SURFACE-ENHANCED RAMAN SCATTERING (SERS) SPECTROSCOPY WITH BOROHYDRIDE-REDUCED SILVER COLLOIDS: CONTROLLING ADSORPTION OF THE SCATTERING SPECIES BY SURFACE POTENTIAL OF SILVER COLLOID

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Dedicated to Professor Otto Wichterle on the occasion of his 80th birthday.

Formation of the Ag colloid/adsorbate SERS-active systems (upon adsorption of the selected adsorbates on the surface of Ag colloidal particles) as a function of (i) $NaBH_4$ to $AgNO_3$ molar ratio in the preparation protocol of Ag colloid, and (ii) aging of the colloid has been investigated by Surface-enhanced Raman scattering (SERS) spectroscopy. Oligomeric synthetic polypeptides, bovine serum albumin, phosphate coadsorbed with CuTMePyP [copper(II) derivative of 5,10,15, 20-tetrakis-(*N*-methylpyridinium-4-yl)porphyrin chloride] and borates in systems with N-containing bases were selected as model adsorbates. Both (i) a decrease of the $NaBH_4$ to $AgNO_3$ molar ratio upon preparation and (ii) aging of Ag colloid affect adsorption of the adsorbates and, consequently, their SERS spectra, in the same manner. Aging of Ag colloid is thus viewed as a slow hydrolysis of BH_4^- anions. The actual concentration of BH_4^- in the system is identified as the most important factor controlling adsorption of all the selected adsorbates on the surface of Ag colloid. As this factor can be related to the surface potential, the conditions controlling adsorption of the selected adsorbates are specified in terms of a more negative and/or more positive surface potential of Ag colloidal particles. A more positive surface potential promotes adsorption of polypeptides, bovine serum albumin and phosphate while observation of spectral features of borates in the SERS spectra of N-containing bases in alkaline solutions is conditioned by a more negative surface potential.

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Surface-enhanced Raman scattering (SERS) spectroscopy takes advantage of the $10^6 - 10^7$ times enhancement of Raman scattering by molecules and/or ions adsorbed on roughened surfaces of metallic Ag, Cu or Au (refs[1,2]). Among a variety of SERS-active surfaces, Ag colloids are most attractive for practical applications of SERS spectroscopy to chemical analysis[3], to investigation of laser dyes[4] and biomolecules[5] etc., chiefly due to the relatively easy and inexpensive way of preparation. At least a partial aggregation of Ag colloidal particles is necessary for observation of an effective surface enhancement of Raman scattering ($10^4 - 10^5$) by the adsorbate located at the surface of the particles, as follows from the comparison between the SERS excitation profiles of the adsorbates and the surface plasmon absorption curves of the aggregated Ag colloids[6]. It is now generally understood that aggregation is induced by adsorption of a particular adsorbate onto the originally isolated Ag colloidal particles which is accomplished under certain conditions. However, the main problem in SERS spectroscopy with Ag colloids is the fact that the conditions under which adsorption occurs have to be tailored for each particular chemical type of adsorbates. This problem is now in focus of interest particularly in SERS spectroscopy of biomolecules. It is our opinion that the differences in the preparation protocols of Ag colloid/biomolecule SERS-active systems may substantially influence the SERS spectra of a particular biomolecule.

Surveying the previous SERS studies published by us[7-9] and others[10-13] we find out that there are generally two ways how to promote adsorption of a particular adsorbate on the surface of the borohydride-reduced Ag colloid: *1*) aging of Ag colloid (i.e. storage in dark for a certain period of time)[9,11], and/or *2*) changes in the preparation protocol of Ag colloid[7-10,12,13]. The latter approach appears to be most effective when the ratio of $NaBH_4$ to $AgNO_3$ in the preparation protocol is altered[7-9,13].

While the potential of Ag electrode can be continuously tuned by the applied voltage, a similar control over the potential of the Ag colloid can be achieved only by means of an external redox system, as demonstrated in ref.[14]. This approach, however, is rather tedious for practical applications and, moreover, involves a danger of chemical interaction between the adsorbate and the components of the external redox system.

In this paper we concentrate our effort on answering the following questions: *1*) Is there a common, rational explanation of the role of both aging of Ag colloid and changing the $NaBH_4$ to $AgNO_3$ molar ratio in the preparation protocol of Ag colloid? *2*) Is there a possible chemical way of tuning the surface potential of the borohydride-reduced Ag colloid? We have selected several biologically important molecules as proteins, synthetic polypeptides, porphyrins and nitrogen-containing bases as model systems for the systematic investigation of the effect of both empirically established factors mentioned above on adsorption of a particular molecule on the surface of Ag colloidal particles as well as on its SERS spectrum.

