Drug Development and Industrial Pharmacy, 34:434–444, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040701831686

informa
healthcare

Diclofenac-Bismuth Complex: Synthesis, Physicochemical, and Biological Evaluation

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Diclofenac-bismuth complexation was attempted by mixing diclofenac sodium (Na) and bismuth-subcitrate aqueous solutions at diclofenac:bismuth molar ratio of 3:1. A solid precipitate was obtained and isolated. The precipitate was characterized for stoichiometric ratio of diclofenac-bismuth complexation using capillary electrophoresis, which showed 1:1 complexation. In addition, nuclear magnetic resonance and Fourier transform infrared analysis were performed for the isolated solid complex and indicated that bismuth was in coordinate bond formation with the carboxylate group of diclofenac. In comparison with diclofenac Na powder, the complex was evaluated as an aqueous suspension for in vitro drug dissolution. The complex exhibited a faster dissolution rate than and similar dissolution extent as diclofenac Na. In comparison with an aqueous solution of diclofenac Na and an aqueous suspension of physical mixture of diclofenac acid (suspended) and bismuth-subcitrate (dissolved), the aqueous complex suspension was evaluated for ulcerogenic effect in rats upon oral administration. The complex led to more gastric ulceration than diclofenac Na, which was not in accordance with the antiulcer properties of bismuth. This antiulcer effect was shown as the physical mixture administration was accompanied with lower gastric ulceration than diclofenac Na administration. These gastric ulceration results were explained in terms of the difference in particle size between solid diclofenac acid formed as a result of the complex breakdown in an acidic medium (0.1 M HCl to simulate the gastric fluid) and that formed as a result of diclofenac Na neutralization. Diclofenac acid particles formed from the complex breakdown were of average size, three times smaller of those formed as a result of diclofenac Na protonation. This difference in particle size was correlated with the higher gastric ulceration associated with the complex than with diclofenac Na in terms of higher coverage of the gastric mucosa with diclofenac, and consequently, higher local ulceration.

Keywords diclofenac; bismuth; complexation; dissolution; ulcerogenic activity

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), although effective in the management of pain and inflammation, are also associated with the development of several gastrointestinal tract (GIT) complications, particularly damage to the upper GIT, namely the stomach and duodenum (Peterson & Killurman, 1996). The effect of this damage appears to have both local and systemic components. Several approaches have been made in the past to overcome the complications associated with NSAIDs. These approaches include enteric coating, prodrugs, polymeric prodrugs, and cotherapies with sucralfate, H_2 antagonists, proton pump inhibitors, and prostaglandin inhibitors (Chandrasekar, Ravichandran, Nanjan, & Suresh, 2001). In addition, a simple approach for this purpose based on drug complexation with metals that have antiulcer properties, such as zinc and copper, has been investigated in several studies. When copper (II)-ibuprofen complex was investigated orally for in vivo anti-inflammatory activity in comparison with free ibuprofen, the complex showed similar anti-inflammatory effects as the free drug (Andrade et al., 2000). However, the gastric irritation was lower for the complex as compared with

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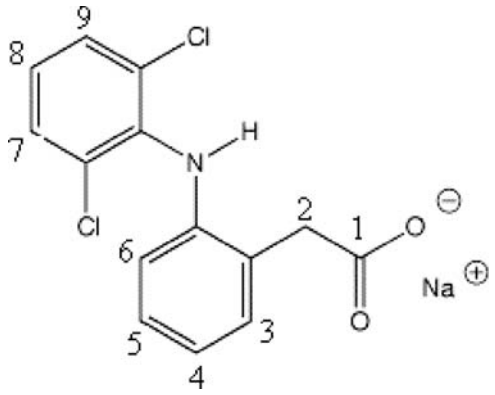

FIGURE 1. Chemical structure of diclofenac Na.

that of ibuprofen. A naproxen-zinc complex showed significant reduction in gastric ulceration in rats than naproxen alone or a physical mixture of naproxen and zinc sulfate (Sharma, Singla, & Dhawan, 2003). A diclofenac-zinc complex showed the same anti-inflammatory and antinociceptive effect as diclofenac alone; however, the gastric lesions induced by the complex were less than with diclofenac alone (Santos et al., 2004).

Diclofenac sodium (Na), [2-[(2,6-dichlorophenyl) amino] phenyl] acetate (Figure 1) is a potent NSAID, therapeutically used in inflammatory and painful conditions of rheumatic and nonrheumatic origin (Sallman, 1986). Diclofenac is a potent inhibitor of cyclooxygenase in vitro and in vivo, thereby decreasing the synthesis of prostaglandins, prostacycline, and thromboxane products (Kovala-Demertzi, 2000). Although diclofenac is usually well tolerated by patients, its use can result in some side effects, particularly those related to GIT (Santos et al., 2004).

Bismuth compounds, mainly bismuth-subcitrate and bismuth-subsalicylate, have proven utility in the treatment of duodenal ulcers, gastritis, chronic diarrhea, traveler's diarrhea, and acute diarrhea in children (Mahony et al., 1999). Bismuth-subcitrate causes an increase in mucus glycoprotein secretion, may also bind to the gastric mucus layer to act as a diffusion barrier to HCl, has bactericidal effect against *Hellicobacter pylori* and prevents its adhesion to epithelial cells, and can inhibit enzymes secreted by *H. pylori,* such as proteases and lipases (Lee, 1991).

The objectives of this study were to convert diclofenac to solid diclofenac-bismuth complex in situ as an aqueous suspension by combining diclofenac Na and bismuth-subcitrate aqueous solutions, to characterize the formed solid complex for drug:metal molar ratio, and, consequently, to determine the stoichiometry of diclofenac-bismuth complexation. The study also aimed to form a suspension of diclofenac-bismuth complex at the determined stoichiometric ratio and to study the formed suspension for drug dissolution in comparison with diclofenac Na powder, as well as to study ulcerogenic effect in rats in comparison with diclofenac Na aqueous solution and an aqueous suspension of physical mixture of diclofenac acid and bismuth-subcitrate.

MATERIALS AND METHODS

Materials

Diclofenac Na and bismuth-subcitrate were gifts from the Arab Pharmaceutical Manufacturing Company, Sult, Jordan. Diclofenac Na was manufactured and supplied by Amoli Organic, PVT Limited, Mumbai, India. Male Wister rats were obtained from Petra University, Amman, Jordan. For all experiments, distilled water was used, and all other chemicals were of pure laboratory grade and used without further purification.

