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Preparation and Characterization of Enrofloxacin/Carbopol Complex in Aqueous Solution

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Since the bitter taste of enrofloxacin apparently limit the patient compliance in the oral formulations of the antibacterial agent, the masking of the taste is essential for the improvement of the therapeutic effectiveness. Therefore, this study was carried out to examine the feasibility of taste masking of enrofloxacin by the retardation of its dissolution rate using the formation of complex between the drug and Carbopol. The complexation between Carbopol and enrofloxacin was confirmed by turbidity, UV spectrophotometry, wide angle X-ray diffraction, and differential scanning calorimetry. The enrofloxacin content in the complexes was 34% (Carbo-enrofloxacin complex I) and 57% (Carbo-enrofloxacin complex II) depending on the preparation method. The dissolution rate of enrofloxacin from the complex increased as the pH was reduced. The dissolution rate of enrofloxacin from the Carbo-enrofloxacin complex I was significantly lower than that of the enrofloxacin powder. Therefore, these observations suggest that Carbo-enrofloxacin complex I can be used to mask the taste of enrofloxacin.

Key words: Carbopol, Enrofloxacin, Ion-pair complex, Dissolution rate, Taste masking

INTRODUCTION

Recently, fluoroquinolones, a group of drugs derived from nalidixic acid, were developed as highly potent antibacterial agents. Fluoroquinolones are used to treat diseases caused by intracellular bacteria, such as Mycobacterium, Mycoplasma, Chlamydia, Legionella, and Brucella (Lizondo *et al.*, 1997). They inhibit the activity of the bacterial DNA gyrase, which is an enzyme that controls the supercoiling of DNA by converting the relaxed covalently closed circular DNA to a superhelical form via an energy-dependent strand breakage and resealing process (Rosenstiel and Adam, 1994; Wolfson and Hooper, 1989). Enrofloxacin, one of these fluoroquinolones, has been used to treat the main bacterial processes affecting farm animals. Although the quantitative evaluation of the bitterness of enrofloxacin has not been reported, its bitter taste has limited its oral administration. Recently, several studies with respect to the quantitative evaluation of the bitterness have been reported (Takagi *et al.*, 2001; Miyanaga *et al.*, 2002; Miyanaga *et al.*, 2003; Tanigake *et al.*, 2003). In these studies, it was observed that the bitterness was proportional to the concentration of the solution. Therefore, minimizing the dissolution rate of enrofloxacin in water would reduce the bitterness of the drug when it is administered.

It was reported that the use of an ion-exchange resin system was effective for retarding the dissolution rate of a drug and masking the taste of bitter drugs (Raghunathan *et al.*, 1981; Borodkin and Sundberg, 1971; Agarwal *et al.*, 2000; Fu Lu and Borodkin, 1989; Fu Lu *et al.*, 1991). The resin forms a complex through a weak ionic interaction with an oppositely charged drug. Consequently, the complex releases a minimal amount of the drug in a neutral medium. After ingestion and exposure the complex to ions in the body, it dissociates and the drug is released. The same concept can be utilized for masking the taste of enrofloxacin. It has been suggested that ionic complexation in a solution is influenced by the polarity of the solvent, and the formation of the complex depends on the solvating ability of the solvent to the ionic groups (Zhang *et al.*, 2001). However, this aspect of dissolution control for enrofloxacin has not been studied in the literature.

Enrofloxacin exists as an acidic cationic form at lower pH (below pH 5) (Lizondo *et al.*, 1997) and Carbopol, a crosslinked poly(acrylic acid), exists in an anionic form in

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aqueous solution due to the dissociation of the carboxylic acid groups. Therefore, negatively charged Carbopol can form an ion pair complex with the positively charged enrofloxacin, as shown in Fig. 1. Carbopol has the advantages over conventional ion-exchange resins (Fu Lu and Borodkin, 1989; Fu Lu *et al.*, 1991) for this application because it swells readily and dissolves in neutral buffers. Moreover, it has been reported to have bioadhesive properties (Harris *et al.*, 1990).

The aim of this work was to study the feasibility of an enrofloxacin complex with Carbopol by an ionic interaction in an aqueous solvent system as a tool for the reduction in the dissolution rate. In this study, we describe physicochemical properties of the enrofloxacin complex by X-ray diffraction, turbidity, differential scanning calorimetry, and its dissolution rate.

MATERIALS AND METHODS

Materials

The enrofloxacin was provided by LG Chemical Co. (Daejeon, Korea). Carbopol® 971 was provided by B. F. Goodrich Co. (Cleveland, OH). All other chemicals were reagent-grade and were used without further purification.

