Surface-enhanced resonance-Raman scattering: an informative probe of surfaces *

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Surface-enhanced resonance-Raman scattering (SERRS) experiments using a citrate-coated silver colloid have been performed. Transmission electron microscopy showed that the colloid consists of nearly mondisperse hexagonal particles with a maximum dimension of 36 nm. For maximum sensitivity, SERRS requires the controlled aggregation of the colloid. The nature of the aggregate formed is dependent on the method of aggregation. The effect of poly-L-lysine on the aggregation procedure was characterised using transmission and scanning electron microscopy. With SERRS, sensitivities down to 6.87×10^{-18} mol dm^{-3} were obtained using rhodamine dye. This corresponds to less than 200 molecules in the beam at any one time and suggests a sensitivity equal to or greater than that of fluorescence. A comparison of the use of SERRS with surface-enhanced Raman scattering (SERS) to follow adsorbate reactivity on a surface at less than monolayer coverage was obtained by studying metal complex formation using a preresonant Schiff base (SERRS) and 2-hydroxy-1-naphthaldehyde excited off resonance (SERS). In the former case, complex formation on the surface at well below monolayer coverage was observed and differences between the surface and the bulk complex characterised. In the latter complex formation was again observed but the bands were broad and problems of contamination, photodecomposition and selectivity evident.

Surface-enhanced resonance-Raman scattering (SERRS) is rapidly emerging as a new and unique analytical tool for use in understanding surfaces.[1-3] Although SERRS is best considered as a single process, it arose experimentally from the combination of two previously studied effects, namely resonance-Raman scattering and surface-enhanced Raman scattering (SERS).

Resonance-Raman scattering[4] arises when the laser excitation frequency is matched or approximately matched to the frequency of a chromophore within a molecule. In most studies the efficiency of the scattering process is improved by three to four orders of magnitude over Raman scattering and since, in the simplest case, the scattering is from one excited state, electronic as well as vibrational information can be obtained. However, resonance-Raman scattering is only one of a number of competing processes including absorption, fluorescence, non-radiative electron transfer and photodecomposition. The relative magnitude of each of these processes is dependent on the nature and method of presentation of the sample and on instrumental parameters such as laser power and the orientation of the laser beam to the sample surface. Thus, resonance-Raman scattering is limited to certain molecules. In addition, it is limited in concentration range. Self-adsorption as well as heating and photodecomposition result if the sample is too concentrated whereas too dilute a sample gives a poor signal-to-noise ratio. Where it is effective, resonance-Raman scattering is very informative and is based on a well understood theory.

Surface-enhanced Raman scattering[5-7] arises from an interaction between an adsorbate and the surface plasmons on a rough metal surface. Silver, gold and copper are commonly used as substrates because their surface plasmons exist in the visible region and, with visible excitation, absorption of the incident radiation by the metal surface is reduced. This effect is also well established for lithium and there are reports that other metals demonstrate SERS but these claims are less well substantiated. The enhancement experienced is dependent on the nature of the surface roughness as well as the metal and the excitation frequency. In addition, the mechanisms producing the enhancement have been the centre of controversy. Of the many mechanisms proposed, two, electromagnetic enhancement and charge transfer or chemical enhancement, are still widely quoted but the contribution to the total enhancement from each mechanism is still not clearly defined. Finally, SERS is very dependent on the conditions of the experiment which, coupled with the high sensitivity, the dependence on angle, surface selection rules and the possibility of photolysis, can lead to uncertainty as to the nature of the surface species detected.

Surface-enhanced resonance-Raman scattering is obtained by using a molecule with a chromophore as the adsorbate and selecting the excitation frequency to match or nearly match that of the chromophore. The enhancement is very much greater than either resonance Raman or SERS enabling detection limits at attomolar levels or lower to be achieved. If excitation is matched to the molecular absorption maximum [Fig. 1(*a*)], we discovered that for azo dyes the surface selection rules expected for SERS are much less effective.[8] The reason is that, as is the case in resonance,[9] the polarisation of the laser excitation alters on interaction with the chromophore during the scattering process. This results in a degradation of the effective input polarisation required for SERS selection rules, making SERRS less sensitive to the orientation of the molecule to the surface. This arrangement makes SERRS readily applicable for methods of quantitative analysis,[8] overcoming one of the problems with using SERS quantitatively where the sensitivity of the signal to any change in adsorbate orientation can result in disappointing reproducibility.

A second possible arrangement illustrated in Fig. 1(*a*) is where the laser excitation is set off the absorption maximum of the adsorbate and at the maximum of the plasmon resonance. For resonance experiments on the molecule, this would be described as a preresonant condition and often SERRS taken in this way is written as SE(R)RS. The polarisation information, although affected, is not lost to the extent found with excitation at the resonance frequency.[2] In this condition, orientation information is obtained but the selectivity of resonance still applies. Thus, it is possible to pick out individual molecules in

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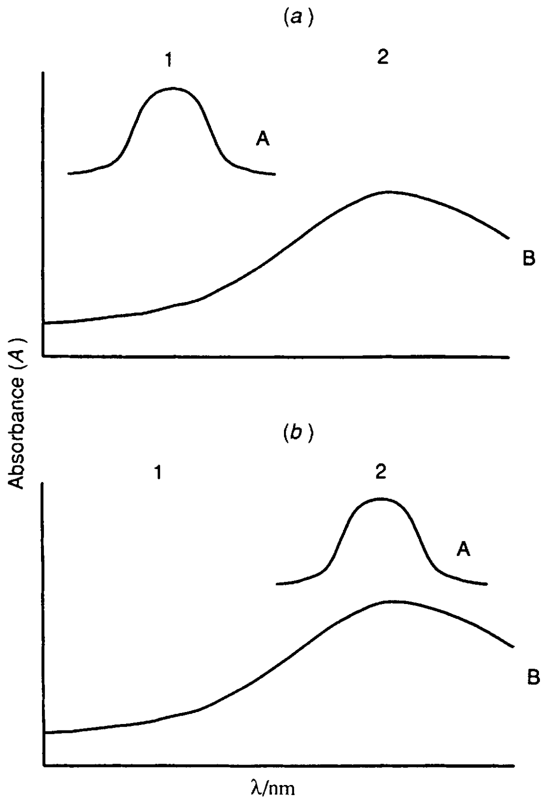

Fig. 1 The different arrangements for SERRS: A, molecular absorbance; B, plasmon absorbance. In (*a*) the molecular absorbance maximum and the plasmon absorbance do not coincide: position 1 represents excitation at the absorbance maximum, 2 that at the plasmon maximum. In *b* the molecular absorbance and the plasmon maximum coincide: position 1 represents excitation away from the absorbance and plasmon maximum where the spectrum has a preresonant component, 2 that at the absorbance and plasmon maximum

the presence of a matrix of interferents but the effect will be more dependent on the angle of the adsorbate to the surface. For surface studies this is a key point and consequently this arrangement may be preferred for surface analysis.

