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Zinc–naproxen complex: synthesis, physicochemical and biological evaluation

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Abstract

Naproxen has a propensity to cause ulcers whereas zinc ions are known to possess an anti-ulcer and anti-inflammatory activity. Therefore, zinc complex of naproxen was prepared by adding zinc sulfate to an aqueous solution of sodium naproxen and its structure was characterized by IR, ^{1}H NMR and ^{13}C NMR, UV, DSC, atomic absorption spectroscopy, and elemental analysis. Anti-inflammatory studies, using the carrageenan-induced hind paw oedema showed that there was a significant difference ($P < 0.05$, ANOVA plotted by Dunnet's test) in the anti-inflammatory activity of naproxen, its zinc complex, and the physical mixture of naproxen and zinc sulfate. In addition, zinc complex of naproxen showed a significant reduction in ulcers (lesion index (LI)) as compared to that of naproxen and physical mixture of naproxen and zinc sulfate. Thus, the use of the complex may be preferable to naproxen alone.

Keywords: Zinc–naproxen complex; Anti-inflammatory activity; Ulcerogenic activity; Naproxen

1. Introduction

Naproxen [(+)-6-methoxy-α-methyl-2-naphthalene acetic acid], a non-steroidal anti-inflammatory agent, is used in painful and inflammation conditions like rheumatoid arthritis, spondilytis, and osteoarthritis but its efficacy is offset by significant incidence of gastrointestinal ulceration (Schoen and Vender, 1989). Naproxen, when orally administered, forms crystals that coat the digestive mucus due to its acidity and low solubility. It dissolves slowly, damaging the stomach walls. It has been reported that complexation with β-cyclodextrin prevents the ulceration by masking the free carboxyl group of the drug and also by enhancing its solubility (Espinar et al., 1991). Various approaches have been used in the past to reduce gastric damage caused by naproxen viz., prodrugs (Shandhag et al., 1992), enteric coating (Aabakken et al., 1992; Huskisson et al., 1992), solid dispersion with polyvinylpyrrolidone (Singla and Nagra, 2000), NO-naproxen (Muscara et al., 2000), microspheres (Calis et al., 2002), lipid emulsion (Nasirideen et al., 1998), and co-compression with buffers (Chakrabarti and Southard, 1997). Metal ion complexation of naproxen is advantageous for the transport of naproxen into the cells as supported by the fact that the transport of organic ligands into the cells can be facilitated by the formation of metal complexes. This concept was invoked to explain the transport and storage of catecholamines (Albert, 1985).

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Zinc ions are known to possess anti-ulcer properties (Fraser et al., 1972; Frommer, 1975) besides having anti-inflammatory activity of their own (Simkin, 1976). Further, low serum zinc levels occur in most rheumatic diseases (Higgs et al., 1979). It has also been reported that zinc complexes of aspirin (Singla and Wadhwa, 1994) and indomethacin (Singla and Wadhwa, 1995) were found to be more potent with reduced ulcerogenic ability than their parent drugs. Hence, it was deemed worthwhile to synthesize and evaluate the zinc complex of naproxen for its physicochemical and biological characteristics.

2. Materials and methods

2.1. Materials

Naproxen was supplied ex-gratis by Divis laboratories, Delhi and Panacea Biotech, Lalru, India. Carrageenan lambda (Type IV), zinc sulfate heptahydrate and 1-octanol were obtained from Sigma Chemical Co., St. Louis, MO, USA; Fluka, A.G.; and E Merck, respectively. Male albino rats (Wistar strain) obtained from central animal house, Punjab University, Chandigarh, India were used for biological studies.

2.2. Methods

2.2.1. Preparation of zinc complex of naproxen

Sodium naproxen, prepared by adding naproxen (30 g or 0.13 mol) to a 0.5 N ethanolic solution of NaOH (300 ml, pH 10.04) and drying under vacuum at 40 °C, was dissolved in water (200 ml). To it, $ZnSO_4{\cdot}7H_2O$ (250 ml, 0.1 M) was added with constant stirring. The precipitates of the zinc–naproxen complex (final yield: 33.67 g or 92.39%, m.p. 225–230 °C), thus, formed were filtered, washed with cold water and dried under vacuum to a constant weight.

2.2.2. Instrumental methods of analysis

Infrared (IR) spectra up to 400 cm^{-1} on Perkin-Elmer 882 spectrophotometer using KBr pellets. ^1H NMR and ^{13}C NMR spectra on varian EM 390-NMR and Bruker AC-300F, (300 MHz) spectrometers using tetramethyl silane as the internal standard and DMSO-d_6 as the solvent were obtained.

Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer. Zinc content was determined using Perkin AAS 3100 atomic absorption spectrophotometer. A Karl–Fischer titrimeter (716-DNS-Titrino) was used for the determination of the water content. Ultraviolet spectra were recorded on a Milton Roy spectronic 1201 UV-vis spectrophotometer while differential scanning calorimetry (DSC) was conducted on a DuPont 2000 apparatus. The sample size used for DSC was about 2 mg for zinc complex, 3.07 mg for naproxen, and 8.29 mg for physical mixture. The scanning speed was 10 °C min^{-1}.

2.2.3. pH-Solubility studies of naproxen and its zinc complex

Solubilities were determined for both compounds at 37 °C as a function of pH in buffer solutions varying in pH from 2 to 8. The buffer solutions were constituted from HCl (0.2 M), sodium hydroxide (0.2 M), potassium hydrogen phthalate (0.2 M), and potassium phosphate monobasic (0.2 M). The pH measurements were carried out with a control dynamic pH meter. An excess of the compound was added to 10 ml of each buffer solution and agitated at 37 °C for 24 h. This was then filtered immediately in test tubes previously placed in a Block thermostat (Grant, BT3, 20–140 °C) maintained at 37 °C, and the equilibrium pH of the filtrate was recorded. The samples (in triplicate) after appropriate dilutions with respective buffer pH were analyzed by ultraviolet spectroscopy (Al-Shammary et al., 1992) using a standard plot of naproxen in the same medium (pH 7.4 A_1^1 67, $r^2 = 0.9995$, pH 5.6, A_1^1 76, $r^2 = 0.999$, pH 1.2, A_1^1 214, $r^2 = 1$ by UV spectrophotometer at 330 nm). In 0.1 N NaOH A_1^1 of 238 ($r^2 = 0.9982$) and in water A_1^1 of 205 was obtained at a wavelength of 271 nm (where A_1^1 is the absorbance of 1% solution in 1 cm cell).

2.2.4. Partition coefficients determination

Partition coefficients (*P*s) of naproxen and its zinc complex was determined in octanol–phthalate buffer (pH 5.6) system. The phthalate buffer was used to simulate gastric pH which has been reported to be in the pH range of 4–6 in the non-fasting state. These phases were mutually saturated before use. The octanol layer was used to prepare the stock solution, 2 ml of which was shaken vigorously, in triplicate, with 4 ml of

saturated phthalate buffer at 37 °C overnight. The mixture was then centrifuged, at 2000 rpm for 20 min and the octanol layer was removed, and analyzed at 330 nm. The stock solution, which was used for partitioning, analyzed in a similar manner. The partition coefficient was determined from the Eq. (1).

$$P = \frac{C_0}{C_i - C_0} \times \frac{V_a}{V_o} \quad (1)$$

where, C_i and C_0 represents the initial (i) and equilibrium (0) solute concentrations of the octanol phase; V_a and V_o represent the volume of aqueous and octanol phases, respectively.

2.2.5. Determination of anti-inflammatory activity

The 'carrageenan-induced hind paw oedema' method (Winter et al., 1962) using young male rats (100–200 g), randomly divided into different groups, each with a minimum of six rats were used. No food was given 16 h before the test while both food and water were given during the test. Test animals were administered orally an aqueous suspension of naproxen (100 mg kg^{-1}), its zinc complex (124.66 mg equivalent to 100 g naproxen and 15 mg zinc ion kg^{-1}) and a physical mixture of naproxen and zinc sulfate (100 mg kg^{-1} and 65.96 mg equivalent to 15 mg zinc ion kg^{-1}). The drugs to be used were finely powdered and suspended in distilled water (5 ml) using carboxy methyl cellulose (CMC) as the suspending agent. The vehicle alone in an equivalent quantity was used as a placebo for the control group. Drug or placebo was orally administered 0.5 h before inducing paw oedema in the right hind paw of each rat by intraplantar injection of 0.1 ml of 1% (w/v) suspension of carrageenan. The volume of the injected paw was measured immediately (0 min) and at 30, 60, 120, 180, and 240 min after the injection using plethysmometer and the amount of paw swelling determined and expressed as percent oedema relative to the initial hind paw volume. The mean percent oedema ± S.E.M. was determined for each time interval and percent inhibition of oedema produced by each drug-treated group was calculated with respect to control as:

$$\%\,\text{inhibition} = \left[\frac{\%\,\text{oedema (control)} - \%\,\text{oedema (drug)}}{\%\,\text{oedema (control)}}\right] \times 100$$

$$\%\,\text{oedema} = \left[\frac{\text{volume of inflammed paw} - \text{volume of control (left) paw}}{\text{volume of control (left) paw}}\right] \times 100$$

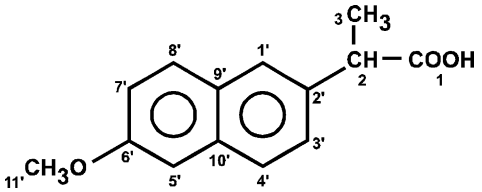

Fig. 1. Structure of naproxen.

