



Trends in
**Applied Sciences
Research**

ISSN 1819-3579



Academic
Journals Inc.

www.academicjournals.com

Experimental and Theoretical Analysis for identifying Sudan Red Molecules Based on Raman Spectroscopy

^{1,2}Zhou Xiaofang, ¹Yan Fang and ^{1,2}P.X. Zhang

¹Department of Physics, Beijing Key Lab of Nanophotonics and Nanostructure, Capital Normal University, Beijing 100037, People's Republic of China

²Institute of Advanced Materials for Photoelectronics, Kunming University of Science and Technology, Kunming, 650051, People's Republic of China

Abstract: In this study Raman spectra and Surface-enhanced Raman scattering (SERS) spectra of four different types of Sudan Red molecules on the dried filter paper coated with silver nano-particles were reported. Raman spectra of these molecules were also calculated using DFT-B3PW91 with lanl2dz program, in which the calculated frequencies are in good agreement with experimental values. Using silver coated filter paper as substrate, SERS can be a powerful technique for detecting trace quantity of Sudan Red and an improved detecting limit up to 10^{-12} g is achieved. It indicates that Raman and SERS techniques are convenient, rapid and highly precise in detecting Sudan Red.

Key words: Sudan Red, Raman Spectra, Surface-enhanced Raman Scattering (sers), theoretical calculation

Introduction

Sudan Red is a synthetic red dye, usually used as industrial color additive, in food, solvent, oil, gas, etc. However, Sudan Red could increase the risk of cancer if consumed over a long period in large quantities. The colorant is prohibited in foods, which is clearly claimed in the Regulations Relating to Food Colorants of the Foodstuffs, Cosmetics and Disinfectant. However, the wicked behavior of using harmful color additive in foods is still widely spreaded in daily life for the purpose of camouflaging poor quality of foods, vegetables and fruits, or simply making the foods look attractive. Therefore, it is significant and immediately necessary to develop an effective and convenient method to detect such kind of carcinogen in daily goods.

Few reports about the measurement of Sudan Red are available currently. High Performance Liquid Chromatography (HPLC) and Gas Chromatogram-Mass Spectrum (GC-MS) are used in identifying them in China and foreign areas (European Commission, 1999; Xiaofan *et al.*, 2001; Huiqin *et al.*, 2005). However, for the former case, it is difficult to declutch the liquid chromatogram, owing to the complex components of the chili oil, flavoring and most other foodstuffs mixing together, while for the GC-MS technique, one has to separate one from another and has to purify it again and again, etc.

SERS, in which probe is photon, has advantages of non damage to samples and *in situ* detection with high-sensitivity and high-resolution. Since it was applied to investigate molecular structures, rapid

Corresponding Author: Yan Fang, Department of Physics, Beijing Key Lab of Nanophotonics and Nanostructure, Capital Normal University, Beijing 100037, People's Republic of China

progress has been made in matters identification, organic and inorganic chemistry, biochemistry and macromolecules, catalyze, petrochemical industry, environmental sciences and so on (Shadi *et al.*, 2001; Arenas *et al.*, 1998; Suh and Moskovits, 1986). It is significant to investigate the interaction between photon and Sudan Red molecule located on surface, as well as its adsorption behavior on the surface, to realize precisely analyzing vibrational modes, interface character and the fine structure of Sudan Red molecules, finally realize the trace quantity detection of Sudan Red.

In this study, Raman spectra and SERS spectra of four solid Sudan red molecules on the dried filter paper coated with silver nano-particles were reported. The differences of SERS spectra result from the different adsorption on the silver coated filter paper and it reflects the different molecular structures. And from another way, we used DFT-B3PW91/lanl2dz to calculate the Raman spectra for these molecules. By analyzing experimental and theoretical results, we obtained more reasonable and reliable detecting and analysis method, which is also expected to be effective for other molecule trace detecting and identification (Zhu *et al.*, 2001; Li *et al.*, 2000).

Materials and Methods

Silver colloid was prepared according to Lee and Meisel's method. One milliliter silver aqueous colloid was dropped onto two layers of quantitative a filter papers (ϕ 7 cm, slow flow rate), then the papers were put in air for about 10 min in order to get uniform thickness of silver and get rid of the water inside. Repeating these procedures, a series of filter papers coated with different amount of silver nano-particles were obtained.