Fig. 1(*b*) illustrates an alternative case in which the molecular chromophore coincides with the surface plasmon maximum. Similar considerations will apply to those discussed for the first case above but a greater increase in sensitivity is likely with excitation at the molecular and plasmon resonance frequency. Orientation dependence will become more apparent, the greater is the difference in frequency between the excitation frequency used and the resonance maximum, but consequently the sensitivity will decrease.

Thus, where SERRS can be applied, it has advantages over either resonance scattering or SERS. The major advantages are as follows.

(1) It is effective in water and air as well as in vacuum and some solvents. The sampling systems which can be applied are quite flexible and the use of fibre optic systems and visible microscopes is routine.[10–12]

(2) It provides vibrational information on an adsorbate and it is possible to obtain electronic information through excitation profiles.[2]

(3) In contrast to resonance, very wide concentration ranges can be studied. In ideal cases detection limits orders of magnitude below those required for monolayer coverage of a surface can be obtained.[13,14]

(4) Many more molecules are effective in SERRS than are effective in resonance particularly since fluorescence is often quenched.[8] The method requires the proximity of a metal surface to the adsorbate causing good energy transfer from the adsorbate to the surface. Thus, fluorescing dyes which are ineffective in resonance studies using visible excitation can give very good SERRS. For example, rhodamine 6G illustrates the value of near-infrared Fourier-transform Raman scattering since it fluoresces strongly in the visible region but gives good Raman scattering in the near infrared. However excellent SERRS with visible excitation is observed.[15]

(5) Photolysis is inhibited with SERRS. The efficiency of SERRS means that in many experiments considerably lower power levels can be used for excitation than are required for either resonance scattering or SERS. In addition, the energy-transfer processes at the surface may also mitigate against photolysis.

(6) The sharp bands obtained enable discrimination of individual species in a mixture. Coupled with the requirement for a chromophore and the high sensitivity associated with SERRS, excellent selectivity is achieved.[8,16]

These advantages enable unique insights to be made into a number of surface problems. However, the technique has the following significant limitations.

(*a*) The requirement for a chromophore limits the applicability.

(*b*) The chromophore is required to be in close proximity to a suitably roughened surface of certain metals and in particular silver, copper and gold.

One effective method for obtaining a rough surface suitable for SERS or SERRS is to aggregate a colloidal suspension. In this case a fractal cluster is obtained and the size of the enhancement is related, at least in part, to the intensity of the fields in the interstices of the aggregates.[13,17] Very sensitive detection is claimed for this method and an enhancement of 10^6 is usually expected for SERS.[18] Methods such as the deposition of silver island films, ruled gratings or roughened electrodes also provide informative studies of SERS-active surfaces. However, to obtain reliability and reproducibility, recent studies in this laboratory focused upon the detailed characterisation and careful development of one particular silver colloid.[19] This paper reports key experiments to indicate the nature of the aggregation process, to probe the sensitivity of the method and to compare the relative merits of SERS and SERRS to follow metal complex formation at the silver surface.

Experimental

Ascorbic acid (Aldrich), 2-hydroxy-1-naphthaldehyde (Fluka), poly-L-lysine hydrobromide, M_r 4000–15 000 (Sigma), rhodamine 6G (BDH), silver nitrate (Aldrich) and trisodium citrate (Johnson Matthey) were of analytical grade; nickel chloride (BDH) was of reagent grade. The Schiff base *N,N'*-bis(salicylidene)benzene-1,3-diamine was a gift from Ciba plc and was checked for purity by chemical analysis.

Raman scattering was collected using an Anaspec modified Cary 81 spectrometer or using a Renishaw 2000 Raman microprobe spectrometer. Excitation was provided for the Cary by a Spectra Physics 2020 argon-ion laser and for the Renishaw 2000 by an omnichrome argon-ion laser. The Cary 81 is a double monochromator with a cooled photon-counting detection system. The Renishaw has a notch filter and single-grating arrangement with a cooled charged coupled device (CCD) detector. In its present configuration, the samples are observed under the microscope. The exciting line used was fixed at 514.5 nm for both instruments.

For quantitative procedures the aggregated colloidal suspension was presented in a glass cuvette and analysed with the Cary spectrometer using a quadrant-mounted shutter arrangement accurately to reposition the cell after each manipulation. This procedure has been used previously[20] and was shown to be accurate and the instrument sufficiently stable to enable quantitative analysis. To study reactions at the surface the colloidal suspension was deposited onto a clean glass microscope slide and allowed to evaporate. Small aggregates of colloid formed and were analysed using the Renishaw 2000 instrument. This method of sampling is less effective for quantitative analysis but the use of the CCD-based machine provided additional sensitivity for the study of reactions.

The silver colloid was prepared according to a modified Lee and Meisel procedure.[21] Before beginning the preparation, all glassware was thoroughly cleaned by treatment with *aqua regia* followed by gentle scrubbing with a soap solution. The vesicles were then thoroughly washed with water. A sample of silver nitrate (90 mg) was suspended in distilled water (500 cm^3) at 45 °C and then rapidly heated to boiling before a 1% solution of trisodium citrate (10 cm^3) was added under vigorous stirring. The suspension was then held at boiling for 90 min. The quality of the resulting sol was checked by determining the λ_{max} and the full width at half height of the absorbance maximum. The λ_{max} should be approximately 404 nm with a full width half height of less than 60 nm. Ethanolic and aqueous ethanol colloids were prepared by centrifuging an aliquot of aqueous colloid, prepared by the method detailed above, at 3000 rpm for 30 min. The colloidal silver falls to the bottom of the centrifuge tube. For ethanolic colloids the supernatant was extracted and replaced with ethanol and for aqueous ethanolic colloids the supernatant was replaced by ethanol–distilled water (1:1 v/v).[11]

Three separate sample preparations were used to provide an aggregated colloid for SERRS.

Acid aggregation. Colloid (2 cm^3) was accurately measured into a cuvette followed by the analyte (150 µl) and nitric acid (0.3% v/v, 35 cm^3). This type of aggregation procedure is dynamic, and the colloid continues to aggregate until the aggregates become so large that they precipitate. This factor makes it impossible to analyse two separate samples under the same aggregation conditions. The relative standard deviation (r.s.d.) associated with this method is high.

Poly-L-lysine aggregation. Colloid (1 cm^3) and distilled water (1 cm^3) were placed in a cuvette, poly-L-lysine (0.01% w/v, 150 µl) was added followed by the analyte (150 µl). This process results in the aggregation process proceeding to a certain point then stopping and remaining constant for days. The r.s.d. associated with this method was lower.

