

# Vibrational study of benzalkonium chloride (1) interaction with metallic ions and surfaces: surface enhanced Raman spectroscopy study of (1) with human serum albumin

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In this paper Raman Spectroscopy is used to study the interaction of microbicide N-Alkyl-N, N-dimethyl-N-benzylammonium chloride (Benzalkonium Chloride or Barquat-80) with metallic ions. The technique Surface Enhanced Raman Scattering applies to this purpose. This technique

applies to the study of the interaction of this compound with Human Serum Albumin (HSA). The observed changes in the spectrum indicate that the alkane chain and the -CH<sub>3</sub> or -CH<sub>2</sub> radicals of barquat attack HAS and the interaction with HSA and Barquat is on the aromatic ring too. Very important features of benzalkonium salts are their bactericidal and antimicrobial properties.

**Key Words:** *Raman spectroscopy; Human serum albumin; Benzalkonium salts*

Barquat-80 (B-80), a quaternary ammonium compound (QAC), is an effective microbicide, fungicide, deodorant and algicide used in general disinfection and general sanitizing. It's handed containing approximately 7% isopropanol and approximately 13% water. The molecule shows a no fixed structure (Figure 1) and the average molecular weight is 364 (1).

Quaternary ammonium compounds are a group of excellent antimicrobial activity, they are mainly used as disinfectant, biocide, and detergent, but also as anti-electrostatic, and very important features of benzalkonium salts are their bactericidal and antimicrobial properties. They are widely used as preservative for ophthalmic, nasal and parenteral products and as topical antiseptics and disinfectants for medical equipment (2). There are studies about the application of Barquat-80 in ocular medicine (3,4) in ophthalmology (5-7) and about several independent decellularization protocols on (porcine) cornea for future clinical use (8) and as cross-linker agents in dentist medicine (9) and are evaluated its bactericidal activity against several bacteria (10-12) and a method was developed for assay determination of Benzalkonium chloride (BKC) in nasal spray formulations (13). Benzalkonium salts (chloride and bromide) inhibit the proliferation of a variety of cells and are effective bactericidal, fungicidal and virucidal agents. They show inhibitor activity against many pathogens virus including human immunodeficiency virus (HIV) and are useful in prevention and treatment of other diseases mainly those originated by viruses and at the skin surface (14) and there are studies about biodegradation of quaternary ammonium compounds (15).

The misuses of QAC, can lead to increasing resistance of microorganisms. For to avoid this serious problem it is suitable the use of new biocides with modified structures instead of the biocides applied so far. New BAC analogues P13-P18 with pyridine rings were synthesized. The new compounds were characterized by NMR, FT-IR and ESI-MS methods. Several features as critical micellization concentrations and antimicrobial properties of novel QACs were examined by determining their minimal inhibitory concentration (MIC) values against several bacteria (16) and there are studies about the biological and medical behavior of BCK (17-19), about the synthesis and biological properties (20) and as preservative used most commonly in eye drops. There are studies that have demonstrated adverse effects on surface epithelial cells for the extend use in eye drops and to decrease in corneal damages by addition of several protective medical substances (7,21-26). BCK could favor drug release in therapies that requires faster but controlled delivery (27). We think that it is important to study the interaction between this molecule and metallic ions because possible arrive, as waste, to environment and may act with metallic ions that are present. In this way,

at the beginning, we have studied the FT-Raman spectrum of this molecule and Surface Enhanced Raman Spectroscopy (SERS) over silver colloids and over mixed silver and magnetic colloids. SERS is a technique that obtains a very increase in the intensity of the Raman signal of a molecule physical or chemical absorbed on a rough metal surface whose size is microscopic particle. When an electromagnetic wave interacts with metal surface, that the fields next this surface change in regard the far field and if it is rough, the plasmons on the surface get turned on and the electromagnetic field is amplified (28-30). This technique is widely used for study the molecules absorbed on metallic surfaces mainly in colloids of silver or copper. SERS allows obtain spectra of substances at very low concentration (31,32). Two theories explain this technique: The electromagnetic theory relies upon the excitation of localized surface plasmons and the chemical theory explains the effect through the formation of charge - transfer complexes (33). At present there are several studies about the SERS enhancement factor of silver sphere nanoparticles depended on temperature (34) and there are recent reviews on the manufacture of SERS substrates and the requirements for characterization of plasmonic materials as SERS platforms (35). Studies about the synthesis of gold nanoparticles are proposed, an instantaneously method using non-toxic reducing agents and applied to SERS (36).