One milligram Azo Dye Sudan dissolved in 10 mL CHCl_3 solvent and 1 drop around 0.04 mL of mixed solution was dropped onto filter paper (Φ 7 cm) and those silver-coated filter papers, then, a series of samples for SERS experiments with different proportion of silver nano-particles and Sudan molecules were prepared.

The Raman spectra were obtained using the RFS 100/s Bruker NIR-FT spectrophotometer. The operating wavelength is 1064 nm. The resolution was 3 cm^{-1} and 180° backscattering geometry was employed. The output laser power was 200 mw in the case of solution and 50 mw in the case of solid sample.

Results and Discussion

Sudan molecules are azo-compound, mainly include four types (Sudan I, II, III and IV), as whose molecular structure laid are shown in Fig. 1. Actually the chemical name for Sudan I is 1-phenylazo-2-naphthol and Sudan II is named as 1-[(2,4-dimethyl phenylene)azo] -2-naphthol, similarly 1-[[4- (phenylazo) Phenyl]azo] for Sudan III and 1-[[2- cymene -4-[(2-methylbenzene)azo]phenyl]azo]-2- naphthol for Sudan IV. The azo unit in these molecules, which has obvious toxicity effect for human apparatus such as liver and kidney, therefore is the origin of the carcinogenicity of these molecules.

Figure 2a presents the Raman of solid powder for the four kinds of Sudan Red molecules. The I aims at that of Sudan I, while II, III and IV refer to the solid Raman of Sudan II, III and IV, respectively. As we can see, owing to the different structures of the four molecules, the resultant Raman spectra are clearly distinguishable. The frequency difference of their Raman peaks indicates the difference of vibrational modes of these molecules, which is the fingerprint to identify different Sudan Red molecules. Actually it is also the basis of trace quantity detection of Sudan Red molecules.

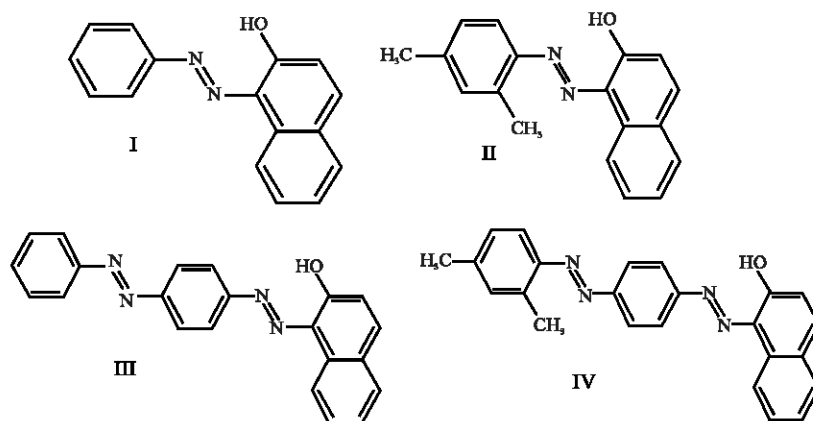


Fig. 1: Structure of Sudan Red molecules

For instance, the molecular structures of Sudan I and II are basically the same, in which Sudan I has exceptional two $-CH_3$ functional clusters and distinguishes itself from Sudan Red II. From this point of view, it is not surprising the Raman spectra of them also show the similar features in some modes, while some other bands show great difference. In comparison with Fig. 2I, Raman peak positions show displacements but also some Raman modes even come to split up seen in Fig. 2II, for instance, 1596 and 1227 cm^{-1} . Taking into account the fact that additional $-CH_3$ functional clusters in Sudan Red I lower the symmetry and modify the select rules, it is not difficult to understand the degeneracy of Raman modes. Same arguments can be applied in explaining the discrepancy between III and IV, between II and III, etc.

It should be noted that usually the quantity of Sudan contained in daily goods is quite small, in order to realize the trace quantity detection and even pico-gram (pg) quantity detecting, SERS of these four kinds of molecules on silver coated filter paper are performed and illustrated in Fig. 2b. Due to poor solubility of Sudan Red molecules in aqueous colloid, it is quite difficult to obtain SERS of Sudan Red in usual way. Using the method aforementioned in Experimental section, one can immediately get the SERS spectra of high quality since the method employed overcomes the shortcomings that aqueous silver colloid usually can't be mixed well with organic solvent.

