Experimental Investigation on Determination of Trace Amounts of Some Colored Compounds by Resonance Raman Spectrometry

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The methodological examinations of resonance Raman spectrometry were carried out in view of the application of the method to the determinations of trace amounts of colored compounds in aqueous solutions. The samples were methyl orange and the two tar food dyes, amaranth and new coccine. In the first place, the details of the experimental conditions determining the sensitivity of this method and the reproducibility of measured intensities were clarified. Then, some problems essential for the analytical application of resonance Raman spectra, such as the detection limits, the selectivity and the additivity of intensities, were examined, and the results were compared with the usual colorimetry. From these results, the possibility of the simultaneous determination of trace amounts of structurally related compounds, such as the two isomers treated in this study, amaranth and new coccine, was suggested.

1. Introduction

It need hardly be said that the vibrational spectrometry, that is, infrared and Raman spectrometry, is one of the most powerful tools in analytical chemistry. One of the main utilities of it can be attributed to its excellent selectivity. However, the vibrational spectrometry has also a distinct disadvantage. Generally speaking, its sensitivity is lower than those of many other analytical methods, and therefore, it is not so effective in the analysis of

trace components.

In relation to this disadvantage, the resonance Raman effect is to be paid much attention to. Although the effect is the current subject of the most vigorous studies in Raman spectroscopy, most of them are limited to the pure scientific aspects, and there are only a few papers concerning the analytical applications of the effect.¹⁻⁶⁾ Moreover, in those papers, the methodological examinations of the basic problems encountered in the microanalytical applications of it can be said to be almost

lacking. However, since the great enhancement of scattering intensities due to the effect may compensate for low sensitivity of the vibrational spectrometry, resonance Raman spectrometry has the possibility of becoming a very promising analytical tool with both excellent selectivity and high sensitivity.

In the present investigation, therefore, we have carried out various methodological examinations in view of analytical applications of resonance Raman spectra. The purpose of this paper is to present the results of the examinations for various experimental conditions to make the analytical utility of the method most effective, and to discuss some important analytical problems such as the detection limits, the fashions of analytical curves, the selectivity, and the additivity of intensities for sample solutions containing different solutes. Through these discussions, we have intended to prove the practical usefulness of the resonance Raman spectrometry in identification and determination of trace components.

2. Experimental

A Japan Spectroscopic Company Model J-800 Laser Raman spectrophotometer was used for the measurements of spectra. As an excitation source, a Spectra-Physics Model 164 Argon Ion Laser was used. The laser was operated at 488.0 nm with about 200 mW of power at samples. A HTV-R-464 photomultiplier was used as a detector. According to the noise levels of spectra, the scans were repeated 4–16 times to improve the S/N ratios. In most cases, the sample cell was rotated to avoid the local heating of sample solutions due to the laser beam irradiation. The rotating speed was 2500 rpm. For the measurements for the colorimetry, a Japan Spectroscopic Company Model UVIDEK-4 Digital Spectrophotometer was used.

In Fig. 1 are shown the samples treated in the present study. The measurements were made all for the aqueous solutions of these samples. In addition to them, we partly used permanganate ion, MnO_4^- , for preliminary examinations.

Fig. 1. The samples treated in this study.
Solvent: Water.

3. Results and Discussion

3.1. The problems for the measurements

Since the present purpose is to obtain the maximum intensities, we must treat here the resonance Raman spectra excited by the laser beam whose frequency is within the range of the absorption bands of samples in the visible region. That is, we must measure the so-called "rigorous" resonance Raman spectra. Moreover, as in the case of usual Raman spectrometry, we must take account of the spectral sensitivity of the instruments used. In fact, considering the later condition, we measured in this study the rigorous resonance Raman spectra excited with the 488.0 nm line even when the maximum absorptions of the samples in question are nearer to the 514.5 nm line than to the 488.0 nm.

Even in the case of the measurements of rigorous resonance Raman spectra, it is not so difficult to obtain only the spectra. However, when we want to apply the spectra to the determination of micro amounts of samples, there occur rather troublesome experimental problems to be solved. First, it is required to

obtain as large intensity enhancement as possible. Second, to obtain the reproducible and stable intensity magnitudes. Thirdly, it is desirable that the relation between intensities and concentrations can be fitted, at least approximately, to linear curves with precisions good enough for practical purposes.

It has been clarified that the following experimental factors should be specially taken care of to satisfy the above-mentioned requirements.

(1) The first factor to be considered is the influence of the local heating of samples due to the laser irradiation and the absorption of it. In Fig. 2 is shown the result of the exami-

Fig. 2. The effect of local heating of samples on intensities. For the 840 cm^{-1} band of MnO_4^- . Concentration: 10^{-3} M .

