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# Surface-Enhanced Raman Spectroscopy as a Probe of Competitive Binding by Anions to Citrate-Reduced Silver Colloids

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Citrate-reduced silver colloids (CRSCs) are used extensively for surface-enhanced Raman scattering (SERS) studies of cations but are typically found to be ineffective for detection of anions unless they are treated with compounds that give them positively charged coatings. In this work CRSCs which were suitable for detection of anions were generated by treatment with aggregating agents that did not bind strongly to the silver surface. Under these conditions the major factor determining the enhancement of added anions was their ability to displace whatever anions were already present. In the case of CRSCs, residual citrate was observed when the colloids were aggregated with sulfate salts, since neither sulfate nor the residual nitrate displaced it. On addition of more strongly binding anions, such as halides, the citrate was displaced and the bands of the added analyte appeared, allowing them to be detected without the need for creation of positively charged coatings. It was found that the relative affinities of the anions, as determined by displacement experiments monitored by SERS, followed the solubilities of their silver salts, presumably because both properties are strongly dependent on the strength of the Ag−anion bonds. The relative affinities determine which anions can be detected in the presence of which others; nitrate, sulfate, and perchlorate are lower in the series than citrate and so are not observed. Displacement experiments show that dipicolinic acid (DPA) and $Cl^-$ have similar (stronger) binding, but they can be displaced in turn by $Br^-$ and $I^-$, which have the highest affinity and lowest solubility. This model allows a broad range of previous observations to be rationalized and allows the experimental conditions suitable for detection of particular new analytes to be designed on rational principles.

## Introduction

Since the first observation of a surface-enhanced Raman signal in 1974,[1] there has been a huge growth in the literature on surface-enhanced Raman scattering (SERS) spectroscopy.[2−6] Although numerous surfaces/substrates have been used to obtain enhanced Raman signals and the range of enhancing metals now includes Ag, Au, Cu, Na, Li, and other transition metals,[7] prepared in a huge array of morphologies, the most common media are the simple Au or Ag colloids whose activity was discovered in the very earliest stages of SERS research. These "simple" colloids continue to be the object of intense scrutiny, and studies on the connection between the particle's size, shape, and aggregation state are still being carried out.[8] Although in many of the most recent studies the emphasis has been on linking the properties of individual particles/clusters with SERS activity, rather than linking average ensemble properties (e.g., average particle diameter) with gross enhancement, much of our knowledge of the SERS effect comes from earlier studies that involved bulk measurements and properties. There have been numerous studies that have investigated the change in signal from Ag colloids on addition of different metal halide salts at various concentrations, and in some of these it has been recognized that there are at least two effects often occurring simultaneously: one is the extent of aggregation of the colloid; the second is modification of the surface chemistry on salt addition (which is "activation" if it leads to a larger signal). Such studies are typically carried out using a single test molecule or set of similar compounds, but since it is extremely unlikely that there will be a single set of experimental conditions that are optimum for all types of compounds, finding the conditions that are best for one particular small subset of analytes does not solve the problem that exactly the same conditions may give a poor SERS signal for a compound that is different from those in the test set. We are interested in finding some general principles that could be used to guide the choice of conditions that would be expected to give the best results for even for new, previously unstudied compounds. To do this it is necessary to establish the underlying reasons why some sets of conditions work particularly well (or badly) for certain sample types. If observations on a diverse range of test compounds and experimental conditions can be incorporated into a simple model, then that model will have genuine predictive power.

The range of potential analytes is vast, but although it is not possible to set very rigid rules, compounds can be classified according to chemical intuition and the most obvious differences are charge, polarity, free coordination sites, etc. At one extreme of the general classes of SERS analytes are the large, aromatic positively charged dye molecules that give huge signals under most experimental conditions (Rhodamine 6G, for example[9,10]). These compounds are currently the most popular targets in single-molecule work for obvious reasons, but because they are such good scatterers they are not so useful for determining what conditions would be best for recording spectra of weaker scatterers. At the opposite extreme lie small, colorless anions that do not have resonance enhancement and are much less widely studied with SERS, despite the fact that they occur much more widely in general chemistry than positive dyes and are important in major environmental issues such as water pollu-

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tion.[11] These anions are thus an important target in their own right and a good complement to the cationic dyes that have been so extensively studied.

