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# Resonance Raman spectroscopic study of free bilirubin and bilirubin complexes with copper(II), silver(I) and gold(III)

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## Abstract

In the present study, the UV-Vis absorption spectra and resonance Raman spectra of free bilirubin and bilirubin complexes with copper(II), silver(I) and gold(III) excited with different wavelengths were measured. From these results and from the band assignments, it is found that complexes of bilirubin-metal have different structures. This is probably due to the difference of ionic charge and ionic radius between the metal ions. © 1997 Elsevier Science B.V.

**Keywords:** Resonance Raman; Bilirubin; Bilirubin complexes

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## 1. Introduction

(4Z,15Z)-Bilirubin IX<sub>a</sub>, commonly called bilirubin (BR), the yellow orange pigment associated with jaundice, is the catabolic product of heme degradation in liver. Primary photoprocesses of BR is an active area of investigation because of its relevance to the phototherapy of hyperbilirubinaemia of the newborn [1].

BR is an unsymmetrically-substituted tetrapyrrole dicarboxylic acid (structure 1a). The low solubility of BR in water is a consequence of the strong internal hydrogen bonds, depicted in structure 1b. The structure has been demonstrated by X-ray crystallography [2]. The intramolecular hydrogen bonds are sufficiently strong enough

that BR is appreciably soluble in aqueous solution only at alkaline pH. Velapoldi and Menis [3] have reported the stabilities of complexes formed between BR and metal ions. It has been shown that adding zinc ions to an alkaline solution of BR results in BR degradation [4], the more linear BR molecule may be forced into the circular biliverdin, that is, a double bond is introduced into the central bridge. The details of structure information of the complexes of BR-metal remain largely unknown.

Raman spectroscopy is a useful tool to study the structure of free BR and complexes of BR with metal ions. Resonance Raman spectroscopy has been used to study changes in intramolecular hydrogen bonding in the complex of BR-albumin [5] and BR-phospholipid [6]. Resonance coherent anti-Stokes Raman spectroscopy has been applied

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to explore the mechanism of binding of BR to human serum albumin [7]. Surface-enhanced Raman spectroscopy has been used to study the adsorption behaviour of free BR and complex of BR-albumin at a silver electrode [8]. Recently, work of Wuhan University, namely surface-enhanced Raman spectroscopy of complexes of BR with iron(II), nickel(II) and cobalt(II) was the first investigation of the effects of differing metal-bonding on Raman spectra of BR [9]. Several

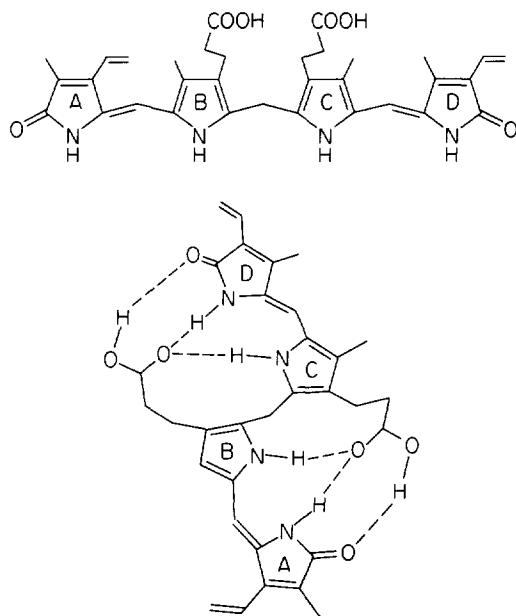
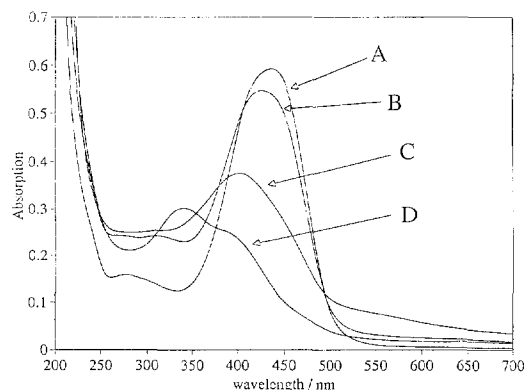


Fig. 1. Electronic absorption spectra of free BR and BR-metal complexes: (A) free BR; (B) BR-Au; (C) BR-Ag and (D) BR-Cu.

