were made as described by Lee and Meisel [11] using solutions of silver nitrate (*Sigma* S6506) and sodium citrate (*Sigma* C8532). The colloid was diluted 8-fold with 0.1M NaCl (*Sigma*) before adding 1:1 to sample serially diluted with the same 0.1M NaCl to wells of the glass bottomed 384 well plate (*Greiner* 781892). Colloid was added last, immediately before scanning with *Identity*. All other chemicals were from *Sigma*. All solutions were made using Nano-Pure water. SERS spectra were analyzed by using Panorama 3 software (*LabCognition*).

SERS Spectra of Selected Polyamines

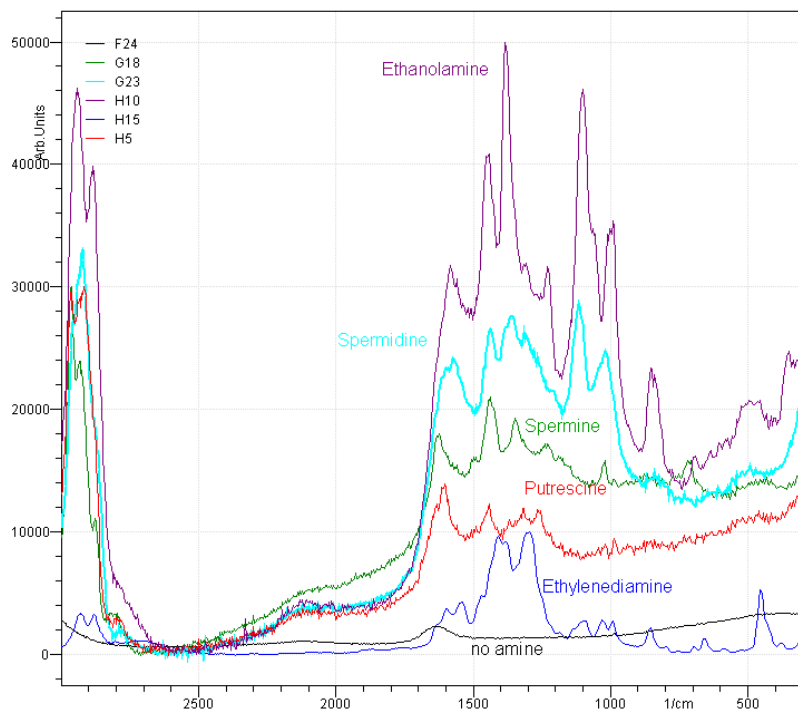


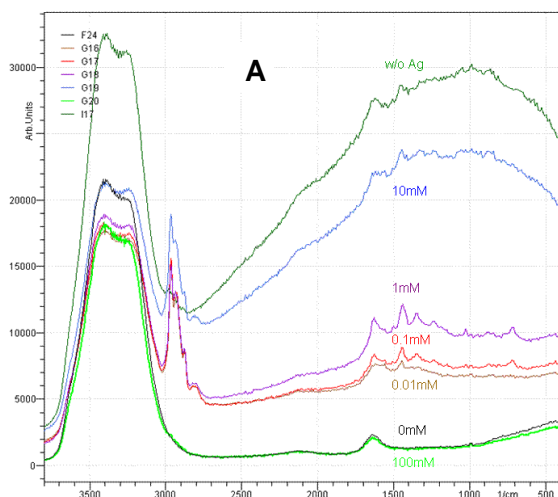
Figure 2. SERS spectra of the five polyamines at 10mM concentrations in 0.1M NaCl. Commercial sources of Polyamines: Spermine x4HCl (*Sigma* S1141); Spermidine base (*Sigma* S0266); Putrescine x2HCl (*Sigma* P5780); Ethanolamine base (*Sigma* E0135); Ethylenediamine base (*Sigma* E1649). *Identity* model RMI-53200-1 was used, total collection time 5 min with 10 co-additions.

