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Preparation, characterization and anti-*Helicobacter pylori* activity of Bi^{3+}-hyaluronate complex

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Abstract

Bi^{3+}-hyaluronate complexes were prepared at pH 11.0–12.0. The complexes were characterized by elemental analysis, ^{13}C NMR, FT-IR, CD, XRD, TGA and XPS. Participation of N and O atoms in coordination with Bi^{3+} has been confirmed. The anti-*Helicobacter pylori* activity in vitro of the complexes are similar with that of CBS, the most utilized bismuth preparation clinically.

Keywords: Bi^{3+}; NaHA; Complex; Characterization; Anti-*Helicobacter pylori* activity

1. Introduction

Hyaluronic acid (HA) is a charged macromolecule, known for more than 70 years. It is structurally the simplest and the most frequent among naturally occurring glycosaminoglycans (Pirc, Arcon, Bukovec, & Kodre, 2000). HA consists of repeating disaccharide units composed of *N*-glucosamine and D-glucoronic acid. HA occurs in the intracellular matrix of most vertebrates, usually as the sodium salt (Lapcik, Lapcik, De Smedt, Demeester, & Chabrecek, 1998). HA is also used clinically because it is able to modulate a number of cellular functions. The pharmacological effects are ascribed either to direct binding on cellular receptors, or to indirect action, e.g., through binding of inflammatory mediators (Lapcik et al., 1998; Pirc et al., 2000). The efficiency of both mechanisms depends on the molecular weight of HA.

Under physiological conditions, HA is a negatively charged polyelectrolyte due to repeating anionic carboxylic sites (Cleland, Wang, & Detweiler, 1982). Each disaccharide unit requires a positive metal ion for the charge neutrality. The interaction of the polyanion with the cations is an important factor for the supermolecular structure (Moulabbi, Broch, Robert, & Vasilescu, 1997). The coordination geometry of the ion exerts a strong influence on the conformation of the macromolecule and its biological activity (Tratar Pirc, Arcon, Kodre, & Bukovec, 2004).

HA has been shown to coordinate to Cu^{2+}, Ag^{+}, Au^{3+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} (Burger et al., 2001; Merce, Marques Carrera, Santos Romanholi, & Lobo Recio, 2002; Pirc et al., 2000; Tratar Pirc et al., 2004). Hyaluronate complexes with heavy metals show microbicidal activity. Gold complexes are used in arthritis therapy; platinum complexes have antitumor activity (Maeda, Takasuka, Suga, Uehara, & Hoshi, 1993). Zinc complexes are explored in treatment of peptic ulcers (Illes et al., 1998).

Helicobacter pylori has been recognized as a common case of chronic, active type B gastritis and peptic ulcer disease. The bacteria were also considered to constitute a risk factor for the development of gastric carcinoma and gastric lymphoma (Wadström, 1995). Bismuth preparations have been used in the treatment of gastrointestinal disease for centuries (Gorbach, 1990; Marshall, 1991). Bismuth compounds showed valid inhibitory activity against *H. pylori* (Dixon, 1995; Iffland, 1994). Among the compounds the most frequently used in treatment are bismuth subsalicy-

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late (BSS), bismuth subnitrate and colloidal bismuth subcitrate (CBS). New bismuth compounds of antibacterial activity have also been investigated (Dittes, Vogel, & Keppler, 1997; Nie et al., 1999; Sandha et al., 1998).

The current work aims to prepare and characterize the Bi^{3+}-hyaluronate complexes and evaluate the anti-*H. pylori* activity of the complexes, with the ultimate objective of finding new effective compounds utilized in the treatment of peptic ulcers.

2. Experimental

2.1. Materials

High molecular weight NaHA (HW NaHA) with *Mr* of 1.24×10^6 Da, medium molecular weight NaHA (MW NaHA) with *Mr* of 6.75×10^5 Da and low molecular weight NaHA (LW NaHA) with *Mr* of 2.40×10^5 Da were obtained from a bacterial strain of *Streptococcus zooepidemicus,* and was provided by Shandong Freda Biochem Co. Ltd. (Jinan, China). All other chemicals were of analytical grade.

2.2. Preparation of the complexes

A quantity of 1.22 g of $Bi(NO_3)_3{\cdot}5H_2O$ and 1.22 g of mannitol were added to 50 ml of distilled water and dissolved under stirring. The pH of $Bi(NO_3)_3$ solution was adjusted to 11.0–12.0 with 10% KOH solution. A quantity of 1.0 g of HW NaHA was added to 100 ml of distilled water and left to swell for several hours. The pH of NaHA solution was adjusted to 9.0–10.0. Then the $Bi(NO_3)_3$ solution was added to NaHA solution and the resulting mixture was stirred for about 20 min. About 2.5 volume of absolute ethanol was added slowly under vigorous stirring. The white precipitate was collected and vacuum dried at 40 °C (BiHA-1). Complexes of Bi^{3+} with MW NaHA and LW NaHA were prepared following the steps (BiHA-2 and BiHA-3).

