A surfactant stabilized sol as substrate for surface enhanced Raman scattering spectroscopy

Information regarding sols dates back to the time of Michael Faraday. Since then, many applications have enriched the world of sol science. Among the innumerable applications of sol systems, the use of silver sol deserves special mention[1]. The discovery of surface enhanced Raman scattering (SERS) on roughened silver surface, in 1973, placed silver in a unique position in the branch of SERS analysis[2]. Thus, during the last two decades scientists have tried to produce silver sol with reproducible particle size using polymers[3,4], ligands[5] and surfactants[6,7] to observe SERS with silver sol. SERS study requires an active substrate for always and the order of enhancement of SERS intensity depends on the micro structure and proximity of the individual particle of the substrate[8,9]. As a result, researchers have unearthed yellow hydrosol, dull red organo sol, Creighton sol[10], etc. But to obtain SERS spectra on silver sol one has to obtain either fractal aggregates[8] or string-like assemblies[9] of sol particles instead of isolated spherical particle. It has been predicted that linear aggregation, if at all produced, would be a green sol[9]. We report here the preparation of a stable green sol of silver with a diameter of ~ 100 nm. The diameter of the sol was determined both by TEM and coulter (N4) counter using light scattering through an angle of 90°. We obtain the green sol system through electrolyte-induced controlled coagulation of surfactant stabilized yellow sol.

Appropriate amount of aqueous silver nitrate and sodium dodecyl sulfate (SDS) solution was mixed and to it was added sodium borohydride solution drop wise with constant shaking. Thus a yellow sol of silver was formed. After ~ 2 min, requisite amount of Na_2HPO_4 solution was added and shaken. A green sol would appear within 1–2 min. In a typical process 0.15 ml (0.01 mol dm^{-3}) $AgNO_3$ and 0.15 ml (0.01 mol dm^{-3}) SDS were mixed and the volume was made up to 3.0 ml. Then 0.1 ml (0.1 mol dm^{-3}) $NaBH_4$ was added to get yellow hydrosol (λ_{max} 400 nm). To this hydrosol 0.025 ml (1.0 ml dm^{-3}) Na_2HPO_4 was added and shaken. After 1–2 min, green sol (λ_{max} ~ 635 nm) appeared and was used as a stock solution for SERS analysis.

To obtain SERS of pyridine 1 ml of stock solution of green sol was taken in a 1 cm quartz cuvette and was mixed with an aqueous solution of pyridine. The final concentration of pyridine in the sol was 5 ppm. The spectrum was reproducible when recorded using the front side excitation technique[10] when examined with a 200 mW Kr laser source even at an interval of 24 h.

Once the sol system is produced, it does not deteriorate upon irradiation of 100–200 mW Kr laser power for 15–20 min unlike the common silver so systems devoid of surfactants. The laser exposed system with or without analyte can be stored for 10 days without any degradation under ambient condition.

Almost all colloidal substrates which are in vogue have poor stability and reproducibility towards SERS studies. On the other hand, our sol system has remarkable reproducibility and stability towards analytes and also laser illumination. This makes the reported sol system a better suited SERS substrate. However

