Normal Raman and SERS spectroscopy of the vitamin E

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Abstract. In this study, surface-enhanced Raman scattering (SERS) spectra of vitamin E were obtained on colloidal silver (Ag). Alpha-(++) tocopherol which is the only form that is recognized to meet human requirements was selected to study. The analytes (±)-tocopherol were dissolved in chloroform (CHCl3) and the silver colloid was poured into the compound. Silver colloid was reduced by hydroxylamine hydrochloride. The analytes were the supernatant after standing the mixture for the reason that chloroform have no signals in surface-enhanced Raman scattering in the Ag colloid, and it would not affect the determination of the (±)-tocopherol. The Normal Raman and SERS spectrum of Vitamin E were contrastively studied to realize how the vitamin E stuck to the silver nanoparticles. The results show the fat-soluble substances can be analysed by SERS. The spectra indicate that the molecules are adsorbed on the surface through the COO- groups by the simultaneous involvement of a and -type coordination. These results suggest some important criteria for consideration in SERS measurements and also provide important insights into the problem of predicting SERS activities for different fat-soluble substances.

Keywords: Surface-enhanced Raman spectroscopy; Raman spectroscopy; Vitamin E; Chloroform (CHCl3); Ag colloid

1. Introduction
The fundamental knowledge on the structure of atoms or molecules we have today is a result of laser spectroscopic researches. In particular, Raman spectroscopy allows one to derive detailed specific information on a molecular level that other laser spectroscopic methods can provide only to a limited extent. So, a Raman spectrum provides a structural ‘fingerprint’ of a molecule.[1~3] The advantages of Raman spectroscopy are its unprecedentedly high specificity and its versatility. However, a great disadvantage in any application of Raman spectroscopy results from the extremely small cross section of the Raman process, which is 12–14 orders of magnitude below fluorescence cross sections. Therefore, in the 1970s, a discovery which showed unexpectedly high Raman signals from pyridine on a rough silver electrode attracted considerable attention[4~6], particularly after experiments in different laboratories gave evidence that the enormously strong Raman signal must be caused by a true enhancement of the Raman scattering (RS) efficiency itself and not by more scattering molecules[7, 8]. Within the next few years, strongly enhanced Raman signals were verified for many different molecules [9] which had been attached to various ‘rough’ metal surfaces, and the effect was named ‘surface-enhanced Raman scattering (SERS)’. The discovery showed promise to overcome the traditionally low sensitivity problem in Raman spectroscopy.

Currently, most SERS techniques use metal aqueous colloids as substrate[10~12], which require the objects studied must be water-soluble compounds, restricting the wild application of SERS technique.

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However, considerable part of the analyte is water-insoluble compound. It’s hard to produce the solution of the fixed concentration. It is unfavorable to use Ag colloid directly to measure their Surface Enhanced Raman Spectroscopy (SERS). There is a method to overcome this embarrassment. First, water-insoluble compounds is dissolved in organic solvents; then, the solution which contain the compound is drip onto the metal substrate surface with the surface-enhanced Raman activity; or the metal substrate is immersed in the sample solution and is got out (taken out) after a certain time; and then the metal substrate with compound is measured by the Raman spectrograph after the solvents dry. The main shortcomings of this method is that the substrate or the compound may occur the carbonation reaction in the laser’s irradiation, which result in strong spectral background and baseline, increasing the noise, weakening the signal and decreasing spectral resolution.

“Vitamin E” is the collective name for a group of water-insoluble compounds with distinctive antioxidant activities. Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity. In this investigation, we selected Alpha- (–) tocopherol which is the only form that is recognized to meet human requirements to study. According to the dissolved characteristic of Vitamin E, a new method was used to determine the SERS spectra of water-insoluble compounds. This method not only can measure the SERS spectra of water-insoluble compounds directly by using Ag colloid, but also overcome the disadvantages of the aforementioned method.