Poly-L-lysine and ascorbic acid aggregation. The same procedure as described for poly-L-lysine aggregation was followed except that, after the addition of the analyte, ascorbic acid (1 mol dm^{-3}, 35 µl) was added. Once again, this process continues to a certain point then stops. The r.s.d. was similar to that for aggregation with poly-L-lysine alone.

A Philips 410 transmission electron microscope was employed to characterise the size and state of colloidal particles under different experimental conditions. It was used to view unaggregated silver colloidal particles, and acid, poly-L-lysine and poly-L-lysine and ascorbic acid aggregated silver particles. The samples were prepared as described above and a small gold foil which had a grid etched upon it was dipped into the sample. The sample was allowed to evaporate on the foil before it was analysed. The grid enabled easy location of the colloidal particles. A Hitachi F4000 field-emission-gun scanning electron microscope was also employed to view the colloidal silver particles. The silver colloid was deposited and dried on a glass slide and covered in a nominal 3 nm coating of chromium before introduction to the microscope.

Aqueous rhodamine 6G solutions (10^{-8}–10^{-16} mol dm^{-3}) were prepared and analysed by SERRS using acid and poly-L-lysine aggregation. The lowest final concentration of the rhodamine 6G present in the aggregated colloid was between 6.87×10^{-10} and 6.87×10^{-18} mol dm^{-3}.

A 10^{-4} mol dm^{-3} aqueous ethanol (50:50 v/v) solution of the Schiff base was prepared and added to aqueous ethanol colloid using both acid and poly-L-lysine aggregation. The nickel complex of the Schiff base was prepared by mixing equimolar amounts of nickel chloride and the Schiff base in an aqueous ethanol solution. A red precipitate of the nickel Schiff-base complex was obtained. The *in situ* formation of the complex was achieved by adding an aliquot of the 10^{-4} mol dm^{-3} aqueous ethanol Schiff-base solution to an aqueous ethanol colloid aggregated with poly-L-lysine. This was followed by the immediate addition of an aliquot of a 10^{-4} mol dm^{-3} aqueous ethanol solution (50:50 v/v) of nickel chloride. The samples were left to stand for 30 min before spectra were measured.

A 10^{-4} mol dm^{-3} ethanol solution of 2-hydroxy-1-naphthaldehyde was prepared and analysed using acid aggregation with an ethanolic colloid. The bis(2-hydroxy-1-naphthaldehydato)silver(I) complex was prepared by adding a 2:1 molar ratio of 2-hydroxy-1-naphthaldehyde to silver nitrate dissolved in distilled water (50 cm^3) at 50 °C. The aldehyde remained in suspension and the suspension was held at 50 °C with continual agitation for 1 h. An ethanolic solution of the resulting complex was prepared and analysed using an ethanolic colloid with acid aggregation. The *in situ* formation of the bis(2-hydroxy-1-naphthaldehydato)silver(I) complex was achieved by adding an aliquot of the 10^{-4} mol dm^{-3} ethanol solution of 2-hydroxy-1-naphthaldehyde to an ethanolic colloid and acid aggregating. In addition, the same reaction was carried out as above but replacing the silver nitrate with nickel acetate. All samples were left to stand for 30 min before spectra were measured except in the case of the silver complex which following the measurement at 30 min was allowed to stand and measurements taken at 4 and 12 h.

Results and Discussion

Surface-enhanced resonance-Raman scattering is a relatively new technique and limited information is available. The potential is explored here by considering the answer to a number of fundamental questions. Selected background information and new key experiments are described in order to provide an answer to each question. The study concentrates on the use of the Lee–Meisel colloid described previously. This is not a unique approach, but the growing understanding of this system enables a good insight into the potential SERRS could achieve if correctly developed.

What is the nature of the metal surface used?

Recently, the nature of the Lee–Meisel colloid used for SERRS has been re-examined using Raman scattering, photoelectron correlation and NMR spectroscopy.[19] The results confirm the previous conclusion that the surface of the silver particles is covered with a layer of citrate with pendant negatively charged groups (Fig. 2). Positively charged molecules such as rhodamine

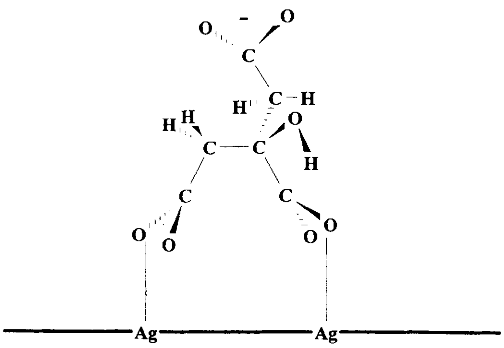

Fig. 2 Idealised representation of the surface of a modified Lee and Meisel silver colloidal particle. The citrate is shown bonded to the silver, present as Ag^I, and there are pendant negatively charged carboxylate groups