Preparation of Solid Diclofenac-Bismuth Complex

Aqueous solutions of diclofenac Na and bismuth-subcitrate were prepared at concentrations of 2% and 0.832%, respectively. The solutions were combined with subsequent stirring for 1 hour and an aqueous suspension was obtained. The molar ratio of diclofenac to bismuth in the mixture was 3:1, which aimed to totally consume bismuth in the complexation process. The formed diclofenac-bismuth solid as suspended solid particles could not be totally separated by filtration (0.45 μm membrane filters) or centrifugation, which was due to the presence of ultrafine particles and the deflocculated nature of the formed suspension. Consequently, the suspension was flocculated by the addition of 10% NaCl solution, and then the suspension was easily filtered using a normal membrane filter. The retained solid was washed twice with distilled water to remove any free diclofenac, and then left to dry at room temperature under vacuum.

Determination of Stoichiometric Ratio of Diclofenac-Bismuth Complexation Using Capillary Electrophoresis

A capillary electrophoresis method was developed to study the stoichiometry of the complex. A standard uncoated capillary with a length of 40 cm (75 μm ID, 365 OD) was employed. The applied voltage was 24 kv and the detection wavelengths were 205 and 275 nm. The running buffer was composed of 870 ml borate buffer (20 mM, pH 8.5) containing dodecyl sulfate (SDS); (1.5 mg %) and 130 ml acetonitrile. The temperature was maintained at 37°C. From diclofenac Na solution (0.01%), dilutions were made in a borate buffer (pH 8.5) so that calibration solutions in the range of 0.002% to 0.01% were prepared. Samples of diclofenac-bismuth complex to be analyzed ($n = 3$) were prepared by dissolving amounts of the prepared diclofenac-bismuth solid complex in a borate buffer (pH 8.5) containing SDS to provide complex concentrations of 4 to 5 mg%.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Proton NMR and ^{13}C NMR were performed using Bruker® NMR spectrometer (Billerica, MA, USA) for diclofenac Na and the prepared diclofenac-bismuth solid complex. The samples were dissolved in deutoriated dimethyl sulfoxide (DMSO) and some samples were run in deutoriated methanol.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed for the prepared diclofenac-bismuth complex using Shimadzu®-FTIR spectrometer (Japan) according to the KBr method. For comparative purposes, FTIR analysis was also performed for diclofenac Na, bismuth-subcitrate, and a physical mixture of diclofenac Na and bismuth-subcitrate.

Suspension Preparation for Dissolution and/or Ulcerogenic

Suspension of In Situ Formed Diclofenac-Bismuth Complex

Aqueous solutions of diclofenac Na and bismuth-subcitrate were prepared at concentrations of 2% and 2.496%, respectively. Diclofenac:bismuth molar ratios for the solutions was 1:1. The prepared solutions were combined with subsequent stirring for 1 hour and an aqueous suspension was obtained.

Suspension of Physical Mixture of Diclofenac Acid and Bismuth Subcitrate

Solid diclofenac acid was obtained by dropping 2 M HCl into diclofenac Na aqueous solution (2%) with continuous stirring until the pH became 1.5. The resulting suspension was filtered using normal filter paper, and the filtered diclofenac acid solid was washed three times with distilled water and then left to dry at room temperature under vacuum for 24 hours. An amount of the dried diclofenac acid was dispersed in distilled water with stirring to form a dispersion having diclofenac concentration equivalent to that in the prepared diclofenac-bismuth complex suspension. The dispersion was further sonicated for 15 minutes and, finally, bismuth-subcitrate was added at equimolar concentration of the added diclofenac acid with subsequent stirring for 0.5 hours.

Dissolution Studies

Dissolution studies were performed for 25 mg diclofenac Na powder and 2.5 ml of the prepared diclofenac-bismuth complex suspension, both having the same diclofenac amount. Type II (paddle) dissolution apparatus (VanKel® dissolution tester, Cary, NC, USA) was used. The dissolution medium was 0.1 M HCl (pH 1.2) for 2 hours, after which the pH of the dissolution medium was raised to 6.8 using appropriate amounts of KH_2PO_4 and NaOH, and then the dissolution was followed for an extra 2 hours. The stirring rate was 50 rpm, and the temperature was kept at 37 ± 0.5°C. Since diclofenac Na and the complex were to be protonated in the acidic medium to produce diclofenac acid before the exposure to the phosphate buffer medium, sink conditions were demonstrated by measuring the solubility of diclofenac acid in the buffer medium. The average value for three solubility determinations was 0.905 ± 0.005 mg/ml. Upon complete drug dissolution, the theoretical drug concentration in the medium was 0.03 mg/ml, which was less than 5% of the determined solubility. Sampling was done with replacement with the dissolution medium at 1 and 2 hours for pH 1.2, and at 15, 30, 45, 60, 90, and 120 minutes for pH 6.8. Withdrawn samples were assayed for dissolved diclofenac using a previously developed high-performance liquid chromatography (HPLC) analysis method (Dorado, Bercz, Caceres, & Lerena, 2003). The HPLC system consisted of a solvent pump (L 7100) and programmable ultraviolet (UV) detector (L 7400; Merck, Germany) set at 282 nm. All chromatographic separations were performed at ambient temperature on a Teknochrom Thecer Extrasil ODS 13 μm 10 × 0.46 cm column (Sharlus Science, Spain). The mobile phase, a mixture of acetonitrile-methanol-tetrahydrofuran-water (22:10:3:65 v/v), included disodium hydrogenphosphate dehydrate (4.5 g/L) and potassium dihydrogenphosphate (2.3 g/L) and had a pH of about 8. The mobile phase was membrane filtered and sonicated before use. The flow rate was maintained at 1 ml/minute, and the injection volume was 20 μl. Upon sample injection of both diclofenac Na and diclofenac-bismuth complex, the HPLC method was unable to differentiate between diclofenac-bismuth complex and free diclofenac Na. This was evident from the similarity of the retention time value of the complex to that of diclofenac Na. Since the complex would not be broken at this mobile phase pH (about 8), this phenomena could be explained according to the fact that the complexed bismuth was interacted with the silanol groups of the column that led to breakdown of the complex to free diclofenac that was eluted and bismuth that remained in the column. Accordingly, the chromatogram peak upon complex injection was taken as a measure of the eluted free diclofenac. Consequently, the HPLC assay results of dissolution for the complex suspension was for the equivalent free diclofenac, regardless of the form of diclofenac dissolved, that is, dissolved complex and/or dissolved free diclofenac. Accordingly, one calibration curve was constructed using diclofenac Na, which was used for dissolution assay of both diclofenac Na powder and the complex suspension. A stock solution of 40 μg/ml diclofenac Na in phosphate buffer (pH 6.8) was prepared. Of this solution, the following dilutions were made: 28, 20, 8, 4, and 2 μg/ml. These solutions were spiked with mefenamic acid as an internal standard added as 20 μg/ml solution (in 0.1 M NaOH) into an equivalent volume of each diclofenac Na solution. Thus, the concentration range of the calibration curve for diclofenac Na was from 1 to 20 μg/ml. Since the HPLC method was previously fully validated (Dorado et al., 2003), partial validation was carried out in our study. The linearity was ensured by constructing three calibration curves covering concentration range of 1 to 20 μg/ml. The average calibration equation was $Y = 0.0913X + 0.2074$ ($r^2 = 0.999$). The precision of the method was examined by obtaining the average determinations of the lowest, middle and highest concentration levels. Relative standard deviation for the lowest concentration level was less than 2%. The lowest limit of quantification was taken as 1 μg/ml. Overall, the method was concluded to be valid for the purpose of our study according to the international guidelines (ICH Q2B, 1996).