2. Experimental

2.1. Instrumentation
For all measurement, the spectra reported in this study were obtained by use of the 514.5-nm line of an argon ion laser as the excitation source. The Raman system was equipped with a BX-41 Olympus microscope and 45x objective. Spectrometer system (Acton spectro@2300i, Princeton Acton, USA), was used with a 1200 gr/mm holographic grating. An air cooled CCD detector (Pixis 256, Princeton Acton, USA) was used for measuring the Raman signal by an integration method. The intensity of the 514.5-nm laser was 8mW at the sample. The Raman spectrum and absorption spectrum were obtained at room temperature. The absorption spectra were detected by spectrophotometer (Lamda 35, PE company, USA). The data processing was operated by using Origin 7.5 software.

2.2. Chemical reagents and Procedure
Colloidal silver nanoparticles were prepared by using hydroxylamine hydrochloride as reducing agent according to Nicolae Leopold and Bernhard Lendl’s method[13]. This method has the advantage of a fast, simple, and is a procedure characterized by a high preparation success rate. What’s more, these nanoparticles have the characteristic of a more uniform distribution of size and shape together with the absence of interferences from remainder oxidation product. Some Notes: The cleanliness of the glassware and purity of the materials used are important factors that determine the reproducibility and stability of the colloids. For this reason, all the glassware used for the sols was previously soaked in a 1:1 HNO₃:H₂O solution and was rinsed several times with deionized water. All materials used were analytical reagent grade or equivalent. Aqueous stock solutions of the compounds were prepared in nanopure water.
The analytes (±)-tocopherol (approx 95% HPLC) were purchased from Sigma. They are used without further purification. The (±)-tocopherol was dissolved in the pure chloroform of the HPLC level chromatograph, stored hermetically in a volumetric flask. The sample was prepared by adding a certain amount of the mixed solution to the equivalent volumes of the Ag colloid, shaking the mixture three minutes. After standing some time, the upper colloids were dripped onto a glass slide, and then the glass slide was placed to the Raman spectroscopy to determine the spectrum.
3. Results and discussion

3.1. Raman and SERS spectra

The Raman spectrum of the (±)-tocoopherol in the viscous oily liquid state is shown in Fig.1(a). It can be seen that two strong bands at 2872 cm$^{-1}$ and 2942 cm$^{-1}$ and four weak bands at 487 cm$^{-1}$, 591 cm$^{-1}$, 1342 cm$^{-1}$ and 1446 cm$^{-1}$ are in good agreement with the calculated frequencies, though there are some differences of the relative intensity. The Raman spectrum shows a group of characteristic hydrocarbon aliphatic stretching bands at 2732 cm$^{-1}$, 2757 cm$^{-1}$, 2872 cm$^{-1}$ and 2942 cm$^{-1}$. Bands at 1591 cm$^{-1}$ and 1612 cm$^{-1}$ can be ascribed to an asymmetric stretching of the COO- group.