EXPERIMENTAL

Materials

Analytical grade chemicals and redistilled deionized water were used for all sample preparations. $NaBH_4$ (Merck) and $AgNO_3$ (Lachema) were used for preparations of silver colloids. L-Lysine-containing oligomeric poly(di-, tri-, tetrapeptides) with an average degree of polymerization about 30: poly(L-lysyl-L-alanine) [Lys-Ala] M_w = 5 470, poly(L-lysyl-L-alanyl-L-alanine) [Lys-Ala-Ala] M_w = 5 470, poly(L-lysyl-L-alanyl-L-alanyl-L-alanine) [Lys-Ala-Ala-Ala] M_w = 6 700, poly(L-lysyl-glycyl-glycine) [Lys-Gly-Gly] M_w = 6 800 and poly(L-lysyl-L-leucyl-L-alanine) [Lys-Leu-Ala] M_w = 7 900 were synthesized in the Institute of Organic Chemistry and Biochemistry. Bovine serum albumin fraction V, {$M_w \approx$ 67 000}, powder (99%, fatty acid and globulin free) was obtained from Sigma. The copper(II) derivative of 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin chloride was synthesized according to ref.[15] from 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrine tetratosylate (Aldrich). Nitrogen-containing bases: 2,2′-bipyridine (bpy) and 2,2′-bipyrimidine (bpm) were obtained from Johnson Mattey.

Preparation Procedures

Preparation of Ag colloids. Ag colloids were prepared by reduction of $AgNO_3$ by $NaBH_4$. Three types of Ag colloids characterized by different values of the $NaBH_4$ to $AgNO_3$ molar ratio have been prepared. In the order of the increasing $NaBH_4$ to $AgNO_3$ molar ratios, the Ag colloids are denoted as CI, CII and CIII. All colloids were prepared according to a common preparation procedure described below. The volumes and concentrations described in the preparation protocol are the characteristics of Ag colloid CI which is designed in this paper for adsorption of polypeptides and proteins. The complete survey of volumes and concentrations of the aqueous solutions of $NaBH_4$ and $AgNO_3$ used for preparations of the colloids is listed in Table I.

Preparation procedure: $1.1 \cdot 10^{-3}$ M aqueous solution of $NaBH_4$ prepared by dissolving 3.1 mg of $NaBH_4$ in 75 ml of water was cooled to 2 °C in an ice-bath. 9.0 ml of $2.2 \cdot 10^{-3}$ M solution of $AgNO_3$ precooled to ca 10 °C was added dropwise to the aqueous solution of $NaBH_4$ upon constant stirring. Stirring continued without interruption for another 45 min to adjust the colloid to laboratory temperature. The resulting silver colloids were bright yellow with the λ_{max} of the surface plasmon absorption at 390 nm and were stable for at least 2 months without addition of stabilizers.

TABLE I
Characteristics of silver colloids

Ag colloid	$NaBH_4$			$AgNO_3$		$NaBH_4 : AgNO_3$ molar ratio
	m, mg[a]	*V*, ml[b]	*c*, mol l^{-1}	*V*, ml[c]	*c*, mol l^{-1}	
CI	3.1	75	$1.1 \cdot 10^{-3}$	9	$2.2 \cdot 10^{-3}$	4.1
CII	3.5	75	$1.2 \cdot 10^{-3}$	9	$2.2 \cdot 10^{-3}$	4.7
CIII	3.5	75	$1.2 \cdot 10^{-3}$	7.5	$2.2 \cdot 10^{-3}$	5.6

[a] The amount of $NaBH_4$ dissolved in 75 ml of water (finally used for colloid preparation); [b] the volume of $NaBH_4$ solution used; [c] the volume of $AgNO_3$ solution used.

Preparation of SERS-Active Systems

Ag-colloid/polypeptide. To 4 ml of Ag colloid CII aged for 30 days and/or CI, 40 μl of 1 . 10^{-4} M aqueous solution of polypeptide were added to form the SERS-active system with a final concentration of polypeptide 1. 10^{-6} mol l^{-1}.

Ag colloid/albumin(aq). 250 μl of aqueous solution of albumin prepared by dissolving of 0.5 mg of albumin in 5 ml of water were added to 4 ml of Ag colloid CI. The concentration of albumin in the system is 25 μg per 4 ml.