Since citrate-reduced Ag colloids (CRSCs) are the most widely used enhancing materials for SERS,[12−16] they are the obvious choice for anion detection. However, most of the published SERS studies on anions have used either electrochemically roughened Ag/Au electrodes,[17−19] colloids that were prepared using different reducing agents (including borohydride[20] or hydrogen peroxide[21]), cellulose acetate films doped with Ag particles,[22] or CRSCs that were treated with surface-modifying agents (such as thiols[23] or poly-L-lysine combined with ascorbic acid[24]) to give them cationic-coated surfaces. To our knowledge there are no published studies on the SERS of anionic analytes using simple CRSCs. In part this is due to the fact that many anionic compounds do not give intense SERS signals with CRSCs and under common conditions the citrate bands are also absent, so that experimental data on this problem can be difficult to obtain. In particular, the lack of signal from added analyte may be attributed to poor binding of the analyte to the surface, although there may be no direct experimental evidence for this, other than the fact that there is no anion SERS signal.

If the general model of low anion SERS intensity being due to poor binding is accepted, it leads naturally to attempts to encourage anionic analytes to adsorb to the enhancing surface by adding materials that form cationic coatings on the surface. This approach has been demonstrated to be effective in promoting SERS detection of some small anions, such as perchlorate, nitrate, and sulfate, that normally do not give a SERS signal.[23] However, in contrast, some previous studies[24] on CRSCs have shown that anions will bind to the surface of silver nanoparticles without any modifying cationic coating. In this paper we attempt to understand this apparent contradiction and, more generally, to disentangle the various factors and conditions that determine whether particular anionic species can be detected by SERS. The semiquantitative model we develop allows a broad range of experimental data on the SERS enhancement of numerous anions and cations to be rationalized.

## Methods

Silver colloids were prepared using the standard Lee and Meisel citrate reduction method.[12] The UV/vis absorption spectra of the colloids typically had $\lambda_{\max} = 406$ nm with a full width half-maximum (fwhm) of 80 nm, but colloids with $\lambda_{\max}$ as long as 412 nm gave equally acceptable Raman spectra. All the spectra were recorded on a compact Raman system of in-house design and construction that used a Brimrose Corporation (BWL-20) frequency-doubled, diode-pumped Nd:$YVO_4$ laser operating at 532 nm and a thermoelectrically cooled CCD detector (Andor Technology, Model DV401) operating at −50 °C, coupled to a 125 mm spectrograph.[25] The spectrometer was calibrated using the standard Raman band positions of a 50/50 v/v toluene/acetonitrile mixture. The samples were held in 96 well microtiter plates, and accumulation times were typically 15 s per spectrum.

## Results

Figure 1 shows a series of spectra of a CRSC to which increasing amounts of citrate were added. The citrate bands that grow to reasonable intensity at the highest concentration ($5 \times 10^{-3}$ mol dm$^{-3}$) are clearly surface-enhanced since they are very much more intense than the spectra of simple aqueous citrate solutions in this concentration range. The bands in the SERS spectra of citrate have been assigned previously.[24] Figure 2 shows a series of spectra illustrating the growth of the citrate signal given by a CRSC in which there was no added citrate but where increasing [$MgSO_4$] was added. The insert shows that the growth in citrate signal is nonlinear with [$MgSO_4$] and that there is a 100 times increase at ca. $1 \times 10^{-1}$ mol dm$^{-3}$ added salt. Even at the highest [$MgSO_4$] there are no SERS or Raman bands near the position of $SO_4{}^{2-}{}_{aq}$, ca. 982 cm$^{-1}$.

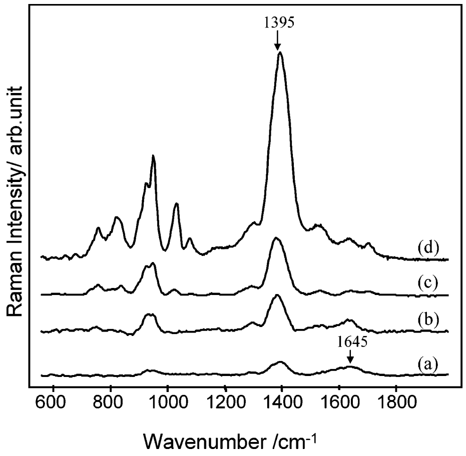

**Figure 1.** SERS spectra of citrate-reduced Ag colloid on addition of additional sodium citrate. (a) Untreated colloid; (b−d) colloid after addition of sodium citrate equivalent to (b) $2.5 \times 10^{-3}$, (c) $5.0 \times 10^{-3}$, and (d) $2.5 \times 10^{-2}$ mol dm$^{-3}$, respectively. The spectra are all shown with the same arbitrary intensity axis, and the bands that grow are characteristic of citrate.

