Colloid Filtration: A Novel Substrate Preparation Method for Surface-Enhanced Raman Spectroscopy

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A new method for preparing a monodisperse layer of metal colloid particles is presented. Metal colloid particles are filtered onto the surface of an aluminum oxide capillary pore surface capture membrane using both vacuum and syringe filtration devices. Scanning electron microscope (SEM) images reveal that the metal particles can be deposited with very little aggregation. Electronic absorption spectra show that the concentration of particles on the surface can be controlled by varying the silver colloid solution concentration. The linear dynamic range of absorbance of particles on the surface is demonstrated to be at least one order of magnitude. Surface-enhanced Raman (SER) activity is observed for the compound 9-aminoacridine (AA) at 514.5 nm using a filtered silver colloid solution. The SER activity of AA is observed to be related to the degree of colloid aggregation on the surface. Surfaces with no aggregation have no SER activity at this wavelength. The syringe colloid filtration technique is a fast, inexpensive method for producing surfaces with a highly controlled metal particle geometry, extremely useful for comparisons between theoretical and experimental results. These surfaces are also ideal for theoretical substrate design.

INTRODUCTION

Most of the experimental research conducted to date in the area of surface-enhanced Raman spectroscopy (SERS) has been directed toward the elucidation of the physical processes underlying the SERS phenomenon or the characterization of the SERS spectra of specific classes of molecules, including nucleic acids (1), drugs (2, 3), and polycyclic aromatic hydrocarbons (4). However, in recent years, a larger percentage of the experimental SERS research has been focused on the use of this relatively new spectroscopy as an analytical technique. The development of a SERS-active substrate which is reproducible, easily prepared, and inexpensive is necessary if this technique is to be used on a routine basis. It is also desirable to have a substrate which closely approximates the models used in the SERS electrodynamic theories so that these calculations can be used in surface design, including optimization of the enhancement as a function of particle size, shape, and composition (5–7). This constrains the surface particle shapes to spheres and spheroids, the only geometries easily treated by current theoretical models (5–7).

Several substrates which have one or two of these characteristics but not all of them have been prepared. Cellulose filter papers (8) and frosted glass slides (9) coated with silver by direct chemical reduction or cellulose filter papers (10) coated by vacuum vapor deposition are easy to prepare and inexpensive; however, the metallic surface microstructures are not spherical (11). Plasma-etched quartz posts (12) coated with silver by vacuum vapor deposition provide the most control over the particle size and, more importantly, particle shape of any SERS active substrate that has been produced. These substrates can be used to rigorously test the most important aspects of the electrodynamic theory. One drawback is that the method of preparing these surfaces

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is complex and requires both plasma-etching equipment and vapor deposition equipment with accurate thickness monitors. The most promising substrate which has been developed for analytical SERS use involves the deposition of silver onto polystyrene spheres which have been spin-coated onto glass slides (13). This type of surface is easy to prepare, inexpensive, and has a surface geometry which can be controlled by varying the silver deposition thickness and the polystyrene sphere size and concentration (14). However, this method requires a spin-coating apparatus and, like the quartz post surface method, a vapor deposition chamber with accurate thickness monitor. This method also produces silver particles with nonmetallic cores. While the electromagnetic theory has been augmented to particles of this type, no attempt was made to use these calculations to optimize the core diameter and the silver shell thickness. Finally, since only the top and sides of the polystyrene spheres are coated, these surfaces are not ideally suited to comparisons with theoretical models (15, 16).

The simplest surface which fulfills all the requirements of an analytically useful SERS substrate is a metal colloid solution. Metal colloids consist of essentially spherical, noninteracting particles. They provide an inexpensive SERS substrate which is easily and reproducibly prepared for many metals, including silver, the metal of choice for most SERS studies. These particles are an ideal substrate for comparison with the theoretical models. However, while silver colloid solutions are stable for up to several months, addition of an analyte which adsorbs to the particle surface destabilizes the colloidal solution, resulting in aggregation and the eventual precipitation of the metal particles (17). This aggregation leads to a highly time-dependent SERS spectrum, making this substrate difficult to use for analytical studies. Several possible solutions to this problem have been presented in the literature, the most prominent of which involves the use of flow injection analysis (FIA). FIA has been used to prepare silver hydrosols on line and to obtain precision of SERS signals of 5% (18). Conditions for the FIA system have been optimized (19) and this technique has been used for quantitative analysis (5, 20). However, although FIA is a practical and useful solution to the problems associated with colloidal SERS, it is not an ideal solution. The idea behind the technique is to allow the flocculation of colloidal particles to occur and to control the mixing rate and mixing time of the analyte and colloid solutions. As the particles aggregate, they initially form strings (21). These strings have a split plasmon resonance, one at the sphere resonance (transverse) and one shifting to longer wavelengths as the string length is increased (longitudinal). By controlling the mixing time and flow rates, FIA attempts to probe the mixture when the maximum number of particle strings are "in resonance" with the excitation radiation, typically the 514.5-nm line of an argon ion laser. Thus, the system is still dynamic, and changes in the solution temperature or analyte concentration, the presence of contaminants, and other external factors could affect the optimum mixing time.