Ag colloid/(phosphate + CuTMePyP) systems. The SERS-active systems were prepared by addition of 40 μl of 1 . 10^{-4} M solution of CuTMePyP in 1 . 10^{-2} M phosphate buffer to 4 ml of the Ag colloids CI, CII, CIII aged for one day and to Ag colloid CII aged for 1 month. The concentration of the porphyrin in the SERS-active system is 1 . 10^{-6} mol l^{-1} , concentration of phosphate is 1 . 10^{-4} mol l^{-1}.

Ag colloid/(N-containing base) systems. The Ag colloid/2,2′-bipyridine, Ag colloid/2,2′-bipyrimidine SERS-active systems were prepared by addition of 10 μl of 1 . 10^{-2} M aqueous solution of bpy and/or bpm to 4 ml of Ag colloid CII. The concentration of the N-containing base in the SERS-active system was 2.5 . 10^{-5} mol l^{-1}.

Apparatus

Surface-enhanced Raman scattering spectra of systems with albumin, synthetic polypeptides and CuTMePyP were measured with a modular spectrometer consisting of a high resolution monochromator THR 1500 (Jobin–Yvon), an additional compact 1/8-m monochromator (Oriel), a photon counting system assembled from NIM moduls (Tennelec and Ortec), and a computer compatible with IBM AT (ref.[16]). Spectra were accumulated with the spectral slit width 6 cm^{-1}, wavenumber step 1 cm^{-1} and the accumulation time 1 s per the spectral point. Excitation was provided with 514.5-nm line of an argon ion laser ILA 120 (Zeiss, Jena). The laser power at the sample was 150 mW. SERS spectra of N-containing bases were obtained with a DILOR XY multichannel Raman spectrometer in conjunction with a Spectra Physics model 2016 argon ion laser. The 514.5-nm line was used for excitation (laser power at the sample 100 mW).

RESULTS AND DISCUSSION

Relationship between Aging of Ag Colloid and Changing the $NaBH_4$ to $AgNO_3$ Ratio in Its Preparation Protocol

As a first step to the investigation of the relationship between aging of Ag colloid and changing of the $NaBH_4$ to $AgNO_3$ molar ratio in its preparation protocol, we formulate a hypothesis on the mechanism of aging of Ag colloid: In the preparation protocol of borohydride-reduced Ag colloid, a large excess of $NaBH_4$ over $AgNO_3$ is generally used[6 – 13,17]. The BH_4^- anion is known to undergo a rather complicated sequence of hydrolytic processes in aqueous solution[18]. The final products of several hydrolytic steps are supposed to be borates[18]. As spectral features of borates were observed in the SERS spectra of the particular adsorbates under certain conditions [e.g. 2,2′-bipyrimidine[19] adsorbed on Ag colloid prepared according to ref.[6] and propylamine at pH ~ 12 (ref.[20])] it is highly probable that the hydrolytic process analogous to that in solution is undergone also by the BH_4^- anions in system with Ag colloid. Thus, by aging of Ag colloid, the concentration of BH_4^- in the system decreases. Provided that this view of aging of Ag colloid is correct, decreasing the $NaBH_4$ to $AgNO_3$ ratio should affect adsorption and SERS spectra of a particular adsorbate in the same manner as aging of the colloid. This deduction is tested by the experiments described in the following sections.

Adsorption of Polypeptides and Proteins on Ag Colloidal Particles

First, the conditions of formation of the Ag colloid/synth. polypeptide SERS-active system have been systematically investigated. The results show that the routinely prepared borohydride-reduced Ag colloid CII (ref.[17]) has to be aged for at least three weeks prior to addition of the polypeptide. A typical SERS spectrum of the SERS-active system prepared by addition of the aqueous solution of Lys-Ala-Ala to Ag colloid CII aged for 30 days [denoted Ag colloid CII (30 days)/Lys-Ala-Ala system] is shown at Fig. 1 (spectrum *a*).

Following the above hypothesis, we have designed and prepared the Ag colloid CI characterized by the lower (4.1) $NaBH_4 : AgNO_3$ molar ratio in comparison to the (4.7) value of this ratio in CII. Testing of Ag colloid CI for adsorption of polypeptides has shown that just one day of aging of Ag colloid CI is sufficient for preparation of a SERS-active Ag colloid CI/polypeptide system. SERS spectrum of the Ag colloid CI (4 days)/Lys-Ala-Ala is shown as an example in Fig. 1, spectrum *b*. SERS spectra in Fig. 1 thus demonstrate that decreasing the $NaBH_4 : AgNO_3$ molar ratio in the preparation protocol of Ag colloid CII (resulting into CI) is equally efficient for adsorption of polypeptides as aging of Ag colloid CII. This analogy between the aged Ag colloid CII and Ag colloid CI corroborates our original presumption that after aging for a certain period of time, the Ag colloid contains less BH_4^- than the freshly prepared one, as a certain fraction of BH_4^- anions hydrolyzed to borates (via several intermediates).