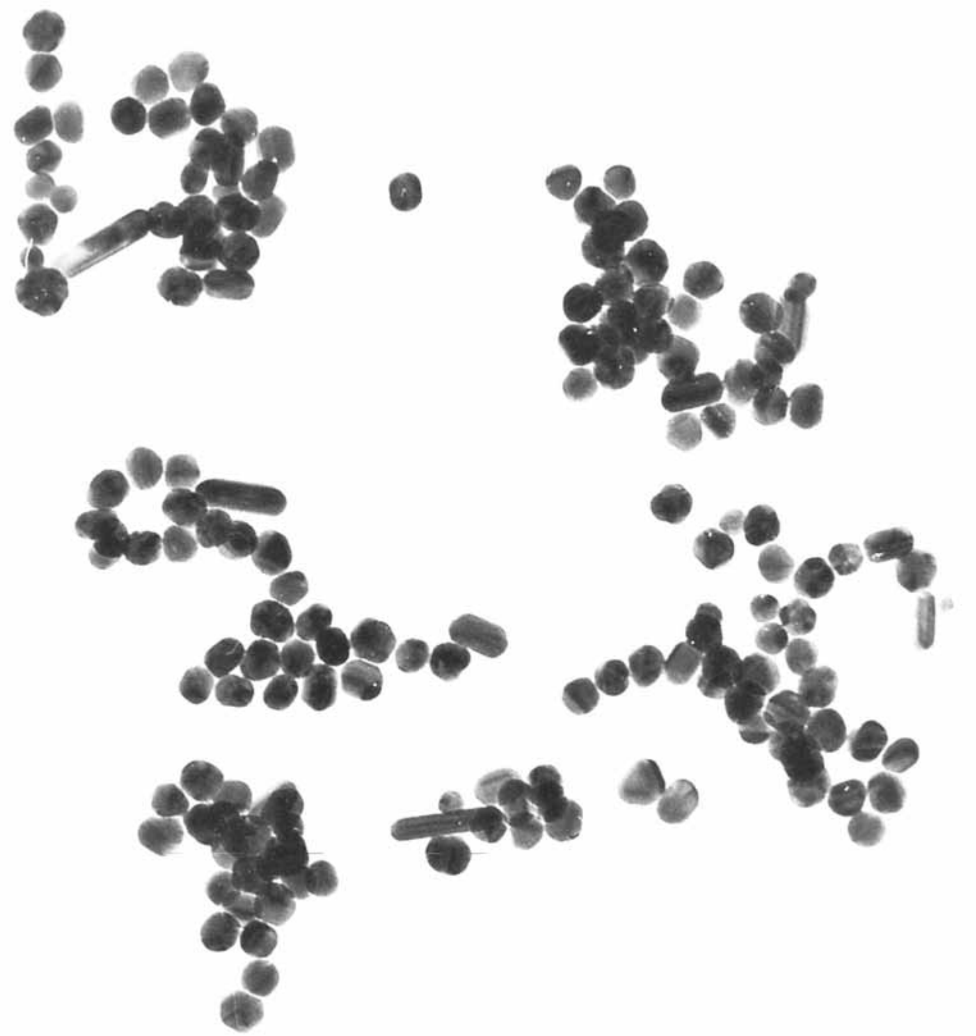

Fig. 3 A TEM photograph of poly-L-lysine aggregated Lee and Meisel modified silver colloid. Magnification = × 150 000

6G readily adhere to the surface and give excellent SERRS. However, negatively charged and some neutral species do not adhere well. This dependence on surface charge gives a selectivity to SERRS which is often not clearly understood.

To achieve more generality, it is possible to coat the surface with a molecule such as poly-L-lysine, which results in pendant positively charged groups on the colloidal surface.[2] This enabled the recording of SERRS for more than 20 negatively charged azo dyes added at an amount equivalent to less than monolayer coverage assuming complete adsorption.[8] The importance of this experiment lies not only in the increased generality and control of the process but also in the fact that SERRS is very sharp and well defined and consequently molecular discrimination of mixtures of dyes without further separation is possible. The precision and accuracy of the method is sufficient for quantitative or semi quantitative analysis.

To achieve an understanding of the nature of the particles and of the aggregation processes, transmission and scanning electron microscopy (TEM and SEM) were used to characterise the nature of colloidal aggregates obtained by different aggregation methods. In the TEM experiments the particles are shown to be hexagons with a largest face dimension of 36 nm (Fig. 3). There are also a few rods. The hexagon dimension is in reasonable agreement with the photoelectron-correlation results where a spherical particle is assumed. These experiments indicate that the surfaces of the particles are well formed and surface adsorption is onto small metal crystallites with reasonably flat faces rather than onto ill defined spheres.

Aggregation of the colloid was achieved by three different methods: addition of poly-L-lysine, of poly-L-lysine and ascorbic acid, and of nitric acid. The TEM images showed that the size and shape of the particles was not affected but that the nature of the aggregates formed differed depending upon the method of aggregation selected. Addition of poly-L-lysine resulted in the formation of small clumps, the size of which increased with time. Each colloidal particle was in proximity with its neighbours *via* more than one face. However, there was a small space between the particles of about 2 nm suggesting that physical contact between the silver in neighbouring particles was prevented by a layer of poly-L-lysine on each particle (Fig. 3). Aggregation with poly-L-lysine and ascorbic acid resulted in larger clumps of colloid. The particles appeared to be fused together indicating that the poly-L-lysine did not separate neighbouring colloidal particles in the same way as the above. The reason for the difference is unclear. A few smaller clumps were also observed (Fig. 4). Aggregation with nitric acid resulted in the formation of small clumps of colloid and some chains and circles. There was no space between the particles. The size of the clumps increased rapidly when the amount of nitric acid used to induce aggregation was increased (Fig. 5).

Scanning electron microscopy (SEM) indicates the three-dimensional nature of the aggregates (Fig. 6). This experiment was carried out starting with a higher concentration of particles in the aggregate enabling more complete aggregation to occur and thereby simulating the conditions required for SERRS. The SEM data allow a connection to be made between the size and shape of the particles in the two-dimensional aggregates viewed in TEM and the three-dimensional aggregates produced in the colloidal suspension.

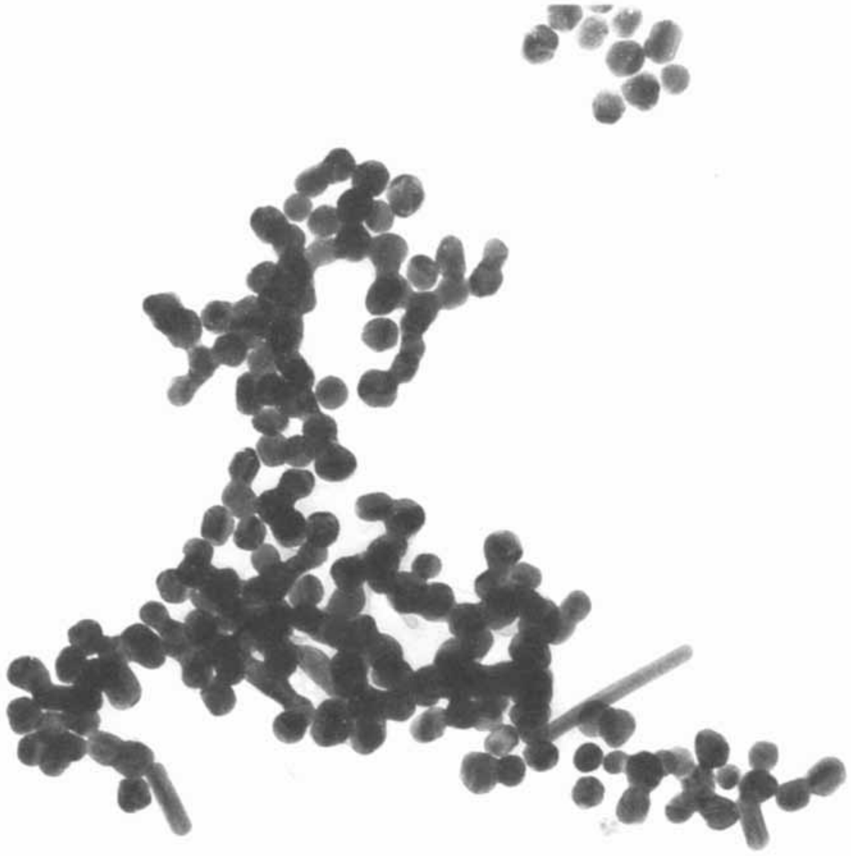

Fig. 4 A TEM photograph of poly-L-lysine and ascorbic acid aggregated Lee and Meisel modified silver colloid. Magnification = × 150 000

In practice, the addition of poly-L-lysine has the key advantage that it can be used to control the extent of aggregation by altering the amount added. Similar experiments are not possible with acid where aggregation continues irrespective of the concentration added. The TEM and SEM results suggest that the poly-L-lysine is present on the surfaces causing a slight spacing between particles and the formation of smaller more stable aggregates. Thus, the picture emerges of well formed hexagonal-shaped particles coated with a layer of poly-L-lysine on the surface which aggregate in a more controlled fashion than is the case with acid.