2.3. Characterizations

The C, H, N contents were determined on a Vario EL III elemental analyzer. The Bi^{3+} content was determined by complexometry (Pharmacopoeia of PRC, 2005). The Na^+ and K^+ contents were determined by a HP 3510 atomic absorption spectrophotometer. FT-IR spectra of isolated powdered Bi^{3+}-hyaluronate complexes and of the starting material NaHA were recorded in the range from 4000 to 400 cm^{-1} on a Nicolet Magna-IR 750 FT-IR spectrophotometer in KBr discs. The CD spectra were recorded in the wavelength range 200–400 nm on a Chirascan Circular Dichroism Spectrometer. ^{13}C NMR spectra were per-

Table 1
Contents determination of Bi^{3+}-hyaluronate complexes

Complex	Analysis found (Calc.)%					
	C	N	H	Bi^{3+}	Na^+	K^+
BiHA-1 $K_{0.08}Na_{0.72}Bi_{0.81}(C_{14}H_{20}O_{11}N)(OH)_{2.23}(H_2O)_{3.7}$	25.54	2.047	3.885	25.6	2.50	0.48
	(25.02)	(2.08)	(4.41)	(25.21)	(2.47)	(0.46)
BiHA-2 $K_{0.08}Na_{0.76}Bi_{0.80}(C_{14}H_{20}O_{11}N)(OH)_{2.24}(H_2O)_{3.5}$	25.46	1.996	3.968	25.2	2.66	0.47
	(25.19)	(2.10)	(4.38)	(25.07)	(2.62)	(0.47)
BiHA-3 $K_{0.08}Na_{0.76}Bi_{0.79}(C_{14}H_{20}O_{11}N)(OH)_{2.21}(H_2O)_{3.5}$	25.61	1.997	4.002	25.2	2.68	0.46
	(25.29)	(2.11)	(4.40)	(24.86)	(2.63)	(0.47)

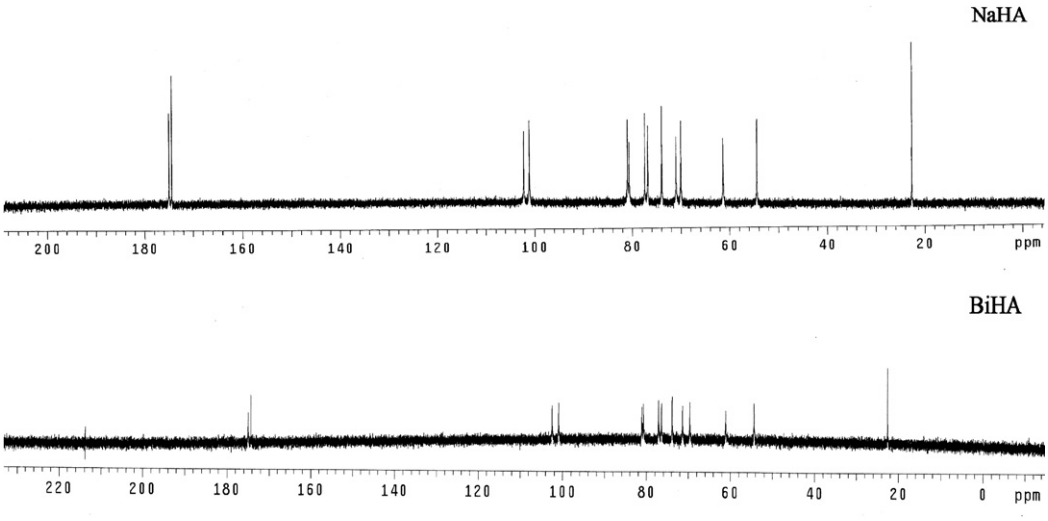

Fig. 1. ^{13}C NMR spectra of LW NaHA and BiHA-3.

formed on a Varian Inova-600 spectrometer in D_2O/NaOD. X-ray diffraction patterns were obtained using a Rigaku D/max-γB X-ray diffractometer. Thermal analysis was performed using thermogravimetric analysis (TGA) on a TG-40 DTA-40M thermal analyzer. X-Ray Photoelectron Spectra were obtained with a PHI-5300 ESCA spectrometer. Binding energies were corrected using the binding energy values for C_{1s} of adventitious carbon fixed at 284.8 eV.

2.4. Testing for anti-H. pylori activity

The minimum inhibitory concentrations (MICs) of Bi^{3+}-hyaluronate complexes and CBS were determined by the agar dilution method (Fukai et al., 2002). The test compounds were dissolved in sterile distilled water and further diluted in a twofold series. *Helicobacter pylori* NCTC11637, 26695 and J99 were incubated on Columbia agar supplemented with 5% sheep blood containing twofold serial dilutions of the substances. Final concentration of the bacteria used was 10^7 colony forming units (cfu)/ml. MICs were determined after 3 days of incubation at 37 °C under microaerophilic conditions. MICs were defined as the lowest concentrations of the test compounds inhibiting visible bacterial growth.