The difference between Raman and SERS spectra is clear for Sudan Red molecules, but the main modes of each kind of molecules in both Raman and SERS show up at the same peak positions. For instance, in Raman Spectra, there are few peaks appearing in Sudan III and IV in the low frequency range, but in Fig. 2b many additional modes turn up, e.g., 879 , 805 , 724 , 628 , 569 , 457 and 375 cm^{-1} . Even in high frequency range, there still exists difference between the Raman and SERS. It is notable of the bands at 1095 , 1130 , 1388 and 1416 cm^{-1} ; the 1172 cm^{-1} mode of Sudan IV also present apparent difference between its Raman and SERS. Actually, we can always find more differences in the SERS spectra of the four kinds of molecules.

It is more reasonable to select SERS to show the differences between one kind of Sudan Red molecule and another, because with more Raman bands appearing in SERS, even some subtle difference can be also digged out through the comparison study of these Sudan Red molecules. Anyhow, the spectrum difference is unambiguous between different Sudan Red molecules except that some peak features are similar to each other e.g., the peaks presented at 983 ($984/998/986$) cm^{-1} band, at 1231 cm^{-1} bands and at 1596 ($1609/1595/1595$) cm^{-1} . This difference between Raman and SERS of the similar Sudan Red molecules can be well explained and analysed by SERS techniques.

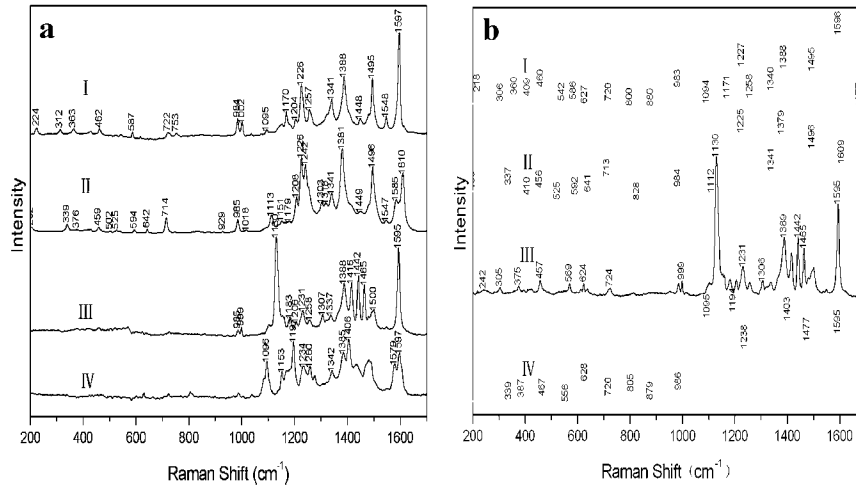


Fig. 2: Raman Spectra from four solid Sudan Red (a) and SERS Spectra from Sudan Red on silver-coated filter paper (b)

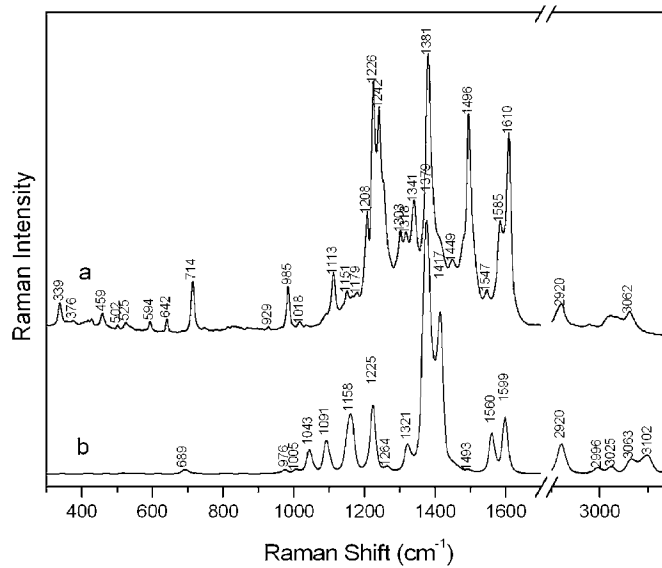


Fig. 3: Raman Spectra from solid Sudan Red I (a) and calculated Raman Spectra of Sudan Red II (b)

Furthermore, we performed the calculation using Gaussian 03 and collected the data of Sudan Red molecules' vibration from literatures, which are listed in Table 1 (Muniz-Miranda, 2002; Ong *et al.*, 2002; Li *et al.*, 1999; Helio and Santos, 2000). The relative intensity of each mode for these molecules and the corresponding assignment are also clearly fingered out. It is noted that the SERS spectra show more additional modes, which is exactly an indication of advantages of SERS techniques.