How sensitive and quantitative is the method?

One of the dyes most studied by SERRS is rhodamine 6G. Experiments with concentrations down to 10^{-12} mol dm^{-3} in the suspension before adsorption occurs have been carried out and a comparison of SERRS, Fourier-transform Raman and infrared absorption has been made.[16,22] It was clear that 10^{-12} mol dm^{-3} was not the detection limit of the method. A recent paper indicates a lower detection limit.[23]

To investigate the sensitivity further the addition of even lower concentrations of rhodamine was investigated. The main problem with such experiments is the presence of contamination with rhodamine. Even vapour from a spillage of a rhodamine solution or from the use of a dye laser containing rhodamine is sufficient to cause detection of the signal after only a few seconds exposure and up to a week after the event. Thus, claims of lower detection limits must be backed by clear evidence of concentration dependence and by replicate analysis. Fig. 7 shows clear evidence that detection can be obtained from rhodamine adsorbed on the silver at levels which are equivalent to 6.87×10^{-18} mol dm^{-3} in solution. Approximately, this corresponds to between 35 and 200 molecules in the beam at any one time. This level already exceeds fluorescence detection limits and indicates that SERRS is one of the most sensitive detection techniques available. It should be noted that the equipment used for this experiment is not optimised to obtain high sensitivity. A modern CCD system as used in the Renishaw instrument will provide at least two to three orders of magnitude more in sensitivity. Thus, further work on these extremely difficult experiments should lead to further improvements in detection limits and possibly to single-molecule spectroscopy by SERRS.

The extent to which quantitative determinations are possible with a variety of systems is difficult to define. Using poly-L-lysine with negatively charged azo dyes at 10^{-8} mol equivalent, a relative standard deviation of about 5% was obtained ($n = 6$). This is reasonable for such low concentrations. The same method was used to obtain an r.s.d. for rhodamine 6G using both acid aggregation and poly-L-lysine aggregation at 6.87×10^{-18} mol equivalent. An r.s.d. of 25% was obtained for acid aggregation and of 37% for poly-L-lysine aggregation. Clearly these values are unacceptable for quantitative analysis at attomolar levels. The difference between the methods can be explained by considering the surface chemistry involved. In acid aggregation the surface of the colloid is negatively charged and the rhodamine is positively charged so that it adsorbs readily onto the surface. The stability of the acid-aggregated colloid is poor leading to a large r.s.d. However, when the surface is coated with poly-L-lysine it has positively charged groups. Therefore the rhodamine would not adsorb on the surface as effectively. Some may remain in solution and the amount that does adsorb may vary from sample to sample depending on the amount of poly-L-lysine added. This is confirmed by the fluorescent background observed with SERRS of rhodamine with poly-L-lysine aggregation and by the high r.s.d. compared to that obtained for the azo dyes. The concentration plot indicates a rather lower scattering of data and clear concentration dependence. With acid, little to no rhodamine remains in solution indicating, as expected, tight binding to the surface. Thus, with one run and careful

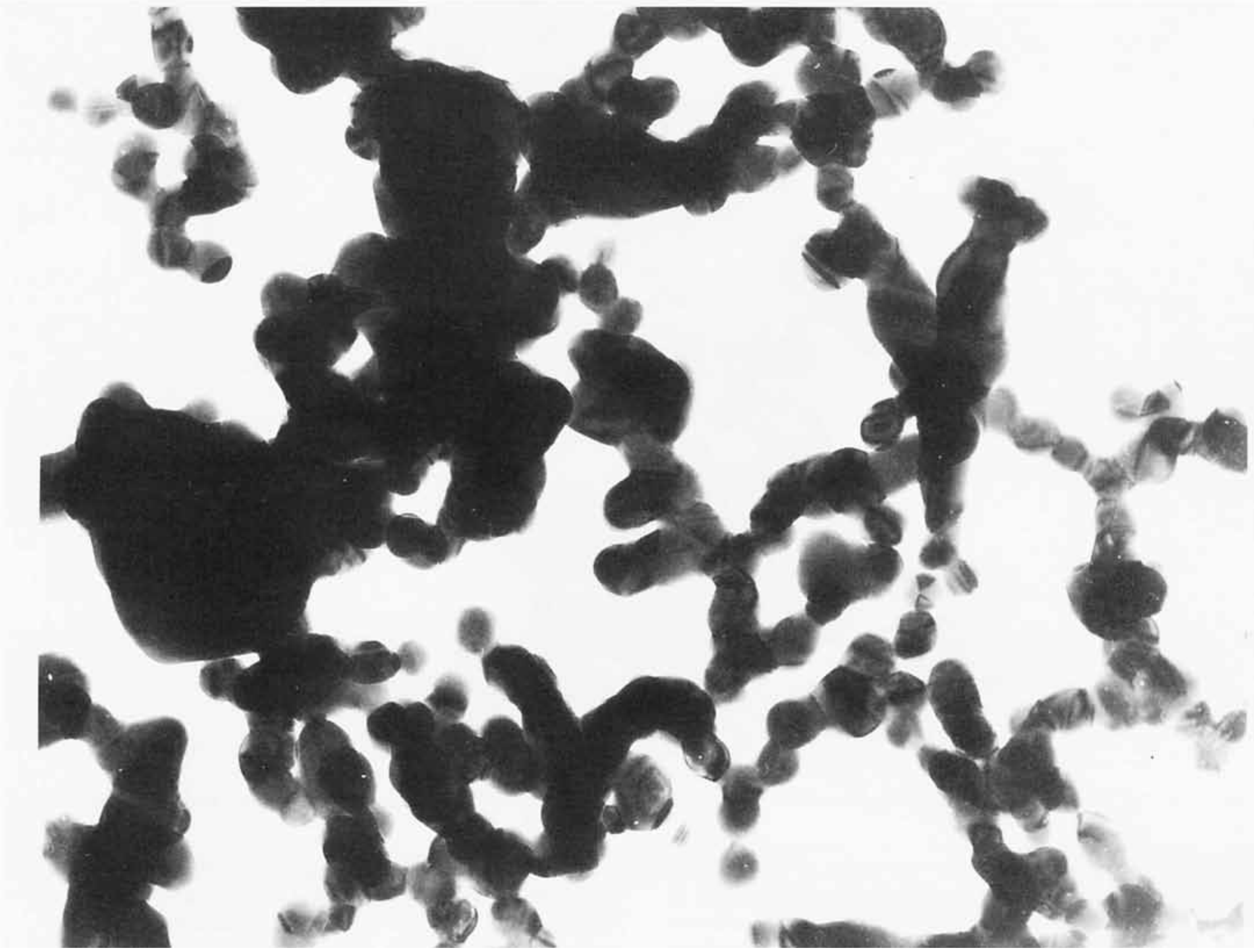

Fig. 5 A TEM photograph of acid aggregated Lee and Meisel modified silver colloid. Magnification = ×250 000