Preparation of Carbopol-enrofloxacin complex

In order to prepare the Carbo-enrofloxacin complex I, Carbopol (0.25 g) was dissolved in 50 mL of distilled water, and the same amount of enrofloxacin was dissolved in 50 mL of a 1% acetic acid solution. The enrofloxacin solution was added to the Carbopol aqueous solution with constant stirring. The formed complex was precipitated and aggregated. The Carbopol-enrofloxacin complex was filtered and washed with distilled water several times. After washing, the complex was dried in a vacuum oven over 12 h at room temperature and ground to a fine powder (< 150 μm) in a mill. This powder was again dried in vacuum oven for 24 h at room temperature.

Carbo-enrofloxacin complex II was prepared in the same way as Carbo-enrofloxacin complex I except enrofloxacin was dispersed in 50 mL of distilled water.

UV spectroscopic analysis

The UV spectroscopic spectra of the Carbopol-enrofloxacin complex, enrofloxacin, and Carbopol in water were measured by UV spectrophotometer (UV-1601, Shimadzu, Japan).

Turbidity measurements

The formation of the complex through ionic interaction between Carbopol 971 and enrofloxacin at various weight ratios of Carbopol 971/enrofloxacin was investigated by monitoring the transmittance of solution at a wavelength of 500 nm by using a spectrophotometer (UV-1601, Shimadzu, Japan). To measure the turbidities, Carbopol (72 mg) was dissolved in 100 mL of distilled water and enrofloxacin (72 mg) was dissolved in 100 mL of 1% acetic acid solution. Carbopol solution and enrofloxacin solution were mixed as a function of various weight ratios (Carbopol/enrofloxacin), and the turbidity was measured.

X-ray diffraction patterns

The wide angle X-ray diffraction patterns of the Carbopol-enrofloxacin complexes, enrofloxacin, Carbopol, and Carbopol/enrofloxacin physical mixture were measured by X-ray diffractometer (D/MAX-3C, Riguku Co., Japan) using Ni-filtered Cu K radiation (35 kV, 15 mA).

Measurement of enrofloxacin content in the complex

Two milligrams of the of the Carbopol-enrofloxacin complex was dissolved in 20 mL of the pH 7.4 phosphate buffer solution. The solution was filtered through a 0.45 μm-syringe filter and diluted 10 times with the pH 7.4 phosphate buffer solution. The diluted solution was analyzed by UV spectrophotometer (UV-1601, Shimadzu, Japan) to determine the enrofloxacin content in the complex at 271 nm.

Differential scanning calorimetry analysis

The differential scanning calorimetry (DSC) thermograms of the Carbopol-enrofloxacin complexes, enrofloxacin, Carbopol, and a Carbopol/enrofloxacin physical mixture were measured by DSC (DSC-2010, TA Instrument, New Castle, DE) at a scan rate of 10°C/min.

Release of enrofloxacin from the complex

The enrofloxacin release rate from the complex was investigated using a dissolution tester (DST 600A, Fine Science Institute, South Korea). The Carbopol-enrofloxacin complexes, enrofloxacin, or the Carbopol/enrofloxacin physical mixture was placed in 500 mL of the dissolution medium and stirred at 100 rpm at 37°C. The pH values of the dissolution medium used were 2.0, 4.0, and 6.0. Aliquots of the medium were withdrawn at predetermined time intervals and equivalent amounts of fresh medium were added. The collected samples were filtered through a 0.45 μm-syringe filter, diluted 10 times with fresh medium, and analyzed by UV spectrophotometer (UV-1601, Shimadzu, Japan) to determine the amount of enrofloxacin released from the complex.

RESULTS AND DISCUSSION

Formation of Carbopol-enrofloxacin complex

A precipitate forms when the carboxylate anionic form

of Carbopol and the acidic cationic form of enrofloxacin are mixed. The formation of the precipitate indicates the formation of an ionic complex between the Carbopol and enrofloxacin, since both components were soluble in the solutions prior to mixing. The formed complex precipitated whether or not enrofloxacin was dissolved in acetic acid solution or suspended in distilled water. Wide angle X-ray diffraction (WAXD), turbidity measurement, and UV spectrum were used to confirm the formation of the complex. The transmittance change of the solution was measured as a function of weight ratio of Carbopol 971 and enrofloxacin (Fig. 2). The transmittance sharply decreased at the weight ratio of 75/25 and reached the lowest value when the ratio was 50/50. Then, transmittance value gradually increased up to the ratio of 25/75. This clearly indicates that Carbopol formed a complex with enrofloxacin by ionic interaction as shown in Fig. 1.

The UV spectra of the Carbopol-enrofloxacin complex was compared with enrofloxacin and Carbopol (Fig. 3).