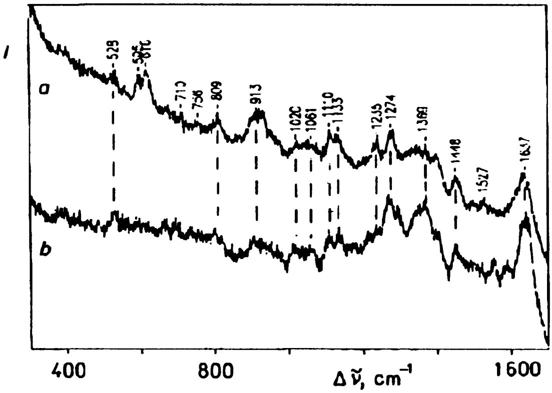

FIG. 1
SERS spectra of Ag colloid/poly(L-Lys-L-Ala-L-Ala) systems: *a* Ag colloid CII (30 days)/Lys-Ala-Ala; *b* Ag colloid CI (4 days)/Lys-Ala-Ala. (Excitation 514.5 nm, power 150 mW)

Preparation of a silver colloid characterized by the even lower $NaBH_4 : AgNO_3$ molar ratio (3.9) has been attempted. The resulting Ag colloid was stable only for a few hours and was thus unsuitable for SERS experiments. This result indicates that when the concentration of BH_4^- anions in the freshly prepared Ag colloid is lower than the particular limit, the isolated Ag colloidal particles are not sufficiently stabilized and tend to aggregate spontaneously into macroscopic particles of metallic silver. The key role of BH_4^- anions in stabilization of the isolated particles of the borohydride-reduced Ag colloid thus emerges as a result of the above experiments.

Adsorption of a globular protein bovine serum albumin on the surface of Ag colloidal particles has been investigated under the conditions analogous to adsorption of synthetic polypeptides, i.e. using Ag colloids CII and CI. The results show that adsorption of albumin and formation of the Ag colloid/albumin SERS-active system can be accomplished only for Ag colloid CI and only after its aging for about 1 month. It is thus evident that in this particular case, aging is the only possibility how to reduce the concentration of BH_4^- anions on the surface of Ag colloidal particles, as the alternative way of reducing the amount of $NaBH_4$ in preparation protocol causes instability of the resulting Ag colloid. The SERS spectrum of the Ag colloid CI (30 days)/albumin is shown in Fig. 2.

The SERS spectrum of albumin in Fig. 2 consists of a variety of rather broad, but well resolved bands. The band at 1 624 cm^{-1} has its counterparts in the SERS spectra of several other species, as e.g. poly(Lys-Ala-Ala) in Fig. 1 and Triton X-100 in ref.[8], and, most importantly, in SERS spectrum of albumin reported by Ahern and Garrell[13]. These bands are attributed to the deformation vibrational mode of H_2O molecules associated with the adsorbate. In refs[8,13] the assignment of this band to δ(HOH) mode of H_2O has been confirmed by experiments with D_2O, in which the typical deuteration shift of this band to ca 1 210 cm^{-1} was observed.

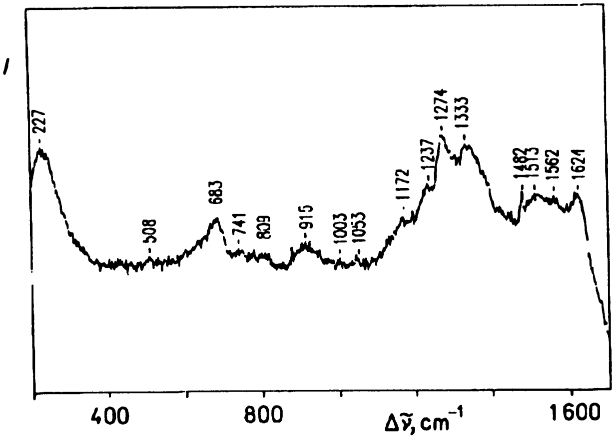

FIG. 2
SERS spectrum of Ag colloid/albumin system: Ag colloid CI (30 days)/albumin