Two other techniques which have been used in an attempt to circumvent the aggregation problem involve the spotting and nebulizing of the colloidal solutions onto a variety of solid supports. These methods, which have been used for the detection of molecules on paper chromatograms (22) and HPTLC plates (23, 24), are closer to an ideal solution to colloid instability, but they still present some problems. The two deposition methods chosen, syringe application and nebulization, do not allow for a control of the distribution of particles on the surface. In fact, the spraying technique actually relies on a color change in the colloidal solution upon application to the surface, due to the longitudinal plasmon resonance of the colloid strings, in order to observe SERS signals. Once again, the technique is relying on the flocculation of the colloidal particles on the surface to "tune" the surface resonance into the excitation line.

In order to obtain SERS spectra and main-

tain the ability to treat a colloidal surface theoretically so that optimum experimental conditions can be calculated, the particles must be deposited onto the surface in a controlled manner while eliminating any flocculation. The laser wavelength used can then be "tuned" to coincide with the single particle resonance, rather than tuning the longitudinal resonance of particle strings into the laser wavelength by means of flocculation. Using a monodisperse surface allows the most control over both the experimental and theoretical aspects of the substrate and assures that all of the metal particles within the probe beam are in resonance with the excitation wavelength.

We present a method for the preparation of a substrate which consists of a monodisperse submonolayer of metal colloid particles by filtration of a silver colloid solution onto the surface of a highly efficient inorganic capillary pore structure surface-capture membrane filter. This technique allows for a high degree of control over surface geometry, while minimizing the problem of particle aggregation. We further present information on the control of the silver particle concentration and extend the technique to aqueous red gold colloids.

MATERIALS AND METHODS

A Sterifil aseptic system 47 vacuum filtration device was purchased from Millipore. Becton-Dickinson 10-ml disposable syringes and Gelman 25-mm Delrin syringe filter holders were obtained from Fisher. Anotec anodiscs (0.02-μm pore size) were obtained from Alltech. Polycarbonate track-etched (PCTE) membranes (0.01-μm pore size) were obtained from Poretics. Raman spectra were obtained with a 0.85-m double-grating spectrometer (SPEX Industries, Model 1403). The spectrometer resolution was set to 10 cm^{-1}. The detector was a cooled RCA C31034 gallium arsenide photomultiplier tube coupled to standard photon counting electronics. Data acquisition, storage, processing, and plotting were under the control of an IBM PC compatible personal computer. All spectra reported represent single scans and are presented without spectral smoothing. Right angle geometry was used for Raman sampling. Laser power at the sample was 20 mW. Scanning electron micrographs of the PCTE membrane were obtained with a JEOL JSM-35C SEM (resolution 15 nm), while the micrographs for the Anopore membranes were obtained with a Hitachi S-4000 field emission SEM (resolution 1.5 nm). The PCTE images were obtained for membranes coated with 10 nm of a gold–palladium mixture to make them conductive. The images of the Anopore membranes required no precoating. The actual magnification of the images presented is different from the value printed on the image since the photograph used to prepare the image was either enlarged (for 120 format, 6×7 cm negatives) or reduced (for 4×5 in. negatives) for purposes of reproduction.

Electronic absorption spectra were obtained using an HP 8450 diode array spectrophotometer and a Perkin-Elmer Lambda-9 spectrophotometer with an integrating sphere.

The silver colloids were produced using the procedure described as follows. A silver nitrate solution was prepared by adding 70 ml of water to 30 ml of a 5.0×10^{-3} M aqueous $AgNO_3$ solution. This solution was ice-cooled and then added dropwise with vigorous stirring to a sodium borohydride solution prepared by adding 120 ml of water to 180 ml of a 2.0×10^{-3} M aqueous $NaBH_4$ solution. The $NaBH_4$ solution was also ice-cooled. The resulting silver hydrosol had an absorption maximum at 400 nm, characteristic of silver particles with diameters between 1 and 50 nm (25). This was experimentally verified during the colloid filtration experiments (see Fig. 1).

The red gold colloidal solution was prepared as follows. Two hundred milliliters of water, filtered through a 0.2-μm filter to remove impurities, is brought to a boil. A 4% gold chloride solution (0.5 ml) is added, followed by 5 ml of a 1% trisodium citrate solution. The mixture is boiled for approximately 5 min. The resulting solution is raspberry red and consists

FIG. 1. Scanning electron microscope image of silver colloid particles vacuum filtered onto a 0.01-μm pore size polycarbonate track-etched membrane at an original magnification of 20,000. The image was taken with a JEOL JSM 35C scanning electron microscope.

of gold particles with a mean diameter of 15 nm.