Recent developments have been made concerning the fabrication of 3D SERS-active hot spot and fabrication of novel 3D plasmonic nanostructures (37). The serum albumins belong to a multigene family of proteins that are of use in the transport, distribution, and metabolism of many endogenous and exogenous ligands: molecules of fatty acids, metals as calcium, zinc and copper, amino acids, hormones, steroids and numerous of therapeutic drugs. Albumins are characterized by a low reserved of tryptophan and methionine and a high content in cystine and charged amino acids. The feature of the albumin molecule is a series of nine loops that repeat in a triplet fashion of large-small-large loops and further grouped as three homologous domains of three loops every one. The three domains also consist of six subdomains. It is included the alignment of 17 disulfide bridges between cystine residue (38-42). The human serum albumin (HSA) (molecular weight 66.439 u.m.a. from composition) consist of 585 amino acids with 35 residues cysteine that form the disulfur bridges at the origin of its tertiary structure and shows a thiol grouping at the level of cysteine (34).

The albumin is the more abundant protein of the plasma, about 60% (43) and plays a key role in transport, distribution and metabolism of metabolites and drugs (44). Recombinant human serum albumin (rHSA) is potential alternatives for human serum albumin (HSA) which may ease severe shortage of HSA worldwide. In theory, rHSA and HSA are the same and analysis

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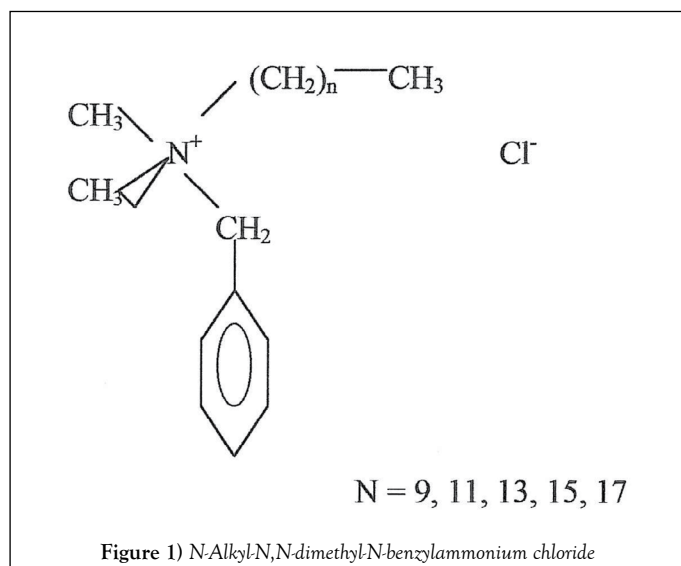


Figure 1) *N*-Alkyl-*N,N*-dimethyl-*N*-benzylammonium chloride

indicated that rHSA and HSA achieved a structural similarity of 99% (45).

The single tryptophan is at residue 214, in loop four. Distribution of other amino acids is uneven, tyrosine is in loops three and six and prolines are in the tip of each long loop. The molecule is not charged uniformly along his length, thus the charge is -9, -8 and +2 in domains I, II and III39.

In this study we have used a silver colloid (named as A), mixed colloid, Ag colloid + magnetic colloid (B) and C, silver colloid using hydroxylamine as reducing agent.

### EXPERIMENTAL

#### Materials

All materials for prepare the colloids:  $\text{AgNO}_3$ ,  $\text{FeCl}_2$ ,  $\text{FeCl}_3$ ,  $\text{HNO}_3$ ,  $\text{Na}_2\text{C}_2\text{O}_4$  and  $\text{NH}_4\text{OH}$ , were purchased from Merck and also  $\text{KNO}_3$ ,  $\text{NaCl}$  in Riedle-De Haën AG and  $\text{Na}_2\text{SO}_4$  from Fluka. HSA from Sigma Chemical Co. (Seelze, Germany) and were used without further purification. All were in grade of purity for analysis. The water solutions were prepared with triply distilled water. Barquat-80 was get in Lonza group.