In order to test for the possible complexation between bismuth and phosphate polyanion in the buffer dissolution medium, the following experiment was performed. Bismuth-subcitrate solutions were added to K-phosphate solutions in the presence and absence of diclofenac Na. The ratio of the combined substances was the same as in the dissolution medium; however, their concentrations were 2,000-fold higher than those in the dissolution medium, which aimed to obtain enough of an amount of a possible formed precipitate for further analysis. A precipitate was obtained regardless of diclofenac Na presence and the precipitates were analyzed for FTIR in comparison with diclofenac Na and diclofenac-bismuth complex.

Determination of Ulcerogenic Effects

This study was conducted in the Experimental Animal Laboratory of the Faculty of Medicine, University of Jordan, Amman, Jordan. All animals were housed, fed, and treated in accordance with the in-house guidelines for animal protection approved by the Faculty of Medicine,. Male Wister rats, weighing 230 to 280 gm, were allowed to acclimatize the room conditions for 1 month and were fed with standard rat pellet feed and tap water at libitum. Preparations tested were distilled water as control, aqueous solution of diclofenac Na (10 mg/ml), the suspension of in situ formed diclofenac-bismuth complexes, and the suspension of physical mixture of diclofenac acid and bismuth-subcitrate. Rats were divided into the following groups with six rats per each test group: group A, the control group for distilled water administration; group B for diclofenac Na solution administration; group C for physical mixture administration; and group D for complex administration. The rats had been fasted for 12 hours before dosing, and they were allowed to eat 2 hours after each dosing. All diclofenac preparations were administered orally at a volume equivalent to 20 mg diclofenac Na/kg once daily for 5 days. Diethyl ether was used to anesthetize the rats for about 2 minutes to allow for dose administration. After 4 hours of the fifth dose, the rats were sacrificed, and their stomachs were opened along the greater curvature, washed with normal saline solution, and then kept in 10% formalin until evaluation. The stomachs were examined for erythema, hemorrhage, and ulceration under dissecting microscope (10 ×). The ulcerogenic scoring procedure was performed by a biologist who was blind to the treatment of each group. Lesions were scored as: 0 (no erythema, hemorrhage, or ulceration), 1 (focal erythema, no hemorrhages or ulceration), 2 (diffuse erythema with petechial hemorrhages), 3 (ulcers < 3 mm in dimension), and 4 (ulcers > 3 mm in dimension). The scores were then added to give the total for each group, which was divided by the number of stomachs examined to obtain the lesion index. In order to correlate between lesion index and the size of diclofenac acid particles administered in the physical mixture suspension formed as a result of the acidic complex breakdown and formed as a result of the acidic diclofenac Na protonation, the following experiment was performed. The complex suspension and diclofenac Na solution were added separately into 0.1 M HCl to simulate the exposure to gastric fluid, with subsequent shaking for 2 hours. The resulting diclofenac acid dispersions, along with the suspension of the physical mixture, were analyzed for diclofenac acid particle size using a laser diffraction particle size analyzer (Malvern, UK).

RESULTS AND DISCUSSION

Determination of Stoichiometric Ratio of Diclofenac-Bismuth Complexation Using Capillary Electrophoresis (CE)

CE is a relatively new analytical technique that is characterized by quantitation ability similar to that of HPLC and a separation mechanism close to that of traditional gel electrophoresis. Thus, small molecules can be separated and quantified using this technique based primarily on differences in charges/mass ratio of different analytes (Jandik & Bonn, 1993). CE is believed to be superior to HPLC for studying complexation or interaction between various types of molecules since there is usually no chromatographic packing. Such packing material may complicate the interaction process by interacting with either of the species being studied, which could alter the equilibrium. The CE method was developed (conditions were described in the experimental section) in order to estimate the percentage of diclofenac and bismuth in the overall weight of the complex, which enabled the estimation of the stoichiometry of the complex. CE was thought of as an ideal technique to separate the negatively charged diclofenac from the neutral complex (assuming 2 diclofenac:1 bismuth complexation) or even positively charged complex (assuming complexation occurred as 1:1). However, detailed study of the system using capillary electrophoresis indicated that the complex was dissociated under the electrophoresis condition to give free diclofenac and bismuth. This finding was in accordance with some published reports that showed dissociation of complexes under electrophoritic conditions (Iki, Hoshino, & Yotsuyanagi, 2000). However, dissociation of the complex was confirmed by injecting a series of solutions containing increasing concentrations of diclofenac Na in the system and a calibration curve was obtained. A similar curve was obtained for solutions containing equivalent concentrations of diclofenac Na but with amounts of bismuth-subcitrate. The obtained electrophorograms (data not shown) were consistently identical in both cases (diclofenac Na and diclofenac-bismuth complex). If the complex was stable under electrophoretic conditions, then a separate peak would be expected to be seen for the complex, or at least a shift in the migration time of the original peak would be expected. Moreover, the slopes of the calibration curves were identical, which further supported that the seen peak in the electropherogram of the complex was due to the dissociated diclofenac. The peak for

the dissociated bismuth ions was expected to appear in the early part of the electropherogram (0–1.5 minutes; before electro-osmotic flow). However, no such peak was observed due to lack of sufficient UV absorptivity of the free bismuth. Accordingly, it was possible to estimate the amount of total diclofenac in a given weight of the complex, because the entire complex would be dissociated to free diclofenac and bismuth under electrophoretic conditions. The average of three trials showed that the percentage of diclofenac in the complex was 60% ± 1.3. The expected theoretical value for diclofenac in the complex in the case of 1:1 complexation is 58%, while in the case of 2 diclofenac:1 bismuth complexation, it is expected to be 74%. Thus, it would be more likely that diclofenac formed a 1:1 complex with bismuth.