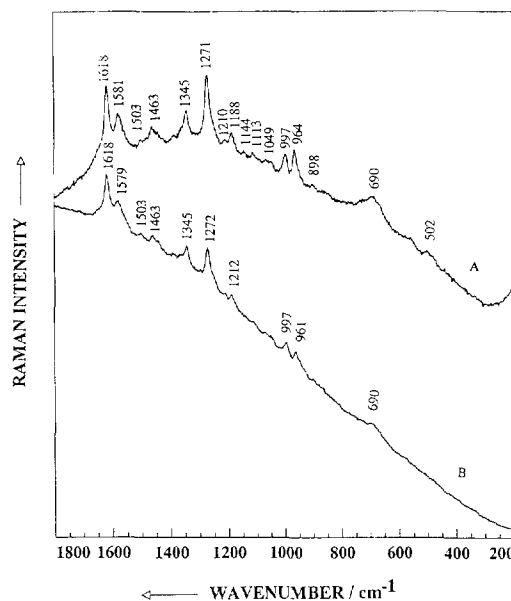


Fig. 2. Resonance Raman spectra of BR at different excitation wavelengths: (A) 514.5 and (B) 488.0 nm.

bands were proposed as marker for complex formation between BR and metal ions.

In the present paper, we report the resonance Raman spectra of free BR and complexes of BR with copper(II), silver(I) and gold(III) at different excitation wavelengths. Also, we interpret the band assignments for free BR and complexes of BR with metal ions. We use these results to describe the metal bonding and conformation of BR in the complexes of BR with metal ions.

## 2. Experimental

BR (bilirubin, p.a.) was obtained from Merck Deionized water was used to prepare solutions. Aqueous solutions of BR were prepared by dissolving BR in 0.05 M NaOH-0.025 M Na<sub>2</sub>HPO<sub>4</sub> solution. Free BR and complexes of BR with metal ions (1:1 molar ratio, BR:M) in solution with pH = 11 were prepared; Concentrations of  $1.0 \times 10^{-4}$  M were used for the measurement of resonance Raman spectra and of  $2.0 \times 10^{-5}$  M for the measurement of UV-Vis absorption spectra. After preparation of the solutions, both the

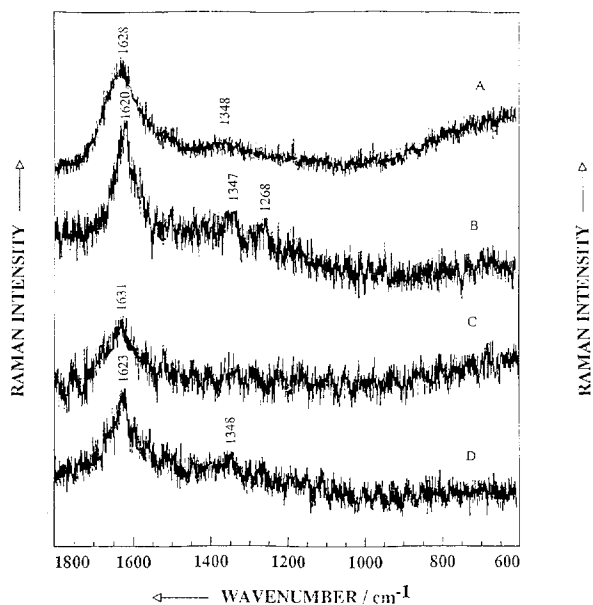


Fig. 3. Resonance Raman spectra of BR at different excitation wavelengths: (A) 300.3 nm; (B) 363.8 nm; (C) 275.4 nm and (D) 335.8 nm.

resonance Raman spectra and the absorption spectra were measured quickly.