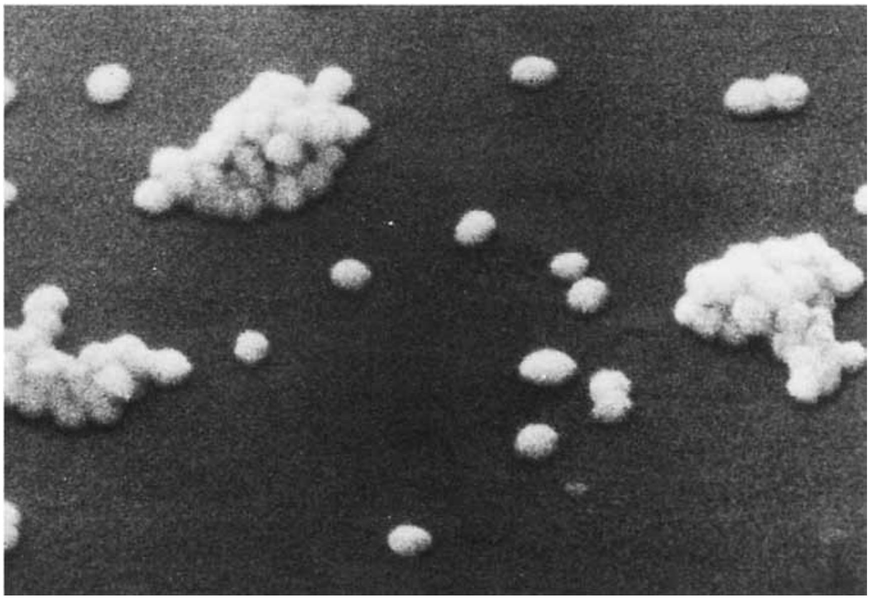

Fig. 6 A SEM photograph of aggregated Lee and Meisel modified silver colloid. Magnification = ×80 000

attention to the time taken between aggregation and measurement, high selectivity can be obtained with reasonable accuracy. However, a method of aggregation analogous to the use of poly-L-lysine which creates greater stability but provides a negative surface charge is required for positively charged dyes.

How informative is the method?

In the work described so far, the main advantage of SERRS has been to identify and quantify the presence of a species at sub-monolayer coverage. However, the vibrational and electronic information obtained should also enable a study of molecular changes associated with reactions on the surface. To probe the potential of the technique for studies on surfaces, two reactions involving the formation of a metal complex were compared. In one the scattering is SERRS and in the other SERS. The Renishaw microprobe was used to maximise sensitivity. A simple colloidal suspension was treated so that reactions were carried out on the surface of the particles in suspension. A drop of the suspension was dried on a microscope slide and individual aggregates investigated using the microprobe. These measurements were on aggregates very similar to those studied by TEM and illustrated in Fig. 3. The sample area used was approximately 2 μm.

In the first example a Schiff base with an absorption maximum at 450 nm was used. To ensure solubility of all reagents the experiments were carried out in aqueous ethanol colloid. The absorption band of the base is broad so that the scattering can be considered as preresonant. An attempt to measure the Raman scattering of an evaporated film of the base on a microscope slide yielded only fluorescence [Fig. 8(*a*)].

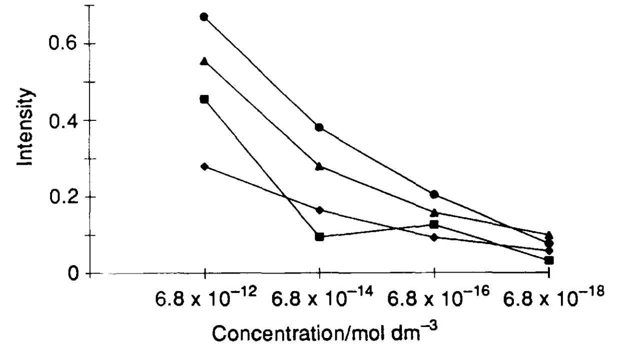

Fig. 7 Relationship of intensity *vs.* concentration for four peaks selected from the rhodamine 6G SERRS spectra using acid aggregation: ■, 612; △, 1510; ◆, 1578; ●, 1650 cm^{-1}

However, on adsorption of the negatively charged base on the surface of poly-L-lysine treated aqueous ethanol colloid a good SERRS spectrum of the base was obtained [Fig. 8(*b*)]. This indicates one of the advantages of SERRS, namely fluorescence has been quenched by the surface. In addition, the lack of a fluorescent background indicates that at the low concentration used there is little unadsorbed base. Mixing equimolar ethanolic solutions of the base with nickel acetate produces a red 1 : 1 nickel complex (Fig. 9) with a broad-band spectrum with absorption maxima at 430 and 470 nm, closer to resonance than is the case with the base. The resonance-Raman scattering from this complex was relatively easily measured with little evidence of fluorescence [Fig. 8(*c*)]. The reaction between the base and the nickel ion can readily be carried out on the surface. Addition of nickel acetate to the colloid containing the base adsorbed on the surface resulted in a change in the SERRS to give a spectrum which is similar but not identical to the resonance-Raman spectrum of the nickel complex [Fig. 8(*d*)]. Thus, complex formation on the surface can be detected. The likely reason that the frequencies from the SERRS of the surface and the resonance spectra of the nickel complex are not identical is that additional co-ordination to give five- or six-co-ordinate nickel is to be expected. This is likely to be with the amine group of poly-L-lysine in the case of the surface treatment. This experiment was carried out with concentrations in the colloidal suspension before adsorption of 10^{-6} and 10^{-8} mol dm^{-3} of the base and nickel acetate, which correspond to approximately monolayer coverage and about a hundredth of mono-