Interestingly, the band of H_2O at 1 640 cm^{-1} was the only spectral feature which Ahern and Garrell[13] observed in the SERS spectrum of albumin 1 h and 24 h after preparation of the SERS-active system. The rather rich spectrum in Fig. 2 is thus in contrast with their observation. However, a SERS spectrum of albumin similar to that in Fig. 2 was obtained in ref.[13] seven days after the SERS-active-system preparation. The differences between the SERS spectrum of albumin reported in this paper and those in ref.[13] thus have their origin in a different time evolution of albumin adsorption. In particular, the comparison of SERS spectra of albumin obtained 24 h after system preparation (i) in this paper (Fig. 2), and (ii) in ref.[13] show that under the conditions of our experiment, albumin is in contact with the surface of Ag colloid both via H_2O molecules associated with the protein, and via the protein itself. In contrast to that, under the conditions described in ref.[13], albumin contacts the surface only via its hydration shell while the direct contact between the protein and the Ag colloidal surface is established only 7 days after preparation of the system.

To pinpoint the source of the different time evolution of albumin adsorption, we focus our attention on the comparison of the preparation protocols of the SERS-active systems in ref.[13] and in this paper. We readily find the chief difference in the preparation protocol of Ag colloid. While Ag colloid CI (Table I) was used for adsorption of albumin in this paper, a silver colloid analogous to CIII, i.e. with a substantially higher $NaBH_4$: $AgNO_3$ molar ratio was used in ref.[13]. It is thus evident that the $NaBH_4$: $AgNO_3$ molar ratio used for preparation of Ag colloid dramatically influences adsorption of albumin on colloidal surface. SERS spectra of both the polypeptides and albumin are assigned and discussed in detail in our forthcoming papers[21,22].

Coadsorption of Phosphate and CuTMePyP on Ag Colloidal Particles

Phosphate buffer is commonly used as a solvent for biomolecules, porphyrins in particular. In our preceding papers[7,23] we have shown that phosphate plays an important role of the preaggregation agent in the Ag colloid/phosphate/$TPPC_4$ system [$TPPC_4$ = 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin] and its presence in the system is the condition necessary for the SERS-active-system formation. On the other hand, in the case of Cu(II)TMePyP [Cu(II)TMePyP = copper(II) derivative of 5,10,15,20-tetrakis-(*N*-methylpyridinium-4-yl)porphyrin chloride] which, owing to the pyridinium-yl substituents, is readily adsorbed on the surface of Ag colloidal particles, preaggregation of Ag colloid by phosphate is not required. Indeed, the simultaneous presence of spectral bands of both CuTMePyP and phosphate in the SERS spectra of the Ag colloid/(phosphate ($1 . 10^{-4}$ M) + CuTMePyP ($1 . 10^{-6}$ M)) system shows that phosphate is coadsorbed with the porphyrin on the surface. This is in contrast to the systems with phosphate and $TPPC_4$, in which, under the same conditions, phosphate is completely replaced by the porphyrin on the surface.

As the broad bands of phosphate observed at 560 – 570 cm^{-1} and approximately 930 cm^{-1} interfere partially with the bands of porphyrin, their elimination or, at least suppression, is required. However, phosphate bands cannot be eliminated from the SERS spectra simply by omitting the phosphate buffer in the system preparation, as it is of importance for dissolution of the porphyrin. Therefore, we investigate the possibility to affect (negatively) adsorption of phosphate on the surface by using Ag colloids with the different $NaBH_4$: $AgNO_3$ molar ratios, i.e. CI, CII, CIII. Following the above hypothesis, we try to establish whether there is an analogy between adsorption of phosphate on the aged Ag colloid CII and the freshly prepared Ag colloid CI. The SERS spectra of the Ag colloid/(phosphate + CuTMePyP) systems with the freshly prepared Ag colloids CI, CII, CIII and the CII colloid aged for 1 month are shown in Fig. 3: spectrum *a*: CIII (1 day)/(phosphate + CuTMePyP); spectrum *b*: CII (1 day)/(phosphate + CuTMePyP); spectrum *c*: CII (1 month)/(phosphate + CuTMePyP) and spectrum *d*: CI (1 day)/ (phosphate + CuTMePyP). As our main goal is to investigate the conditions under which the SERS-signal of phosphate is suppressed with respect to that of the porphyrin, we compare the intensities of the phosphate bands in each of the SERS-active-system spectra to the 390-cm^{-1} band of porphyrin. The spectra in Fig. 3 are thus arranged in the increasing order of the relative-intensity ratios of the ca 560-cm^{-1} band

