Raman spectroscopy as a detection method for liquid-separation techniques

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Raman spectroscopy has considerable identification power but is, nevertheless, seldom used for detection in liquid-separation methods such as column liquid chromatography and capillary electrophoresis. This is mainly due to its poor analyte detectability. This review gives attention to the improved performance effected by instrumental developments (e.g., laser technology and liquid-core waveguide detector cells) and the proper use of analyte-enrichment techniques. However, the main emphasis is on the benefits that derive from using special Raman modes, such as resonance Raman and surface-enhanced (resonance) Raman spectroscopy. The most promising on-line and at-line set-ups are highlighted, and the detectability *vs.* identification dilemma is explained. To achieve a real breakthrough, relevant real-life applications will be crucial.

Keywords: Capillary electrophoresis; Column liquid chromatography; Resonance Raman spectroscopy; Raman spectroscopy; Surface-enhanced (resonance) Raman spectroscopy; Thin-layer chromatography

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1. Introduction

In recent decades, Raman spectroscopy (RS) has become a powerful analytical technique that is, however, mainly used for stand-alone applications. Relatively little attention has been devoted to hyphenating RS and separation techniques, primarily those that use a liquid eluent or carrier stream [i.e. column liquid chromatography (LC), thin-layer chromatography (TLC) and capillary electrophoresis (CE)]. Generally speaking, the poor analyte detectability provided by RS – illustrated by an almost complete absence of appealing applications – is considered the main cause of this lack of interest. However, one should not underestimate the considerable instrumental developments of recent years (e.g., laser technology, detectors, microscopes, fiber optics and holographic filters) [1]. To this should be added that RS can provide detailed vibrational information for identification and confirmation purposes, can be used in aqueous solutions, and is, therefore, the method of choice in bioanalytical/medical studies.

In other words, there is a real need for a critical evaluation of the state-of-the-art of RS-based detection for liquid-separation methods to answer the question whether this can become a valuable addition to the present list of hyphenated and hypernated systems [2]. This is a question of special interest because of novel approaches in this area, based on the use of advanced RS modes, such as resonance RS (RRS), surface-enhanced RS (SERS) and their combination, SERRS.

In this article, we review the literature on both on- and at-line coupling of the various modes of RS detection to liquid-separation methods. We will also briefly discuss the frequently overlooked options on the separation end of the hyphenated systems.

2. Basics of RS

RS is based on the scattering of light by matter, which includes solids, liquids and gases. Such scattering is mainly elastic, where the scattered radiation has the same frequency as the incident light, or, for a minor part, inelastic, where the scattered radiation has a different frequency. This can be explained by a simplified Jablonski diagram (Fig. 1), which shows several singlet (S) electronic states of a molecule and, superimposed, some vibrational states. All vibrational states have a stack of associated rotational levels (not shown), but, in the condensed phase,

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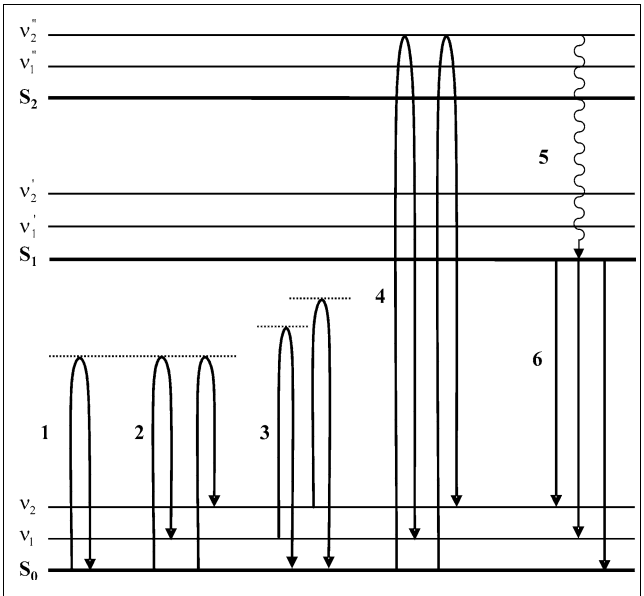