#### Preparation of colloids

Silver colloid (A) was prepared by Lee method (46): To 50 ml of boiling  $\text{AgNO}_3$  aqueous solution  $10^{-3}$  M, vigorously stirred, are added 1 ml of aqueous sodium citrate (1%), drop by drop and remain boiling and stirring during one hour. Magnetic colloid was prepared by Massart method (47) a little modified: A mix that consist of 10 ml of  $\text{FeCl}_3$  aqueous solution, 1 M and 2.5 ml of  $\text{FeCl}_2$  2 M dissolved in  $\text{HCl}$  2 M was added drop by drop to 125 ml of  $\text{NH}_4\text{OH}$  0.7 M and it was vigorously stirred. A solid precipitate of  $\text{Fe}_3\text{O}_4$  is obtained that is washed with  $\text{HNO}_3$  2 M. This solid is separated by means of a centrifuge and abundant triply distilled water is added for obtain the peptization. Mixed colloids were prepared dissolving solid  $\text{AgNO}_3$  into 50 ml of aqueous solution of magnetic colloid at calculated concentration and, when it is boiling, 1 ml of sodium citrate was added at 1% of concentration. We have named as B colloid. The silver colloid using hydroxylamine as reducing, named C colloid, was prepared by Lendl and method (48), that consist of dissolve 0.017 g of  $\text{AgNO}_3$  in 90 ml of triply distilled water, also 0.021 g of  $\text{NH}_4\text{OHCl}$  are added to 5 ml of water and 4.5 ml of  $\text{NaOH}$  0.1 M also are added. All this mixture is emptied quickly and stirring on  $\text{AgNO}_3$  solution.

#### Instrumentation

The absorption spectrum recorded with a double beam Cintra 5 UV visible absorption spectrophotometer using a 10 mm silica cell. FT-Raman spectrum was recorded with Bruker RFS 100/S by using a ND: YAG laser source at 1064 nm, the output power was 150 mW. The sample was placed into a 10 mm silica cell. The final spectrums were the result of 1000 scans accumulations and power about 300 mW. Raman spectrum was recorded with a Renishaw R.M. 1000 by using a laser source at 782 nm and placing the liquid sample on a glass sheet and evaporating until obtain a solid residue and objective 100. We have obtained also microscopic pictures of internal structure of samples.

### DISCUSSION

#### Absorption spectra of pure Barquat CB-80 and $10^5$ M over (A) and © colloid

We have gotten the absorption spectrum of Barquat 80% and  $\text{pH}=5.0$  at  $10^6$  of dilution, that shows one peak at 215 nm and a shoulder about 257 nm that disappears in solution. The spectra of Barquat at concentration  $10^5$  M and on (A) colloid and  $\text{pH}=5.3$ ; on (C) colloid containing 0.1% of magnetic colloid and  $\text{pH}=5.0$ ; on (C) colloid containing 0.5% of magnetic colloid and on (C) colloid containing 1% of magnetic colloid and  $\text{pH}=4.7$  only show a highest value about 200 nm. These indicate the interaction with the metal of colloid. The spectrum is not visible at as low concentration  $10^5$  M.

#### FT-Raman spectra of Barquat CB-80 and SERS

In the Table 1, are indicated the possible main vibrations frequencies of FT-Raman spectrum of Barquat-80 and FT-SERS of aqueous solution  $10^5$  M  $\times 10$  over A colloid and over mixed colloid (B) 1% and Raman spectrum on Ag colloid (C). The mixed colloid formed for silver colloid over 1% of magnetic colloid contains particles of  $\text{Fe}_3\text{O}_4$  recovered of particles of silver that present interaction with the light. This fact pointed that both spectra, over silver colloid (A) and over mixed colloid (B), present few differences and had been our object of study in older works. It's observed that the strong band, in the spectrum of alone Barquat, at  $2891\text{ cm}^{-1}$ , is not present in the other three spectra and the very strong band at  $2852\text{ cm}^{-1}$  appears as a shoulder at  $2848\text{ cm}^{-1}$  in SERS over silver colloid and at  $2856\text{ cm}^{-1}$  in SERS over mixed colloid and on C colloid do not appear. Both bands correspond to  $-\text{CH}_2$  symmetric stretching, that is to say, that are corresponding to paraffin chains. The bands at  $1004$  and  $1032\text{ cm}^{-1}$ , corresponding to aromatic chain, appear practically equal in FT-SERS on A and B colloids that in alone Barquat, only change the intensity, but on C colloid the intensity does not change. In the two first peaks, the intensity decreases in FT-SERS on Ag colloid and in FT-SERS of mixed colloid and, in Raman spectra on Ag colloid, the intensity do not change but the peaks are little shifted (at  $1039\text{ cm}^{-1}$  and  $1003\text{ cm}^{-1}$ , respectively). The shoulder at  $2931\text{ cm}^{-1}$ , corresponding to  $-\text{CH}_3$  anti-symmetric stretching, appears strong in FT-SERS on Ag colloid and medium  $2938\text{ cm}^{-1}$  in FT-SERS on mixed colloid and in Raman spectra on Ag colloid do not appears. The strong peak at  $1448\text{ cm}^{-1}$ , also corresponding to  $-\text{CH}_2$  anti-symmetric, changes the intensity and, on C colloid, the band is about  $1456\text{-}1453\text{ cm}^{-1}$ . The medium peak at  $1302\text{ cm}^{-1}$  in alone barquat-80, possible to  $-\text{CH}$  methane deformation, changes the intensity and lightly its value and the band at  $873\text{-}888\text{ cm}^{-1}$ , only appears in spectrum on C colloid. As we stated in the previous paragraph, Barquat shows an  $\text{N}^+$  linked to an aromatic radical and also linked to two  $-\text{CH}_3$  groups and to a very long paraffin chain, which has from 10 to 18 carbon atoms. It makes the union of this positive nitrogen with the negative surface formed for the citrate anion, which recover the metallic atoms of silver, guides the long paraffin chain far away of the negative surface, due to stereo reason, and  $-\text{CH}_3$  groups and aromatic chain are located next to the surface. This fact is the reason of the weak bands corresponding to paraffinic chain.