Vacuum Filtration

A 47-mm-diameter PCTE membrane was placed into the Sterifil vacuum filtration unit. Initial tests used an aspirator to create the vacuum necessary for filtration. Subsequent tests used a mechanical pump to create a stronger vacuum. This experiment was also performed using a 47-mm-diameter Anopore membrane. For this membrane, only the aspirator was used for creating the vacuum. For both experiments, 20 ml of the silver colloid solution was filtered.

Syringe Filtration

A 25-mm-diameter PCTE membrane was placed into the Gelman syringe filter holder. Two milliliters of the silver hydrosol was filtered through the membrane using a syringe pump. This procedure was repeated using a 25-mm-diameter Anopore membrane using both the silver and gold colloid solutions. For this membrane the pressure was applied both with a syringe pump and by hand.

Absorption Spectra

The Anopore membranes are somewhat transparent to visible light. It was possible to obtain absorption spectra of both the blank membrane and a coated membrane (silver colloid) for wavelengths as low as 400 nm by simply placing a blank membrane in the reference beam and a coated membrane in the sample beam of the HP spectrophotometer. To obtain data below 400 nm, the spectra were obtained while the membranes were wet. Filling the pores with a solution with an index of refraction closer to that of the membrane than air renders the membrane more transparent (26). Additional absorption data were obtained by reflection using the Perkin-Elmer

instrument by first measuring a blank membrane placed over a sample port in the back of the integrating sphere and then placing a coated membrane in the same place. The overall absorption spectra were obtained by background subtraction.

Reagents

All chemicals used were analytical reagent grade or equivalent. Demineralized water was used throughout. 9-Aminoacridine hydrochloride monohydrate was purchased from Aldrich and was used without further purification.

RESULTS AND DISCUSSION

Polycarbonate Track-Etched Membranes

PCTE membranes are frequently used in biological laboratories for filtration of cells, bacteria, and other micrometer-sized particles for purposes of SEM imaging. These membranes are essentially flat on a submicrometer scale and have a narrow pore size distribution. The smooth surface provides a high contrast between the sample and the particles on the surface, making it an ideal support for SEM images (21). They are available in pore sizes ranging from 10 nm to several micrometers, with the intermediate pore sizes used for biological purposes.

Figure 1 shows the PCTE membrane coated with silver colloid particles using the vacuum filtration technique. The membrane is covered with thin black lines which conform to the pattern of the support mesh in the Sterifil unit (essentially concentric circles). This SEM image confirms that aggregation is prevalent using vacuum filtration on the PCTE membrane. This SEM image also confirms that the silver colloid particle sizes are in agreement with the absorption spectra results. The particles in Fig. 1 are between 5 and 25 nm. No image of a clean membrane is given because the contrast between the colloid particles and the smooth PCTE surface, coupled with the small percentage of the membrane surface area covered, provides a good view of both the coated and uncoated membrane. The membrane produced using the syringe filtration technique has the same degree of aggregation. SERS activity was tested with 1 μl of a 30 ppm AA solution using the Raman microprobe. The result is also shown in Fig. 1.

Colloid filtration using the PCTE membrane presents a number of problems. Filtration using an aspirator did not provide enough vacuum for filtration. The mechanical pump created a sufficient vacuum to filter the colloid solution, but 4 h was required to filter 20 ml of the hydrosol. These problems are not surprising considering the low pore density of the PCTE membrane, as seen in Fig. 1. A second practical problem is that the 2-μl aqueous aliquots of AA typically used to probe the SERS activity do not readily absorb into the membrane. Over 20 min elapsed between the "spotting" of the analyte solution on the membrane complete evaporation of the solvent. It is likely that the solvent is evaporating rather than being taken into the membrane. Finally, the SERS activity to AA is low due to the excessive aggregation of the colloid particles on the surface. These problems imply that this membrane is not practical as a support for colloid filtration.

Anotec Anopore Alumina Membranes

The Anotec Anopore inorganic filter membranes make the syringe filtration method feasible and are discussed in some detail. Anopore inorganic membranes were invented in the laboratories of Alcan International. Anotec Separations was formed by Alcan to further develop this technology. These membranes are composed of aluminum oxide and have a highly ordered, honeycomb structure with sieve-like capillary pores with diameters of 20, 100, and 200 nm (27). Porosity exceeds 50% by volume, resulting in very high flow rates when compared to other filters with comparable pore sizes. The water flux rate at 25°C and 10 psi for a 100-nm pore size filter membrane with a 25-mm diameter is 8 ml min^{-1} cm^{-2}. A comparable flow rate for a polycar-

bonate track-etched membrane, which also has sieve-like capillary pores, is less than 0.2 ml min^{-1} cm^{-2}, mainly due to the difference in pore density. For example, a 200-nm pore size PCTE membrane has 3×10^8 pores cm^{-2}, whereas a 200-nm pore size Anopore membrane has 3×10^9 pores cm^{-2}. A direct comparison of the Anopore and PCTE membranes is given by Jones *et al.* (28). Anopore membranes are also used in epifluorescence (29) and in general separations (30).