A model Lambda 19 UV-VIS-NIR spectrometer (Perkin-Elmer) was used to record the absorption spectra. Resonance Raman spectra were taken by using the 647.1 nm line of a krypton ion laser (model 2025, Spectra-Physics), the 514.5 nm and 488.0 nm lines of an argon ion laser (model 166, Spectra-Physics), and the 363.8 nm, 335.8 nm, 300.3 nm and 275.4 nm lines of an argon ion laser (model 2085, Spectra-Physics) with power ranging between 40 and 70 mW for excitation. The scattered light was analyzed with a model 1404 0.85 M double monochromator (Spex), and detected with a model 9000 Cooled COD (Photometrics) for 647.1, 514.5 and 488.0 nm excitation, and a model CC200 Cooled CCD (Photometrics) for 363.8 nm, 335.8 nm, 300.3 nm and 275.4 nm excitation. Most of the spectra were acquired with 4–10 s integration time. The spectral resolution was  $1 \text{ cm}^{-1}$ .

Table 1

Wavenumbers and assignments of resonance Raman spectra of BR with different excitation wavelengths

Excitation wavelength (nm)						Assignment [9,10]*
514.5	488.0	363.8	335.8	300.3	275.4	
1618	1618	1620	1623	1628	1631	Str C=C, C=O, C–N
1581	1579					Str C=C, C=O, C–N
1506	1503					Str C=C, C=O
1463	1463					Bend C–H
1345	1345	1347	1348	1349		Def C–C, str C–C, C–N
1271	1272	1268				Tors C–C, C–N, C=O
1210	1212					Str C=C, N–H
1188						Str C–C, bend C–C
1144						Str C–C
1113						Def C–H
1049						Str C=C, C–N
997	997					Str C–C, C–N
964	961					Tors C=C, C–C, C–N
898						str C=C, C–C, N–H
690	690					Str C–C
502						Rock C=O
						Bend C=O

\* Bend, bending; def, deformation; rock, rocking; str, stretching; tors, torsion.

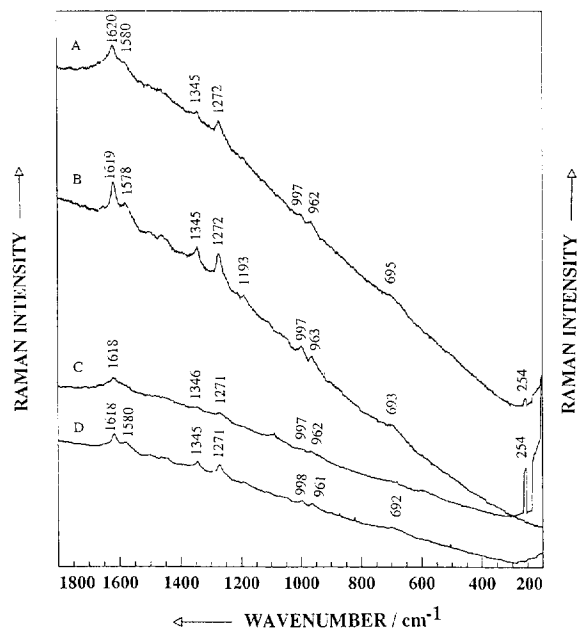


Fig. 4. Resonance Raman spectra of complexes of BR-Ag and BR-Au at different excitation wavelengths: (A) BR-Au, 514.5 nm; (B) BR-Au, 488.0 nm; (C) BR-Ag, 514.5 nm and (D) BR-Ag, 488.0 nm.

### 3. Results and discussion

The absorption spectra of free BR and complexes of BR with metal ions are shown in Fig. 1. The absorption peak of BR is shifted to the violet region in the presence of metal ions. The peak shift and the decrease of absorption caused by metal ions are of order  $\text{Cu(II)} > \text{Ag(I)} > \text{Au(III)}$ . Two new absorption peaks are observed in the presence of Cu(II). One is strong at 340 nm and another is weak at 670 nm. We shall refer the appearance of new absorption peaks and the violet shift of the absorption peak to the formation of complexes of BR with metal ions. The suggestion has been verified by electrochemical experiments. The composition of these complexes (ratio of BR to metal) was found by Equimolar Series Graph [9], to be 1:1.