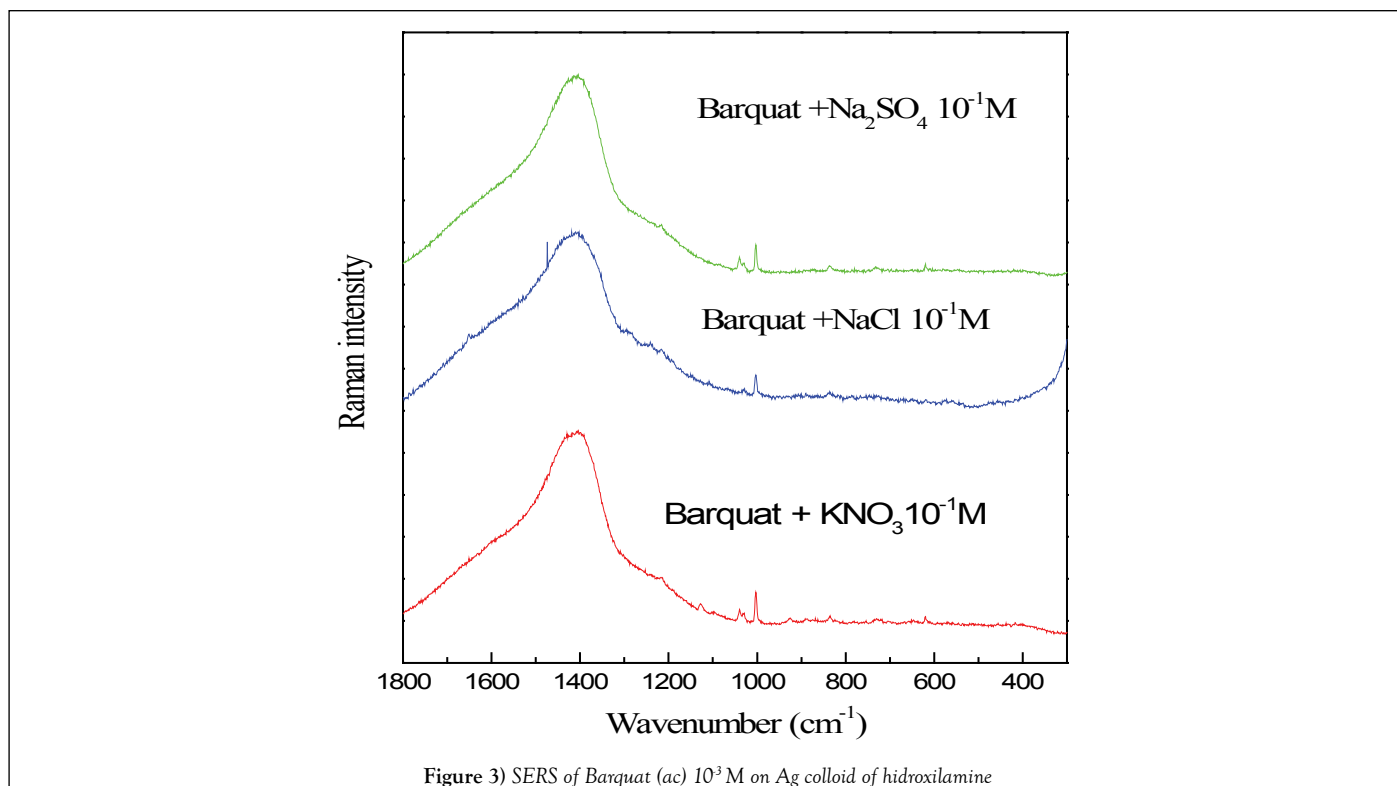
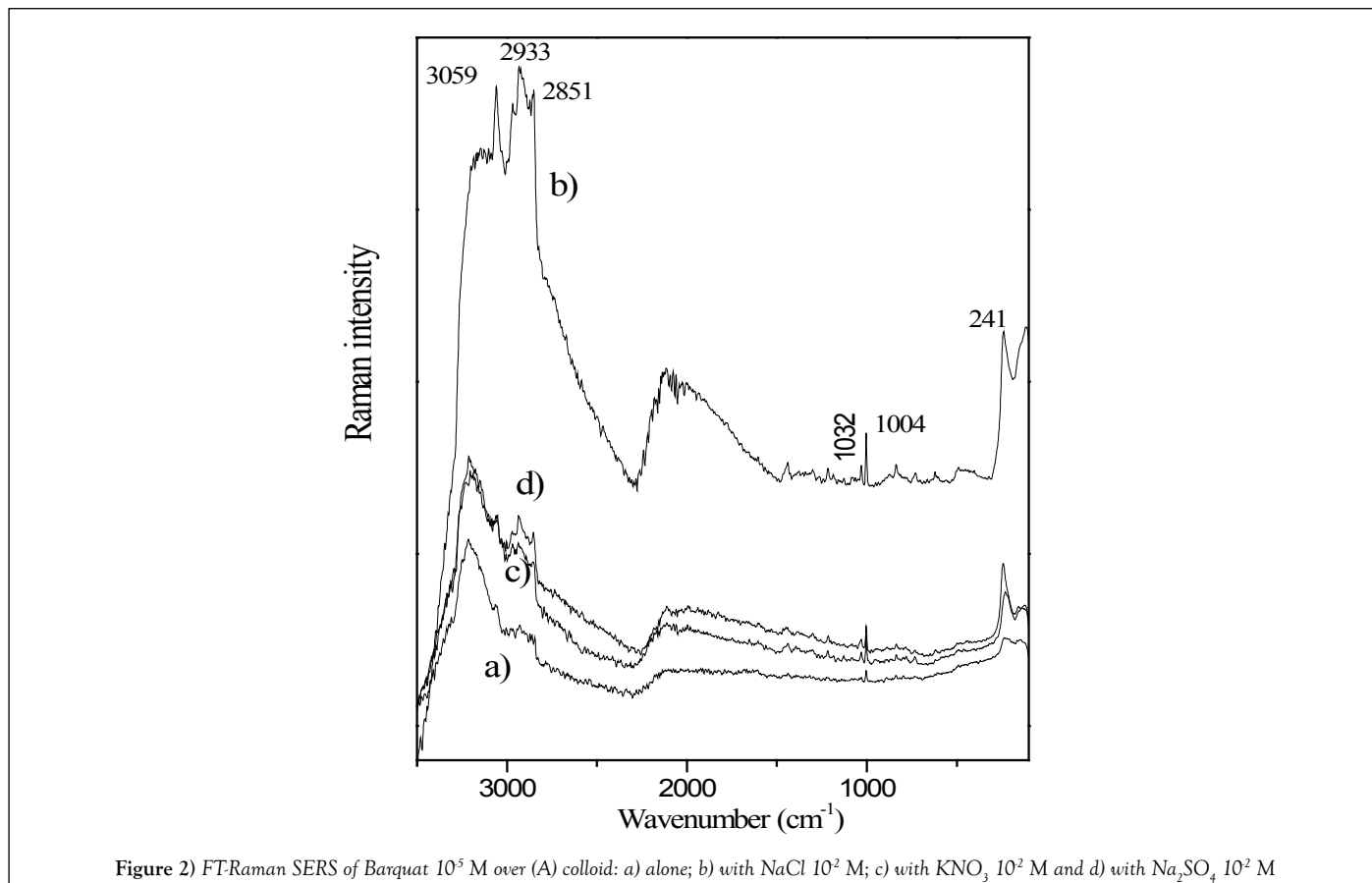
#### FT-Raman of SERS of Barquat-80 over silver colloid (A) after addition of $\text{NaCl}$ , $\text{KNO}_3$ and $\text{Na}_2\text{SO}_4$