2.2.6. Determination of ulcerogenic effects

The rats (150–200 g) kept under fasting state (12 h) were administered orally an aqueous suspension of the drug corresponding to naproxen 29 mg kg^{-1} twice a day over a 2-day period. The rats were then sacrificed the day after giving the final dose. To determine the gastric mucosal damage, rat stomachs were removed, opened along the length of greater curvature and cleaned of the debris, washed and examined under a microscope (10×) and the ulcers were scored as: 0 (normal colored stomach), 0.5 (red-coloration), 1 (spot ulcers), 1.5 (hemorrhagic streaks), 2 (ulcers >3 mm but <5 mm), and 3 (ulcers >5 mm). The mean ulcer score for each animal was expressed as ulcer/lesion index (LI).

3. Results and discussion

3.1. Characterization of naproxen and its zinc complex of naproxen

The structure of naproxen is shown in Fig. 1. Examination of the IR spectra of naproxen and zinc–naproxen (Fig. 2) revealed the loss of the carboxylic –OH stretching (3490–2800 cm^{-1}) in the spectrum of the zinc complex probably due to deprotonation of naproxen, and showed distinctive shoulders characteristic of the carboxylic acid dimer (2700–2500 cm^{-1}) and peaks at 1550 and 1400 cm^{-1} corresponding to asymmetrical and symmetrical carboxylate anion stretching, respectively, as compared to that at 1700 cm^{-1} (carbonyl of –COOH) in the IR spectrum of naproxen (Fig. 2). The shifts occurred