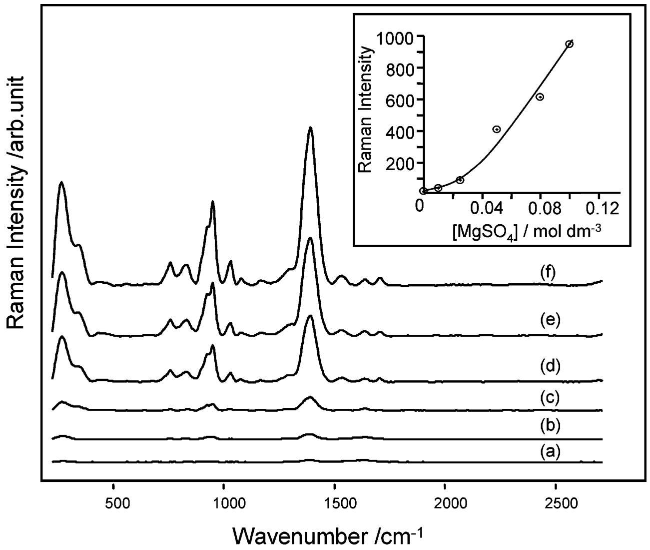

**Figure 2.** SERS spectra illustrating growth of citrate signal from a CRSC that was induced by addition of $MgSO_4$. (a) Untreated colloid; (b−f) colloid after addition of $MgSO_4$ equivalent to (b) $1 \times 10^{-2}$, (c) $2.5 \times 10^{-2}$, (d) $5 \times 10^{-2}$, (e) $8 \times 10^{-2}$, and (f) $1 \times 10^{-1}$ mol dm$^{-3}$, respectively. The insert shows that the growth in citrate signal is nonlinear with [$MgSO_4$] and that there is a 100 times increase at ca. $1 \times 10^{-1}$ mol dm$^{-3}$ added $MgSO_4$. Even at the highest [$MgSO_4$] there are no $SO_4{}^{2-}$ bands at the ca. 982 cm$^{-1}$ position of $SO_4{}^{2-}{}_{aq}$.

In Figure 3, spectra taken on the sequential addition of sulfate, DPA, and Cl$^-$ to a CRSC are shown. Addition of sulfate led to aggregation and increased enhancement of citrate bands (spectrum b), while subsequent DPA addition gave spectrum c, where the citrate bands were replaced by those of DPA. The DPA features were, in turn, replaced by the strong AgCl band when Cl$^-$ was added (spectrum d).

Figure 4 shows that at high DPA concentration ($1.5 \times 10^{-3}$ mol dm$^{-3}$/250 ppm in solution) a strong DPA signal could be

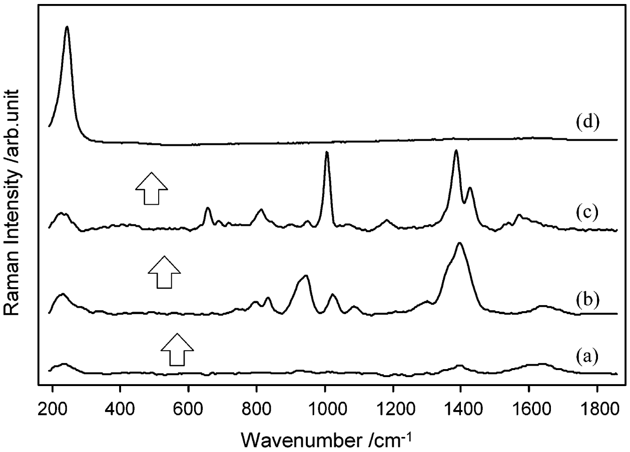

**Figure 3.** SERS spectra taken on sequential addition of sulfate, DPA, and $Cl^-$ to CRSC. (a) Untreated colloid. (b) $MgSO_4$ added to the colloid (a) to give a solution $5 \times 10^{-4}$ mol $dm^{-3}$ in $MgSO_4$ but displaying only citrate bands. (c) DPA added to the colloid (b) to give a solution $2.7 \times 10^{-5}$ mol $dm^{-3}$ in DPA that displays only the characteristic SERS bands of DPA. (d) NaCl added to solution (c) to give a $Cl^-$ concentration of $5 \times 10^{-3}$ mol $dm^{-3}$, DPA signal replaced with the strong AgCl band at 245 $cm^{-1}$.