The Figure 2 displays SERS of Barquat-80 over silver colloid (A) at concentration  $10^5$  M and  $\text{pH}=5.3$ : a) alone; b) with  $\text{NaCl}$   $10^2$  M; c) with  $\text{KNO}_3$   $10^2$  M and d) with  $\text{Na}_2\text{SO}_4$   $10^2$  M. All these compounds increase the profile of spectra. It's observed that the larger increase appears for addition of  $\text{NaCl}$  and it is possible, due to interaction between  $\text{Cl}^-$  anion and  $\text{Ag}^+$  forming a larger negative sheet over the surface, that fixed more intense the  $\text{N}^+$ .

We have studied FT-Raman SERS of Barquat of  $10^5$  M over (A) colloid with  $\text{Na}_2\text{SO}_4$   $10^2$  M and over (C) colloid with  $\text{Na}_2\text{SO}_4$   $10^1$  M (Figure 3). The spectrum over © colloid, obtained using  $\text{Na}_2\text{SO}_4$  as gathering substance, shows more intense that on (A) colloid. The main bands are the same in both spectra but the spectrum on A colloid shows the peaks at  $1324$  and  $1386\text{ cm}^{-1}$  appointed to vibrations of benzene more intense that on C colloid.

#### SERS of albumin + barquat over (A) colloid

Table 2 shows Raman SERS of Albumin  $2.8 \times 10^5$  M +  $\text{KNO}_3$   $3 \times 10^3$  M (x5), barquat  $7 \times 10^6$  M +  $\text{KNO}_3$   $3 \times 10^3$  M and albumin + Barquat 4/1 +  $\text{KNO}_3$   $3 \times 10^3$  M (x5). It is noted the main peaks of SERS of mixed albumin and Barquat in relation 4/1 and are compared with the SERS of albumin and Barquat at the same concentration,  $2.8 \times 10^5$  M for albumin and  $7 \times 10^6$  M for Barquat) and addition of  $\text{KNO}_3$   $3 \times 10^3$  M. In the Figure 4, it is mainly observed that the intensity of the spectrum of the mixture Albumin-Barquat



is lower than the intensity of alone Barquat (the spectrum of the mixture is multiplied by 5). Table 2 shows some differences in the peaks of alone Barquat in respect to the mixture: the medium peak at  $2926\text{ cm}^{-1}$  of Barquat appears strong in the mixture; the shoulder at  $2892\text{ cm}^{-1}$  disappears in the mixture and it is noted that the weak band at  $1382\text{ cm}^{-1}$ , corresponding to  $-\text{CH}_3$  anti-symmetric deformation in alone Barquat, is observed strong at  $1386\text{ cm}^{-1}$  in the mixture and joined to  $1378\text{ cm}^{-1}$  in alone albumin and the peak of Barquat at  $1127\text{ cm}^{-1}$ , corresponding to  $(-\text{C}-\text{C}-)$  alkane stretching or

$\delta$  (CH) aromatic, decreases in the bands of the mixture. We think that these changes indicate that the alkane chain and the  $-\text{CH}_3$  or  $-\text{CH}_2$  radicals attach HAS. The aromatic peak, in Barquat, at  $1604\text{ cm}^{-1}$ , is weaker in the mixture, the also aromatic weak peak at  $1083\text{ cm}^{-1}$  in Barquat possible is at  $1075\text{ cm}^{-1}$  with the same intensity in the mixture. It is also observed that the medium band at  $831\text{ cm}^{-1}$  in albumin disappears or is strong at  $837\text{ cm}^{-1}$ , joined to the point of alone Barquat and the peaks at  $736\text{ cm}^{-1}$  (weak) in albumin,  $734\text{ cm}^{-1}$