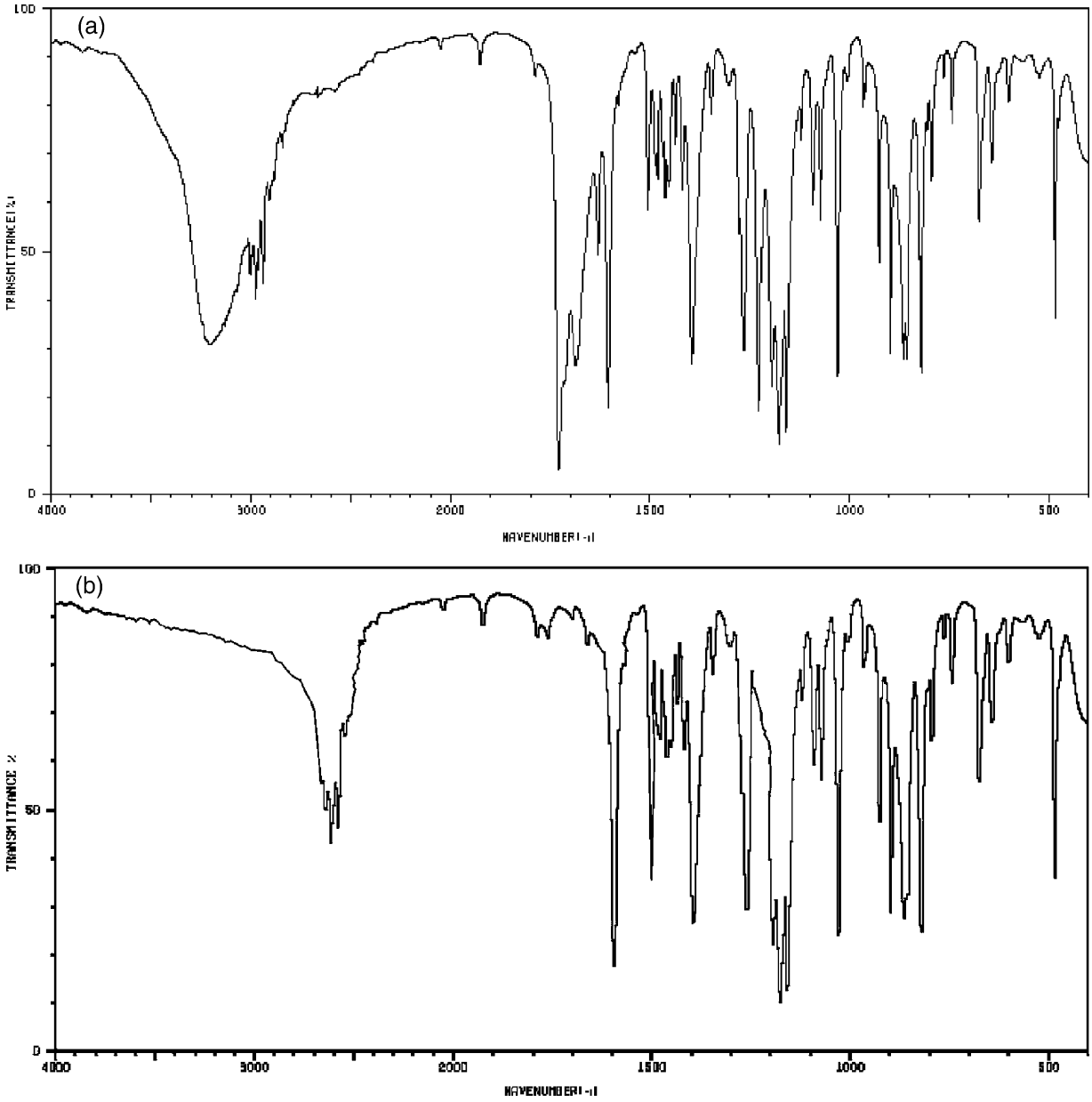

Fig. 2. IR spectra of (a) naproxen and (b) zinc–naproxen complex.

towards longer wavelength, which may be due to donation of electrons to the metal (Williams et al., 1976). Comparison of the ^{1}H NMR spectra of the two compounds showed the disappearance of the peak for proton of carboxyl group at δ: 9.91 ppm and slight shift in the peak of 2C–H (3.84–3.72 ppm) and $3C–H_3$ (1.57–1.46 ppm) in the case of zinc–naproxen (Fig. 3). There was an additional peak at δ: 3.40 ppm indicating the presence of two water molecules. The formation of the zinc–naproxen complex was further confirmed by comparing ^{13}C NMR spectra of these compounds which showed a strong shift in the

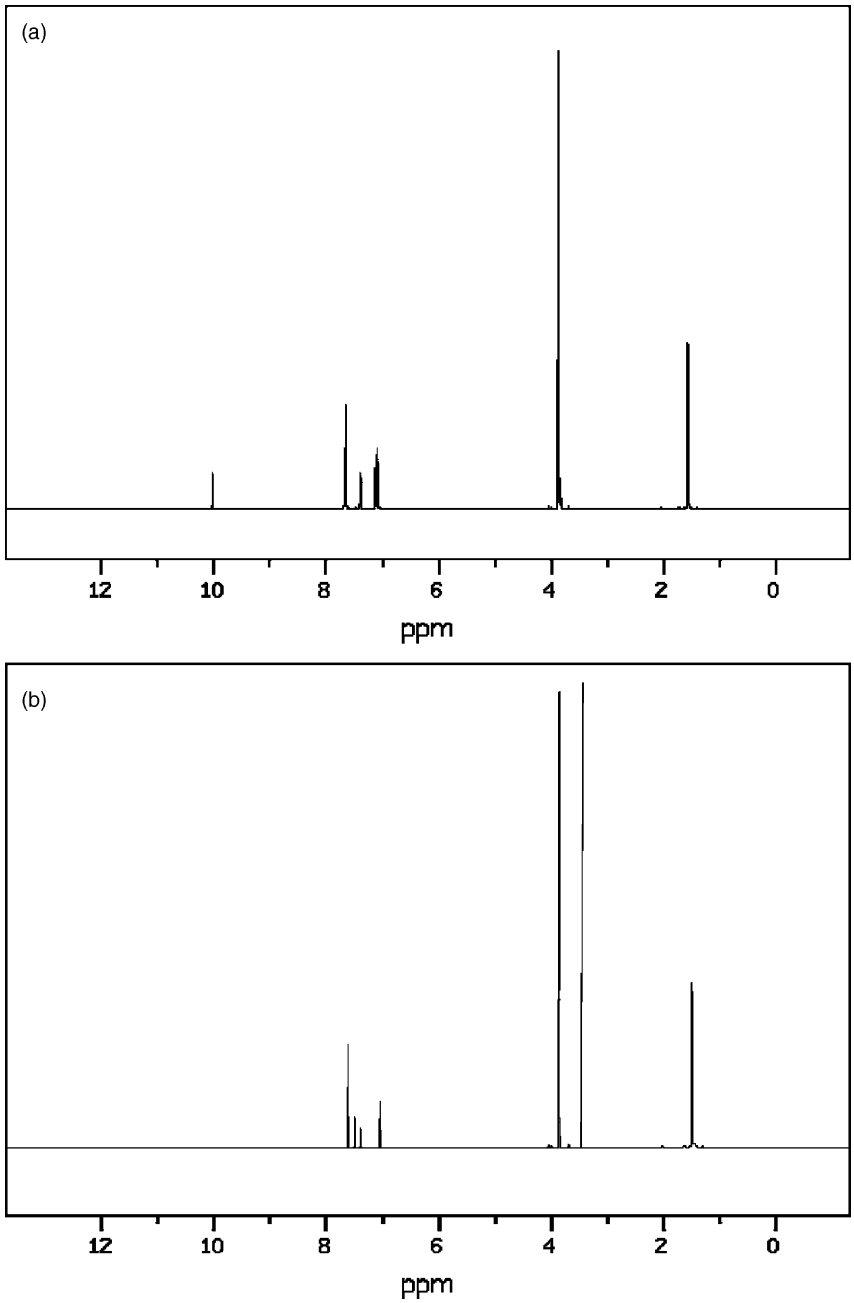

Fig. 3. ^{1}H NMR spectra of (a) naproxen and (b) zinc–naproxen complex.