The resonance Raman spectra of free BR are shown in Fig. 2 and Fig. 3. The Raman shifts and their assignments are compiled in Table 1. There are shoulders on many bands. Some bands are

sufficiently broad that they are probably composed of two or more unresolved components. We have listed the strongest bands, and noted the presence of shoulders where appropriate. It was impossible to obtain resonance Raman spectra of free BR with 647.1 nm excitation because free BR did not absorb at this excitation wavelength. With excitation of 514.5 nm and 488.0 nm, most vibrational modes of lactam and pyrrole can be observed in BR molecules. The  $1618 \text{ cm}^{-1}$  band is due to a lactam C=C, C=O, C–N and pyrrole C=C stretching; mode. The band at  $1579\text{--}1581 \text{ cm}^{-1}$  is assigned to a lactam C=C and C=O stretching mode. The band at ca.  $1503\text{--}1506 \text{ cm}^{-1}$  is identified as vinyl C–H bending mode. There is an unresolved band appearing at  $1463 \text{ cm}^{-1}$ . This band is a coupled pyrrole-lactam bridging carbon deformation and lactam C–C, C–N stretching mode. The band at  $1345 \text{ cm}^{-1}$  is a lactam ring torsion mode. The  $1271\text{--}1272 \text{ cm}^{-1}$  band is assigned to a vinyl coupled, bridge C=C and lactam N–H stretching mode. The  $1049\text{--}1212 \text{ cm}^{-1}$  region contains several closed spaced

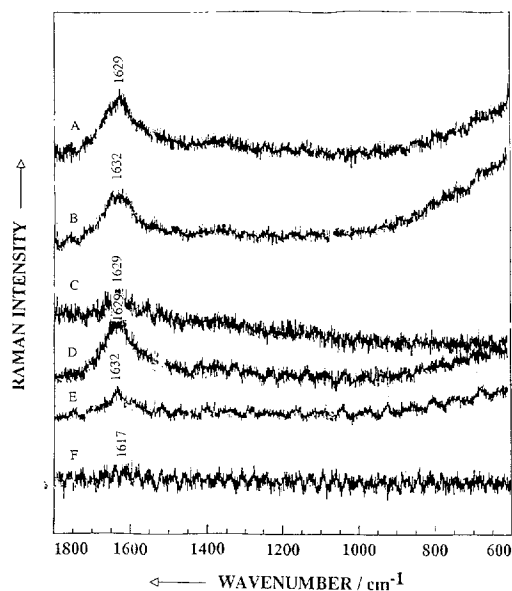


Fig. 5. Resonance Raman spectra of complexes of BR-Ag and BR-Au at different excitation wavelengths: (A) BR-Au, 300.3 nm; (B) BR-Au, 275.4 nm; (C) BR-Au, 335.8 nm; (D) BR-Ag, 300.3 nm; (E) BR-Ag, 275.4 nm and (F) BR-Ag, 363.8 nm.

Table 2

Wavenumbers and assignments of resonance Raman spectra of complex BR-Au with different excitation wavelengths

Excitation wavelength (nm)						Assignment [9,10]
514.5	488.0	363.8	335.8	300.3	275.4	
1620	1619	1617	1629	1629	1632	Str C=C, C=O, C-N
1580	1578					Str C=C, C=O, C-N
1462						Str C=C, C=O
1345	1345					Def C-C, str C-C, C-N
1272	1272					Tors C-C, C-N, C=O
	1193					Str C=C, N-H
997	997					Str C-C
962	963					Tors C=C, C-C, C-N
695	693					Str C=C, C-C, N-H
						Rock C=O

For abbreviations see the footnotes of Table 1.