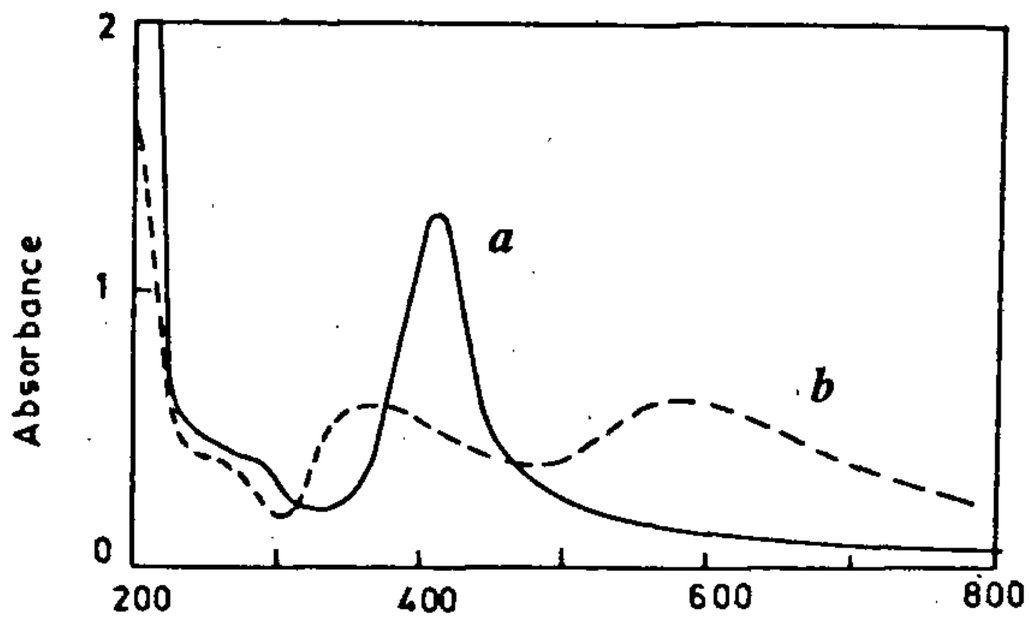

Figure 1. UV-visible spectra of (*a*) yellow hydrosol, (*b*) green sol.