absorption of carbon of –COOH from 177.66 to 179.79 ppm indicating that donation of electrons from the carboxylate anion to zinc causes deshielding of the carbon of –COO in the zinc complex and slight shift in the absorption of carbon nuclei adjacent of –COO group in the zinc complex, i.e. C-2 (45.91–46.21 ppm) and C-3 (19.07–19.21 ppm) which is also indicative of interaction of the carboxyl group with zinc (Fig. 4).

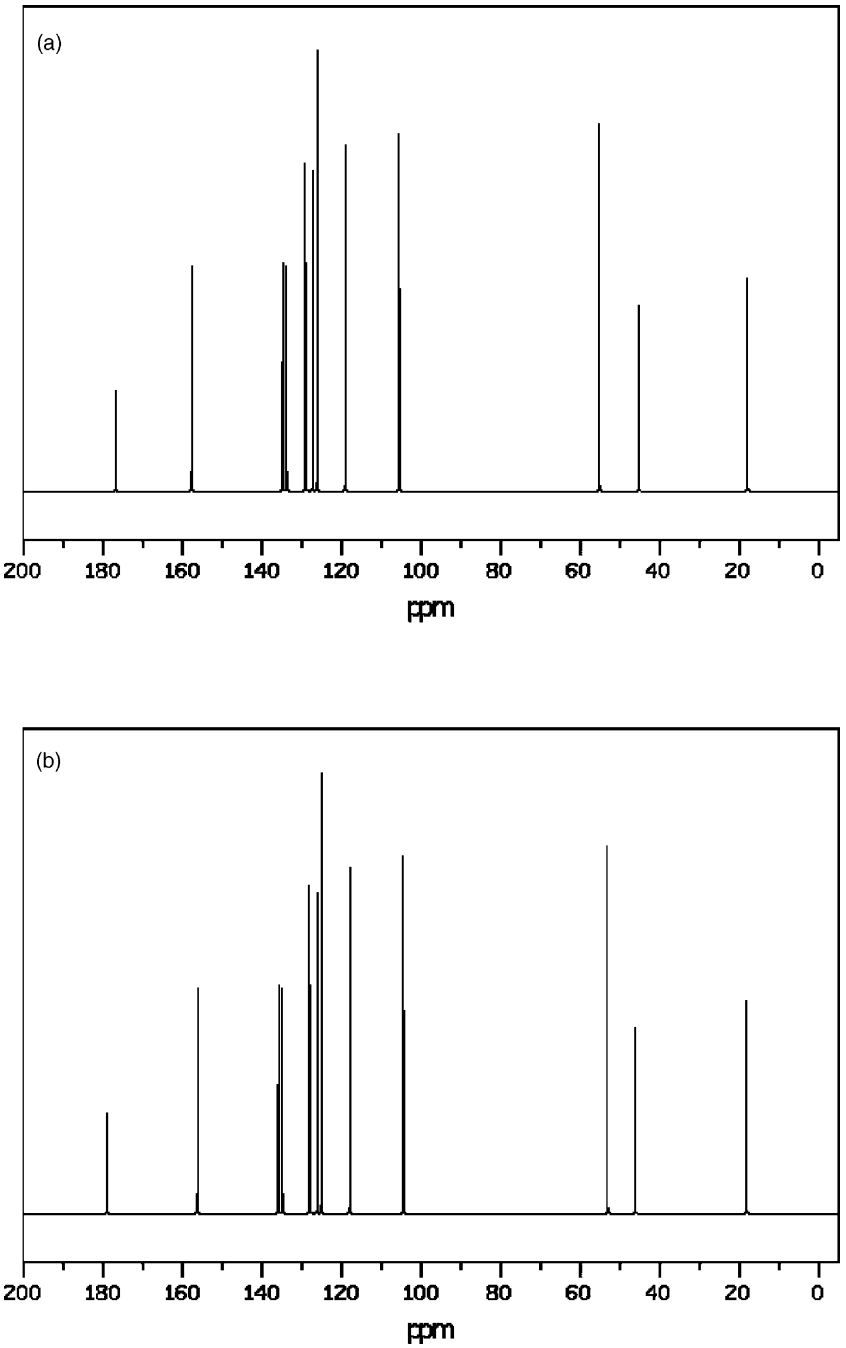

Fig. 4. ^{13}C NMR spectra of (a) naproxen and (b) zinc–naproxen complex.