bands. They are due to coupled C-C stretches on the lactam ring and vinyl side chain C-C bending ( $1210\text{--}1212\text{ cm}^{-1}$ ), C-C stretching on the pyrrole ring ( $1188\text{ cm}^{-1}$ ), pyrrole inplane methyl deformation ( $1144\text{ cm}^{-1}$ ), vinyl C=C, exo C=C, lactam and pyrrole C-N stretching ( $1113\text{ cm}^{-1}$ ) and pyrrole ring C-C and C-N stretching ( $1049\text{ cm}^{-1}$ ) modes. The  $997\text{ cm}^{-1}$  band is attributed to coupled bridge C=C, C-C torsion and lactam C-N torsion modes. The  $961\text{--}964\text{ cm}^{-1}$  band is also a lactam, C=C, C-C and N-H stretching mode. The band at  $898\text{ cm}^{-1}$  is identified as pyrrole C-C stretching mode. The band with position at cat  $690\text{ cm}^{-1}$  is a propionic acid C=O

rocking mode and the band at  $502\text{ cm}^{-1}$  is assigned to a lactam C=O bending mode. Apparently, the intensity of the lactam mode is stronger than that of the pyrrole mode.

By contrast, with UV laser excitation, only a few bands were observed. A new broad band appeared in the  $1628\text{--}1631\text{ cm}^{-1}$  region. This is

Table 3

Wavenumbers and assignments of resonance Raman spectra of complex BR-Ag with different excitation wavelengths

Excitation wavelength (nm)				Assignment [9,10]
514.5	488.0	300.3	275.4	
1618	1618	1629	1632	Str C=C, C=O, C-N
	1580			Str C=C, C=O, C-N
1346	1345			Str C=C, C=O
1271	1271			Tors C-C, C-N, C=O
1098				Str C=C, N-H
997	998			Bend C-H
962	961			Tors C=C, C-C, C-N
	692			Str C=C, C-C, N-H
				Rock C=O

For abbreviations see the footnotes of Table 1.

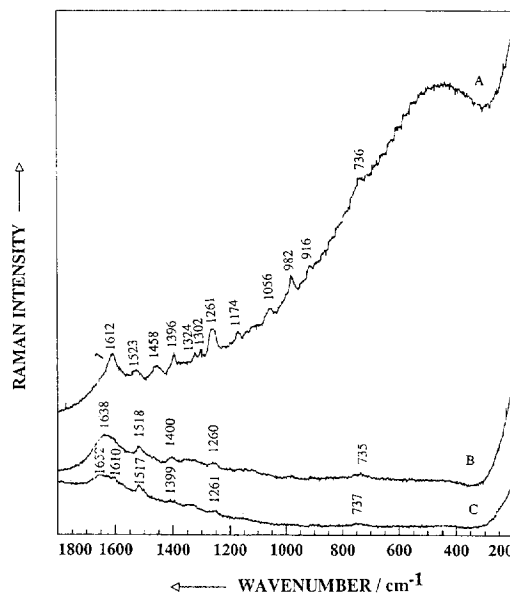


Fig. 6. Resonance Raman spectra of complex of BR-Cu at different excitation wavelengths: (A) 647.1 nm; (B) 514.5 nm and (C) 488.0 nm.

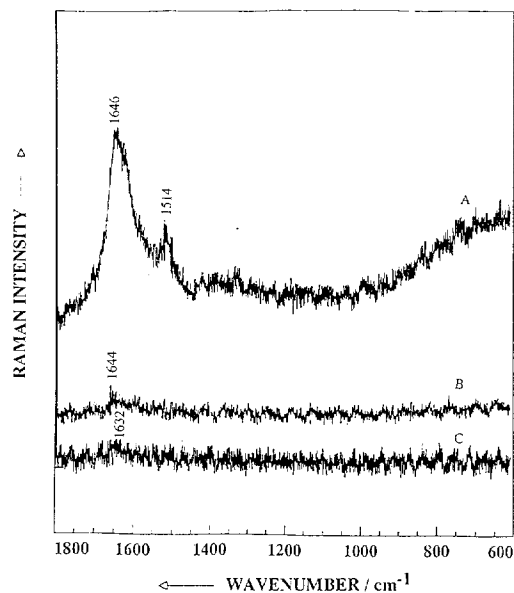


Fig. 7. Resonance Raman spectra of complex of BR-Cu at different excitation wavelengths: (A) 300.3 nm; (B) 275.4 nm and (C) 335.8 nm.

also a lactam C=C, C=O, C–N and F pyrrole C=C stretching mode. These results reflect the influence of different excitation modes on resonance Raman spectra of BR.