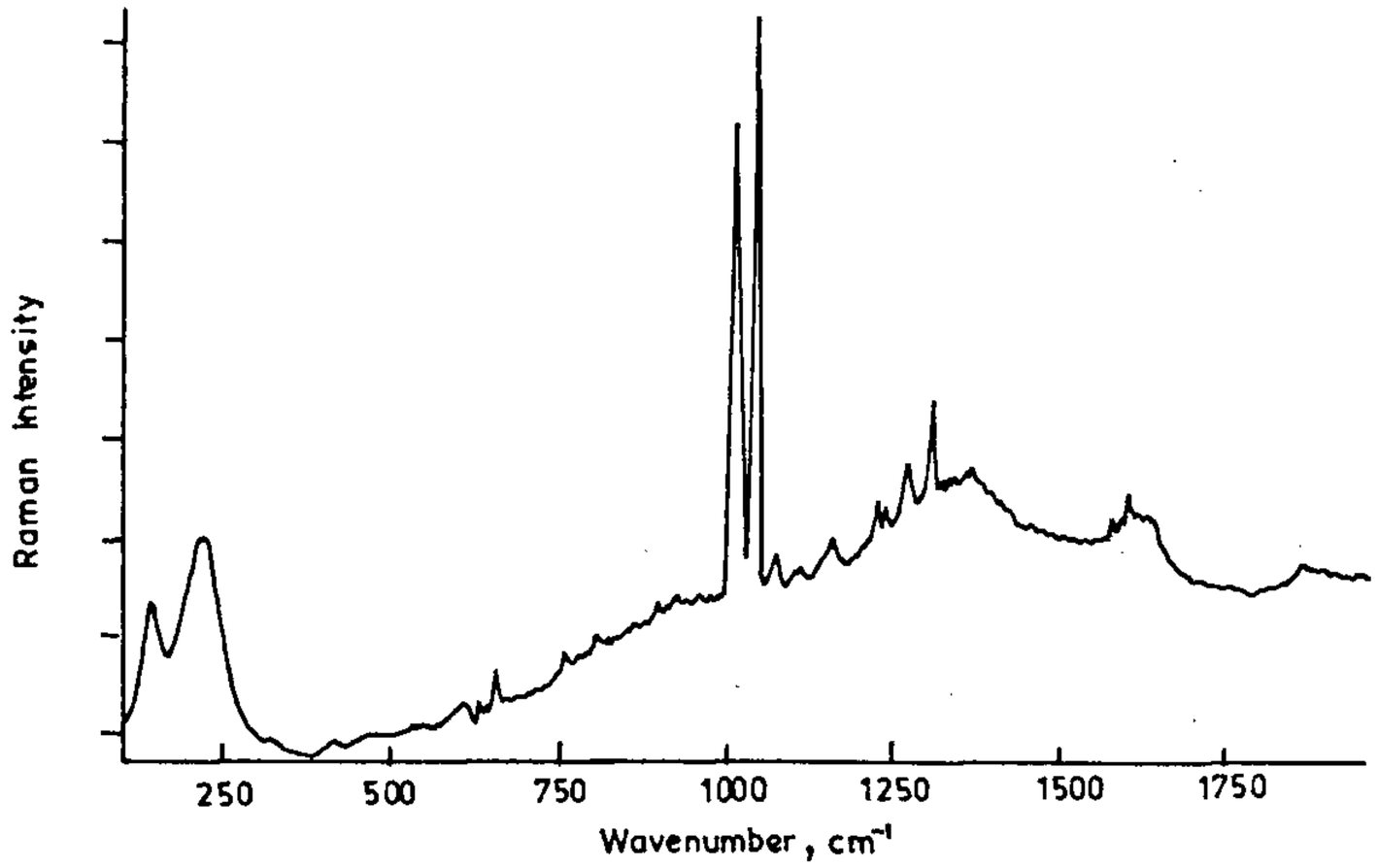

Figure 2. Surface enhanced Raman scattering spectrum of pyridine (5 ppm) adsorbed on the sol excited with 647.1 nm; Kr laser. Conditions: $[AgNO_3] = [SDS] = 5 \times 10^{-4}$ mol dm^{-3}; $[Na_2HPO_4] = 8 \times 10^{-3}$ mol dm^{-3}.

in our case the order of enhancement of SERS intensity due to pyridine is comparable to that of the best silver aggregates reported earlier.

It has been observed that Kr and He–Ne lasers enhance the SERS spectra of pyridine adsorbed onto the green sol, to the maximum extent and hence best suited for the excitation purpose unlike Ar laser. This is because the wavelength for green absorption due to fractal aggregates of silver resonates with the Kr and He–Ne lasers. The He–Ne laser which was available could generate only 20 mW power. Hence we have used Kr laser for its high power efficiency.

The SERS results were obtained in Prof. A. J. Creighton's laboratory at the University of Kent at Canterbury, England.

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Species identification of snake-head fishes by nuclear DNA RFLP: Its taxonomic implications

The snake-heads (genus *Channa*, family Channidae) are commercially important air-breathing fishes. They occur in freshwater marshes, swamps and ditches of the Indian sub-continent[1]. Three species of snake-head fishes namely, *Channa punctatus*, *C. striatus* and *C. gachua*, which occur sympatrically in this locality were studied. The natural populations of these species show a declining trend owing to (i) the modification and encroachment of their habitat by developmental activities, and (ii) their over-exploitation. Inter-specific hybridization has been attributed to be yet another cause of decline of populations in some species of fishes like apache trout[2,3]. Environmental changes and habitat modification have led to increased frequency of hybridization in various groups of fishes including Cyprinidae, Salmonidae, Cyprinodontidae, Cichlidae, Catostomidae, Escocidae, Poeciliidae, Antherinidae, Centrarchidae and Percidae[4].

Although these species are sympatric, there is no report on hybrids. Unlike cyprinids, salmonids and other groups of fishes, are these species not susceptible to natural hybridization (due to the stringent reproductive isolation mechanism!) or has the natural hybridization between these species escaped the notice in the absence of suitable markers that would have helped in identifying the hybrids? This study was undertaken to find a species-specific molecular marker, that might allow detection of natural hybridization between these species.