(a) In the case of using the usual cell,
(b) In the case of using the rotating cell.

nations of the intensity changes with time for 840 cm^{-1} band of MnO_4^- . The concentrations of the solution is 10^{-3} M . It is seen from this figure that when the usual cylindrical cell is used, the intensities decrease after a short time to become about an order of magnitude smaller. This corresponds to the phenomenon that, owing to the local heating, the laser beam running in the sample diffuses, and as a result, the scattering light does not focus into a clear image on the entrance slit. On the other hand, it is seen that when the rotating cell technique is used, such influence becomes markedly smaller. Of course, the influence in question is to become smaller when the concentrations of the

absorbing sample are lower. However, in order to obtain the maximum and reproducible intensity, it is desirable to use the rotating cell technique.

(2) The second factor is related to the setting of the cell to the cell rotating instrument. That is, if the cell is not set in such a way that the central axis of the cell is coincident with that of the rotating apparatus, the rotation becomes the precession movement. This results again in the diffuse image on the slit. It has been clarified that the measured intensities change with the factor of about three, whether we are sufficiently careful in the setting of the cell to minimize the precession movement or not.

(3) The third factor is that it is necessary to radiate the sample very close to the cell wall to minimize the reabsorption of scattering light. Moreover, the reproducibility is required to this operation.

3.2. The detection limits

On the basis of the experimental technique described in the preceding section, we have examined the detection limits of resonance Raman spectrometry. It has been clarified that the limiting factor determining the sensitivity in this case is the background from sample solutions. The main cause of this background seems to come from the fluorescence. In the present study, we have adopted the so-called "quench-drench method" to decrease the background, that is, we radiated the sample solutions with the laser beam for about an hour or less before the measurements.

In Fig. 3 are shown the spectra of methyl orange in the $1500\text{--}1300\text{ cm}^{-1}$ region for the solutions of the concentrations in the vicinity of the detection limit. These bands are the most intense ones and are assigned to $\text{N}=\text{N}$ or ring stretching vibrations. From this figure, we have concluded that the detection limit in this case is shown by the spectrum (b), that is, about $2 \times 10^{-8}\text{ M}$ (7 ppb).

The colorimetric method is used in many cases for the determination of samples like those treated here. Thus, it is interesting to compare the results for resonance Raman spectrometry with those for usual colorimetry. As a result of experimental examinations, it

Fig. 3. The resonance Raman spectra of methyl orange in the 1500–1300 cm^{-1} region.

(a) 5×10^{-8} M, (b) 2×10^{-8} M. (a or b)–1: Raw data, (a or b)–2: Processed by smoothing.

Table I. The detection limits of resonance Raman spectrometry and those of colorimetry.

Samples	Resonance Raman Spectrometry	Colorimetry
Methyl Orange	2×10^{-8} M (7 ppb)	2×10^{-7} M (65 ppb)
Amaranth	4×10^{-8} M (24 ppb)	2×10^{-7} M (120 ppb)
New Coccine	4×10^{-8} M (24 ppb)	2×10^{-7} M (120 ppb)

has been proved that in the case of colorimetry using a 1 cm cell, the lower limit of concentrations below which no significant signal can be obtained is about 2×10^{-7} M for methyl orange.

In Table I are summarized the detection limits for the three samples obtained by resonance Raman spectrometry and colorimetry. It is seen from this table that as far as the samples examined here are concerned, the sensitivity of resonance Raman spectrometry is almost an order of magnitude higher than that of usual colorimetry. However, it will be necessary to mention here that the sensitivity of

colorimetry becomes higher when the cell with longer path length is used and the background absorptions of the solvent are small enough. Considering such circumstances, it may be more appropriate to conclude that the sensitivity of resonance Raman spectrometry is comparable to or somewhat higher than that of colorimetry. This conclusion is not contradictory to that for the aqueous solutions of some industrial dyes in the paper by Haverbeke et al.⁶⁾, published quite recently.

3.3. The analytical curves

As is well known, in the case of resonance

Raman spectrometry, we can not expect the linearity of analytical curves over the wide range of concentrations, since the relation between the intensities and the concentrations is determined by the two competing factors, that is, the increase of intensities with higher concentrations and the lowering of them by the increase of absorptions. However, in the comparatively narrow range of fairly low concentrations, we may expect approximately linear relations between intensities and concentrations. As an example, the analytical curve for methyl orange solutions in the concentration range of the order of 10^{-8} M is shown in Fig. 4. This

Fig. 4. The analytical curve for the 1390 cm^{-1} band of methyl orange in the concentration range, $(2-8) \times 10^{-8}$ M.

$$I = 0.12 + 0.88C_m, S_c(\text{for the concentration}) = 0.26 \times 10^{-8} \text{ M.}$$

result clearly shows that even in the concentration range of the same order of the detection limit, the analytical curve can be fitted to a linear line with a precision good enough for the practical purposes. It has been confirmed that the experimental data in the concentration range from 2×10^{-8} M to 1×10^{-6} M are fitted to the linear line shown in Fig. 4. This range seems to be sufficient for the purpose of the usual analysis of trace components. It need, however, be emphasized here that, strictly speaking, this linear relation is an approximate one and is based on no theoretical foundation.