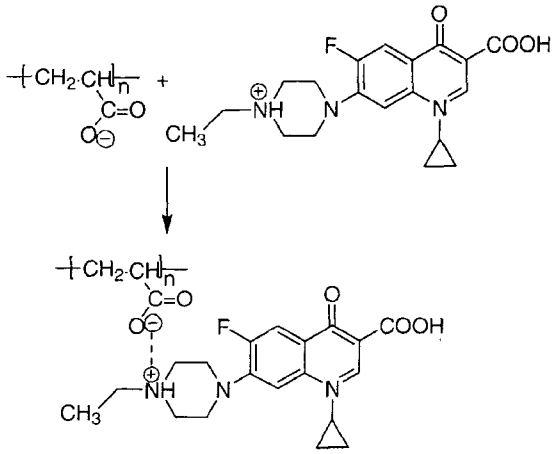

Fig. 1. Scheme of the complexation of enrofloxacin with Carbopol

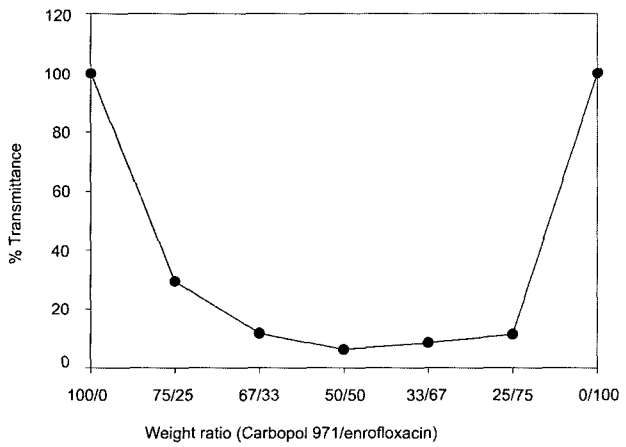

Fig. 2. Transmittance change of the solution in which Carbopol 971 and enrofloxacin were complexed

The λ_{max} of enrofloxacin appeared at 272.4 and 322.4 nm. The λ_{max} of the complex appeared at 223.0, 275.2 and 317.8 nm. However, no λ_{max} was observed in case of Carbopol. As shown in Fig. 3, a new λ_{max} appeared at 223.0 nm in the complex and the λ_{max} of enrofloxacin at 272.4 and 322.4 nm shifted to 275.2 and 317.8 nm, respectively. These results suggest that a complex was formed between Carbopol and enrofloxacin.

Fig. 4 shows the WAXD patterns of Carbopol, enrofloxacin, Carbo-enrofloxacin complex I, Carbo-enrofloxacin complex II, and a Carbopol/enrofloxacin physical mixture. Carbopol exhibited a broad pattern of a typical amorphous polymer, whereas enrofloxacin exhibited characteristic peaks between 2θ = 7.40 and 27.65° due to its crystalline properties. The Carbopol/enrofloxacin physical mixture also

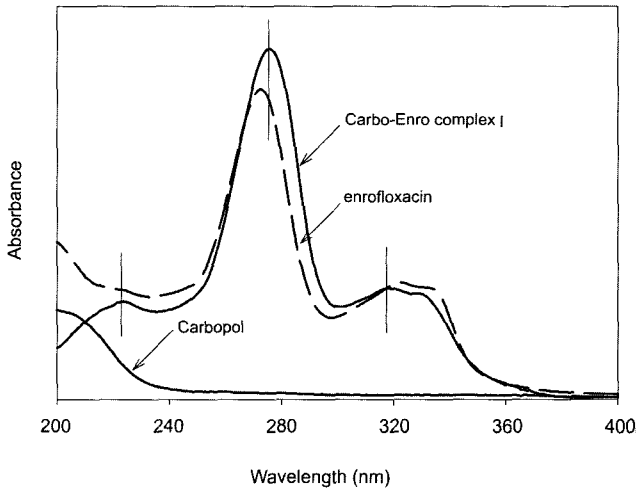

Fig. 3. UV spectra of Carbo-enrofloxacin complex I, enrofloxacin, and Carbopol

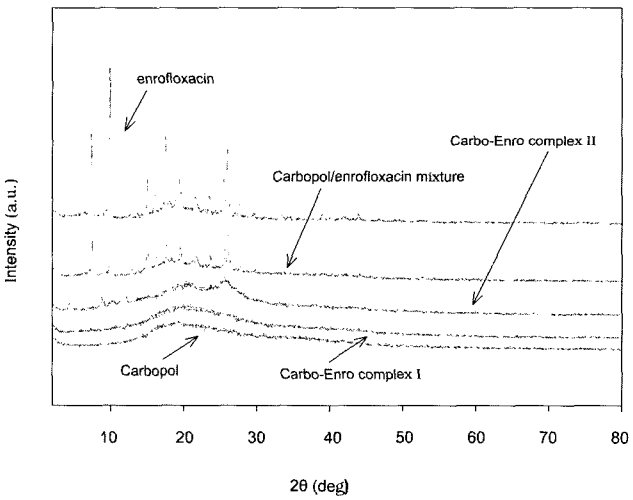

Fig. 4. Wide angle X-ray diffraction (WAXS) patterns of Carbo-enrofloxacin complex I, Carbo-enrofloxacin complex II, enrofloxacin, Carbopol, and their physical mixture