Table 2
^{13}C chemical shifts for LW NaHA and BiHA-3

		Chemical shifts/ppm	
		NaHA	BiHA-3
GlcA	C-1	102.139	102.488
	C-2	70.926	71.385
	C-3	73.855	73.834
	C-4	80.545	80.746
	C-5	77.334	77.120
	C=O	174.318	174.297
GlcNAc	C-1	101.013	100.933
	C-2	54.348	54.367
	C-3	80.875	81.106
	C-4	69.930	69.583
	C-5	76.712	76.376
	C-6	61.262	61.004
	C—Me	22.580	22.592
	C=O	174.896	174.942

3. Results and discussion

3.1. Characterization of Bi^{3+}-hyaluronate complexes

The results of contents determination are shown in Table 1. The complexes could readily dissolve in water to form an alkaline colloidal solution with high viscosity (pH 9.5–9.9 in 1 mg/ml of solution). Each complex contains Na^+, K^+, Bi^{3+}, hyaluronate ($C_{14}H_{20}O_{11}N$, disaccharide unity), OH^- and H_2O (9.5%~10.1%). The mechanism of the complexes formation could be described as follows:

$$Bi^{3+} + \text{mannitol} + OH^- \rightarrow Bi^{3+} - \text{mannitol} - OH^-$$

$$Bi^{3+} - \text{mannitol} - OH^- + \text{hyaluronate} \rightarrow Bi^{3+} - \text{hyaluronate} - OH^- + \text{mannitol}$$

Because of the high viscosity of NaHA and BiHA, the signals of ^{13}C NMR in neutral condition were weak and of poor resolution. The signals of BiHA and NaHA in

Table 3
The characteristic frequencies for the symmetric and asymmetric vibrational modes in NaHA and BiHA

	ν_{asym} (cm^{-1})	ν_{sym} (cm^{-1})	Δ (cm^{-1})
HW NaHA	1630	1413	217
BiHA-1	1617	1412	205
MW NaHA	1632	1415	217
BiHA-2	1618	1410	208
LW NaHA	1628	1413	215
BiHA-3	1620	1411	209

Separation (Δ) of the vibrational frequencies is also given.

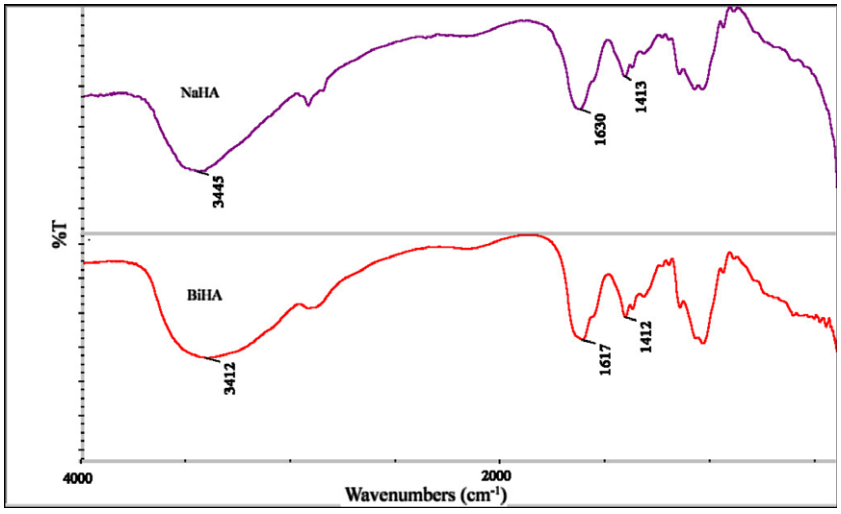

Fig. 2. FT-IR spectra of BiHA-1 and corresponding starting material HW NaHA.

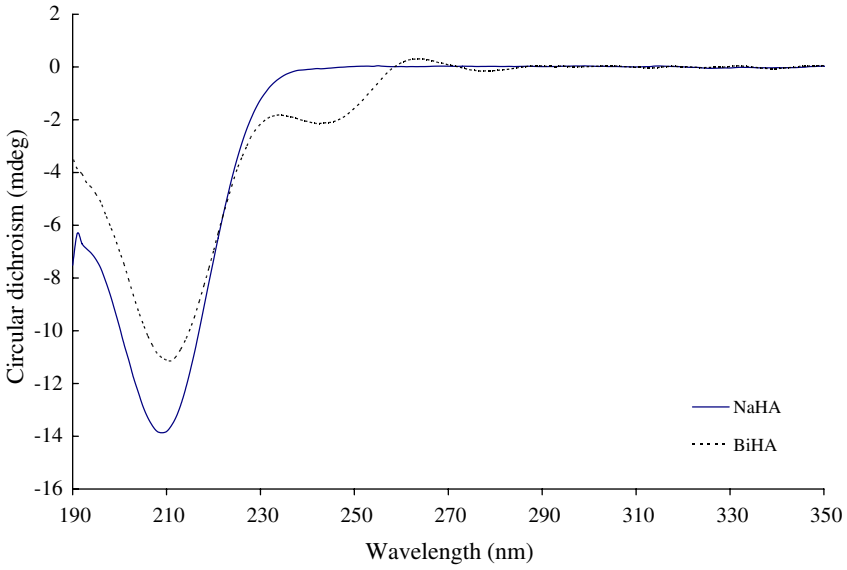

Fig. 3. CD spectra of BiHA-1 and corresponding starting material HW NaHA.