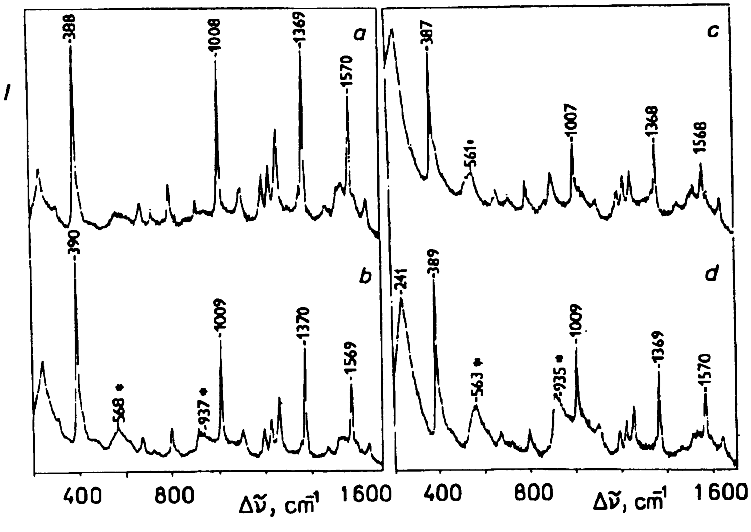

FIG. 3

SERS spectra of Ag colloid/(phosphate + CuTMePyP) systems: *a* Ag colloid CIII (1 day)/(phosphate + CuTMePyP); *b* Ag colloid CII (1 day)/(phosphate + CuTMePyP); *c* Ag colloid CII (1 month)/ (phosphate + CuTMePyP); *d* Ag colloid CI (1 day)/(phosphate + CuTMePyP). The characteristic bands of phosphates are marked by asterisks of phosphate to the 390-cm^{-1} band of CuTMePyP. The particular values are: 0.15 for spectrum *a*; 0.42 for *b*; 0.65 for *c*; and 0.9 for *d*. Thus in spectrum *a* of the system with Ag colloid CIII, SERS signal of phosphate is very weak and does not interfere with the bands of porphyrin. The signal of phosphate then increases systematically in order CIII (spectrum *a*) < CII (spectrum *b*) < CI (spectrum *d*) for systems with the 1 day aged Ag colloids.

The 0.65 value of the relative intensity ratio of phosphate to the porphyrin obtained for spectrum *c* of the system with the CII colloid (1 month) falls within the 0.42 and 0.9 values of this ratio obtained for freshly prepared CII and CI colloids, respectively. Aging of Ag colloid CII has a similar effect on adsorption of phosphate as using a freshly prepared Ag colloid CI.

The following conclusions are drawn:

1) Adsorption of phosphate on the surface of Ag colloidal particles in the Ag colloid/(phosphate+CuTMePyP) SERS-active system is dramatically affected by the value of the $NaBH_4$: $AgNO_3$ molar ratio used for preparation of the particular Ag colloid. Adsorption of phosphate is promoted by the low BH_4^- concentration on the surface of Ag colloidal particles as observed for Ag colloid CI. On the other hand, Ag colloid CIII with the highest content of BH_4^- anions is most suitable for suppression of the SERS signal of phosphate. This result is immensely important for SERS spectroscopy of porphyrins in systems with phosphate buffer. For example, we can now rationalize our earlier empirical observation[23] that to obtain a SERRS (Surface-enhanced resonance Raman scattering) spectrum of the porphyrin, Ag colloid CIII has to be used for preparation of the Ag colloid/phosphate/$TPPC_4$ system in which phosphate acts as a pre-aggregation agent. The surface potential of the Ag colloid CIII enables only a week bonding interaction between the phosphate and the Ag surface thus promoting the replacement of the phosphate by the porphyrin on the surface.

2) The analogous effect of aging of Ag colloid (CII) and decreasing the $NaBH_4$: $AgNO_3$ molar ratio (CII → CI) on adsorption of phosphate is proved. In both cases, adsorption of phosphate is promoted.