The resonance Raman spectra of complexes of

BR with gold(III) and silver(I) are shown in Fig. 4 and Fig. 5. Their Raman shifts and band assignments are listed in Table 2 and Table 3. There are some differences in band intensity and shift in comparison with free BR. A new weak band appears at  $1098\text{ cm}^{-1}$  in complex of BR-Au. The band is a propionic acid methylene bending mode. Some medium and weak intensity band disappear. Generally, the band intensity of the complex of BR-Au is stronger than that of BR-Ag.

Fig. 6 and Fig. 7 show the resonance Raman spectra of the complex of BR-Cu excited with Vis and UV light, respectively. Table 4 presents the Raman shifts and band assignments. The resonance Raman spectra of BR-Cu is entirely different from those of free BR, BR-Au, and BR-Ag. Most of the vibrational modes of the lactam ring disappear and some new vibrational modes of pyrrole, propionic acid and big ring appear. The band at cat  $1632\text{--}1652\text{ cm}^{-1}$  is a lactam C=O stretching mode. The band at  $1612, 1456$  and  $916\text{ cm}^{-1}$  band are pyrrole C–C sketching modes. The band appearing at  $1517\text{--}1523\text{ cm}^{-1}$  is assigned to pyrrole C=C and C–C stretching modes. The band at cat  $1396\text{--}1400\text{ cm}^{-1}$  is a methyl C–H deformation mode. The bands at  $1324$  and  $982\text{ cm}^{-1}$  are vinyl C–H rocking modes and those appearing at  $1302$  and  $1260\text{--}1261\text{ cm}^{-1}$  are due to propionic acid C–H twisting modes. The

Table 4

Wavenumbers and assignments of resonance Raman spectra of complex BR-Cu with different excitation wavelengths

Excitation wavelength (nm)						Assignment [11,12]
647.1	514.5	488.0	335.8	300.3	275.4	
1612	1638	1652	1632	1646	1644	Str C=O
1523	1518	1517				Str C–C
1456						Str C=C, C–C
1396	1400	1399				Str C–C
1324						Def C–H
1302						Rock C–H
1261	1260	1261				Wag C–H
1174						Wag C–H
1056						Twis C–H
982						Rock C–H
916						Rock C–H
736	735	737				Rtr C–C
						Str C–C, def C–C

Twis, twisting; wag, wagging. For further abbreviations see the footnotes of Table 1.

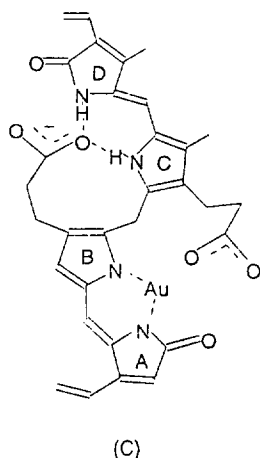
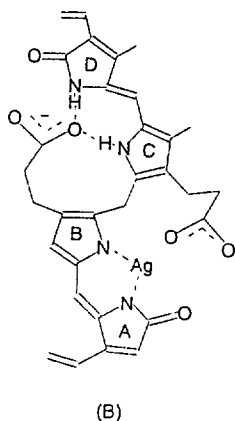
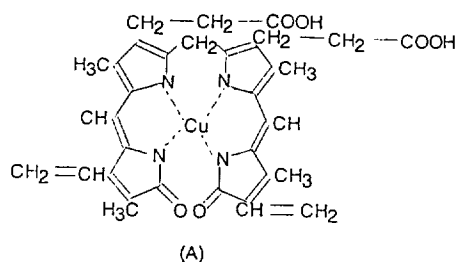


Fig. 8. Structural diagrams of BR-metal complexes: (A) BR-Cu; (B) BR-Ag and (C) BR-Au.

band at  $1174\text{ cm}^{-1}$  is a propionic acid C–H twisting mode. The band appearing at  $1056\text{ cm}^{-1}$  is attributed to a propionic acid C–H rocking mode and the one at  $735\text{--}737\text{ cm}^{-1}$  is a big ring coupled stretching C–C and deformation C–C mode. The

difference of the Raman spectra of BR with Cu ions from those with Ag and Au ions indicates that the complexation manner of a Cu ion with BR differs from that of an Ag or Au ion with BR.