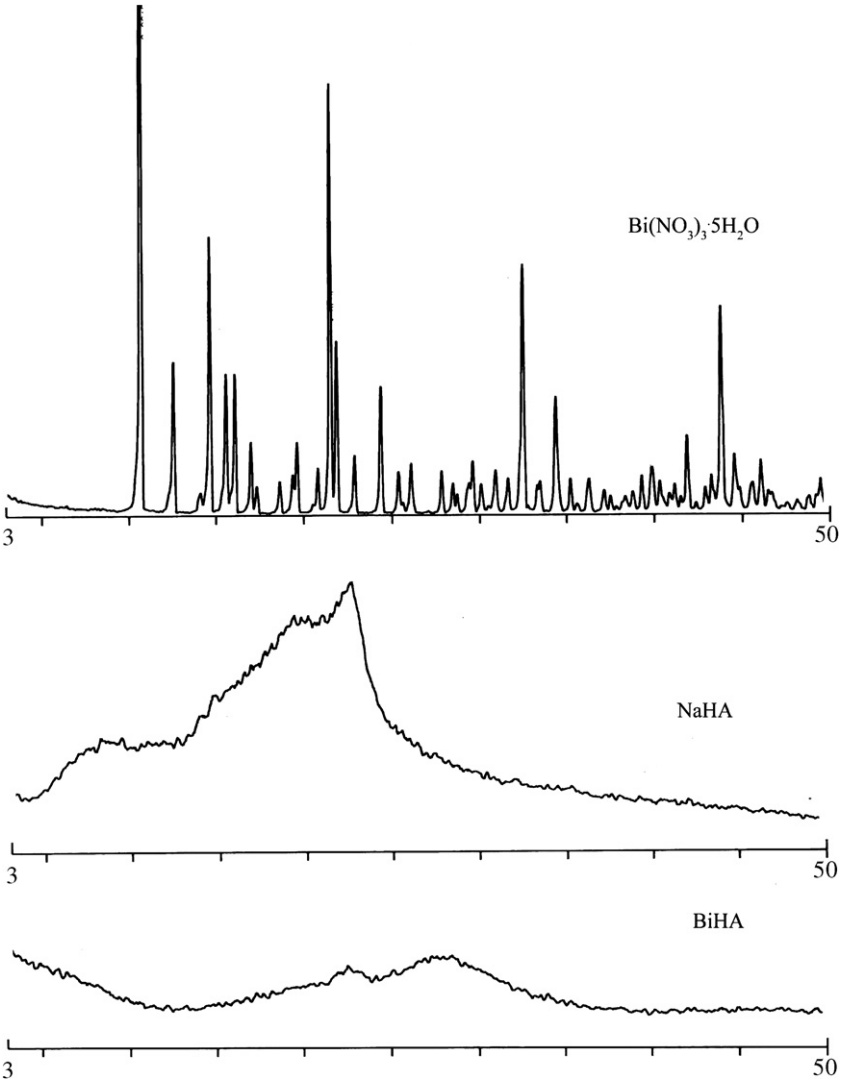

Fig. 4. X-ray diffraction of $Bi(NO_3)_3{\cdot}5H_2O$, LW NaHA and BiHA-3.

Table 4
Thermal analysis (TGA) of LW NaHA and BiHA-3

	Temperature range (°C)	Corresponding weight loss (%)
LW NaHA	41.1–135.5	21.11
	135.5–223.2	1.66
	223.2–253.3	30.01
	253.3–368.3	18.88
	368.3–496.9	4.44
	41.1–496.9	76.09
BiHA-3	41.0–131.9	22.08
	131.9–214.8	2.40
	214.8–238.6	28.80
	238.6–494.5	12.48
	41.0–494.5	65.76

alkaline condition were clear and distinct, consistent with the reports (Bociek, Darke, Welti, & Rees, 1980). As shown in Fig. 1 and Table 2, the chemical shifts of BiHA-3 were similar with those of NaHA. This supports the results of contents determination that there was no residual mannitol in the complexes. Such observations also indicate that complexation occurs without significant depolymerization.

FT-IR spectra of the complexes and corresponding starting materials NaHA were measured (see Fig. 2). The spectra of NaHA agree well with those obtained by Gilli et al. (Gilli, Kacurakuva, Mathlouhi, Navarini, & Paoleti, 1994). The spectra of all three complexes are similar with that of NaHA, so the same assignations can be adopted.

The FT-IR spectra of BiHA-1 exhibit many alterations from that of NaHA. The major differences are: (1) The wide peak at 3445 cm^{-1}, corresponding to the stretching vibration of —NH_2 group and —OH group, shifted to lower frequency (3412 cm^{-1}), indicating that the —NH_2 group and —OH group were involved in complexation (Wang, Du, & Liu, 2004). (2) The carboxyl absorption

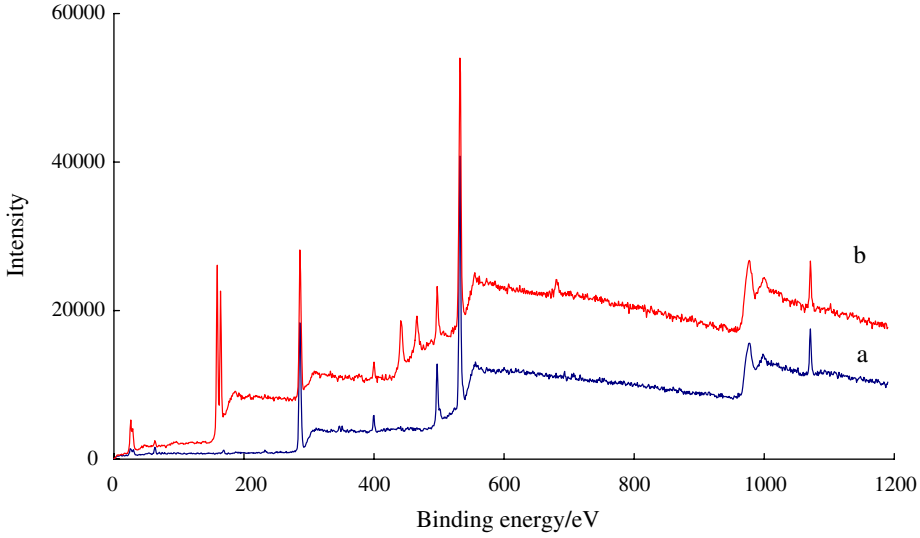

Fig. 5. XPS survey spectra of LW NaHA (a) and BiHA-3 (b).