Interference of Borates in SERS Spectra of N-Containing Bases

SERS-spectral features of borates, namely three rather broad bands at 615, 923 and 1 380 cm^{-1} have been observed in the SERS spectrum of 2,2′-bipyrimidine (bpm)[19]. Moreover, Sbrana et al.[19] suggested that the bands at 615, 930 and 1 390 cm^{-1} observed in the SERS spectrum of 2,2′-bipyridine (bpy) and attributed to byproducts by Kim et al.[24] are in fact the bands of borates. Both bpm[19] and bpy[24] were investigated in the system with Ag colloid prepared according to ref.[6]. In the preparation protocol of this particular colloid, the $NaBH_4$: $AgNO_3$ molar ratio is 6.0, i.e. substantially higher than that of the Ag colloids CI – CIII reported in this paper (Table I).

To investigate the effect of the $NaBH_4 : AgNO_3$ molar ratio in the preparation protocol of Ag colloid on the interference of borate bands, we have measured SERS spectra of both bpm and bpy in systems with Ag colloid CII. The results are shown in Fig. 4: spectrum *a* Ag colloid CII/bpy; spectrum *b* Ag colloid CII/bpm. The SERS spectrum of CII/bpy system in Fig. 4*a* does not show any spectral features of borates, in contrast to the SERS spectrum in ref.[24]. Furthermore, although the typical borate bands can clearly be observed in the SERS spectrum of CII/bpm system in Fig. 4*b*, their relative band intensities with respect to the bpm bands are evidently lower in the spectrum in Fig. 4*b* than in that reported in ref.[19]. The spectra in Fig. 4 thus demonstrate that the interference of borate bands in the SERS spectra of the N-containing bases can be effectively suppressed by reducing the $NaBH_4 : AgNO_3$ molar ratio in the preparation protocol of Ag colloid. This approach is alternative to suppression of borate bands by addition of Cl^- reported by Sbrana et al.[19] and appears to be more suitable for SERS-spectral investigation of adsorbates, the adsorption and SERS spectra of which are strongly affected by presence of Cl^- anions as, e.g. 2,2′-bipyridine[24].

CONCLUSIONS

The above experiments have shown that for all the adsorbates investigated, aging of the borohydride-reduced Ag colloid and reducing the $NaBH_4 : AgNO_3$ molar ratio in its preparation protocol affect adsorption of the particular adsorbate on the surface in the same manner. Our hypothesis explaining aging of Ag colloid as hydrolysis of BH_4^- thus appears to be correct.

Concentration of BH_4^- anions is thus proved to be the common, rational basis of the analogy between aging of the Ag colloid and reducing the $NaBH_4 : AgNO_3$ molar ratio

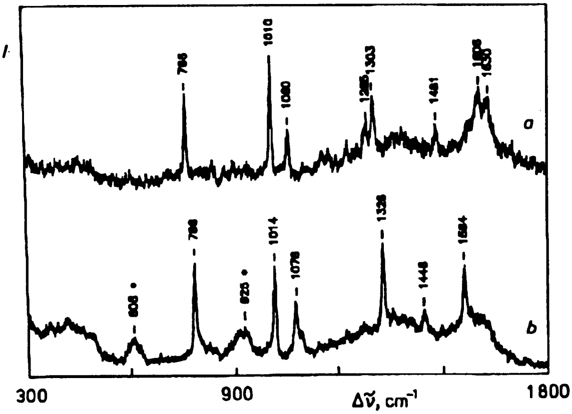

FIG. 4
SERS spectra of Ag colloid/(N-containing base) systems: *a* Ag colloid CII/2,2′-bipyridine; *b* Ag colloid CII/2,2′-bipyrimidine. The bands of borates are marked by asterisks. (Excitation 514.5 nm, power 100 mW)

in its preparation protocol. Furthermore, we try to find out how the concentration of BH_4^- anions in the system can be related to the surface potential of Ag colloid. The solution of the problems is found by application of the results obtained by Blatchford et al.[25] for citrate-reduced Ag colloid to the interpretation of our data with the borohydride-reduced Ag colloid. Blatchford et al.[25] have shown that the electrochemical potential in the system with citrate-reduced colloid (probed by an Ag electrode) can be controlled by concentration of the aqueous solution of $NaBH_4$ added to the system. With the increasing amount of $NaBH_4$ added, the electrochemical potential of the system (as probed by a silver electrode) shifts to more negative values and vice versa. In the interpretation of their experiment, Blatchford et al.[25] point out that although the electrochemical potential of a bulk Ag electrode cannot be directly quantified as the surface potential of Ag colloid, it is highly probable that both types of potential follow the same trend upon addition of $NaBH_4$ to citrate-reduced Ag colloid.

In our case of the borohydride-reduced Ag colloid, the concentration of BH_4^- in the system can be directly tuned in both directions of the more negative and the more positive potential by increasing/decreasing the $NaBH_4 : AgNO_3$ molar ratio, and in the direction of the more positive potential by aging of Ag colloid.