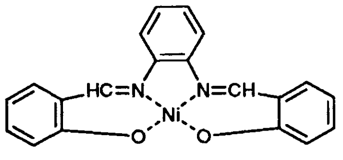

Fig. 9 Structure of the nickel Schiff-base complex

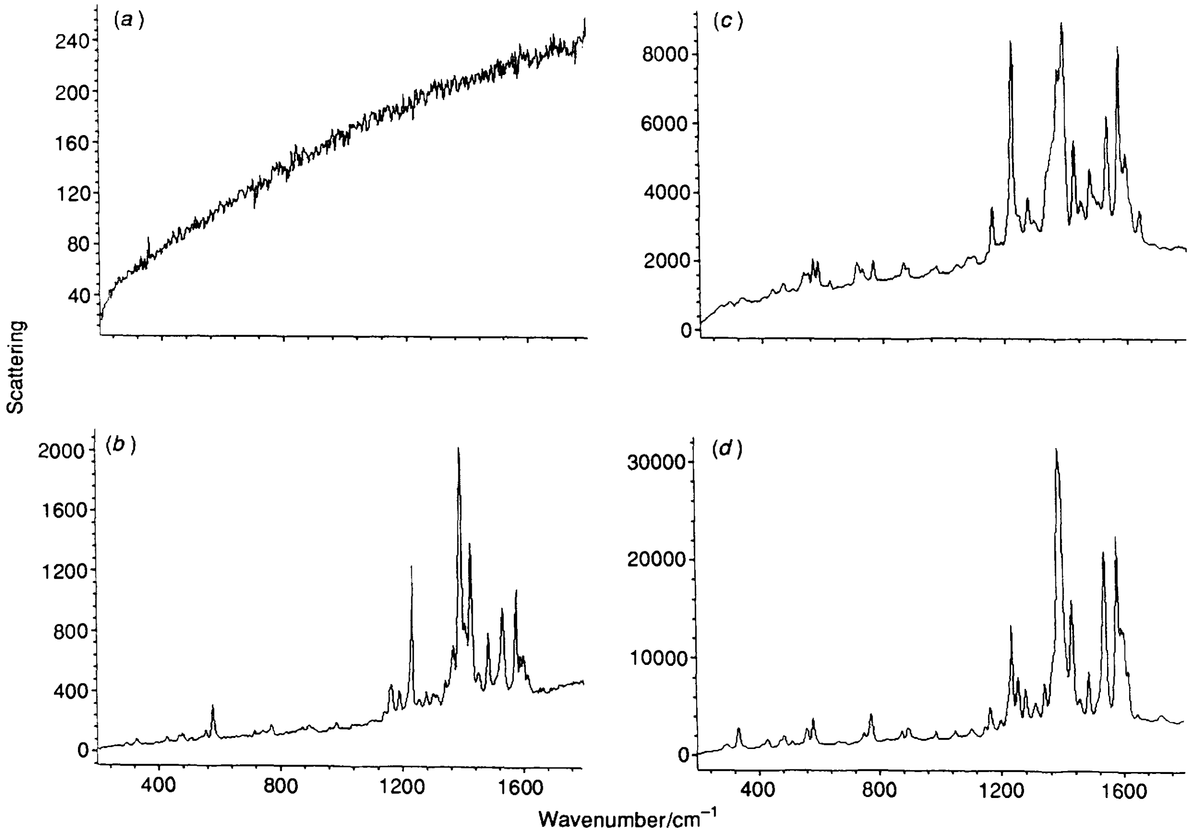

Fig. 8 (*a*) Excitation of the Schiff base taken from a microscope slide indicating the dominance of fluorescence over resonance-Raman scattering. (*b*) The SERRS of the Schiff base. (*c*) Resonance-Raman spectrum of the nickel Schiff-base complex. (*d*) The SERRS of the nickel Schiff-base complex

layer coverage. The results of the two experiments were very similar.

Comparing the SERRS and resonance spectra of the complexes, in addition to differences in wavenumber of 2–5 cm^{-1} in some bands, there are clear differences in relative intensity. For example, the bands just below 1600 cm^{-1} and the band at 1290 cm^{-1} have quite different relative intensities in the two spectra. The differences in frequency indicate some slight differences in the nature of the complex probably due to changes in co-ordination in the fifth position. Further theoretical and spectroscopic investigation is required to separate the effect on intensity of the difference in the fifth co-ordination position from the effect due to orientation. Assignments on the basis of calculations for 2-hydroxy-1-naphthaldehyde indicate that the bands below 1600 cm^{-1} and the band at 1290 cm^{-1} are due to stretching modes of the aromatic rings with the strong band at 1390 cm^{-1} due to a stretch involving large displacements of the carbon and oxygen of the chelate ring.[24] Since the bands just below 1600 cm^{-1} may be assigned to quadrant stretching modes, it is tentatively postulated that the relatively high intensities of the surface complex may reflect the fact that the plane of the molecule is vertical to the surface. Quadrant stretching modes in this orientation will have a large polarisability component vertical to the surface. The information is clear but the effect smaller than was found for the azo dyes, where there was a greater separation between the absorption maximum and the excitation frequency.[2] The degree of depolarisation is dependent on the molecule and hence more cases require to be characterised before general principles can be established. However the relative balance of surface and molecular enhancement will determine the dependence of the signals on orientation and hence the applicability or otherwise of quantitative methods and surface selection rules.

In a second example the spectra are taken with excitation well away from the absorbance maximum of either ligand or complex. An ethanolic solution of 2-hydroxy-1-naphthaldehyde was incubated with an ethanolic colloid. The resultant spectra indicated that the aldehyde was successfully adsorbed [Fig. 10(*a*)]. The absorption maximum is in the ultraviolet so that the spectrum of the aldehyde taken with 514.5 nm excitation is SERS not SERRS. If the sample is left in the beam with high power some photodecomposition is observed with broad non-specific signals growing in. This is the only case where this is observed. It is relatively common in SERS but we have not observed it in SERRS. However at low powers time-stable signals could be obtained. Since this aldehyde may well complex with silver ions on the surface of the colloid, experiments were carried out to see if a silver complex of 2-hydroxy-1-naphthaldehyde could be detected. The Raman spectrum from a crystal obtained by evaporating an ethanolic solution of a 2 : 1 silver complex of the aldehyde on a microscope slide (the structure of the complex is displayed in Fig. 11) was compared to SERS spectra taken from the complex adsorbed onto the colloid and for the aldehyde adsorbed onto the colloid [Fig. 10(*c*) and 10(*b*)]. There are clear differences in the spectra. In particular the band at 1363 cm^{-1} for the bulk crystal identified previously as being affected by chelation due to large displacements of atoms in the chelate ring[24] was shifted for the complex at the surface compared to the aldehyde suggesting that the latter had not formed a complex on the surface. Incubation of the aldehyde with the colloid for periods of up to 12 h did not result in the formation of the complex despite the fact that the analogous complex can be made on a bare copper electrode.[24] This suggests that the polymeric citrate coating is preventing reaction. There are differences in the spectra between the silver complex adsorbed onto the colloid and the Raman spectra of the crystal of the complex. A likely cause for these changes is a change from four-co-ordination in the bulk to five-co-ordination on the surface due to the formation of surface-to-complex bonds.