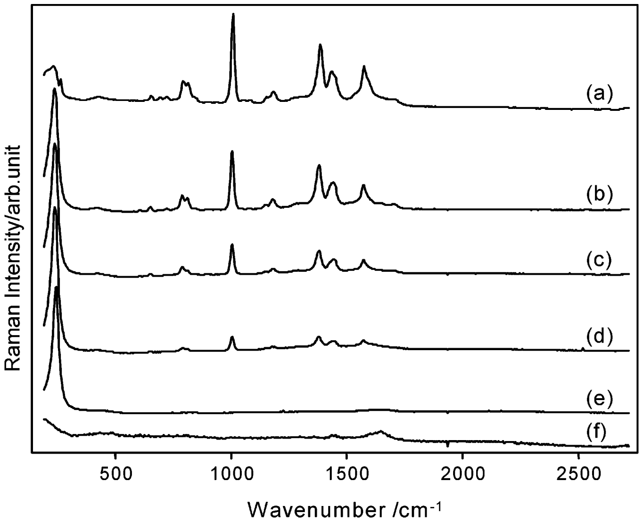

**Figure 4.** Effect of adding $Cl^-$ and $I^-$ to SERS spectrum of high concentration, 250 ppm ($1.5 \times 10^{-3}$ mol $dm^{-3}$) DPA. (a) 185 $\mu$L of Ag colloid mixed with 185 $\mu$L of 500 ppm DPA; (b) 10, (c) 20, and (d) 30 $\mu$L of 0.01 mol $dm^{-3}$ $MgCl_2$ added to (a); (e) 30 $\mu$L of 0.10 mol $dm^{-3}$ $MgCl_2$ added to (a); (f) 40 $\mu$L of 0.1 mol $dm^{-3}$ NaI added to (a). Traces b−d were normalized on the Ag−Cl band, while trace f was expanded to show that the only feature present was the weak water band at 1645 $cm^{-1}$.

observed even when no other aggregating agent was added. Addition of increasing concentrations of $MgCl_2$ led to the gradual replacement of the SERS signal of DPA by that of AgCl, which has a single strong band at ca. 245 $cm^{-1}$. The addition of $I^-$ (spectrum f) also removed the DPA bands but no strong new features grew to replace them.

The effect of adding anions on the intensity of the strongest SERS citrate band in a $SO_4^{2-}$-aggregated CRSC is shown in Figure 5a. Addition of $ClO_4^-$ and $NO_3^-$ up to $5 \times 10^{-3}$ mol $dm^{-3}$ in the solution had essentially no effect on the citrate signal, but $Cl^-$ addition caused dramatic loss of citrate. In contrast, Figure 5b shows that the SERS signal from DPA is gradually reduced by addition of $Cl^-$ but, as above, addition of $ClO_4^-$ and $NO_3^-$ up to $5 \times 10^{-3}$ mol $dm^{-3}$ in the solution had essentially no effect.

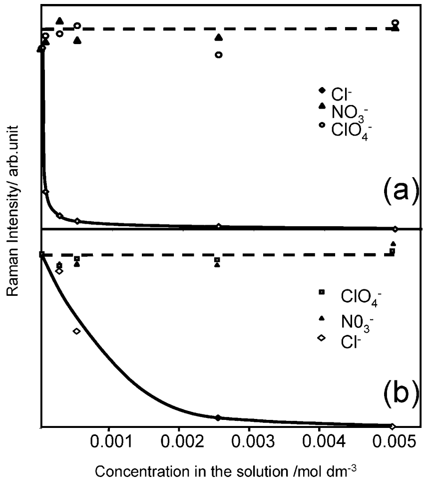

**Figure 5.** Effect of adding anions ($Cl^-$, $ClO_4^-$, $NO_3^-$) on intensity of the strongest SERS bands of citrate- and DPA-containing colloids. (a) Intensity of the 1395 $cm^{-1}$ citrate band in a sulfate-aggregated CRSC (5 $\mu$L of $1 \times 10^{-1}$ mol $dm^{-3}$ $MgSO_4$ in 380 $\mu$L of colloid). (b) Intensity of the 1010 $cm^{-1}$ DPA band in a similar sulfate-aggregated CRSC that also contained $6 \times 10^{-4}$ mol $dm^{-3}$ (100 ppm) DPA.

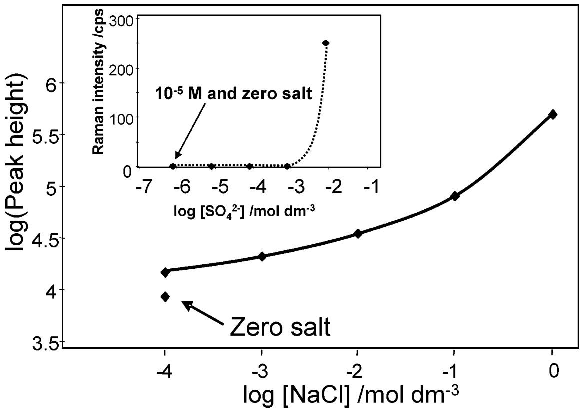

**Figure 6.** Increase in SERS signal (1175 $cm^{-1}$) of crystal violet, CV ($3.3 \times 10^{-7}$ mol $dm^{-3}$), on addition of $Cl^-$. Note the increase in signal on addition of just 20 $\mu$L of $1.0 \times 10^{-4}$ mol $dm^{-3}$ $Cl^-$ to 375 $\mu$L of CV/colloid solution. The insert (discussed in the text) shows the effect of $SO_4^{2-}$ addition on the signal (1010 $cm^{-1}$) from DPA; the log scale indicates $SO_4^{2-}$ concentration in the mixture after addition; the arrow marks two overlaid points corresponding to addition of 0 and $10^{-5}$ mol $dm^{-3}$ $MgSO_4$.