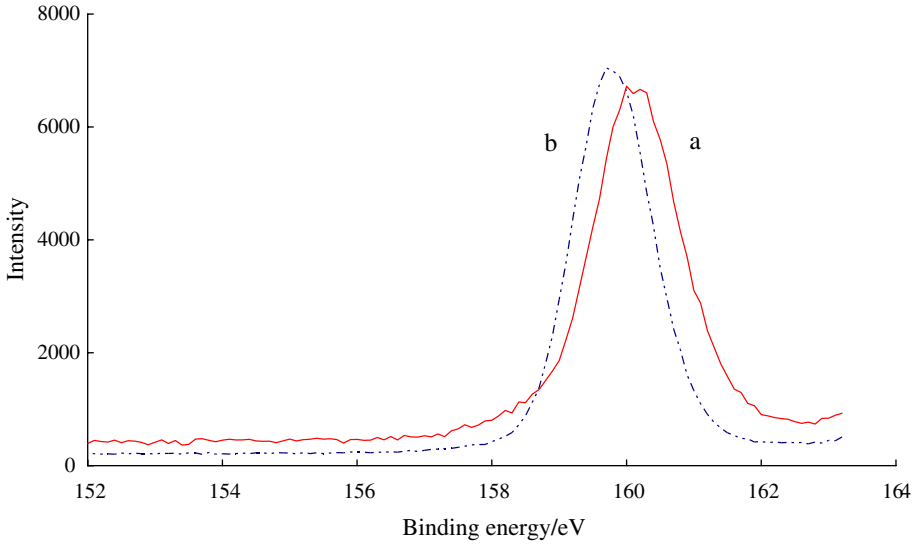

Fig. 6. $Bi_{4f7/2}$ spectra of $Bi(NO_3)_3{\cdot}5H_2O$ and BiHA-3.

bands are shifted significantly, indicating the bonding of Bi^{3+} ion to the carboxyl group. The separation of the two vibrational frequencies (Δ) in complexes are slightly less than in corresponding starting material NaHA (see Table 3), thus a bridging coordination can be deduced (Nakamoto, 1997).

Fig. 3 shows that complexes of Bi^{3+} with hyaluronate change appreciably in the CD properties compared with NaHA. For the complex a small positive CD in the region of 260–265 nm is found. According to reports (Figueroa & Chakrabarti, 1978), a ligand-to-Bi^{3+} charge-transfer transition can account for the positive CD band. The difference in CD property also indicates the conformational change owing to the formation of the complexes.

The X-ray diffraction spectra of $Bi(NO_3)_3{\cdot}5H_2O$, LW NaHA and BiHA-3 are shown in Fig. 4. $Bi(NO_3)_3{\cdot}5H_2O$ have 11 typical crystal peaks at 2θ of 10.70°, 12.58°, 14.66°, 15.58°, 16.10°, 21.50°, 21.86°, 24.36°, 32.48°, 34.38° and 43.78° and numerous small peaks between 10° and 45°. NaHA consists of three peaks at 2θ of 8.24°, 19.26° and 22.60°, among which the peak at 22.60° is relatively sharper. BiHA contains two broad diffraction peaks at 2θ of 22.52° and 27.86° which are hardly appreciable. These results indicate that BiHA is of poor crystallinity than NaHA.

The thermal stability and degradation behavior of LW NaHA and BiHA-3 were evaluated by TGA under nitrogen atmosphere. As shown in Table 4, NaHA decomposed in five stages. First, between 41.1 and 135.5 °C, NaHA lost