Figure 2 shows a blank Anopore membrane at an original magnification of 35,000. Figure 3 shows two images of this membrane coated with silver colloid particles using the syringe filtration technique. Figure 3b is split to show the results of backscattering detection. The backscattering is obtained using an optional attachment to the Hitachi S-4000 instrument and differentiates between the atomic masses of the objects visualized. The white particles on the left half of the image, which also appear in the backscattered image (right half of the image), are shown to consist of atoms with a different mass than aluminum or oxygen, the primary constituents of the membrane. These particles, not present on a blank membrane, are silver colloid. Since the concentration of the silver relative to the aluminum oxide of the membrane was very low, energy-dispersive spectroscopy did not reveal a silver peak. Vacuum filtration was initially used with the Anopore membrane, but the syringe filtration technique outlined above was found to be much simpler and less expensive. It should be noted that the silver colloid solution prepared using the technique outlined under Materials and Methods is much too concentrated for filtration purposes. Using the vacuum filtration technique and 20 ml of the colloid, the membrane appearance after filtering is solid black. The blank membrane is white. Using 2 ml of this solution with the syringe filtration technique results in a mirror-like silver surface on

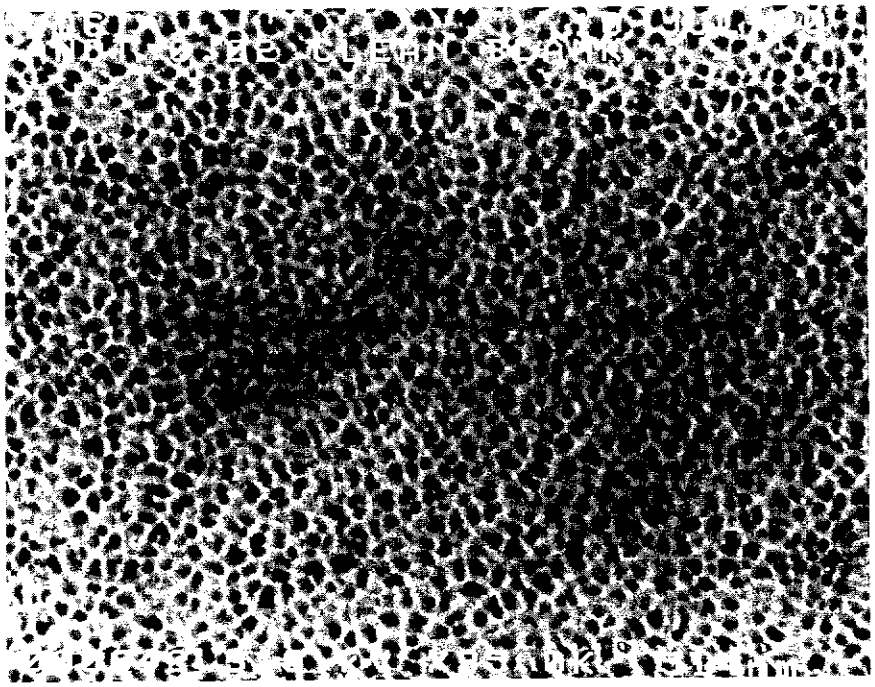

FIG. 2. Scanning electron microscope image of a blank Anopore aluminum oxide filter membrane with a pore size of 0.02 μm at an original magnification of 35,000. The image was taken with a Hitachi S4000 field emission tip scanning electron microscope.