Atomic absorption spectrophotometry and elemental analysis of the hydrated zinc–naproxen complex showed it to possess a ligand-metal ratio of 2:1 with two molecules of water which was further confirmed by Karl–Fischer testing which gave the water content equal to 6.72% (calculated for $2H_2O$: 6.43%). Anal: calculated for $(C_{14}H_{13}O_3)_2$ $Zn{\cdot}2H_2O$:C, 60.04; H, 5.36; Zn, 11.61; found C, 59.46; H, 5.808; Zn, 11.82; molecular weight 559.3 (Rast method) (calculated: 559.6). The thermal curves obtained for naproxen, its physical mixture with zinc sulfate and zinc–naproxen complex are shown in Fig. 5. The endothermic peak of the parent drug at 155.49 °C, though present in its physical mixture at or around the same temperature, disappears completely in the case of its zinc complex with the appearance of new endothermic peaks, thus, corroborating the formation of a new compound. Two new peaks were observed in the zinc complex. The first peak at 103.00 °C was broader probably due to loss of water of crystallization of the complex, as broader endotherms indicate a slow change in heat capacity and cover behaviors like dehydration. The second peak at 234.19 °C was sharper and might be due to melting with decomposition of the complex (Willard et al., 1986).

3.2. Physicochemical properties of zinc complex of naproxen

3.2.1. pH-solubility profile

The solubility of naproxen and its zinc complex in different pH buffers at 37 °C indicates that the solubility of the zinc complex was more than that of naproxen till pH 6 (Table 1). The low solubility of acidic NSAIDS not only delays the absorption but gastric irritation and bleeding may also result from the erosive action of the crystals of the drug or possibly from its acidity (Espinar et al., 1991). Hence, the increased solubility of the zinc–naproxen complex at gastric pH as compared to naproxen prevents this erosion action, and resistance in dramatic fall in pH at higher pH values makes the zinc complex as advantageous as buffered preparations. Also it was observed that zinc–naproxen complex caused an increase in pH of most acidic buffers used in this study making it as advantageous as buffered preparations.

Table 1
Solubility of naproxen and Zinc–naproxen at different pH

pH	Solubility ($mg\,ml^{-1}$)	
	Naproxen	Zinc–naproxen
2.04	0.026	0.034
3.08	0.04	0.059
3.90	0.058	0.096
4.62	0.134	0.237
5.52	0.531	0.833
6.00	1.609	2.09
6.55	5.044	4.6
6.85	7.891	7.67

3.2.2. Partition coefficient

Partition coefficient using octanol–phthalate buffer (pH 5.6) were determined for naproxen and its complex and were found to be 3.19 and 2.36, respectively. The decrease in partition co-efficient of the latter can be attributed to its higher solubility in phthalate buffer as compared to the former.

3.3. Biological activity of zinc complex of naproxen

The effect of complexation with zinc on the anti-inflammatory activity and ulcerogenic effect of naproxen was studied in albino rats (Wistar strain). The biological evaluation was necessary to see whether the zinc complex was a better alternative to its respective parent drug.

3.3.1. Anti-inflammatory activity

Since the oedema of the rat paws induced by carrageenan injection develops slowly and its peak effect is attained in 4 h (Winter et al., 1962), this study was carried out for 4 h. Fig. 6 shows the inhibition of oedema after oral administration of naproxen ($100\,mg\,kg^{-1}$), its zinc complex ($124\,mg\,kg^{-1}$ equivalent to $100\,mg\,kg^{-1}$ of naproxen) and naproxen ($100\,mg\,kg^{-1}$)-zinc sulfate ($62.42\,mg\,kg^{-1}$) mixture against control. Carrageenan-induced oedema is a biphasic event. The early hyperemia being due to the release of histamine and serotonin (Vinegar et al., 1969) and the delayed oedema being due to the release of prostaglandins and superoxide anions (Frechilla et al., 1990). One way ANOVA followed by Dunnet's *t*-test when carried at each time interval showed that percent oedema obtained with zinc–naproxen