Figure 6 shows the intensity of the SERS signal (1175 $cm^{-1}$) of crystal violet, CV ($3.3 \times 10^{-7}$ mol $dm^{-3}$), on addition of increasing concentrations of $Cl^-$. Particularly noticeable is the increase in signal on addition of just $1.0 \times 10^{-4}$ mol $dm^{-3}$ $Cl^-$. In contrast, the insert to Figure 6 shows that $SO_4^{2-}$ addition does not have any noticeable effect on the SERS signal (1010 $cm^{-1}$) of DPA until a very high concentration ($8 \times 10^{-3}$ mol $dm^{-3}$) has been added.

## Discussion

Citrate-reduced silver colloids are not normally used to enhance the Raman spectra of anions, but they can be used to give SERS spectra of anions under appropriate conditions. For example, Figure 1 shows that the SERS spectrum of citrate could be obtained simply by adding a high concentration ($5 \times 10^{-3}$ mol $dm^{-3}$) of citrate to the colloid. Interestingly, even the spectrum recorded in the absence of added citrate (Figure 1a) did have a weak feature at ca. 1395 $cm^{-1}$, which is at the same position as the strongest citrate band, but assignment of this band was ambiguous because a second broad weak feature of similar intensity was also observed at 1645 $\mathrm{cm}^{-1}$. When pairs of broad bands in this general region were previously observed in SERS experiments, it was convincingly argued that they were due to amorphous carbon created on the Ag surface by high intensity laser irradiation. The SERS spectrum of amorphous carbon[26] adsorbed to the Ag surface does have the same general features as those in Figure 1a, but the similarity between this pair of broad weak bands observed here and those from amorphous carbon is purely coincidental.

If amorphous carbon was the source of the two features, then it would be expected that their relative band intensities would remain approximately constant under different experimental conditions, even if the absolute intensity changed. However, we have found that the relative intensities can be changed simply by centrifuging the colloid. In the uncentrifuged colloid they are of similar intensity (Figure 1a), but centrifugation and removal of 95% of the supernatant gives a sample where the colloid particle:aqueous solvent ratio is dramatically increased. This is reflected in the 1395:1645 $\mathrm{cm}^{-1}$ ratio, which changes from 1.08 to 2.5 in the centrifuged colloid (spectrum not shown). The decrease in the relative intensity of the 1645 $\mathrm{cm}^{-1}$ band, coupled with the fact that it has the same position, width, and absolute Raman intensity as the aqueous solvent, makes its assignment as the weak water band unambiguous. Since the 1645 $\mathrm{cm}^{-1}$ band is due to the aqueous solvent rather than surface carbon, this implies that the 1395 $\mathrm{cm}^{-1}$ band is also not due to surface carbon but is from residual citrate which is present in such low concentration in the untreated CRSC that even the strongest (1395 $\mathrm{cm}^{-1}$) band is only just detected.

The fact that the intensity of the strongest citrate band in the untreated CRSC is so weak that its intensity is similar to that of the water band is not unexpected since many analytes give weak spectra with unaggregated colloids and it is often necessary to aggregate the colloids before the dominant, intense SERS signals are generated. However, the vast majority of examples in the literature are of cases where aggregation of CRSCs enhances the spectra of low concentration analytes but does not also dramatically increase the intensity of the citrate bands which, as a consequence, are hardly ever observed. Indeed the lack of interference by citrate bands in SERS measurements is so common that the absence of citrate signals is typically not even commented upon. There is one published study in which the SERS signals of low concentration citrate were observed and characterized in detail, in that case nitric acid was used as the aggregating agent.[24] The spectra in Figure 1 show that it is possible to obtain citrate SERS spectra without using strong acid (which it would generally be preferable to avoid in solutions of organic analytes) but the data are ambiguous in that it is not clear whether the growth in the citrate signals at higher concentrations is due to: increased aggregation; higher concentrations of citrate adsorbing on the surface of the enhancing particles; or a mixture of both effects. However, the situation is much clearer if the citrate concentration is kept constant and a different aggregating agent is used.