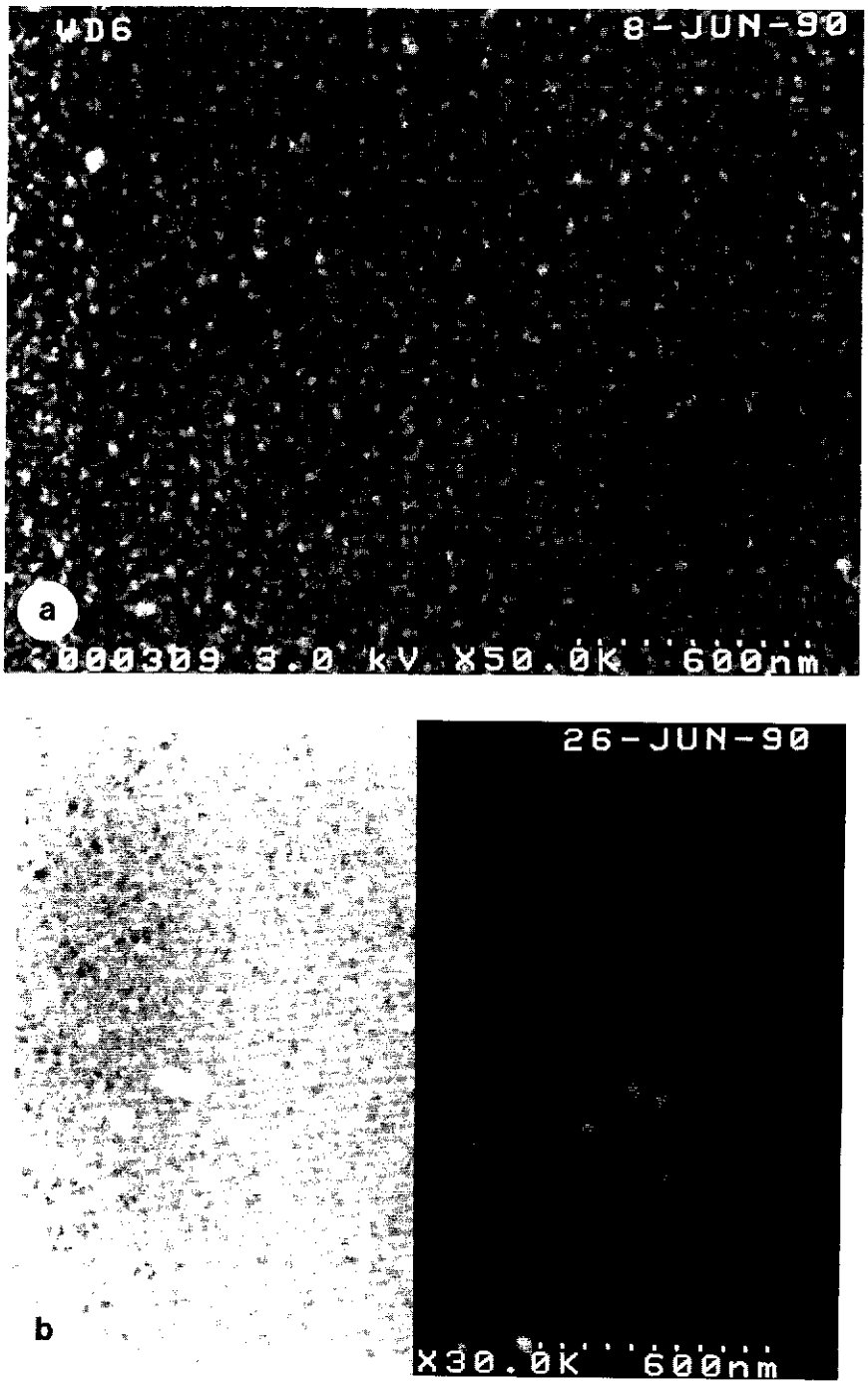

FIG. 3. (a) Scanning electron microscope image of silver colloid filtered onto the surface of an Anopore membrane with a pore size of 0.02 μm using syringe filtration. (b) Backscattering image of a surface prepared as in (a) with an original magnification of 30,000. The white particles in the backscattered image (right half) correspond to the white particles in the SEM image (left half), indicating that they are of different atomic mass than the aluminum oxide membrane.

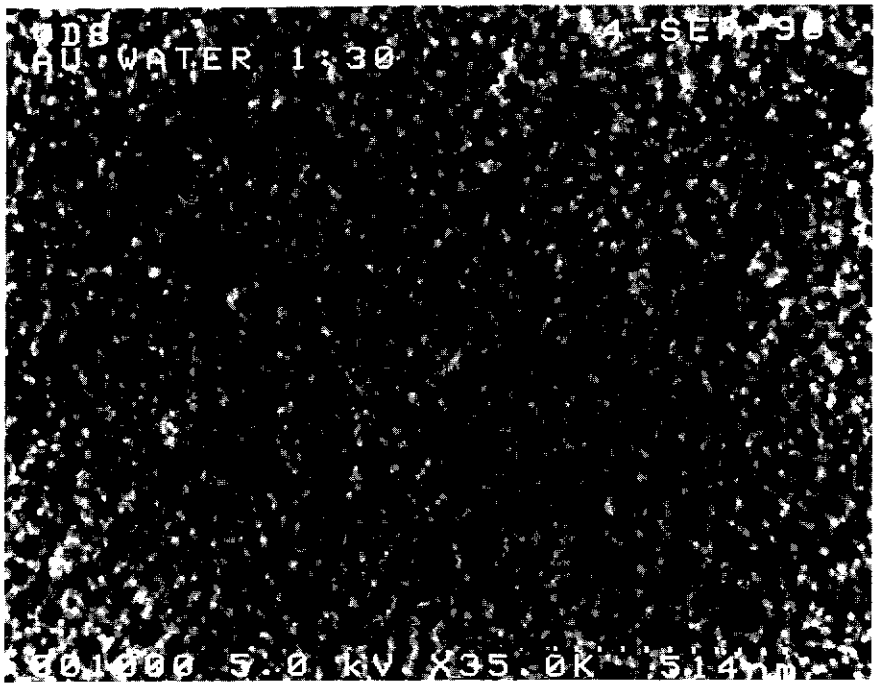

FIG. 4. Scanning electron microscope image of red gold colloid filtered onto an Anopore membrane with a 0.02-μm pore size using syringe filtration with an original magnification of 35,000.