NMR Spectroscopy

Proton and ^{13}C-NMR spectra were obtained for both diclofenac Na and diclofenac-bismuth complex in deuterated DMSO. For diclofenac Na, the ^{1}H spectrum (Figure 2) could be divided into three distinct regions of signals (a doublet at 3.5 ppm, aromatic proton region at 6.2 to 7.6 ppm, and a singlet at 10.2 ppm). A similar NMR spectrum for diclofenac Na in DMSO has been previously reported (Kenawi et al., 2005). The peak integration ratios for the three groups of signals were 3.5 :7 :1, respectively. This is different from the expected ratio of 2 (CH_2 protons):7 (aromatic protons):1 (amino proton). A simple explanation for this might be that the methylene protons appeared as doublet and with a higher integration ratio than expected due to impurities from the solvent. Accordingly, the singlet at 10.2 ppm could only be due to the amino proton. This explanation was that adopted by Kenawi and colleagues (2005). However, the authors in this study are not in favor of that explanation for the following reasons. Firstly, the aromatic protons are unlikely to resonate at 10.2 ppm (Silverstein, Bassler, & Morrill, 1991). The expected resonance value for an aromatic proton lies in the range 3–5 (Silverstein et al., 1991). Secondly, when the spectrum of diclofenac Na was recorded in methanol, a completely different spectrum (than that of diclofenac Na in DMSO) was obtained (Figure 3). The spectrum in methanol appeared to have three major signals, that is, at 3.48, 4.8, and 6 to 7.2, with integration ratios of 2:1:7, respectively. The spectrum of diclofenac Na in methanol, therefore, exactly matches theoretical expectations in terms of the resonance values as well as the peak integration ratios. Thus, the seven protons in the range 6 to 7.2 are obviously the aromatic ones, the two protons at 3.5 are the methelyne protons, and, finally, the signal at 4.8 can only be attributed to the amino proton. If it is well accepted that the signal of the amino proton appeared at 10.2 in DMSO and at 4.8 in methanol, then

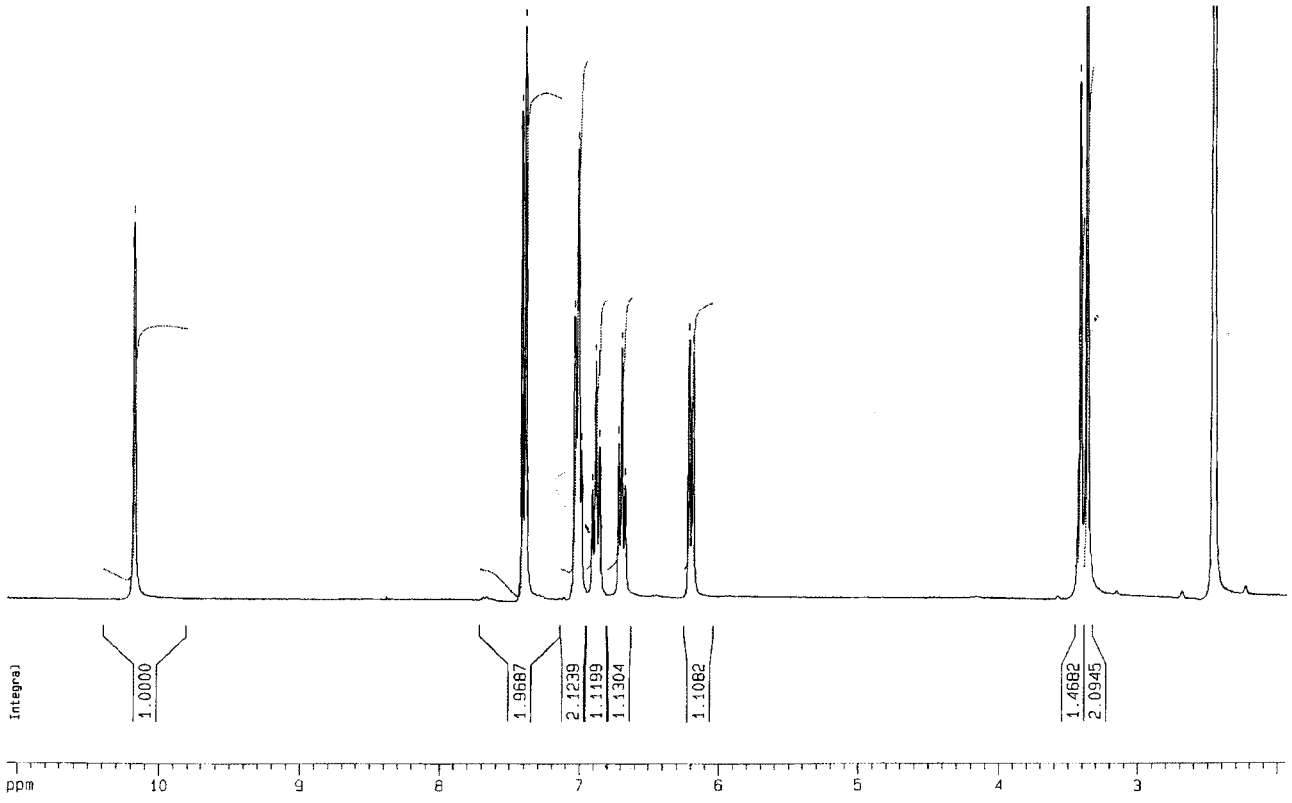

FIGURE 2. ^{1}H-NMR spectrum for diclofenac Na in DMSO.