Restriction fragment length polymorphism (RFLP) of ribosomal rRNA gene has been useful for systematic analysis in fishes at the species level[5,6], species and hybrid identification in carps and catfishes[7,8]. In the hybrids, the restriction fragments of ribosomal rRNA gene were found to inherit biparentally[7,8]. Therefore,

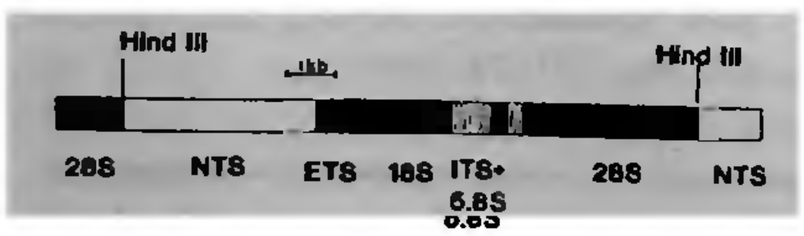

Figure 1. A diagrammatic representation of ribosomal RNA gene structure. The 5.8S, 18S and 28S are the regions coding for 5.8S, 18S and 28S rRNAs. ETS, External transcribed spacers; ITS, Internal transcribing spacer; NTS, Non-transcribing spacer.

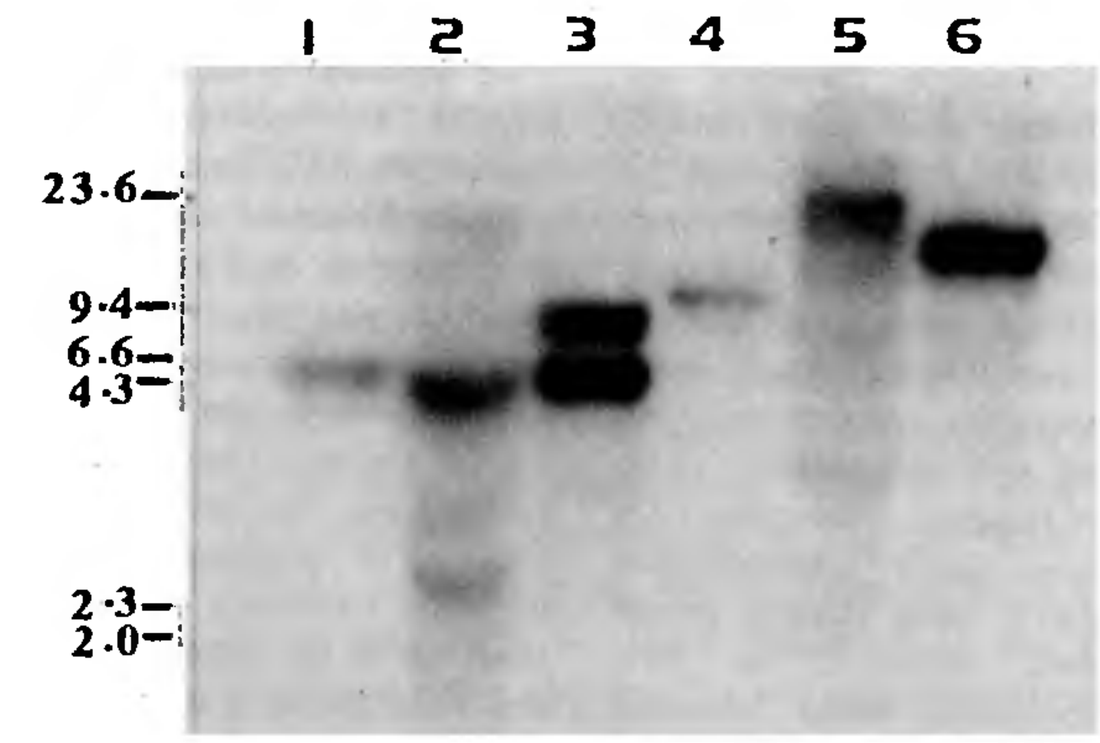

Figure 2. Ribosomal RNA gene RFLP in *Channa*. Restriction enzyme digested nuclear DNA was subjected to electrophoresis and Southern hybridized using labelled pXlr 101 probe. *Eco*RI digests (lanes 1–3): 1, *Channa punctatus* (Cp); 2, *C. striatus* (Cs); 3, *C. gachua* (Cg). *Hin*dIII digests (lanes 4–6): 4, Cp; 5, Cs; 6, Cg DNA. The approximate positions of the lambda *Hin*dIII size markers are indicated.