the membrane. The images in Fig. 3 result from diluting the stock silver colloid solution prepared above 1:39 with water. Figure 4 shows an Anopore membrane coated with red gold colloid particles. The concentration of the stock gold colloid solution was also too high. The coated image results from a dilution of the stock colloid solution by 1:79 with water. Figure 5 shows the UV–Vis spectra of the silver colloid in solution (a) and on the surface of the membrane (b). Both spectra show an absorption maximum at 400 nm, and both have the characteristic yellow color of silver colloid particles. The presence of a shoulder at longer wavelengths in the spectrum of the filtered colloid indicates the presence of particles on the surface with nonspherical geometries (31), most likely small aggregates of particles which formed during the filtration process, similar to those observed in the backscattering image in Fig. 3b. No attempt was made to optimize the colloid concentration or the filtration rate for this measurement.

As noted in the procedure description above, it was necessary to keep the membrane wet during the collection of the absorption spectra so that light below 400 nm could be collected. Since the absorption maximum of aqueous colloidal silver is between 385 and 400 nm, the data below 400 nm are very important. The spectrum in Fig. 5 was taken by placing a wet blank membrane in front of the reference beam and placing a wet, freshly prepared membrane in front of the sample beam of the HP spectrometer. As the data are collected over a period of 10 s, the membranes begin to dry nonuniformly and some aggregation of the deposited colloid could occur. Thus it is uncertain if the "aggregation peaks" observed in Fig. 5 are a result of the colloid

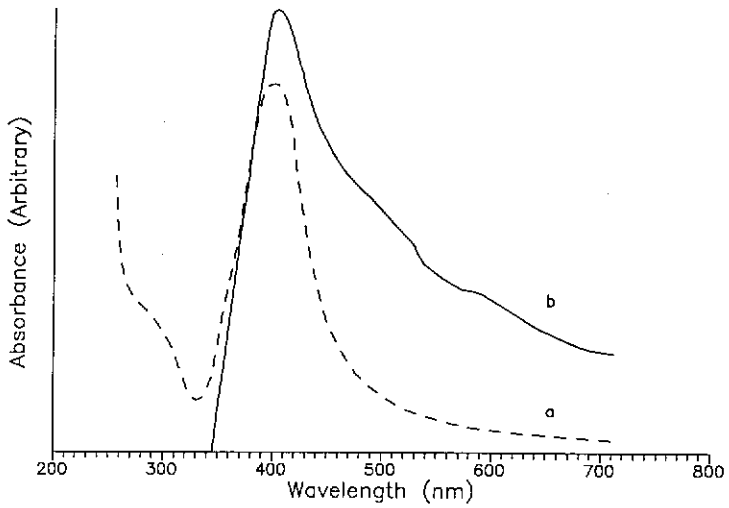

FIG. 5. Electronic absorption data of silver colloids. (a) Aqueous colloid; (b) colloid filtered onto an Anopore membrane (0.02-μm pore size) using an HP 8450 diode array spectrometer.

filtration process or of the nonuniform drying of the wet membrane during the data collection period. Placing the wet membrane between two quartz windows would not ensure that postfiltration aggregation was eliminated.

In order to look at this problem more carefully and to study the effects of the initial colloid concentration on the absorption intensity, a Perkin-Elmer Lambda-9 spectrophotometer with an integrating sphere was used. This is a single-beam instrument, so the background and sample spectra were collected separately and background subtraction was used to obtain the final spectra. Figure 6 shows a typical absorption spectrum of both a blank membrane and a membrane coated with silver colloid via syringe filtration. Both membranes were dry. The low absorbance of the colloid absorption relative to the background is readily evident. Thus, the results of the background subtraction must be observed with the realization that errors can be significant when the small colloid absorption signal is retrieved off of the large background absorption of the blank membrane. Figure 7 shows the result of dilutions of the stock silver colloid solution. The stock solution, referred to as the 30-ml solution, since 30 ml of the 5×10^{-3} M silver nitrate solution was used to prepare it, was diluted 1:9 with water. This "3-ml" solution is the highest concentration of silver used with the Perkin-Elmer instrument. The 3-ml solution was diluted 1:1, 1:4, and 1:9 with water to yield 1.5-, 0.6-, and 0.3-ml solutions, respectively. Figure 8 shows the result of a Beer's law plot for these spectra. These data represent the background subtracted absorbance values versus concentration. The background was estimated by drawing a base line between the minimum near 325 nm and the region for each spectrum to the right of the peak where the background was level (470, 480, 570, and 585 nm for the 0.3-, 0.6-, 1.5-, and 3.0-ml solutions, respectively). These data indicate that Beer's law is obeyed, with deviations occurring at the lowest concentration. The line drawn through the data represent a linear least-squares fit (with a correlation coefficient of 0.999) for the three largest concentrations. The deviation at the lowest concentration is not surprising, since the signal is the lowest there and is more likely to be affected by errors from the background subtraction.