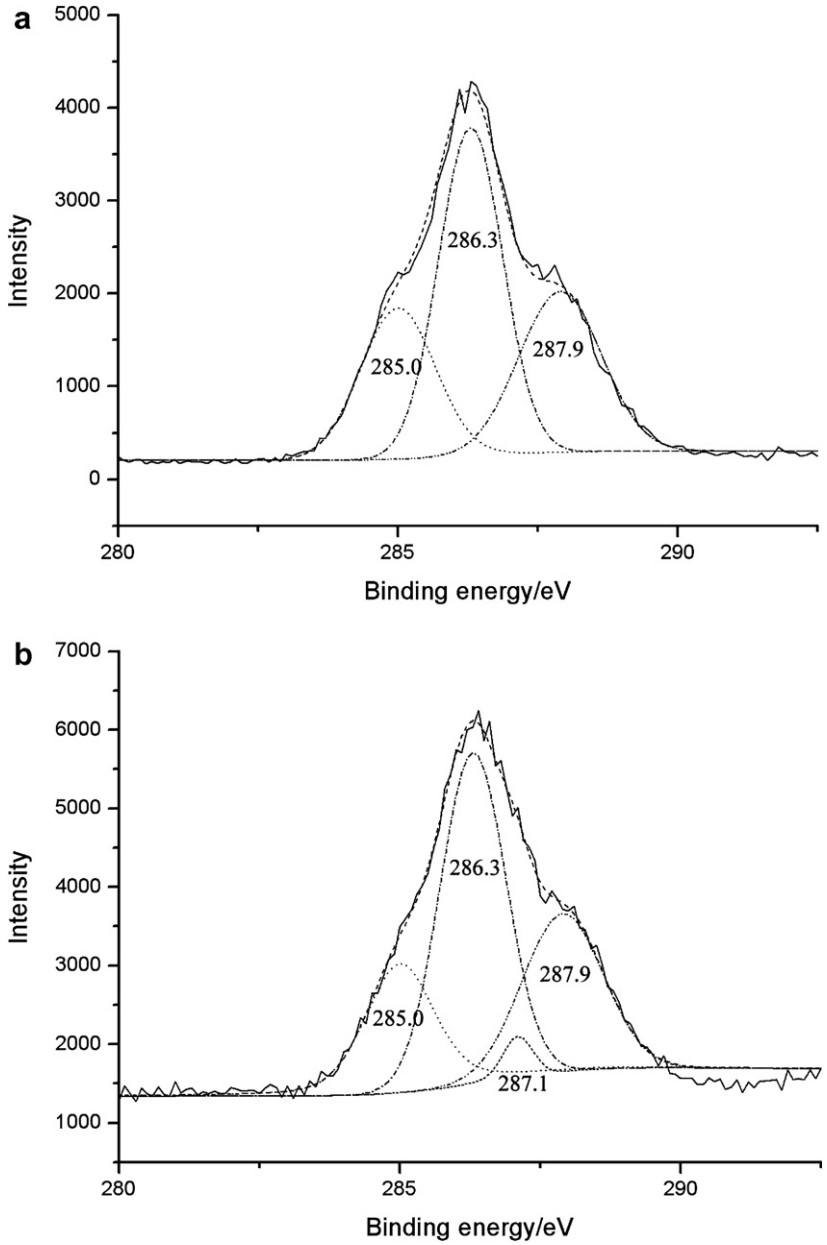

Fig. 7. C_{1s}, N_{1s} and O_{1s} spectra of LW NaHA and BiHA-3 after peak fitting. (a) C_{1s} spectra of LW NaHA; (b) C_{1s} spectra of BiHA-3; (c) N_{1s} spectra of BiHA-3; (d) O_{1s} spectra of BiHA-3.

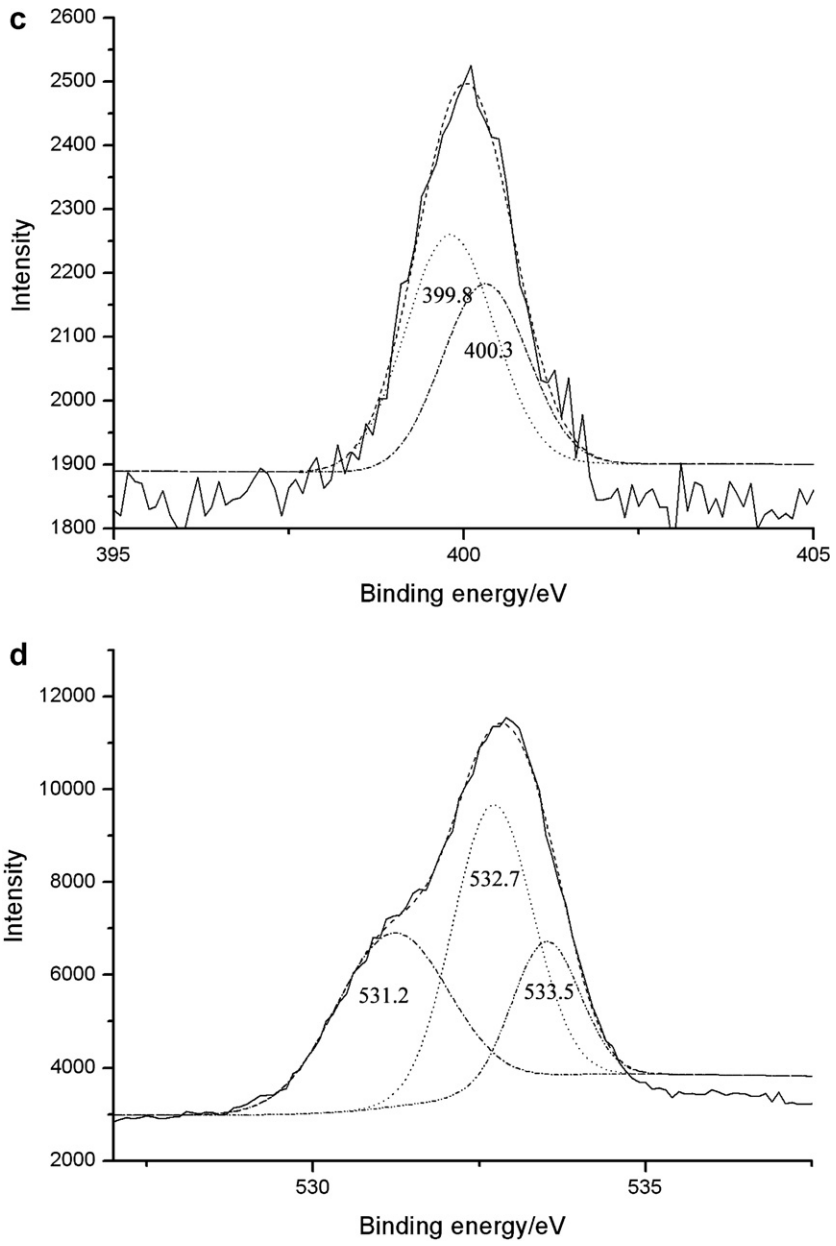

Fig. 7 (*continued*)

21.11% of its weight, including the loss of water. Between 135.5 and 223.2 °C, the weight loss was very few. In the subsequent stage from 223.2 to 253.3 °C, NaHA lost 30% of its total weight. In the stage of 253.3–368.3 °C and 368.3–496.9 °C, NaHA lost 18.88% and 4.44% of its total weight, respectively.