CONCLUSIONS

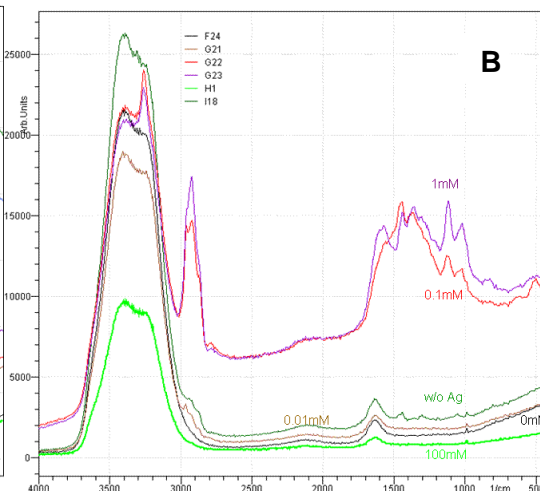
- Polyamines demonstrate strong SERS signals within the areas of 400-1800 cm^{-1} , 2800-3000 cm^{-1} and 3200-3400 cm^{-1} (Figure 2). Although the pattern depends on the concentration of the polyamine (see, for example, Figure 3E), it remains quite characteristic for each type of the polyamine and therefore could be used for the polyamine identification in a complex mixture.
- Identity* Raman Plate Reader can be successfully used for monitoring biologically active polyamines (spermine, spermidine, putrescine) in aqueous solutions both by traditional Raman technology, and SERS method. Using SERS method helps extending the limit of detectable polyamine concentration below 10ppb when strong peaks around 2800-3000 cm^{-1} are used for comparison (Figure 3A-3C).
- Straight concentration dependence of SERS signal was confirmed for the 1300-1500 cm^{-1} peaks of spermine within the concentration range 0.3-30mM (figure not shown).

APPLICATION NOTE

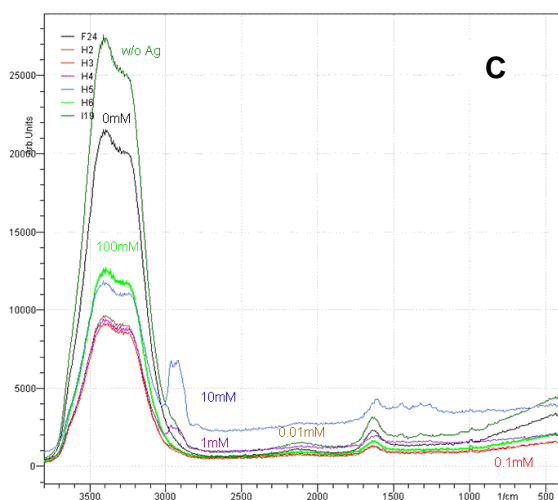
Effect of Spermine concentration on SERS Signal.



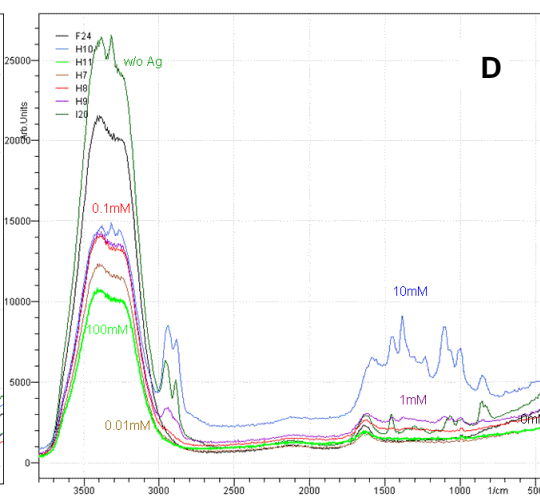
Effect of Spermidine concentration on SERS Signal.



Effect of Putrescine concentration on SERS Signal.



Effect of Ethanolamine concentration on SERS Signal.



Effect of Ethylenediamine concentration on SERS Signal.