In a previous study on dipicolinic acid (DPA), we found that sulfate aggregation was particularly effective for enhancing DPA signals.[27] The same is also true for citrate, and Figure 2 shows that even CRSCs with no extra added citrate give very intense citrate SERS signals on aggregation with sulfate. The growth of the citrate signal with [$\mathrm{MgSO_4}$] addition is significant, and there is a 100 times increase when 5 $\mu$L of $1 \times 10^{-1}$ mol $\mathrm{dm}^{-3}$ $\mathrm{MgSO_4}$ is added to 380 $\mu$L of colloid. The requirement for such high concentrations of $\mathrm{MgSO_4}$ implies that the enhancement is arising from simple aggregation of the colloid. The UV/vis spectra reflect this aggregation with the expected broadening and appearance of significant absorption at long wavelengths.

The data in Figures 1a and 2 highlight the fact that citrate is always present in CRSCs, whether it is observed or not, and any attempt to record SERS spectra of anions sets up a direct competition between citrate and the incoming analyte. Since the resulting signal will depend on which species occupy the surface sites and are enhanced, it is important to be able to observe and quantify the competition between the various anionic species present in the solution. The sulfate-treated colloids are excellent for this purpose because they are aggregated, and thus give good SERS signals, but the sulfate does not interfere with the measurements since it does not give a strong SERS signal itself. Presumably this is because the sulfate has a very low affinity for the silver surface.

A good example of this competitive adsorption is shown in Figure 3, where data from experiments in which up to five different anions were present simultaneously and where three negatively charged analytes—dipicolinic acid, citrate, and chloride—were eventually all competing for the same surface sites in the presence of a high concentration of sulfate aggregating agent and the residual nitrate from the preparation which is always present but gives no signal under any of the conditions used here. Figures 2a and 3a show that the spectrum of the untreated CRSC is essentially featureless on this intensity scale (although expansion of the intensity axis would reveal weak citrate bands), but once the colloid is aggregated with $\mathrm{MgSO_4}$, clear citrate bands appear. Although the citrate bands are intense, they are easily replaced by other analytes. Figure 3c shows that addition of just 10 ppm ($6 \times 10^{-5}$ mol $\mathrm{dm}^{-3}$) DPA to the solution used for Figure 3b causes almost compete removal of citrate signal, which is replaced by the characteristic DPA bands. In contrast, addition of lower concentration ($<$1 ppm/$6 \times 10^{-6}$ mol $\mathrm{dm}^{-3}$) DPA gives solutions in which only the original citrate bands are detectable (not shown). This clearly indicates that an important factor setting the detection limit is the requirement for the incoming analyte to displace the citrate that is present on the Ag surface. Although $6 \times 10^{-5}$ mol $\mathrm{dm}^{-3}$ DPA is sufficient to replace the citrate on the surface, it can itself be displaced by $\mathrm{Cl^-}$, which has a higher affinity for the silver surface (see below). Figure 3d shows that the spectrum generated by addition of $\mathrm{Cl^-}$ ($5 \times 10^{-3}$ mol $\mathrm{dm}^{-3}$ in the mixture) to the sulfate-aggregated DPA colloid is now dominated by the strong AgCl band lying at 245 $\mathrm{cm}^{-1}$, so despite the fact that the citrate, sulfate, and DPA anions (along with residual nitrate) are still present in the solution, only $\mathrm{Cl^-}$ is detectable.

It should be emphasized that there will always be competition between the various anions for surface sites, so that although sulfate aggregation allowed the replacement of residual citrate by low concentration DPA bands to be observed (Figure 3), the same reaction would still be expected to take place in samples without sulfate aggregation (although in that case the equilibria might not be detectable by SERS). For example, when the DPA concentration was kept at the same ($6 \times 10^{-5}$ mol $\mathrm{dm}^{-3}$) level that was used for Figure 3 and the series of experiments was repeated, except that no sulfate was added, then neither citrate nor DPA signals were sufficiently strong to be detected. Indeed, the only signal observed with significant intensity in the entire experimental sequence was the Ag−Cl band generated after $\mathrm{Cl^-}$ addition and there was essentially no indication of a series of complex equilibria in the spectra (data not shown). However, if a high concentration of DPA is used, some of the equilibria can be observed.