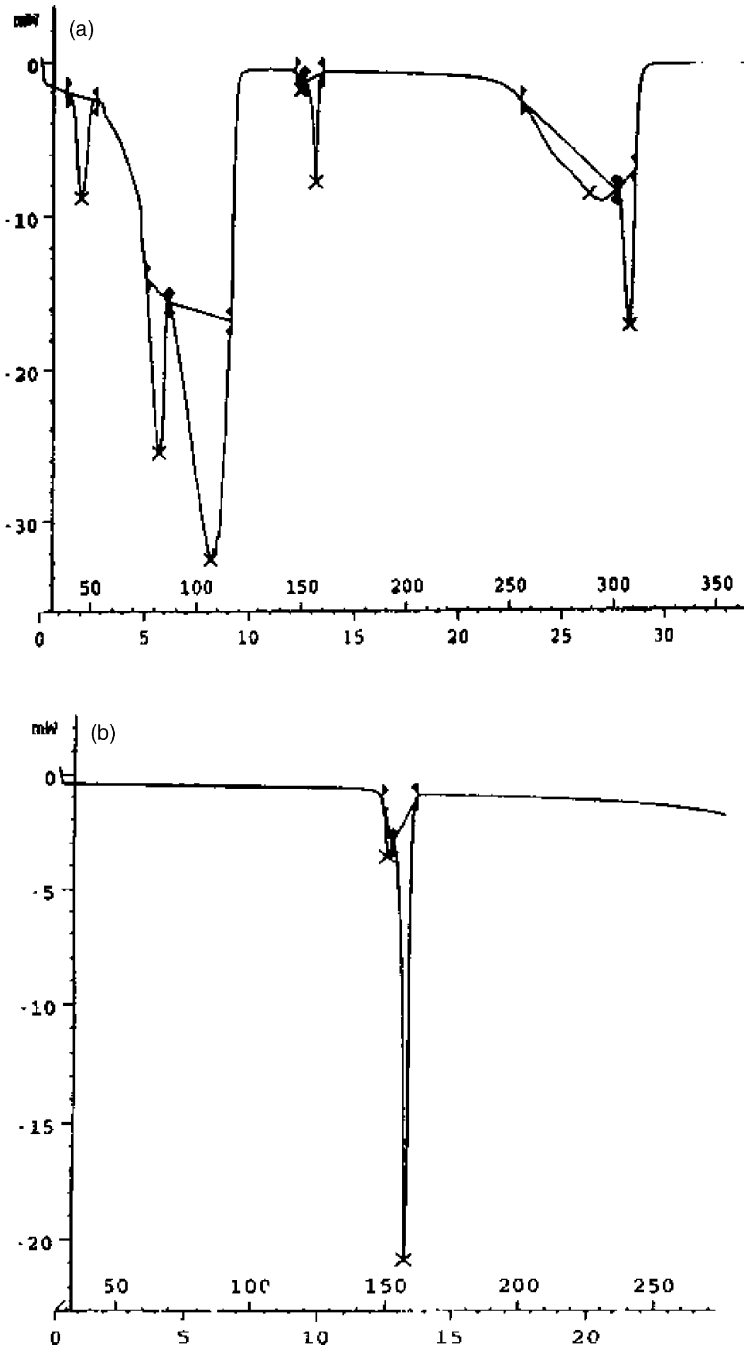

Fig. 5. DSC curves of (a) physical mixture of naproxen and zinc sulphate; (b) naproxen; and (c) zinc–naproxen.

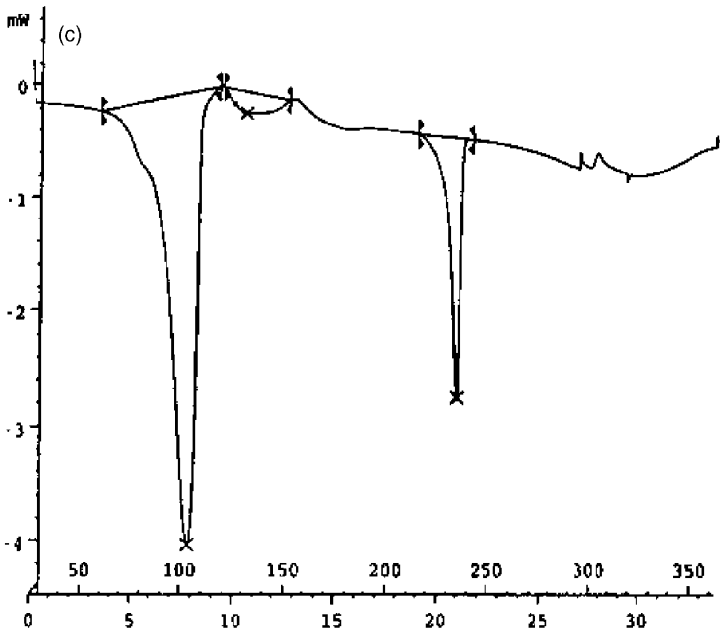

Fig. 5. (*Continued*).

complex was significantly less ($P < 0.05$) than the control, naproxen, and its physical mixture with zinc sulfate treatment in the second phase of oedema.