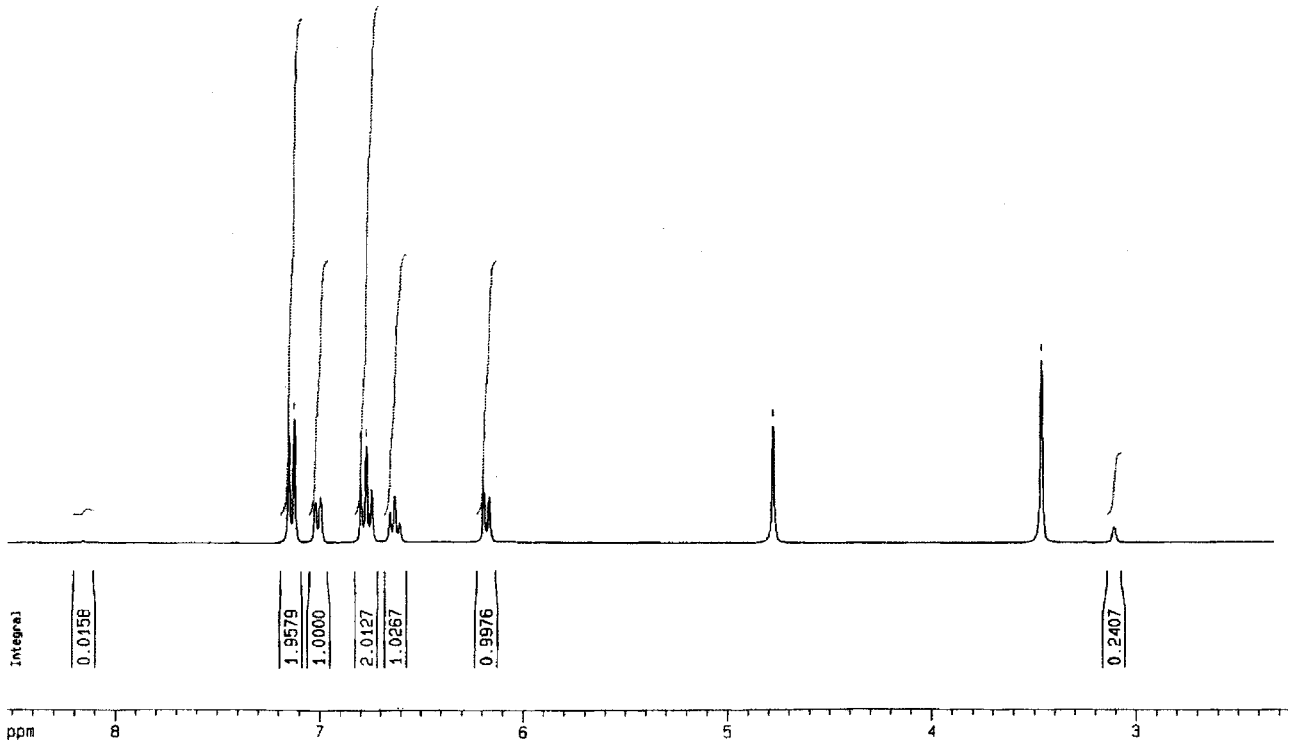

FIGURE 3. ^{1}H-NMR spectrum for diclofenac Na in methanol.

this would be a huge shift in the resonance value that was unlikely to be attributed to a simple solvent effect.

A plausible interpretation for the obtained spectra could be that the doublet at about 3.5 ppm (seen in DMSO) corresponds to the coupling of the two protons numbers 1 and 2 (numbering of proton as in Figure 1). However, for this signal to be seen as a doublet, the rotation of the two protons (1 and 2) must be restricted around the methylene axes. This condition is attained only if the carboxyl end of the molecule is bound to the amino group, making a ring structure. This can be achieved if the amino proton is involved in intramolecular hydrogen bond formation with the free carboxylate group. It is likely that under the high polarity of DMSO, sodium ions were dissociated by DMSO, leading to a free carboxylate that attracted the amine proton—already not well accommodated because of the two adjacent aromatic rings—to form a carboxylic acid. Thus, the proton appeared to be closer to the carboxylate group than the amino group and, consequently, exhibited a signal at 10.2 ppm, which can only be seen for carboxylic protons (Silverstein et al., 1991). These findings were also supported by the ^{13}C spectrum for diclofenac Na in DMSO (Figure 4) when a characteristic carboxylic acid carbon was observed at 176 ppm. Unlike DMSO, methanol did not appear to dissociate sodium ions from diclofenac Na. Consequently, there was no intramolecular hydrogen bonding between the carboxyl and amino groups. This allows for rotation of the methylene group around its axis, making the two protons (1 and 2) chemically equivalent and their signal appear as a singlet. On the other hand, the ^{1}H-NMR of diclofenac-bismuth complex in DMSO (Figure 5) showed no peak at about 10 ppm, contrary to the spectrum of free diclofenac Na. This could be explained as a result of involvement of the carboxyl group in the coordinate bond formation with bismuth, thus preventing the amino proton from being transferred to the carboxyl, and thus appearing as broadband within the area of the aromatic protons (Silverstein et al., 1991). In conclusion, the carboxylate oxygen is coordinately attached to bismuth with no possible interaction between carboxylate and amino groups, allowing movement around the methylene axis. Consequently, the two methylene protons would be closer to equivalence and their signal would appear as a broad singlet instead of a sharp doublet (at about 3.8 ppm). The previous conclusion was also supported by ^{13}C spectra of diclofenac Na (Figure 4) and the complex (Figure 6), both in DMSO. A singlet was observed for diclofenac Na at 45 ppm and seen as a negative signal in distortionless enhancement through polarization transfer (DEPT) experiment, which supports the presence of a methylene with two protons. A singlet was also observed for diclofenac Na at 176 ppm in the same spectrum, which is typical of a carboxylic acid carbon. In comparison, the signal corresponding to methylene and carboxylate carbons in the complex spectrum appeared significantly broader, which supports the idea of free rotation around the methylene axis as a result of complex formation. These observations again supported a complex formation between diclofenac and bismuth where carboxylate oxygen seems to accommodate the coordinate bond with bismuth, as evidenced by the absence of the acidic proton (about at 10.2).

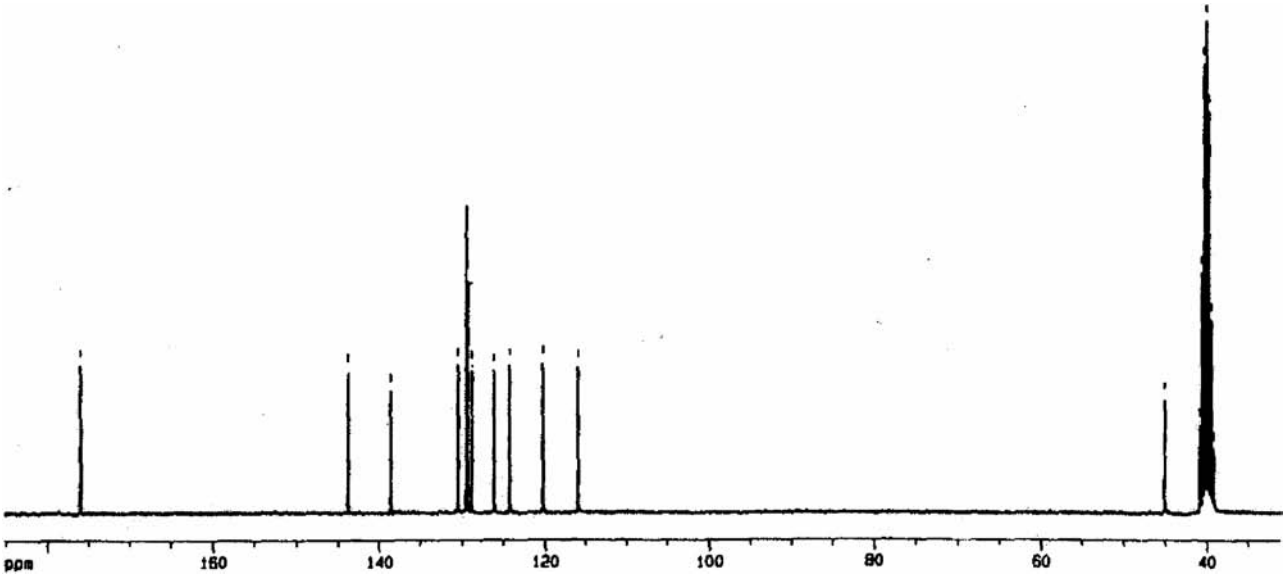

FIGURE 4. ^{13}C-NMR spectrum for diclofenac Na in DMSO.