The SERS activity of an Anopore membrane coated with silver colloid particles using the syringe filtration technique has been tested

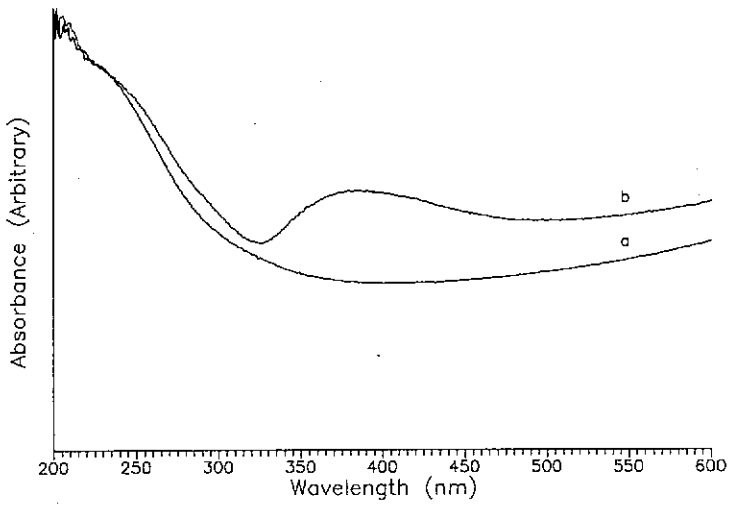

FIG. 6. Electronic absorption spectra of a silver colloid solution filtered onto an Anopore membrane (0.02-μm pore size). Data obtained are from reflected light collected in an integrating sphere of a Perkin-Elmer Lambda 9 spectrometer. (a) Blank membrane; (b) membrane with a 3-ml aqueous silver colloid filtered onto the surface.

with 9-aminoacridine (AA). The use of AA also allows the activity of this substrate to be compared to other substrates which have been prepared in this laboratory using different techniques (11). Figure 9 shows a spectrum of a 200-nl volume of a 300 ppb aqueous solution of AA using the 514.5-nm line from an argon ion laser. The laser power at the sample was 20 mW. The total amount of AA in the analyte spot, which was approximately 0.75

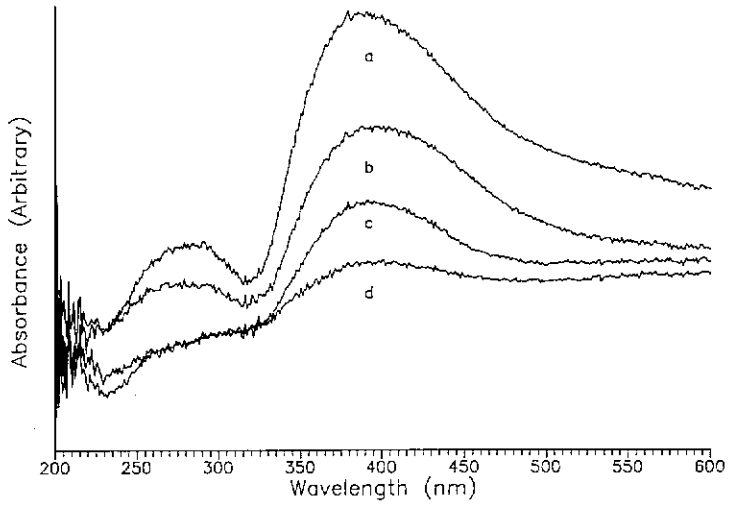

FIG. 7. Background corrected electronic absorption spectra of four dilutions of a 3-ml aqueous silver colloid filtered onto an Anopore membrane (0.02-μm pore size). Data obtained are from reflected light collected in an integrating sphere of a Perkin-Elmer Lambda 9 spectrometer. (a) 3.0-ml solution; (b) 1.5-ml solution; (c) 0.6-ml solution; (d) 0.3-ml solution.

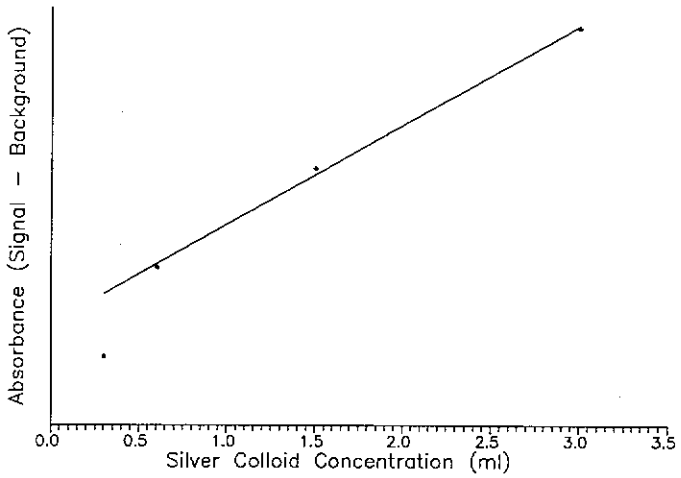

FIG. 8. Beer's law plot of the data in Fig. 7. Data are background subtracted absorbances, where the background at the peak was estimated by a linear fit of points to either side of the peak. The best fit line is plotted for the data corresponding to a, b, and c of Fig. 7.