We have shown in this paper that a stable silver colloid with the very positive value of the surface potential required for adsorption of albumin can be prepared by aging of Ag colloid but not, alternatively, by reducing the $NaBH_4 : AgNO_3$ molar ratio. The most probable explanation of this observation is that reduction of the $NaBH_4 : AgNO_3$ molar ratio was accomplished by reduction of the total amount of borohydride in the system, while in the case of aging, the concentration of BH_4^- anions is reduced by replacement of a certain fraction of total amount of BH_4^- present in the system by hydrolytic products (finally borates). The above results point to the important role of borohydride and its hydrolytic products and oxidation products* in stabilization of the isolated particles of a silver colloid against a spontaneous aggregation into macroscopic Ag particles. Borohydride and borate anions thus form the anionic part of the electric bilayer enve-

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* Several reactions are involved in the rather complicated mechanism of oxidation of BH_4^- anions to borates. The argentometric titration leading to macroscopic metallic silver is described by equation:

$$8\,Ag^+ + BH_4^- + 8\,OH^- \rightarrow 8\,Ag + H_2BO_3^- + 5\,H_2O\,, \qquad (A)$$

while the first /quite fast/ step of borohydride oxidation is supposed to be reaction:

$$BH_4^- + 2\,OH^- \rightarrow 2\,e + BH_3(OH)^- + H_2O\,. \qquad (B)$$

The $BH_3(OH)^-$ can also act as reducing agent and is oxidized via series of reactions to the final borate anion. It is difficult to determine correctly the real stoichiometric excess of $NaBH_4$ over $AgNO_3$ used for preparation of Ag colloid. With respect to reaction (*A*) the excess of $NaBH_4$ to $AgNO_3$ is 30 – 40 times over the stoichiometry while in the case of reaction (*B*) this excess is 8 – 10 times over stoichiometry.

loping each of the Ag colloidal particles, in which they compensate (and most probably also stabilize)* the positively charged silver active sites (most probably of the Ag_n^{m+}) type**. The controlled aggregation of the isolated colloidal particles into colloidal aggregates which is necessary for formation of a SERS-active system, can be accomplished only by perturbation of the anionic (borohydride/borates) part of the electric bilayer by adsorption of an adsorbate. From the above view of the process, it is evident that the redox potentials of the systems borohydride/borate are the factors influencing the actual surface potential of the particles of Ag colloid, and consequently, adsorption of a particular chemical type of adsorbate.

Following the above arguments, we discuss the "suitability" of Ag colloids for adsorption of the particular type of adsorbate in terms of the surface potential. We are well aware of the fact that the exact value of the surface potential of a particular Ag colloid is not known and so far cannot be determined experimentally. However, we have proved that the surface potential of the borohydride-reduced Ag colloid can be tuned chemically to more positive and/or more negative values. In the terms of the surface potential of Ag colloid, our results can be summarized as follows:

1) Adsorption of albumin and the synthetic polypeptides is promoted by a more positive potential of Ag colloid.

2) Adsorption of phosphate is also promoted by a more positive potential of Ag colloid. In turn, a more negative potential is required for suppression of phosphate coadsorbed with a biomolecule on the surface of Ag colloid.

3) Interference of borates in the spectra of N-containing bases is increased by a more negative potential of Ag colloid. A more positive potential is thus required for suppressing interference of borates in the SERS spectra of the particular, strongly basic species. However, it follows from ref.[20] that observation of borates in the SERS spectra of bases is also markedly dependent on pH of the system being strongly promoted in alkaline solutions pH > 11. The results so far obtained by us and others[19,20] indicate that observation of borate bands in the SERS spectra of other species is not a common, but rather special phenomenon.

In conclusion, the surface potential of Ag colloid is proved to be an important tool for controlling the adsorption of the scattering species on the surface of Ag colloidal particles in a SERS-spectroscopic experiment. For a deeper understanding of the role of the surface potential in adsorption of chemically different adsorbates, the nature of the adsorbate–surface interaction has to be elucidated by a detailed analysis of the SERS and/or SERRS spectra obtained. Such an analysis is the subject of our forthcoming papers.

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* Stabilization of Ag_n^{m+} clusters during the preparation of Ag colloid by certain anions was reported by Ershov et al.[26].

** Roy and Furtak[27] have given evidence of the presence of Ag_n^{m+} clusters on the surface of Ag electrode.

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