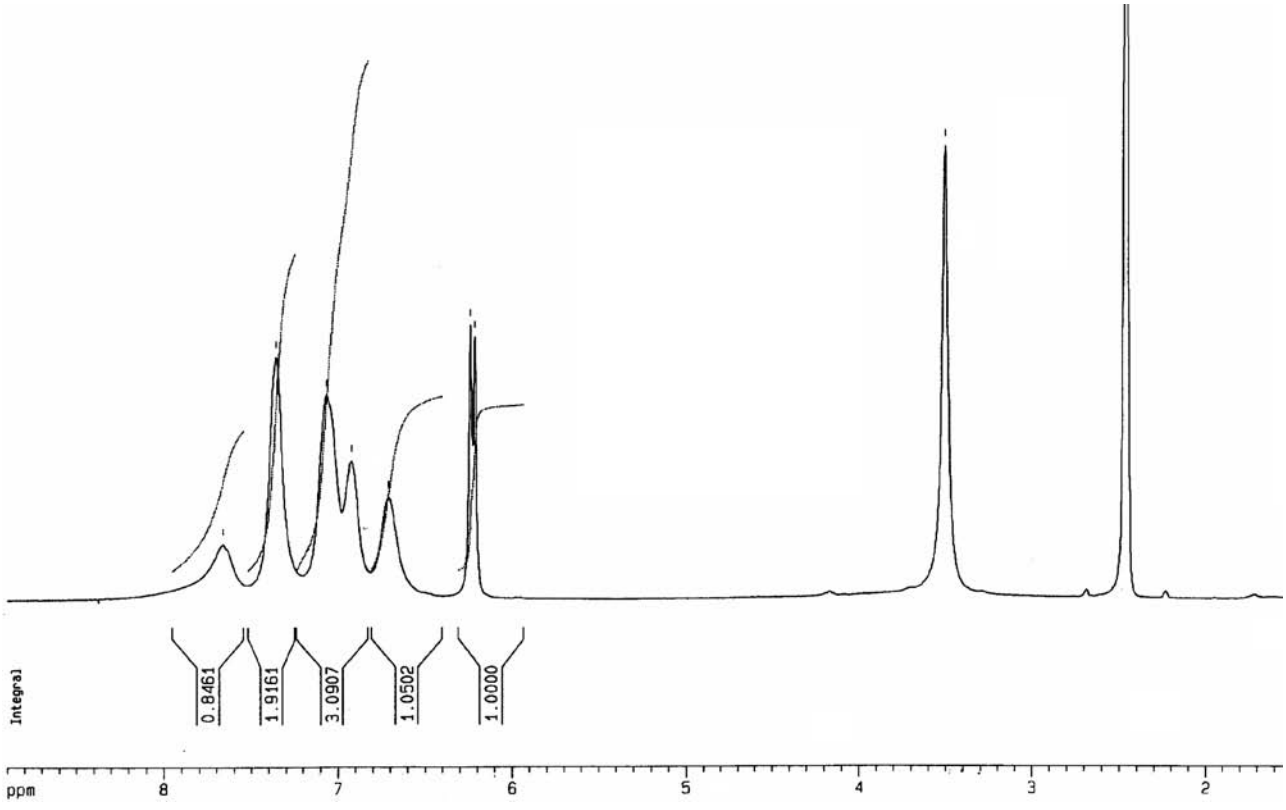

FIGURE 5. ^{1}H -NMR spectrum for diclofenac-bismuth complex in DMSO.

FTIR Spectroscopy

Complex formation was also evident from FTIR spectra (Figures 7 and 8), since those obtained for the complex was significantly different from those of free diclofenac Na and/or the physical mixture of diclofenac Na and bismuth-subcitrate. The major change in the spectra was the shift in the carbonyl absorption band to a higher frequency (1694 cm^{-1}), likely as a result of complex formation. The carbonyl stretching band of

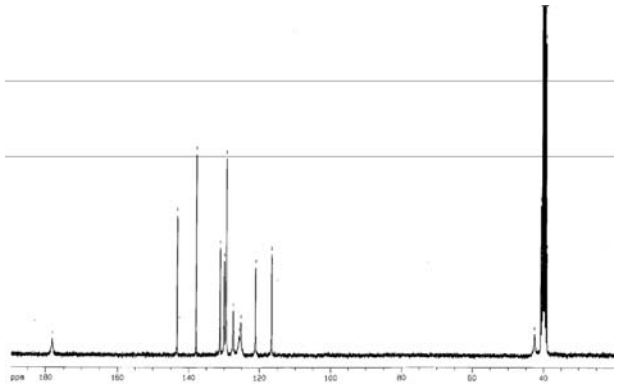

FIGURE 6. ^{13}C-NMR spectrum for diclofenac-bismuth complex in DMSO.

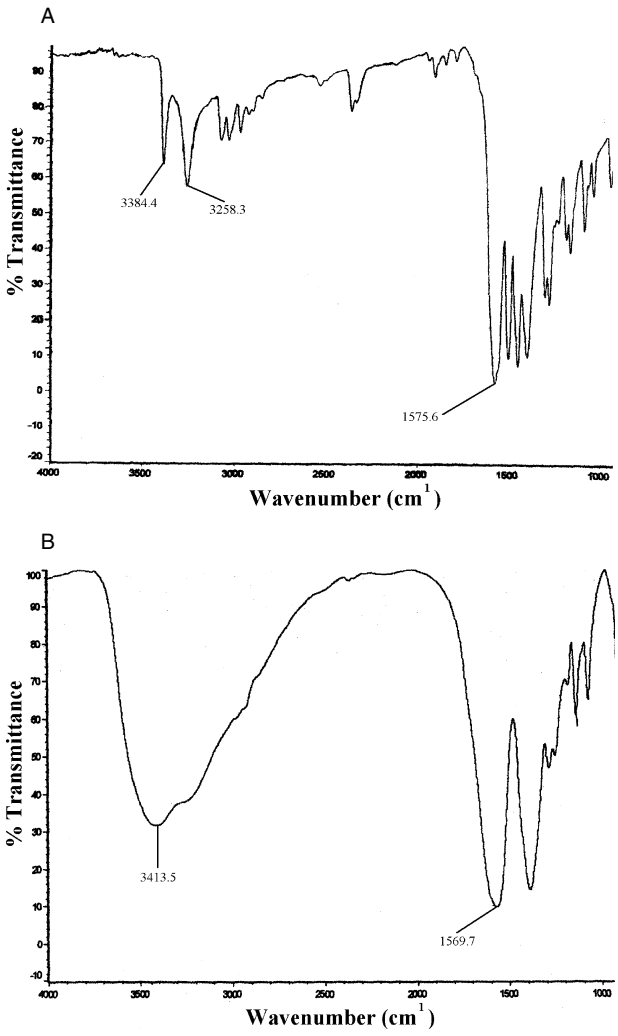

FIGURE 7. FTIR spectra of (A) diclofenac Na and (B) bismuth-subcitrate.