**TABLE 1: Solubility of Some Ag Compounds**

| compound | solubility (g/100 cm$^{-3}$) |
|---|---|
| AgI | $2.8 \times 10^{-7}$ |
| AgBr | $8.4 \times 10^{-6}$ |
| AgCl | $8.9 \times 10^{-5}$ |
| Ag citrate | $2.8 \times 10^{-2}$ |
| $Ag_2SO_4$ | $5.7 \times 10^{-1}$ |
| $AgNO_3$ | 122 |
| $AgClO_4$ | 557 |

As shown in Figure 4, $3 \times 10^{-3}$ mol dm$^{-3}$ (500 ppm) DPA gives a signal that is easily observed without addition of further aggregating agents (Figure 4a). On addition of Cl$^-$ (Figure 4b−e), gradual loss of the DPA bands and growth of the Ag−Cl band are seen. The DPA can also be displaced by other halides. Figure 4f shows the result of treating a $1.5 \times 10^{-3}$ mol dm$^{-3}$ (250 ppm) DPA solution with KI. In this case there is again a complete loss of DPA bands although the expected Ag−I band lies at 170 cm$^{-1}$, which is outside the accessible range of the spectrometer.

The competition between different analytes is reminiscent of the equilibria that have been observed for Ag and Au roughened electrodes modified with cationic thiols. In those systems the factor that determines which analyte binds most effectively to the surface, and thus gives the largest signal intensity, is the strength of the interaction between the analyte and the compounds used to create the surface-modifying layer. However, the cationic thiol layer itself is strongly bound to the surface, so the SERS signals invariably contain a significant number of bands associated with the modifying layer, along with the analyte bands that grow on top of this highly structured background signal. The situation is different in the sulfate-aggregated CRSCs studied here because the strong background citrate signal deceases on addition of the analytes, which displace the citrate from the surface. Indeed, the data in Figures 3 and 4 show examples where one analyte displaces another from the surface, and they highlight the fact that it is clearly important to quantify or at least rationalize the relative affinity of the various anionic analytes for the surface. Although surface potential has previously been cited as an important factor in determining the relative affinities of charged analytes for colloid particles and in electrodes changing the potential can alter binding of small anions,[18,28] the most important factor in the CRSCs would be expected to be the free energy change associated with replacing one anion bound to the silver surface by a different anion. We have found that even in the absence of detailed thermodynamic information on this process it is possible to correlate the relative affinities, as measured by SERS, with the solubility of the silver salts of the various anions. Presumably, this is because the same underlying thermodynamic parameters govern both processes. At the simplest level, one could argue that the difficulty in observing SERS of sulfate is consistent with the high solubility of silver sulfate in aqueous solution since the high solubility implies there is a strong thermodynamic driving force toward formation of solvated silver and sulfate rather than retaining the silver−sulfate bonds in solid $Ag_2SO_4$. There is thus an obvious implication that, if a surface silver−sulfate complex formed, it would have low stability because dissolution to form aqueous ions would be favored. In contrast, it is difficult to displace chloride from the silver surface due to the high thermodynamic stability of the surface AgCl.

Table 1 lists the solubilities of a wide range of silver salts, and it is clear that all the trends in the observed SERS behavior follow the changes in solubility of the silver−anion salts. For example, the observation that no surface-enhanced sulfate bands are observed from colloids that have been aggregated with even high concentrations of sulfate is also true for nitrate[24] and perchlorate,[29] the two other high-solubility salts in Table 1. Conversely, citrate (a medium-solubility salt) can be observed by SERS but the spectrum is easily replaced by one dominated by Ag−Cl (low solubility) on Cl$^-$ addition. Finally, it has previously been observed that Cl$^-$ can in turn be replaced by Br$^-$ [30] (similarly, in this work we have displaced Cl$^-$ by I$^-$) and SCN$^-$ displaced[31] by Cl$^-$, Br$^-$, and I$^-$.

In effect, there is a hierarchy in the anions that determines which anions can be detected in the presence of which others. Those at the bottom of the series, below citrate, will not be observed even in aggregated CRSCs because they are unable to displace the residual citrate. Hence nitrate, perchlorate, and sulfate can be used as aggregating agents that facilitate observation of citrate bands, but they cannot themselves be detected. Indeed, in standard CRSCs prepared by the Lee and Meisel method,[12] in addition to the residual citrate (which can be observed when $SO_4^{2-}$ aggregation is used but is normally not detected), there will also be ca. $1 \times 10^{-3}$ mol dm$^{-3}$ residual $NO_3^-$ from the $AgNO_3$ starting material, but this is never observed, even with sulfate aggregation, because it is displaced by the citrate.