As for the complex, the thermal characteristic was different from that of NaHA. BiHA decomposed in four stages. Between 41.0 and 131.9 °C, BiHA lost 22.08% of its total weight, including the loss of water. In the stage of 131.9–214.8 °C, BiHA lost 2.40% of its total weight. Between 214.8 and 238.6 °C, BiHA lost 28.8% of its total weight. In the last stage from 238.6 to 494.5 °C, BiHA lost 12.48% of its total weight. Compared with NaHA, the thermal stability of BiHA declined, for the major weight loss of BiHA appeared at lower temperature than NaHA. These results may be interpreted by the conformational change and the descent of crystallinity in the formation of the complex.

X-ray photoelectron spectroscopy (XPS) was used to characterize NaHA, $Bi(NO_3)_3{\cdot}5H_2O$ and BiHA, as shown in Figs. 5 and 6. As shown in Fig. 5, the survey spectra of NaHA contains three main absorption peaks at about 286, 400 and 533 eV corresponding to C_{1s}, N_{1s} and O_{1s}, respectively. The survey spectra of BiHA contains five main absorption peaks at about 286, 400, 533, 159 and 164 eV corresponding to C_{1s}, N_{1s}, O_{1s} and Bi_{4f}, respectively. As shown in Fig. 6, the binding energy of $Bi_{4f7/2}$ in BiHA decreased by 0.9 eV compared with that in $Bi(NO_3)_3{\cdot}5H_2O$, indicating the coordination of Bi^{3+} with ligands. In the

Table 5
Binding energy and chemical state of C, O, N and Bi in LW NaHA, $Bi(NO_3)_3{\cdot}5H_2O$ and BiHA-3

	Element	Binding energy/eV	Binding energy after peak fitting/eV	Chemical state
LW NaHA	C	286.3	285.0	C—C, C—H
			286.3	C—O—H, C—O—C
			287.9	C=O
	N	399.8		C—N, N—H
	O	532.7		C—O—H, C—O—C, C=O
$Bi(NO_3)_3{\cdot}5H_2O$	$Bi_{4f7/2}$	159.7		Bi—O, Bi—N
BiHA-3	C	286.4	285.0	C—C, C—H
			286.3	C—O—H, C—O—C
			287.9	C=O
			287.1	C—O—Bi, C—N—Bi
	N	400.1	399.8	C—N, N—H
			400.3	N—Bi
	O	532.9	532.7	C—O—H, C—O—C, C=O
			531.2	O—H
			533.5	O—Bi
	$Bi_{4f7/2}$	158.8		Bi—O—C, Bi—N—C

Table 6
MICs of Bi^{3+}-hyaluronate complexes and CBS

	MICs (μg/ml, calculated as bismuth content)		
	NCTC11637	26695	J99
BiHA-1	5	5	5
BiHA-2	5	5	5
BiHA-3	5	5	5
CBS	5	5	5

process, the Bi^{3+} ions provided empty orbits and accepted lone-pair electrons donated by O and N atoms.

By peak fitting, the binding energy and chemical states of C, O, N and Bi are shown in Fig. 7 and Table 5. It could be seen that the binding energy of C_{1s} after peak fitting are 285.0, 286.3 and 287.9 eV corresponding to different chemical states in NaHA. After coordination with Bi^{3+}, two new chemical states with binding energy of 287.1 eV appeared in BiHA. Compared with NaHA, the new chemical state of N with binding energy of 400.3 eV appeared, owing to the complexation with Bi^{3+} in BiHA. In NaHA, the binding energy of O_{1s} is 532.7 eV. In BiHA, two chemical states of O_{1s} with binding energy of 531.2 and 533.5 eV appeared, owing to the involvement of OH^- and complexation with Bi^{3+}, respectively.

3.2. Testing for anti-H. pylori activity

As shown in Table 6, the Bi^{3+}-hyaluronate complexes showed valid anti-*H. pylori* activity. The MICs of the complexes against three *H. pylori* strains are both identical with that of CBS, the most used bismuth preparation in eradication treatment of *H. pylori*, despite the high molecular weight and complicate structure of the complexes.

4. Conclusion

Bi^{3+}-hyaluronate complexes were prepared by the reaction of NaHA with $Bi(NO_3)_3$ in alkaline condition. The complexes were characterized by elemental analysis, FT-IR, ^{13}C NMR, CD, XRD, TGA and XPS. All the analysis confirmed that in Bi^{3+}-hyaluronate complexes, Bi^{3+}coordinated with N and O atoms. The stability of the complexes decreased than that of NaHA.

The anti-*H. pylori* activity of Bi^{3+}-hyaluronate complexes in vitro were similar with that of CBS despite the high viscosity and complicate structure of the complexes.