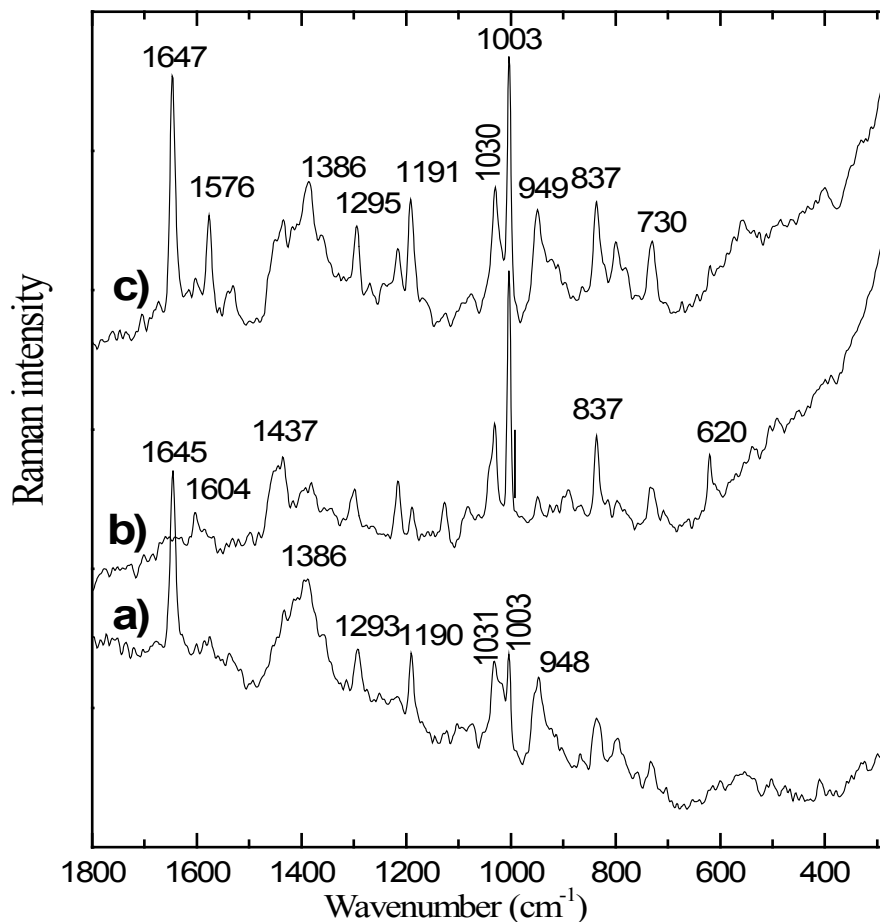


Figure 4) Raman SERS: a) Albumin  $2.8 \times 10^{-5} \text{ M} + \text{KNO}_3 3 \times 10^{-3} \text{ M} (\times 5)$ ; b) barquat  $7 \times 10^{-6} \text{ M} + \text{KNO}_3 3 \times 10^{-3} \text{ M}$  and c) albumin + Barquat 4/1 +  $\text{KNO}_3 3 \times 10^{-3} \text{ M} (\times 5)$

TABLE 1

Possible main vibrational frequencies of FT-Raman spectrum of Barquat – 80, FT-SERS on Ag colloid, FT-SERS on mixed colloid and Raman spectra on Ag colloid

FT-Raman Spectrum of Barquat-80	FT-SERS on Ag-colloid (A)	FT-SERS on mixed colloid (B)	Raman spectrum on Ag colloid ©	Assignments
3064 $\text{cm}^{-1}$ (s)	3058 $\text{cm}^{-1}$ (m)	3073 $\text{cm}^{-1}$ (m)		Aromatic C-H stretch
2953 '' (sh)	2968 '' (sh)			-CH <sub>3</sub> anti-symmetric stretch or methyl. C-H assym or symm stretch
2931 '' (sh)	2931 '' (s)	2938 '' (m)		-CH <sub>3</sub> anti-symmetric stretch
2891 '' (s)				-CH <sub>2</sub> symmetric stretch or methane C-H stretch
2852 '' (vs)	2848 '' (sh)	2856 '' (sh)		-CH <sub>2</sub> symmetric stretch
1605 '' (m)	1605 '' (w)	1632 '' (w)		Aromatic $\nu$ (C-C)
1448 '' (s)	1443 '' (w)	1448 '' (m)	1456-1453 $\text{cm}^{-1}$ (w)	-CH <sub>3</sub> anti-symmetric stretch or methyl C-H assym or symm stretch
1381 '' (w)	1386 '' (w)	1400 '' (w)		-CH <sub>3</sub> symmetric deformation or methyl C-H assym or symm stretch
	1324 '' (sh)		1318 '' (vw)	-CH methine deform
1302 '' (m)	1282 '' (vw)	1295-1305 '' (vw)	1292 '' (vw)	-CH methine deform or $\delta$ ip (arom C-H)
1032 '' (m)	1032 '' (w)	1032 '' (w)	1039 '' (m)	Aromatic C-H in-plane def. or methine skeletal C-C vib
1004 '' (s)	1004 '' (m)	1004 '' (m)	1003 '' (s)	Aromatic C-H in-plane def. or methine skeletal C-C vib or amines
873 – 888 '' (w)			870 '' (w)	$\nu_{\text{C,N}}$ Aromatic $\delta_{\text{oop}}$ (C-H) or methine skeletal C-C vib.
837 '' (w)	839 '' (w)	837 '' (w)	836 '' (w)	Aromatic $\delta_{\text{oop}}$ (C-H)
784 '' (vw)			783 '' (vw)	Aromatic $\delta_{\text{oop}}$ (C-H) or methine skeletal C-C vib
732 '' (w)	727 '' (vw)	735 '' (vw)	737-724 '' (w)	Aromatic $\delta_{\text{oop}}$ (C-H) or -(CH <sub>2</sub> ) <sub>n</sub> - sim $\delta$ (C-H)
620 '' (w)			619 '' (w)	

TABLE 2

SERS: a) Albumin  $2.8 \times 10^{-5} \text{ M} + \text{KNO}_3 \ 3 \times 10^{-3} \text{ M}$  (x5); b) Barquat  $7 \times 10^{-6} \text{ M} + \text{KNO}_3 \ 3 \times 10^{-3} \text{ M}$  and c) albumin + Barquat 4/1 +  $\text{KNO}_3 \ 3 \times 10^{-3} \text{ M}$  (x5)