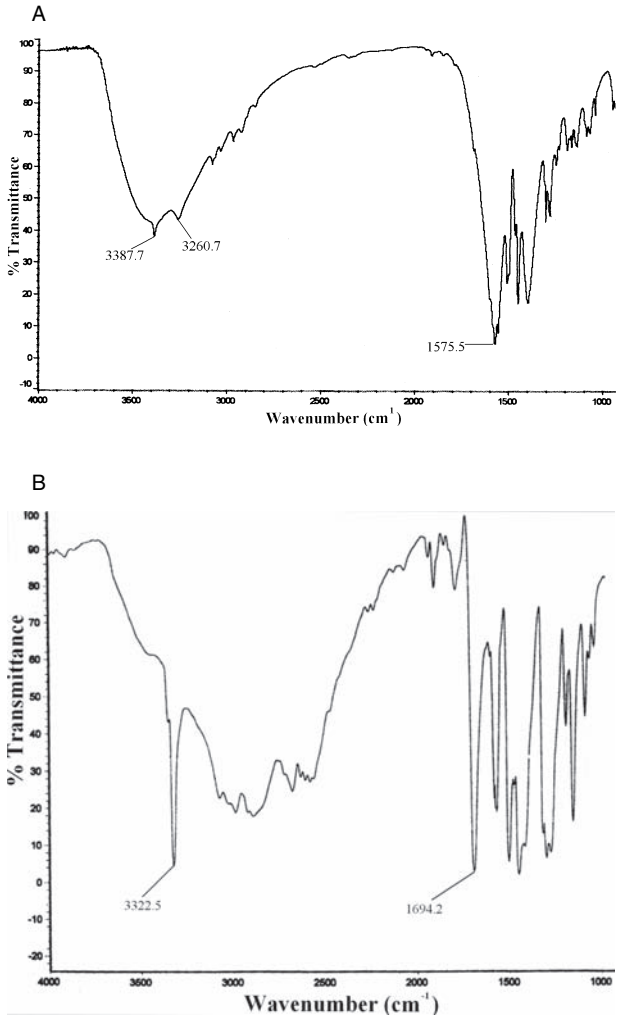

FIGURE 8. FTIR spectra of (A) physical mixture of diclofenac Na and bismuth-subcitrate and (B) solid diclofenac-bismuth complex.

diclofenac in the spectra of diclofenac Na and the physical mixture appeared at 1575 cm^{-1}. The same shift of carbonyl stretching to higher wave number accords with the formation of bonds between the carbonyl oxygen and bismuth (Silverstein et al., 1991).

Dissolution Studies

The dissolution profiles of the acidic presoaking of diclofenac Na powder and the suspension of in situ formed diclofenac-bismuth complex in 0.1 M HCl with subsequent dissolution in a pH 6.8 medium are reported in Figure 9. The drug release percentages in 0.1 N HCl for diclofenac Na powder and the suspension were 0.03% and 9.68% at 1 hour, and 5.24% and 10.87% at 2 hours, respectively. These low dissolution values could be attributed to the conversion of diclofenac Na to diclofenac acid and the breakdown of diclofenac-bismuth complex to diclofenac acid and free bismuth, both as a

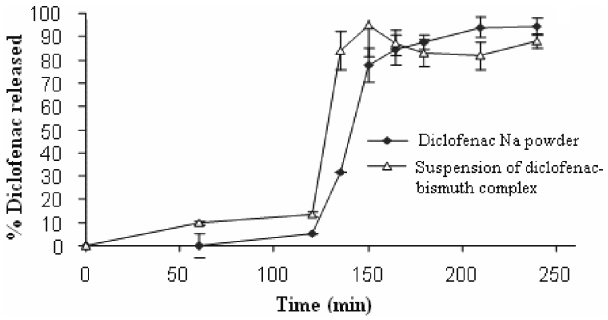

FIGURE 9. Dissolution profiles for aqueous suspension of in situ formed diclofenac-bismuth complex and diclofenac Na powder in a pH 6.8 buffer (2 hours) after acidic soaking in 0.1 M HCl (2 hours).

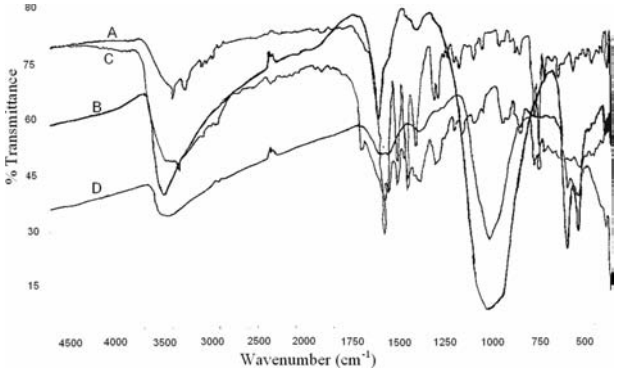

FIGURE 10. Overlaid FTIR spectra of (A) diclofenac Na, (B) the precipitate of mixed aqueous solutions of diclofenac, K-phosphate and bismuth-subcitrate, (C) diclofenac-bismuth complex, and (D) the precipitate of mixed aqueous solutions of K-phosphate and bismuth-subcitrate.

result of diclofenac protonation. However, the suspension had a higher dissolution rate than the diclofenac Na powder. Indeed, diclofenac Na powder was observed to form large lumps during the acidic soaking, while the acidic medium containing the complex suspension was turbid, indicating a smaller size for the formed diclofenac acid. In the pH 6.8 medium, the dissolution rate was also higher for the complex suspension than for the diclofenac Na powder, which is consistent with the observed finer diclofenac acid particle size obtained from the complex suspension compared with diclofenac Na powder during the acidic presoaking. However, both diclofenac Na powder and the complex suspension attained almost the same dissolution extent at 2 hours with almost 100% drug release, which was attributed to the high ionization of diclofenac acid at pH 6.8 (pKa of diclofenac is 4.0). This complete drug dissolution was expected for diclofenac Na, but not for the complex, because the medium at the end of dissolution was a clear solution for diclofenac Na while it was turbid with fine solid particles for the suspension. The existence of suspended particles in the medium of the suspension would contradict the complete drug dissolution if the solid particles in the medium resulted from recomplexation of bismuth and the ionized diclofenac, which was a probability to be taken into consideration. However, these solid particles could arise from a competitive complexation between bismuth and the phosphate polyanions of the buffer. The FTIR spectra of the precipitates obtained upon mixing bismuth-subcitrate solutions with K-phosphate solutions in the presence and absence of diclofenac Na are shown in Figure 10. These spectra matched each other with a shared wide band between 3,000 and 3,500 cm^{-1} and distinctive peaks at 1,580 and 1,000 cm^{-1}. However, they showed the absence of the characteristic peaks for diclofenac Na at 3,384, 3,258, and 1,912 cm^{-1}, and for diclofenac-bismuth complex at 3,322 and 1,694 cm^{-1}. Accordingly, diclofenac was not involved in the precipitate formation and, thus, these precipitates were bismuth-phosphate particles, consistent with the complete drug dissolution obtained from the complex suspension. The favored complexation between bismuth and phosphate in the presence of diclofenac Na could be explained based on the higher acidic strength of the phosphate group compared with that of the carboxylate group of diclofenac. The previous in vitro dissolution results for the complex suspension could provide a prediction of what could happen in vivo upon its oral administration. The complex is likely to be broken down in the stomach into solid diclofenac acid and free bismuth. If diclofenac acid and bismuth remained within the same vicinity of drug absorption upon gastric emptying into the small intestine, the favored complexation of bismuth with the intestinal phosphate polyanions, rather than with the ionized diclofenac, would allow for the absorption of diclofenac in free form. This suggests that the pharmacological action of diclofenac would not be changed when the drug is administered orally as bismuth-complex.