To evaluate the enhancement efficiency of this system, a simple formula is employed to calculate the enhancement factor (Zhixun and Yan, 2005).

$$G = (I_{\text{SERS}}/N_{\text{surf}}) / (I_{\text{Raman}}/N_{\text{bulk}}) \quad (1)$$

Here $(I_{\text{SERS}}/N_{\text{surf}})$ represents the SERS intensity contributed by Sudan Red molecules adsorbed on substrates and $(I_{\text{Raman}}/N_{\text{bulk}})$ the normal Raman intensity of the species in solution exposed to laser light. Choosing several modes as the typical bands, one obtains an enhancement factor G as high as 10^5 .

To confirm the affectivity of the Raman technique for detecting and identifying Sudan Red molecules, using DFT-B3PW91 with lanl2dz, (Frisch *et al.*, 2002) we calculated the Raman spectra of Sudan II as shown in Fig. 3b; Raman Spectra of solid Sudan red II, is also shown in Fig. 3a. The

Table 1: The assignment of vibrational modes for Sudan Red

Sudan Red I		Sudan Red II		Sudan Red III		Sudan Red IV		Assignment
Solid	SERS	Solid	SERS	Solid	SERS	Solid	SERS	
1597(s)	1596(s)	1610(s)	1609(s)	1595(s)	1595(s)	1597(s)	1595(s)	v(NN) and v ₂ (CC)
		1585(m)	1583(m)			1579(m)	1582(m)	
1548(m)	1548(w)	1547(w)	1548(w)		1550(w)	1550(w)	1548(w)	v(NN) and v ₁₂ (CC)
1495(ms)	1595(s)	1496(s)	1496(s)	1500(w)	1502(w)	1481(m)	1477(m)	
		1480(w)			1465(m)		1465(m)	
1448(w)	1451(w)	1449(w)	1448(w)	1442(m)	1421(m)	1435(m)	1432(m)	
				1416(m)	1416(m)	1406(s)	1403(s)	Ring dilation vibration
1388(ms)	1388(ms)	1381(w)	1379(w)	1388(s)	1389	1385(s)	1384(s)	v(CN) and v ₁₂ (CC)
1341(m)	1340(m)	1341(m)	1341(m)	1337(w)	1336(w)	1342(m)	1342(m)	β(C-H)
		1318(w)	1317(w)					
		1303(w)	1302(w)	1307(w)	1306(w)	1307(w)	1315(w)	v ₂₁ (CC)
1257(m)	1258(m)	1242(ms)	1255(m)	1258(w)	1256(w)	1260(m)	1275(m)	In-planering and β(OH)
			1241(ms)				1257(m)	
1226(ms)	1227(ms)	1226(s)	1225(s)	1231(w)	1231(m)	1234(m)	1238(m)	
1204(m)	1207(m)	1208(m)	1208(m)	1206(w)	1206(w)	1197(s)	1194(s)	β(C-H) and β(OH)
1170(m)	1171(m)	1179(s)		1183(w)	1181(w)	1172(m)	1181(m)	
							1166(m)	
		1151(w)	1150(w)	1130(s)	1130(s)	1153(m)	1152(m)	v ₁₂ (CC)
1095(w)	1094(w)	1113(m)	1112(m)	1103(w)	1103(w)	1096(s)	1095(s)	v ₂ (CC) and v(CN)
						1039(w)	1038(w)	β(CH ₂)
1002(m)	999(m)	1018(w)	1015(w)	999(w)	999(w)			Ring breathing vibration
984(m)	983(m)	985(m)	984(m)	985(w)	985(w)	987(w)	986(w)	β(C-H)
		929(w)					966(w)	Benzene ring breathing
			870(w)				879(w)	v ₂₁ (CC) and v(CN)
753(w)	800(w)					806(w)	805(m)	β(C-OH)
722(w)	720(w)	714	713(m)		724(w)	724(w)	720(w)	v ₁₂ (CC)
		627(w)	642(w)		624(w)	630(w)	628(m)	
587(w)	586(w)	594(w)	592(w)		569(w)			
462(w)	460(m)	459(w)	456(m)	521(w)	457(m)		467(w)	
363(w)	409(w)		410(w)	437(w)	375(w)		387(w)	
312(w)	360(w)	339(w)	337(m)	381(w)	305(w)		339(w)	δ(C-C)

Note: s: strong; m: medium; w: weak; ms: medium strong; mw: medium weak

Table 2: The comparison of theoretical and experimental results for Sudan II

Theoretical calculation	3102	3062	2920	1599	1560	1493	1417	1379	1321	1264			
Experimental	3063	2920	1610	1585	1547	1496	1449	1381	1341	1318	1303	1242	
Theoretical calculation	1225		1158	1091	1005	976		689					
Experimental	1208	1226	1179	1151	1113	1018	985	929	714	642	594	459	339

calculated and experimental results can be also found in Table 2. It is easy to find that both results are in good agreement with each other in some extent.