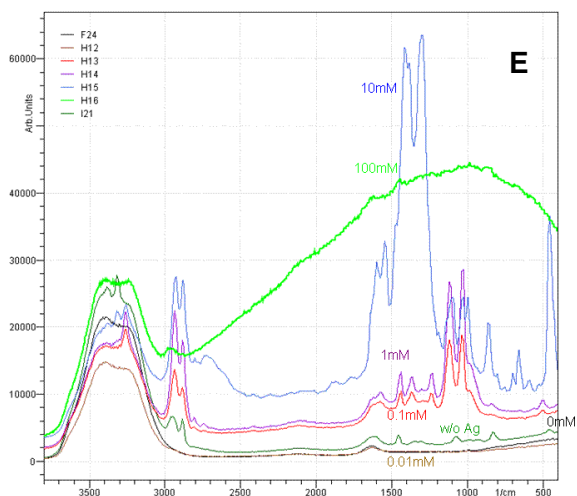


Figure 3. SERS spectra of the five chosen polyamines at different concentrations (0.01-100 $\mu\text{g/ml}$) in 100mM NaCl. Spermine (A); Spermidine (B); Putrescine (C); Ethanolamine (D); Ethylenediamine (E). Identity model RMI-53200-1, total collection time 5 min with 10 co-additions.

APPLICATION NOTE

REFERENCES

1. A. A. Ancheta, L. Hawel and C. V. Byus, "Acute Increases in Intracellular Putrescine Lead to the increase in Steady-State Levels of *c-fos*, *c-jun*, *RING3*, and *ID-1* mRNAs" in: *Polyamine Cell Signaling*, J.-Y. Wang and R. A. Casero Jr., Eds, Totowa, NJ: Humana Press, pp.25-40 (2006).
2. S. M. Oredsson, "Polyamine-Dependent Early Cellular Signals and Cell Proliferation" in: *Polyamine Cell Signaling*, J.-Y. Wang and R. A. Casero Jr., Eds, Totowa, NJ: Humana Press, pp. 41-50 (2006).
3. J.-Y. Wang, "Cellular Signals Mediating Growth Arrest after Polyamine Depletion" in: *Polyamine Cell Signaling*, J.-Y. Wang and R. A. Casero Jr., Eds, Totowa, NJ: Humana Press, pp. 51-74 (2006).
4. S. Venkiteswaran, T. Thomas and T. J. Thomas, "Role of Polyamines in Regulation of Sequence-Specific DNA Binding Activity" in: *Polyamine Cell Signaling*, J.-Y. Wang and R. A. Casero Jr., Eds, Totowa, NJ: Humana Press, pp. 91-122 (2006).
5. C. A. Hobbs and S. K. Gilmour, "Role of Polyamines in the Regulation of Chromatin Acetylation" in: *Polyamine Cell Signaling*, J.-Y. Wang and R. A. Casero Jr., Eds, Totowa, NJ: Humana Press, pp.75-90 (2006).
6. A. K. Verma, "Polyamines and Cancer" in: *Polyamine Cell Signaling*, J.-Y. Wang and R. A. Casero Jr., Eds, Totowa, NJ: Humana Press, pp.313-328 (2006).
7. N. Iyandurai and R. Sarojini, "Structural Analysis of DNA Interaction with Spermine Studied by Raman and Infrared Spectroscopy" *J. Appl. Sci. Res.* **5**, 1149-1154 (2009).
8. A. M. Amorim da Costa, M. P. M. Marques and L. A. E. Batista de Carvalho, "Raman spectra of putrescine, spermidine and spermine polyamines and their N-deuterated and N-ionized derivatives" *J. Raman Spectr.* **34**, 357-366 (2003).
9. D. L. Drapcho, I. Zlatkin, F. Inscore, C. Shende, A. Sengupta, H. Huang, and S. Farquharson, "High-Throughput Trace Analysis Using SERS-Active Microtiter Plates with a Raman Plate Reader" *Spectroscopy* **25**, Suppl., 42-50 (2010) (request a copy).
10. Igor Zlatkin, Sarah Mendelowitz, David Drapcho "Analysis of DNA-polyamine interactions using SERS method in Identity Raman Microplate Reader". XXII International Conference on Raman Spectroscopy, Boston, MA, USA, poster 099 (2010) (request a copy).
11. P. C. Lee and D. Meisel, "Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols" *J. Phys. Chem.* **86**, 3391-3395 (1982).

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