Vitamin E is a drug which is widely prescribed for the antioxidant effect. Traditional methods for its identification are the time-consuming and complicated tests. Therefore, the development of a method...
that can allow a rapid identification of the drug is desirable. Fig. 1(b) shows the SERS spectrum of the mixture containing (±)-tocopherol and chloroform adsorbed on Ag colloids. The frequencies at which the bands appear are included in the spectrum to facilitate future reference. In order to study the adsorption process of Vitamin E on Ag colloids, the SERS spectrum of (±)-tocopherol was compared with the normal Raman spectrum of (±)-tocopherol. The SERS spectral profile corresponds very well with the normal Raman spectrum and the enhanced signals clearly indicate the achievement of SERS activity for Ag colloids. From these spectra, some notable phenomena should be taken into account. The relative intensities of Raman signals have significant differences between the normal Raman spectra and the SERS spectra though the experimental processes were the same, and some of the intensities of Raman signals are obviously enhanced in the SERS spectra. C-C stretching vibration appearing at 1342/1381/1446 cm\(^{-1}\) in the normal Raman spectrum of pure liquid (Fig.1(a)) were observed. In the SERS spectra, at 1343/1384/1462 cm\(^{-1}\) spectrum, the C-C stretching vibration appearing was observed. The \(\nu(C - O)\) band appeared at 1247 cm\(^{-1}\) both in the normal Raman spectrum and in SERS. From these results we propose that (±)-tocopherol is adsorbed on a silver electrode through C-C bonds. Both electromagnetic enhancement and charge transfer enhancement contribute to the intensity of the spectra. Charge transfer enhancement is indicated both by the perturbation from the normal Raman spectrum of (±)-tocopherol and by the high sensitivity obtainable. There are, however, wave number shifts in some of the major bands and significant differences in the intensities of the weaker bands. First, only 0.1×10\(^{-6}\) mol·L\(^{-1}\) (±)-tocopherol can obtain a good SERS spectrum, which indicates that the surface-enhanced Raman scattering is very strong. Second, compared with the normal Raman spectra, this SERS spectra appear some new peaks, increasing the amount of information. As we know, the SERS effect is mainly independent of some variables, such as dielectric constants and polarizability. However, due to its high sensitivity, it may be affected easily by some other factors, such as the adsorption behaviour and the distance from the metal surface to the samples, which can bring on changes of surface selection rules and the interaction between molecules and substrates. Due to the absorption function, the vibrations of some molecules are more obvious, and then some new peaks appear in the spectrum.

3.2. Influences from background substrates
(±)-tocopherol is insoluble in water, so it can’t directly adsorb on Ag colloids. To solve this problem, the pure chloroform was used as the intermediate. In order to learn whether the chloroform would influence experimental results, the SERS spectrum of the chloroform was studied. The chloroform was mixed with Ag colloids at the volume ration of 1:1, and the mixture was shaken for three minutes. After standing layer, the upper colloids were dripped onto a glass slide, and then the glass slide was placed to the Raman spectroscopy to determine the spectrum. Fig.2 displays the Raman spectrum of the mixture containing the chloroform and the Ag colloids (fig.2(b)) and the Raman spectrum of the Ag colloids (fig.2(a)). As can be seen from the figure, both of the Raman signs of the SERS spectrum of the chloroform and the Raman spectrum of the Ag colloids are quite weak. Because the pure chloroform have the very weak SERS signs, the addition of the chloroform have little influence to the Raman spectra of the analyte, and have no affect to the accuracy of identification of analyte. There are no any obvious peaks in the SERS spectrum of the pure chloroform, indicating that the pure chloroform have no SERS effect and does not interfere with the determination of vitamin E. What’s more, the SERS of the Ag colloid substrates rarely have any peak. Therefore, the differences between the Normal Raman spectrum and the SERS spectrum mainly comes from the SERS effect which is attributed to the analytes adsorbed on the Ag colloids.
Figure 2. (a). Raman spectrum of the Ag colloids.
(b). the SERS spectrum of the pure chloroform solution.

4. Conclusion
The experimental results clearly demonstrate that an analytical method based on SERS provides high spectral selectivity and identification power as well as high analytical sensitivity and identification power for the study of the (±)-tocopherol. The method proposed in this investigation has the extremely important significance to determine the SERS spectrum of the water-insoluble compounds. The increasing kinds of compounds can be determined by using the SERS spectrum in the future. This provides some advantages in SERS applications. In this experiment, the (±)-tocopherol was used as an example water-insoluble analyte to verify this method is feasible. From the results, the SERS spectra of the (±)-tocopherol is quite a bit different from the normal Raman spectra of the (±)-tocopherol. The attribution of the peaks was illustrated by comparing SERS spectra with NR(Normal Raman) spectra, and the mechanism and orientation of adsorption were also discussed according to the peaks which was absent in NR spectrum but observed in SERS spectrum. To verify the feasibility of the method for the quantitative analysis, SERS spectra at the same condition of the (±)-tocopherol were collected. In the SERS spectra, at 1343/1384/1462 cm$^{-1}$ spectrum, the C-C stretching vibration appearing were observed. The intensity of this peak was enhanced compared with the normal spectra.

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