Ulcerogenic Study

A total of five rats were examined in groups A and D due to the death of one rat in each group as a result of the anesthesia prior to dosing. In groups B and C, no deaths occurred, and six rats were examined. In Group A, two stomachs showed diffuse erythema in the absence of hemorrhage or ulceration, and the remaining stomachs were completely unremarkable. In Group B, two rats showed focal erythema in the absence of petechial hemorrhage or ulceration, two rats showed erythema accompanied by streaks and petechial hemorrhage in the absence of ulcerations, and two rats showed multiple ulcers, < 3 mm in total dimensions. In Group C, two stomachs were completely unremarkable, one stomach showed focal erythema without petechial hemorrhage, and three rats showed diffuse erythema with petechial hemorrhage in the absence of ulceration. In Group D, one rat showed erythema and a focus of petechial hemorrhage, one rat showed diffuse erythema accompanied by three petechial hemorrhages, one rat showed four ulcers with < 3 mm diameter, and two rats showed multiple ulcers > 3 mm.

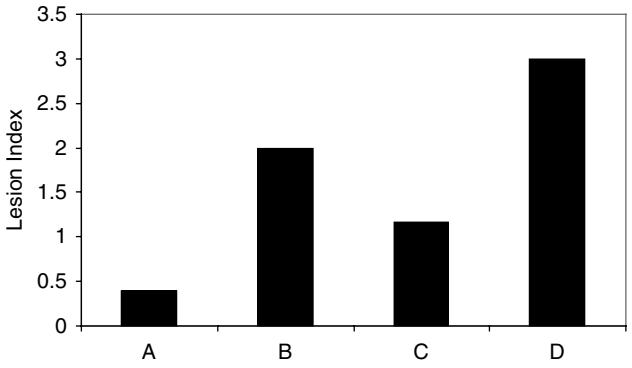

FIGURE 11. Lesion index for rat groups. Group A: control; group B: diclofenac Na; group C: physical mixture of diclofenac acid and bismuth-subcitrate; and group D: diclofenac-bismuth complex.

The lesion indices for the groups were calculated and plotted in Figure 11, which shows that the ulcerogenic effect was in the ascending order of distilled water, diclofenac-bismuth physical mixture, diclofenac Na, and then diclofenac-bismuth complex. The higher ulceration associated with the complex suspension than with diclofenac Na solution was not in accordance with the known protective effect of bismuth against gastric ulceration. This protective effect can explain the least ulceration shown for the suspension of diclofenac acid-bismuth physical mixture among the three diclofenac preparations. According to the dissolution studies, diclofenac-bismuth complex is likely to be broken into diclofenac acid and free bismuth in the acidic gastric fluid. Consequently, a physical mixture of diclofenac acid and free bismuth should have formed in the stomach upon the complex suspension administration. Thus, both the complex suspension and the physical mixture suspension should be chemically equivalent for their gastric local effect. However, the suspension containing the physical mixture was less ulcerogenic than the complex suspension, indicating that the effect of bismuth was confounded with another variable. This confounding variable may also have made diclofenac Na solution less ulcerogenic than the complex suspension. The possible differences in particle size between the administered diclofenac acid in the suspension of the physical mixture and diclofenac acid formed from the complex breakdown and diclofenac Na neutralization upon gastric exposure was thought to be as this confounding variable. A smaller size of diclofenac acid could lead to higher coverage of the stomach mucosa with the ulcerogenic diclofenac and this effect could exceed the protective effect of bismuth. Simulating the exposure to gastric fluid, soaking of the complex and diclofenac Na in 0.1 M HCl formed diclofenac acid particles with average size ($n = 3$) of 22.9 ± 0.5 and 66.1 ± 0.6, respectively, corresponding to 61.3 ± 1.5 μm for diclofenac acid in the suspension of the physical mixture. Accordingly, diclofenac acid formed from the complex was almost three-fold smaller than that formed from diclofenac Na, and the latter was of similar size to that in the suspension of the physical mixture. The finest diclofenac acid size obtained from the complex could be correlated with the highest gastric ulceration associated with its administration and could have dominated the antiulcer effect of bismuth complexed with diclofenac. On the other hand, similarity in size between diclofenac acid in the physical mixture suspension and that formed from diclofenac Na solution as a result of the acidic neutralization, if simulated, the in vivo performance would make bismuth as the only discriminating factor for the gastric ulceration effect of the two preparations. This would explain the higher ulcerogenicity of diclofenac Na solution with no bismuth.

CONCLUSION

In situ diclofenac-bismuth complexation can be achieved by mixing diclofenac Na and bismuth-subcitrate aqueous solutions to give an aqueous suspension of the complex. This complexation appeared to occur at 1:1 diclofenac:bismuth molar ratio by coordinate bonding between bismuth and the carboxylate group of diclofenac. Our ulcerogenic study showed that upon oral administration to rats, physical mixing of diclofenac acid and bismuth-subcitrate was effective in reducing the gastric ulceration of diclofenac, which was attributed to the antiulcer effect of bismuth. On the other hand, diclofenac-bismuth complex was more ulcerogenic than diclofenac Na. The formation of diclofenac acid in gastric fluid as a result of complex breakdown and diclofenac Na protonation with different particle size between the two forms could be linked to the difference in their gastric ulceration. Finer diclofenacc acid size could lead to higher coverage of the gastric mucosa with diclofenac and more local ulceration of the gastric mucosa. The formation of diclofenac acid in gastric fluid was simulated by soaking diclofenac-bismuth complex and diclofenac Na in 0.1 M HCl with subsequent particle size measurement of the formed diclofenac acid particles. Three-fold finer diclofenac acid size was obtained from the complex soaking than from diclofenac Na soaking.

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