These experiments were carried out at a concentration of 10^{-6} mol equivalent before adsorption. In contrast to the quantitative experiments, the scales on the *y* axis cannot be

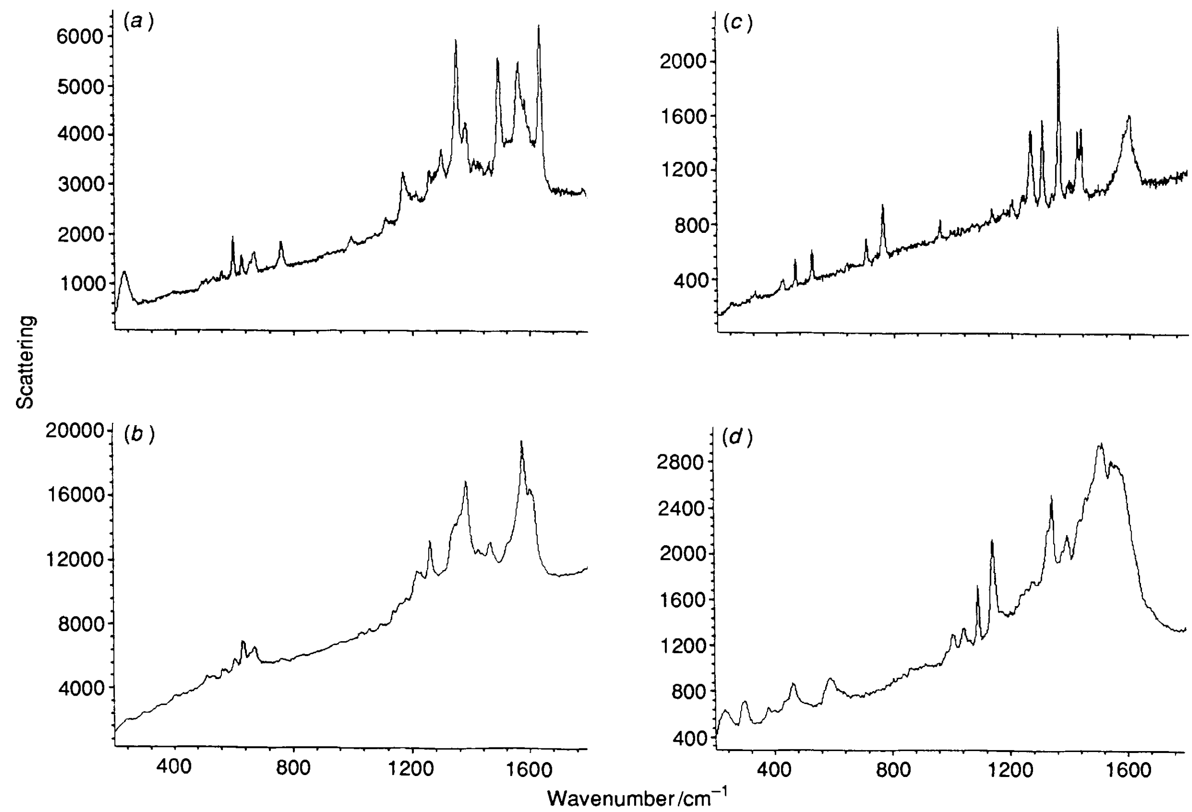

Fig. 10 (*a*) The SERS of a 2-hydroxy-1-naphthaldehyde ethanol solution using acid aggregation and an ethanolic sol. (*b*) The SERS of a silver–2-hydroxy-1-naphthaldehyde complex ethanol solution using acid aggregation and an ethanolic sol. (*c*) Raman spectrum of a silver–2-hydroxy-1-naphthaldehyde complex. (*d*) The SERS of nickel-2-hydroxy-1-naphthaldehyde complex

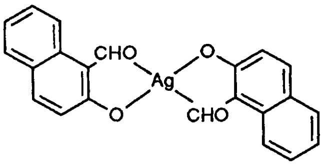

Fig. 11 Structure of the silver–2-hydroxy-1-naphthaldehyde complex

compared between samples because they are very dependent on the number and nature of the aggregates studied and instrument alignment but the sensitivity is high enough to enable complex formation to be observed. However, the bands are broad and the possibility of the presence of more than one species either through contamination or photodecomposition cannot be ruled out.

To complete the study and to ensure that the broad bands were not unique to silver, the *in situ* reaction was carried out with the same aldehyde and with nickel acetate in aqueous ethanolic colloid. The absorption maximum of the complex nearest to 514.5 nm is at 420 nm but it is a weak band. An attempt to measure the spectrum of a 2:1 complex by drying a solution of it on the microscope slide and measuring the spectrum as before failed due to fluorescence. However the SERS spectrum of the complex [Fig. 10(*d*)] from dried colloid previously treated with a solution of the complex could be obtained. It is similar to the spectrum obtained from a colloid containing the adsorbed ligand and subsequently treated with nickel acetate. Both are completely different from the spectrum of the aldehyde [Fig. 10(*a*)]. The spectrum is broad but the method again indicates the formation of the complex.

Overall the SERRS study provides a more effective approach to the investigation of surface reactions due to a combination of sensitivity, selectivity, band width and freedom from contamination. The newer more flexible sampling arrangememts of modern Raman spectrometers make such experiments relatively simple. Surface-enhanced resonance-Raman scattering also contains electronic information which could be obtained by measuring an excitation profile. However, the single monochromator and CCD instruments are less suitable for this purpose and hence profiles are not measured here. They remain an important additional source of information unique to SERRS.

Conclusion

Surface-enhanced resonance-Raman scattering is a rapidly developing technique. It has considerable advantages over the parent techniques of SERS and resonance-Raman scattering. In particular, it applies to a wider range of molecules than resonance and is more robust and more sensitive than SERS. It can now compete with and possibly surpass fluorescence in terms of detection limits.

Compared to other surface techniques, it has the advantage that it can be applied under a very wide range of conditions including experiments carried out under water and in air. Recent equipment improvements make it reliable and well within the compass of a well equipped laboratory.

The method will never be ubiquitous as very carefully designed experiments which are compatible with the experimental parameters are required. However, where it is correctly used, the information obtained is unique and its value as a surface probe is very significant providing in the main information on bonding and ligand orientation.

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