Conclusions

Raman spectra and SERS spectra of four kinds of solid Sudan Red molecules were reported. The differences of SERS spectra result from the different adsorption on the silver coated filter paper and it reflects the different molecular structures. Using DFT-B3PW91 with lanl2dz, we calculated the Raman spectra of these molecules and found that the calculated Raman frequencies are in good agreement with experimental values. By analyzing experimental results assistant with theoretical calculations, we obtained more reasonable and reliable detecting and analysis method. It is proved SERS is a powerful technique for detecting Sudan Red when using silver coated filter paper as substrate and an improved detecting limit up to 10^{-12} g is achieved. Actually the method we used in SERS experiments can be also applied in trace quantity detecting and fingerprint identification for other matters.

Acknowledgements

The authors are grateful for the support of this research by the National Natural Science Foundation of China and the Natural Science Foundation of Beijing.

References

- Arenas, J.F. *et al.*, 1998. SERS of acrylic acid and methyl derivatives on silver sols, *J. Raman Spectroscopy*, 29: 585-591.
- European Commission, 1999. Health and Consumer Protection Directorate-General, Directorate D-Food Safety, Production and Distribution Chain, Analysis and Dosage of the Colorants Sudan and Bixin in Chili Powder and Pepper-Base Products[S], 03 99.
- Frisch, M.J. *et al.*, 2002. Gaussian 03, Revision B.05, Gaussian Inc., Pittsburgh, PA, 2003.
- Hélio, F. and D. Santos *et al.*, 2000. Quantum mechanical investigation of the Tautomerism in the Azo Dye Sudan. *Intl. J. Quan. Chem.*, 80: 1076-1086.
- Huiqin, W. *et al.*, 2005. Rapid Determination of Sudan Red 1 in Food by GC-MS/SIM. *J. Instrumental Analy.*, 24: 1-5.
- Li, W.H. *et al.*, 1999. Surface-enhanced Hyper-Raman Spectroscopy (SEHRS) and surface-enhanced Raman spectroscopy studies of pyrazine and pyridine adsorbed on silver electrode. *Chem. Phys. Lett.*, 305: 303-310.
- Li, W.H. *et al.*, 2000. Surface-enhanced hyper-Raman scattering and surface-enhanced Raman scattering studies of electroreduction of phenazine on silver electrode. *Chem. Phys. Lett.*, 327: 153-161.

- Muniz-Miranda, M., 2002. Adsorption mechanism of 2-amino, 5-nitropyrimidine on silver substrates, as detected by surface-enhanced Raman scattering. *Vib. Spectrosc.*, 29: 229-233.
- Ong, S.Y. *et al.*, 2002. Time-resonance raman spectroscopy and density functional study of 2-fluorenylitrene and its dehydroazepine products. *Chem. Eur. J.*, 8: 2163-2171.
- Shadi, I.T. *et al.*, 2001. Semi-quantitative trace analysis of nuclear fast red by surface enhanced resonance Raman scattering. *Anal. Chim. Acta*, 450: 115.
- Suh, J.S. and M. Moskovits, 1986. Surface-enhanced raman spectroscopy of amino acids and nucleotide bases adsorbed on silver. *J. Am. Chem. Soc.*, 108: 4711-4718.
- Xiaofan, Q. *et al.*, 2001. Study surface of fruits by Micro-raman spectroscopy. *Chinese J. Light Scattering*, 13: 41-45.
- Zhu, P. *et al.*, 2001. Transient-resonance raman and density functional theory investigation of 4-biphenylnitrenium, 2-fluorenylnitrenium and diphenylnitrenium ions. *Chem. Eur. J.*, 7: 4928-4935.
- Zhixun, L. and F. Yan, 2005. SERS of gold/C₆₀ (/C₇₀) nano-clusters deposited on iron surface. *Vibrational Spectrosc.*, 39: 151-156.