It is possible to make semiquantitative estimates of binding affinities by titrating CRSC solutions with competing analytes. Figure 5 shows that the intensity of the citrate signal from a sulfate-aggregated colloid is barely perturbed on sequential addition of up to $5 \times 10^{-3}$ mol dm$^{-3}$ (in solution) of $ClO_4^-$ or $NO_3^-$. In contrast, because chloride has a much higher affinity for the surface, addition of even small amounts of chloride (much lower than are normally used for aggregation) results in replacement of the citrate on the surface and observation of only Ag−Cl vibrations. Figure 5a shows that more than 75% of the citrate signal is removed on addition of sufficient NaCl to make [Cl$^-$] in the solution $5 \times 10^{-5}$ mol dm$^{-3}$. The concentration of Cl$^-$ required for monolayer coverage of the Ag particles can be estimated as ca. $2 \times 10^{-5}$ mol dm$^{-3}$ (assuming all Cl$^-$ binds, colloid particles are 50 nm spheres, and the ionic radius of Cl$^-$ is 1.81 Å). Despite the considerable uncertainty with this estimate, these two values are certainly close enough to suggest that no great excess of Cl$^-$ is required to replace the surface citrate by Cl$^-$. This means that the equilibrium constant for the displacement reaction

$$\mathrm{cit_{surf}}^- + \mathrm{Cl_{aq}}^- \rightleftarrows \mathrm{cit_{aq}}^- + \mathrm{Cl_{surf}}^-$$

must lie to the right. Unfortunately, it is not possible to estimate the value of the equilibrium constant from this experiment with any degree of certainty because the citrate concentration is not known. The same very effective removal of citrate is also observed for DPA addition (Figure 3c), but again no quantitative analysis is possible.

In cases where the affinities are closer there is some possibility for altering the binding of competing species by changing the concentration. Figure 5b includes a plot illustrating displacement of DPA by Cl$^-$. In contrast to the citrate discussed above, there is a more equal competition for binding sites between the DPA and Cl$^-$, so simply adding sufficient Cl$^-$ to provide monolayer coverage does not lead to replacement of the surface DPA. Indeed, the plot shows there is approximately 50% displacement when the DPA and Cl$^-$ are at similar concentrations (DPA = 100 ppm, i.e., $6 \times 10^{-4}$ mol dm$^{-3}$, Cl$^-$ = $5 \times 10^{-4}$ mol dm$^{-3}$). This implies that the binding affinities of DPA and Cl$^-$ are very similar. Consistent with this is the observation that nitrate and perchlorate addition has little effect on the DPA signal.

The efficacy of the sulfate-aggregated colloids is not limited to the small set of anions discussed for illustrative purposes above. We have also recorded strong SERS signals from $<10^{-5}$ mol dm$^{-3}$ level picolinic acid, CN$^-$, $Cr_2O_7^{2-}$, CNS$^-$, and anionic dyes. Indeed, the only anions for which the method was ineffective were the $SO_4^{2-}$, $ClO_4^-$, and $NO_3^-$ ions that have very soluble silver salts.

Finally, this picture can also account for the somewhat surprising observation that, in experiments using positively charged analytes, addition of Cl$^-$ at much lower concentration than would be required to give significant aggregation does lead to a sudden jump in the signal. For example, Figure 6 shows a case where addition of NaCl at slightly greater than the concentration required for monolayer coverage gave a 2 times increase in the absolute intensity of the SERS signal from crystal violet (CV).

Presumably in this case the replacement of citrate in the surface layer by Cl$^-$ changed the binding affinity of CV and this led to a change in intensity that was not due to aggregation but can be attributed to the "activation" effect in SERS. In contrast, where the added ions do not adsorb to the Ag surface, only the effect of aggregation is observed. For example, the insert to Figure 6 shows that, with DPA, addition of low $SO_4^{2-}$ concentrations has no effect until the concentration is sufficiently high ($2 \times 10^{-2}$ mol dm$^{-3}$ in the solution) to aggregate the colloid.

## Conclusions

We have found that it is possible to obtain strong SERS spectra of a broad range of anions, including DPA, CN$^-$, $Cr_2O_7^{2-}$, citrate, and halides using CRSCs aggregated with $MgSO_4$. Aggregation is vital for observation of strong SERS signals from these analytes at low (parts per million) concentrations, but it was found that many aggregating agents adsorb to the surface of the colloid particles and thus prevent adsorption and detection of incoming analytes. In general, within these systems there is always competitive adsorption among the anions that are present. Even in the untreated CRSC there are both nitrate and citrate ions competing for the surface, but the citrate binds more strongly and displaces any surface nitrate. If aggregating agents containing $SO_4^{2-}$, $ClO_4^-$, and $NO_3^-$ are chosen, they do not displace the residual citrate, which gives a strong signal. However, more strongly binding analytes will displace the citrate and will be observed. There is a simple correlation between the solubility of the Ag salts of the anions and their ability to compete for surface sites, with the least soluble salts being the most strongly binding. The anions' binding equilibria can also affect the signals from cationic analytes since replacing one type of surface anion by another may alter the binding of cations to the surface of the colloid. In general, these observations mean that the experimental conditions suitable for detection of particular new analytes can be designed on rational principles.

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