This significant difference in the anti-inflammatory activity can be attributed to the inhibition of the prostaglandin biosynthesis by naproxen as well as by zinc (Nugteren et al., 1966; Todd and Clissold, 1990). In addition, zinc also exerts its anti-inflammatory action through different mechanisms (Chvapil et al., 1972; Karl et al., 1973 and Yamamoto and Takahashi, 1975), which has been confirmed by the use of zinc sulfate in rheumatic arthritis (Simkin, 1976) and in psoriatic arthritis (Clemmenson et al., 1980). Finally, superoxide dismutase, which plays a key role in the protection of tissues against toxic effects of superoxide anions, has been shown to be a zinc metalloenzyme (Dreno et al., 1990).

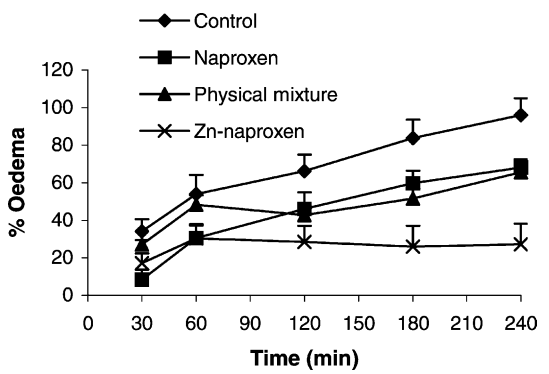

Fig. 6. Effects of oral administration of naproxen, zinc–naproxen complex and physical mixture of naproxen and zinc sulphate on carragenan-induced paw oedema in rats.

Thus, it can be concluded that its zinc complex will be more effective in the second phase as compared to the first. However, the superiority of the zinc complex may be attributed to the enhanced gastrointestinal absorption of both ligand (naproxen) and zinc in the complexed form (Evans and Jhonson, 1980). These findings are also in agreement with the earlier reports (Singla and Wadhwa, 1994).

3.3.2. Ulcerogenic effects

Since the effective dose for 50% inhibition of the biological response (ED_{50}) of naproxen in the carrageenan oedema test was 29 mg kg^{-1} (Otterness and Gans, 1988), the LI was determined after the oral administration of naproxen, its zinc complex and the naproxen-zinc sulfate physical mixture (all in a dose corresponding to 29 mg kg^{-1} of naproxen and/or

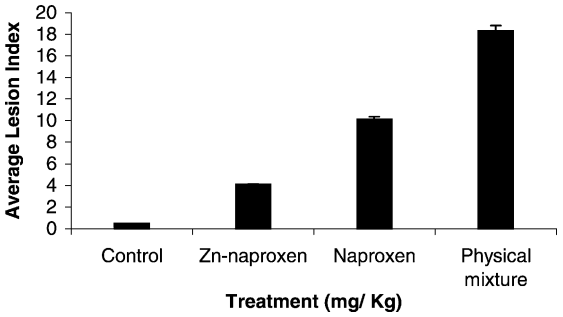

Fig. 7. Lesion index in rats (each bar represents the average±S.E.).

14.5 mg kg^{-1} of zinc). As shown in Fig. 7, the complex was the least damaging with an average LI of about one fifth and one third of that of the physical mixture and the parent drug, respectively. A significant reduction in the LI ($P < 0.05$, Dunnet's *t*-test) of the zinc complex as compared to the physical mixture and naproxen can be attributed to masking of free carboxyl group of naproxen (Espinar et al., 1991). Although the exact cytoprotective effect of zinc is not clearly understood, it is believed that zinc in complexed form is better tolerated (Reynolds, 1993), and a reduction in the LI of the complex form may be due to its direct gastroprotective action through an increase in mucus synthesis (Cho et al., 1985), reduction in back diffusion of protons (Wong et al., 1986), and inhibition of mast cell degranulation (Guth and Hall, 1996). Moreover, the presence of zinc ions in the molecule probably stabilizes the mucosal membrane (Chiu et al., 1983). The acute damage to the gastric mucosa caused by the physical mixture may be due to the ulcerogenic effect of naproxen along with the corrosive effects of overdosage of zinc sulfate on gastric mucosa, which, by its conversion to zinc chloride in the stomach, is highly astringent (Reynolds, 1993).

4. Conclusion

Naproxen due to its acidity and low solubility damages the stomach wall. Hence, the carboxyl group of naproxen was masked by making its zinc complex. Zinc complex of naproxen was characterized by IR, ^{1}H NMR and ^{13}C NMR. The complex was found to possess ligand to metal ratio (2:1). The solubility of Zinc–naproxen complex was found to be more than that of naproxen. Using carrageenan-induced hind paw oedema method, anti-inflammatory activity of Zinc–naproxen, naproxen, and physical mixture was observed. Zinc complex was found to be more effective than naproxen. Also, a significant reduction in the LI of the zinc complex was observed as compared to the physical mixture and naproxen which can be attributed to masking the free carboxyl group of naproxen.

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