mm in diameter, is 60 pg, of which approximately 25% is illuminated by the laser beam using the macroscopic sample chamber. Under ideal conditions, little or no SERS signal should be detected, since the 514.5-nm line is outside the absorption profile of the unaggregated silver hydrosol. However, as mentioned above and shown in Fig. 5, there is some apparent aggregation on the membrane with the silver concentrations used for both the absorption spectrum and the SERS spectrum in Fig. 9. Careful study of the absorption spectrum indicates that a maximum in the data occurs near 515 nm. The colloid aggregates on the surface which have longitudinal resonances in this region are likely the source of the SERS signal observed (32). Little SERS intensity has been observed at 514 nm for

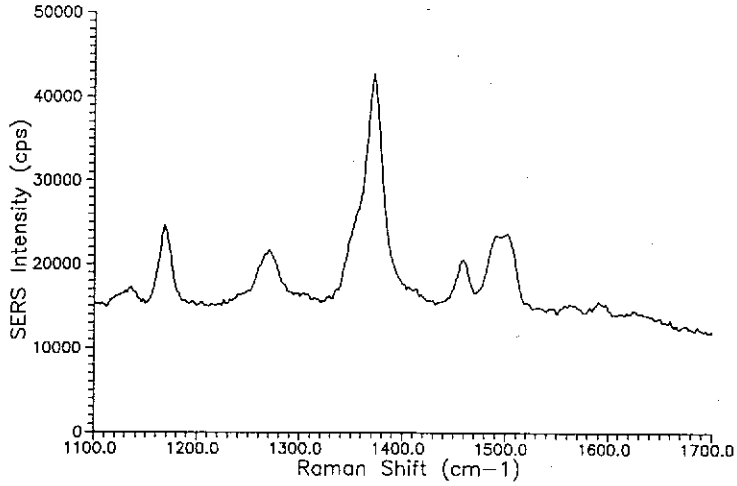

FIG. 9. SERS spectrum from a 0.2-μl sample of a 300 ppb AA solution on an Anopore membrane coated with silver colloid particles. The substrate was prepared by filtering 2 ml of a 3-ml silver colloid solution onto the surface.

membranes coated with more dilute silver solutions, where aggregation is less apparent (Fig. 3). The data from the Perkin-Elmer instrument are not sufficient to determine if the longer wavelength features disappear as the colloid concentration is decreased.

The ability to prepare a surface of noninteracting spherical metal colloid particles allows for a direct comparison of experimental SERS data with theoretical calculations. The filtered colloid substrate does not conform precisely to the electrodynamic model because of effects of the filter. Thus comparisons with calculations will not be exact, but the trends with respect to composition, size, and shape should be comparable with theoretical calculations. This will allow theoretical optimization of parameters such as the particle size and metal to use for optimum SERS enhancement at a given wavelength, making theoretical substrate design feasible.

SUMMARY

The use of colloid filtration in the preparation of SERS-active substrates can yield uniform, monodisperse layers of colloidal particles. Cellulose and other depth capture membranes are not appropriate supports for this technique due to their inefficient retention and the difficulties of probing the metal particles within the depth of the membrane. The PCTE surface capture membrane is also an inappropriate support for syringe filtration since the flow rates are too slow to permit smooth, uniform filtration in a reasonable amount of time and due to poor absorption of analyte aliquots. A number of characteristics of the Anopore membranes make them an ideal support for the preparation of SERS-active substrates by colloid filtration. The ability to capture virtually 100% of all particles greater than the rated pore size makes them highly efficient for isolating colloidal particles. The surface capture property of the Anopore membrane allows all the colloid particles trapped to be illuminated by the probe beam. The fast flow rate allows for the rapid preparation of SERS substrates. The chemical inertness of the membranes to many organic solvents also allows nonaqueous colloids to be used. The membranes are transparent to visible light with wavelengths longer than 400 nm. The thickness of the membranes is small enough, typically 60 μm, that the electronic absorption of the colloids on the membrane can be measured directly by standard UV/Vis instruments. The membranes can be made transparent to wavelengths shorter than 400 nm by wetting them with water. The ability to prepare a surface of noninteracting spherical metal colloid particles allows for a direct comparison of experimental SERS data with theoretical calculations. This will allow theoretical optimization of parameters such as the particle size and metal to use for optimum SERS enhancement at a desired wavelength, making theoretical substrate design feasible. Finally, the ability to reproducibly prepare a SERS-active substrate in under 5 min with only a few dollars in equipment makes this a viable technique for routine analytical use.

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