Albumin $2.8 \times 10^{-5} \text{ M} + \text{KNO}_3 \ 3 \times 10^{-3} \text{ M}$ (x5)	Barquat $7 \times 10^{-6} \text{ M} + \text{KNO}_3 \ 3 \times 10^{-3} \text{ M}$	Albumin + Barquat (4/1) + $\text{KNO}_3 \ 3 \times 10^{-3} \text{ M}$	Assignments
3209 $\text{cm}^{-1}$ (s)(br)	3211 $\text{cm}^{-1}$ (s)(br)	3213 $\text{cm}^{-1}$ (s)(br)	
3060 '' (sh)	3060 '' (m)	3052 '' (w)	Aromatic C-H stretch
	2969 '' (sh)		Alkanes $\nu$ (C-H)
2932 '' (m)	2926 '' (m)	2927 '' (s)	$-\text{CH}_3$ anti-symmetric stretching or Alkanes $\nu$ (C-H)
	2892 '' (sh)		$-\text{CH}_3$ anti-symmetric stretching or Alkanes $\nu$ (C-H)
1645 '' (s)	1645 '' (m)	1647 '' (s)	Amines $\delta_{\text{NH}}$
	1604 '' (w)	1600 '' (vw)	Aromatic $\nu$ (C-C)
1572 '' (vw)		1576 '' (m)	Aromatic $\nu$ (C-C) or amines $\delta_{\text{NH}}$
1540 '' (vw)		1532 '' (w)	Aromatic $\nu$ (C=C)
	1437 '' (m)	1438 '' (sh)	$-\text{CH}_3 \ \delta_{\text{C-H asim}}$
1378 '' (s)	1382 '' (w)	1386 '' (s)	$-\text{CH}_3 \ \delta_{\text{C-H asim}}$
1293 '' (m)	1299 '' (m)	1295 '' (m)	Amines $\nu$ (C-N)
	1216 '' (w)		Amines $\nu$ (C-N)
1190 '' (m)	1191 '' (w)	1191 '' (s)	Amines $\nu$ (C-N)
	1127 '' (w)	1127 '' (vw)	(-C-C-) stretching or $\delta$ (C-H) aromatic
	1083 '' (w)	1075 '' (w)	Aromatic $\delta_{\text{p}}$ (C-H)
1031 '' (s)	1031 '' (s)	1030 '' (s)	Aromatic C-H in-plane def. or methane skeletal C-C vib
1003 '' (sh)	1003 '' (s)	1003 '' (s)	Aromatic C-H in-plane def. or methane skeletal C-C vib. or amines $\nu_{\text{C-N}}$
948 '' (s)	948 '' (s)	949 '' (s)	Aromatic $\delta_{\text{p}}$ (C-H)
831 '' (m)	837 '' (s)	837 '' (s)	Aromatic $\delta_{\text{oop}}$ (C-H)
802 '' (m)		790 '' (m)	Aromatic $\delta_{\text{oop}}$ (C-H)
736 '' (w)	734 '' (m)	730 '' (m)	Aromatic $\delta_{\text{oop}}$ (C-H)
	620 '' (m)		

(medium) in Barquat assigned to aromatic  $\delta_{\text{oop}}$  (C-H), possible are observed at  $730 \text{ cm}^{-1}$  (medium). All these peaks point that the interaction with HSA and Barquat is also on the aromatic ring.

### CONCLUSION

SERS of Barquat-80 using mixed colloids shows the increase of the numeral values of spectrum owing to the presence of two metallic ions:  $\text{Ag}^+$  and  $\text{Fe}^{3+}$  and the profile of spectrum indicates that  $\text{N}^+$  of the molecule of Barquat-80 is fixed over the surface that has positive electric charge and its molecular structure determines that the aromatic ring and the  $-\text{CH}_3$  radicals are placed next the metallic surface, but the paraffinic chain is placed far the surface. The presence of NaCl increases to fix the molecule on the surface. The higher values of FT-SERS spectra over magnetic colloid than over silver colloid possible is due to more intense interaction between molecule of Barquat-80 and the metallic surface. The more intense spectrum of Barquat over C colloid points that the molecule is absorbed with more force on this colloid, only the bands that correspond to vibrations of benzene point that the interaction of this radical with C colloid is weaker than in the others colloids. The interaction between Barquat - 80 and albumin looks in the spectrum because the intensity is lower in the mixture that in the Barquat-80 and this molecule joined to albumin by alkane chain and  $-\text{CH}_3$  or  $-\text{CH}_2$  radicals and with a noteworthy interaction of the aromatic points.

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