3.4. The selectivity and the additivity of intensities

As has already been mentioned in the beginning of this paper, resonance Raman spectrometry is expected to have the excellent selectivity in addition to the high sensitivity. We have examined this problem for the mixed solutions of the two tar food dyes, amaranth and new coccine. As is seen from Fig. 1, these two dyes are mutually isomers and have the closely related molecular structures. For these samples, the absorption bands in the visible region overlap almost completely with each other and no separate signal of each component can be obtained, that is, the usual spectrophotometric method has no selectivity.

Usually, the food dyes of this kind are used as mixtures consisting of two or more components and the analysis of each component is carried out by colorimetry, using together with the chromatographic technique, or by the special means such as the dual-wavelength spectrophotometric method⁷⁾. Either of the methods are rather tedious and the sensitivity of them is rather lower than resonance Raman spectrometry.

In Fig. 5 are shown the resonance Raman spectra of amaranth, new coccine and their mixture in the $1300-1400\text{ cm}^{-1}$ region, where the most intense resonance Raman bands are observed. These spectra demonstrate clearly

Fig. 5. The resonance Raman spectra of amaranth, new coccine and their mixture in the $1400-1300\text{ cm}^{-1}$ region.

(a) —: amaranth, (b) ----: new coccine, (c) —: mixture

that in the case of resonance Raman spectroscopy, the completely separate signal for one of the components can be obtained even for the isomers such as treated here. This result can be presented as a clear evidence of the excellent selectivity of the method in question.

In the next, we have examined the spectra in view of the determination of each of the components. In general, in the case of resonance Raman spectroscopy, the signal intensity of a component is to be affected by the coexistence of another components having the absorption bands in the wavelength range of the measurements. Such influence comes from the circumstances that the excitation laser beam and the Raman scattering light are absorbed by the coexistent components and weakened. However, if the concentrations of the coexistent components are very low, such influence will be neglected, provided that a certain degree of errors can be allowed for.

As is shown in Fig. 5, the band at 1350 cm^{-1} comes from amaranth only, and so, we have examined about this band in the first. The results are shown in Fig. 6. These results clearly prove that there is no appreciable difference between the two analytical curves shown in this figure. This fact shows that we can use the analytical curve for pure amaranth at least when $(0-1)\times 10^{-6}\text{ M}$ of new coccine coexists.

Fig. 6. The analytical curves for the 1350 cm^{-1} band of amaranth.

(a) —○—: amaranth only, $I=0.32+0.97C_m$, S_b (for the inclination)=0.04, S_a (for the intercept)=0.23, (b) —x—: amaranth+ 10^{-6} M of new coccine, $I=0.11+0.96C_m$, $S_b=0.02$, $S_a=0.12$.

Fig. 7. The analytical curves for the 1370 cm^{-1} band of new coccine and amaranth. (a) —○—: new coccine only, $I=0.22+0.99C_m$, $S_b=0.02$, (b) —x—: new coccine+ 10^{-6} M of amaranth, $I=4.00+0.93C_m$, $S_b=0.05$, (c) —○—: amaranth only.

In the next, we have examined the similar problem for the band at 1370 cm^{-1} . In this case, not only amaranth but also new coccine contributes to the intensities of this band. Here, we encounter the problem of the additivity of intensities in resonance Raman spectroscopy. In Fig. 7, are shown the analytical curves for new coccine only and for new coccine when 10^{-6} M of amaranth coexists. To verify the additivity of intensities is equivalent to examining whether the curve for pure new coccine and the one in the case of the coexistence of amaranth are mutually parallel or not. It is seen from this figure that the obtained two lines can be assumed to be parallel to a fairly good approximation. Thus, it can be concluded that the additivity of intensities is realized when $(0-1)\times 10^{-6}\text{ M}$ of amaranth coexists, and that by use of these curves and those shown in Fig. 6, the simultaneous determination of the components in the mixed solutions is possible, allowing for a certain degree of errors.

4. Conclusion

The experimental results so far described demonstrate clearly the high sensitivity and the excellent selectivity of resonance Raman

spectrometry, and suggest the usefulness of the method for the identification and the determination of samples for which the usual spectrophotometric analysis is difficult, if not impossible.

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