BR has two similar planar pyrromethenone groups, A–B and C–D. The molecule generally adopts an internally hydrogen-bonded conformation. Since all the skeleton carbon atoms of BR bond in  $sp^2$  orbital hybridization, the molecule is basically in a planar form. When metal ions were added, the complexes are also basically in a planar configuration. But the structure of complexes of BR with metal ions depends on their ionic charge and ionic radius. The ionic radius of a silver ion ( $1.26\text{ \AA}$ ) and a gold ion ( $0.85\text{ \AA}$ ) are larger than that of a copper ion ( $0.72\text{ \AA}$ ). When silver or gold ions were added, they interact with one pyrromethenone group only (Fig. 8B and C); whereas, when copper ions were added, a copper ion can interact with two pyrromethenone groups, which leads to the formation of a circular complex which is analogous to the structure of the porphyrin big ring (Fig. 8A). These structure differences of the complexation of BR with different metal ions are reflected by the above mentioned Raman spectra changes with different metal ions. In the structure of complex of BR with a Cu ion (see Fig. 8A), the N–H band should not be observable. This is confirmed by the disappearance of the N–H stretching bands at about  $1268\text{--}1272$  and  $961\text{--}964\text{ cm}^{-1}$  in Fig. 6. These bands are strong in the complexes of BR with Ag and Au ions (see Fig. 4). The different structures of the complexes of BR with different metal ions shown in Fig. 8A and C are further supported by the following facts: (i) the appearance of the new big ring mode in the BR-Cu complex; (ii) no such mode in the BR-Ag and BR-Au complexes; (iii) the similarity of both absorption and Raman spectra of BR-Ag and BEL-Au complexes to the free BR and (iv) the appearance of the new absorption peaks in UV-Vis absorption spectra of BR-Cu complex.

#### 4. Conclusion

Resonance Raman spectra of free BR and complexes of BR with copper(II), silver(I) and gold(III) excited with different excitation wavelengths as well



as absorption spectra have been obtained. The resonance Raman spectra of complexes of BR with metal ion, have been reported for the first time. The information about vibrational modes of free BR and complexes of BR with metal ions indicates that different structure complexes are formed between BR and different metal ions.

### Acknowledgements

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### References

- [1] A.F. McDonagh, L.A. Palma, D.A. Lightner, *Science* 208 (1980) 145.
- [2] R. Bonnett, J.E. Davies, M.B. Hursthouse, *Nature (London)* 262 (1976) 326.
- [3] R.A. Velapoldi, O. Menis, *Clin. Chem.* 17 (1971) 1165.
- [4] J. Fog, B. Bugge-Asperheim, *Nature (London)* 203 (1964) 756.
- [5] Y.-Z. Hsieh, M.D. Morns, *J. Am. Chem. Soc.* 110 (1938) 62.
- [6] B.-J. Yang, M.D. Morns, *Biochemistry* 30 (1991) 688.
- [7] H. Murakawa, J. Abe, A. Seki, H. Takahashi, *J. Mol. Struct.* 297 (1993) 41.
- [8] Y.-Z. Hsieh, N.-S. Lee, R.-S. Sheng, M.D. Morris, *Langmuir* 3 (1987) 1141.
- [9] J. Chen, J.-M. Hu, Z.-S. Xu, R.-S. Sheng, *Spectrochim. Acta* 50A (1994) 929.
- [10] B. Yang, R.C. Taylor, M.D. Morns, X.-Z. Wang, J.-G. Wu, B.-Z. Yu, G.-X. Xu, R.D. Soloway, *Spectrochim. Acta* 49A (1993) 1735.
- [11] K. Smit, J. Matysik, P. Hildebrandt, F. Mark, *J. Phys. Chem.* 97 (1993) 11887.
- [12] S. Sunder, H.J. Bernstein, *J. Raman Spectrosc.* 5 (1976) 351.