Figure 1. Simplified Jablonski diagram. S_0, S_1 and S_2 are singlet electronic states; v_1, v_2 are vibrational states. The following processes are depicted: (1) Rayleigh; (2) Stokes Raman; (3) anti-Stokes Raman; (4) resonance Stokes Raman; (5) radiation-less relaxation by internal conversion and vibrational relaxation; and, (6) fluorescence.

this rotational structure is lost. If elastic scattering – denoted as Rayleigh scattering – takes place, the molecule is excited to a virtual state (dotted line) and immediately returns back to the ground state (**1**). If the molecule follows an alternative pathway and returns to a higher vibrational state of the electronic ground state, the scattering is inelastic and called Stokes Raman (**2**). Molecules that are already in a higher vibrational state of S_0 can undergo another mode of inelastic scattering and return to the ground state by producing a photon with a higher frequency than the incident radiation (anti-Stokes Raman) (**3**). In both Raman scattering processes, the energy differences between incident and scattered light correspond to vibrational energy levels in the electronic ground state of the molecule. According to the Boltzmann distribution, the population of the lowest vibrational state at room temperature is (much) greater than that of the excited vibrational states, and the intensity of the Stokes Raman bands is therefore (much) stronger than that of the anti-Stokes Raman bands. Hence, in analytical practice, attention is exclusively on Stokes Raman.

Although (large) molecules have a large number of vibrational states ($3N - 6$, where N is the number of atoms), not all of these are Raman active (i.e. can show up in a Raman spectrum). A vibration will be Raman active only if the polarizability of the molecule changes during the vibration. Functional groups such as –C–X (X = F, Cl, Br or I), –C–NO_2, –C–S–, –S–S–, –C═C–, –C≡C– and –C≡N, exhibit strong polarizability changes and therefore give strong Raman signals. Nonetheless, Raman scattering has an extremely low probability (cross section) (e.g., compared to electronic absorption transitions). Despite the instrumental improvements of recent decades, the detectability of conventional RS is still rather poor, with limits of detection (LODs) for small organic molecules typically in the mM region. To improve the detectability special Raman modes can be used (i.e. RRS and SE(R)RS). Their principles are briefly discussed below.

2.1. RRS

To obtain resonance-signal enhancement in RS, the excitation wavelength has to be tuned to an electronic absorption band of the analyte molecule. As a result, the molecule is excited to a higher vibronic state (e.g., a vibrational level of S_1 or S_2) and immediately returns to one of the vibrational levels of S_0 by emission of a Stokes Raman photon (**4** in Fig. 1). A 10^2–10^3-fold signal enhancement can be expected and values as high as even 10^8 have been reported [3]. In general, only totally symmetric vibrations that vibronically couple with the electronic states involved will be enhanced. Hence, RRS spectra are often relatively simple, since, in practice, only vibrations related to the chromophoric part of the molecule are observed; the other Raman-allowed vibrations are not intensified and therefore mostly too weak to be detected.

RRS is closely related to fluorescence emission, since both phenomena are triggered by the absorption of a photon (Fig. 1). However, there is a difference in lifetime. In fluorescence, after excitation, the molecule first relaxes to the vibrational ground state of S_1 via internal conversion and vibrational relaxation (**5**). Next, the molecule emits a fluorescent photon and returns to a vibrational level of the electronic ground state (**6**); typical fluorescence lifetimes are in the low ns range. By contrast, in RRS, the molecule relaxes immediately (i.e. without radiation-less decay) to a vibrational level of the electronic ground state. Consequently, there is also an obvious difference in energy of the emitted photon; except for excitation to the lowest vibrational state of S_1, fluorescence is observed at longer wavelengths than RRS. The shape of the spectra is also different; fluorescence spectra of analytes in liquid or frozen solutions are typically broad-banded due to inhomogeneous broadening, whereas Raman – and also RRS – bands are sharp. The fluorescence background can easily overwhelm the RRS spectra; fluorescence quantum yields are usually several decades higher than those of RS and, furthermore, not only the analyte, but also all kinds of impurity fluorescence can play a role. Because of the large differences in quantum yields, even trace amounts of fluorophores can already compete successfully with RS or RRS. In order to make RRS successful, suppression