References

Bociek, S. M., Darke, A. H., Welti, D., & Rees, D. A. (1980). The ^{13}C NMR spectra of hyaluronate and chondroitin sulphates. Further evidence on an alkali-induced conformation change. *European Journal of Biochemistry, 109*(2), 447–456.

Burger, K., Illes, J., Gyurcsik, B., Gazdag, M., Forrai, E., Dekany, I., et al. (2001). Metal ion coordination of macromolecular bioligands: Formation of zinc(II) complex of hyaluronic acid. *Carbohydrate research, 332*(2), 197–207.

Cleland, R. L., Wang, J. L., & Detweiler, D. M. (1982). Polyelectrolyte properties of sodium hyaluronate. 2. Potentiometric titration of hyaluronic acid. *Macromolecules, 15*(2), 386–395.

Dittes, U., Vogel, E., & Keppler, B. K. (1997). Overriew on bismuth(III) and bismuth(V) complexes with activity against *Helicobacter pylori*. *Coordination Chemistry Reviews, 163*, 345–364.

Dixon, J. S. (1995). *Helicobacter pylori* eradication: Unravelling the facts. *Scandinavian Journal of Gastroenterology, 30*, S48–S62.

Figueroa, N., & Chakrabarti, B. (1978). Circular dichroism studies of copper(II)-hyaluronic acid complexes in relation to conformation of the polymer. *Biopolymers, 17*(10), 2415–2426.

Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S., & Nomura, T. (2002). Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life Sciences, 71*(12), 1449–1463.

Gilli, R., Kacurakuva, M., Mathlouhi, M., Navarini, L., & Paoleti, S. (1994). FTIR studies of sodium hyaluronate and its oligomers in the amorphous solid phase and in aqueous solution. *Carbohydrate Research, 263*(2), 315–326.

Gorbach, S. L. (1990). Bismuth therapy in gastrointestinal diseases. *Gastroenterology, 99*(3), 863–875.

Iffland, R. (1994). Bismuth. In H. G. Seiler, A. Sigel, & H. Sigel (Eds.), *Handbook on metals in clinical and analytical chemistry* (pp. 269–281). New York, NY: Marcel Dekker, Inc.

Illes, J., Matuz, J., Neszmelyi, E., Forrai, G., Stefko, B., Saghy, K., et al. (1998). Use of zinc hyaluronate against peptic ulcer. Patent WO/1998/048815.

Lapcik, L., Jr., Lapcik, L., De Smedt, S., Demeester, J., & Chabrecek, P. (1998). Hyaluronan: Preparation, structure, properties, and applications. *Chemical Reviews, 98*(8), 2663–2684.

Maeda, M., Takasuka, N., Suga, T., Uehara, N., & Hoshi, A. (1993). Antitumor activity of a new series of platinum complexes: Trans(+/−)-1,2-cyclohexanediammineplatinum(II) conjugated to acid polysaccharides. *Anticancer Drugs, 4*(2), 167–171.

Marshall, B. J. (1991). The use of bismuth in gastroenterology. The ACG Committee on FDA-related matters. American College of Gastroenterology. *The American Journal of Gastroenterology, 86*(1), 16–25.

Merce, A. L., Marques Carrera, L. G., Santos Romanholi, L. K., & Lobo Recio, M. A. (2002). Aqueous and solid complexes of iron(III) with hyaluronic acid. Potentiometric titrations and infrared spectroscopy studies. *Journal of Inorganic Biochemistry, 89*(3–4), 212–218.

Moulabbi, M., Broch, H., Robert, L., & Vasilescu, D. (1997). Quantum molecular modeling of hyaluronan. *Journal of Molecular Structure (Theochem)*, 477–508.

Nakamoto, K. (1997). *Infrared and Raman spectra of inorganic and coordination compounds* (5th ed.). New York: Willy.

Nie, Y., Li, Y., Wu, H., Sha, W., Du, H., Dai, S., et al. (1999). Colloidal bismuth pectin: An alternative to bismuth subcitrate for the treatment of *Helicobacter pylori*-positive duodenal ulcer. *Helicobacter, 4*(2), 128–134.

Pharmacopoeia of PRC. (2005). Part II, 628. Beijing: Chemical Industry Press.

Pirc, E. T., Arcon, I., Bukovec, P., & Kodre, A. (2000). Preparation and characterisation of copper(II) hyaluronate. *Carbohydrate Research, 324*(4), 275–282.

Sandha, G. S., LeBlanc, R., Van Zanten, S. J., Sitland, T. D., Aqocs, L., Burford, N., et al. (1998). Chemical structure of bismuth compounds determines their gastric ulcer healing efficacy and anti-*Helicobacter pylori* activity. *Digestive Diseases and Sciences, 43*(12), 2727–2732.

Tratar Pirc, E., Arcon, I., Kodre, A., & Bukovec, P. (2004). Metal-ion environment in solid Mn(II), Co(II) and Ni(II) hyaluronate. *Carbohydrate Research, 339*(15), 2549–2554.

Wadström, T. (1995). An update of *Helicobacter pylori*. *Current Opinion in Gastroenterology, 11*, 69–75.

Wang, X., Du, Y., & Liu, H. (2004). Preparation, characterization and antimicrobial activity of chitosan–Zn complex. *Carbohydrate Polymers, 56*, 21–26.