showed the characteristic peaks of enrofloxacin. However, in the case of the Carbo-enrofloxacin complex I, the characteristic peaks of enrofloxacin disappeared. The disappearance of the characteristic peaks of enrofloxacin suggests that enrofloxacin exists in an amorphous form and a complex was formed between the Carbopol and enrofloxacin. In contrast, the weak characteristic peaks of enrofloxacin at 2θ = 9 and 25.5° appeared in the Carbo-enrofloxacin complex II. It seemed that when the enrofloxacin suspension was mixed with the Carbopol solution, some of the enrofloxacin dissolved due to the acidic pH of Carbopol solution and then formed a complex. However, some of the drug remained undissolved and mixed alongside the complex, resulting in the appearance of the weak characteristic peaks of enrofloxacin.

Thermal property of the complexes

Fig. 5 shows the DSC thermograms of Carbopol, enrofloxacin, Carbo-enrofloxacin complex I, Carbo-enrofloxacin complex II, and a Carbopol/enrofloxacin physical mixture. Although the glass transition temperature (Tg) of Carbopol was not clearly observed, the melting peak of enrofloxacin was observed in the physical mixture. However, the melting peak of enrofloxacin disappeared in the case of the Carbo-enrofloxacin complex I. In case of the Carbo-enrofloxacin complex II, a melting peak of enrofloxacin was observed, indicating some of enrofloxacin exist in crystal form in the Carbo-enrofloxacin complex II.

Enrofloxacin content in the complex

Table I shows the drug content of the enrofloxacin in the Carbo-enrofloxacin complex I and the Carbo-enrofloxacin complex II. The drug contents of the enrofloxacin in the Carbo-enrofloxacin complex I and the Carbo-enrofloxacin complex II were 34% and 57%, respectively. It should be noted that some of the enrofloxacin in the Carbo-enrofloxacin complex II did not form a complex with the Carbopol as discussed previously, which is why there was a higher drug content.

Table I. Enrofloxacin content of Carbo-enrofloxacin complex I and Carbo-enrofloxacin complex II (n=3)

	Enrofloxacin content (%)
Carbo-enrofloxacin complex I	34 ± 2
Carbo-enrofloxacin complex II	57 ± 1

Release of enrofloxacin from the complex

The effect of pH on the enrofloxacin release rate from Carbo-enrofloxacin complex I is shown in Fig. 6. The enrofloxacin release rate from the complex increased as the pH decreased. The release of a drug from the complex was reported to be an equilibrium phenomenon where the counterions displace the drug from the carboxylic acid sites of the polymer (Miyanaga *et al.*, 2002). At a lower pH environment, the enrofloxacin release rate was promoted by the higher solubility of the enrofloxacin and the Carbopol equilibrium lying primarily toward the undissociated carboxylic acids. Despite of slow release rate of enrofloxacin at pH 6.0, the complexation between Carbopol and enrofloxacin would not significantly reduce the bioavailability of enrofloxacin. As can be seen in Fig. 6, 60% of the drug was released at pH 2.0 after 1 h, indicating that the most of the administered drug will be dissociated and dissolved in the stomach.

The enrofloxacin release rate from the Carbopol-enrofloxacin complexes were compared with that from the enrofloxacin powder at pH 2.0, 4.0, and 6.0 (Fig. 7). The

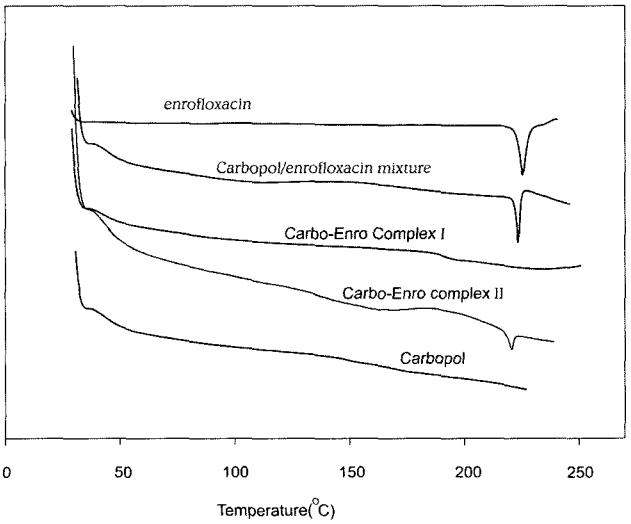

Fig. 5. DSC thermograms of the Carbo-enrofloxacin complexes, enrofloxacin, Carbopol, and their physical mixture

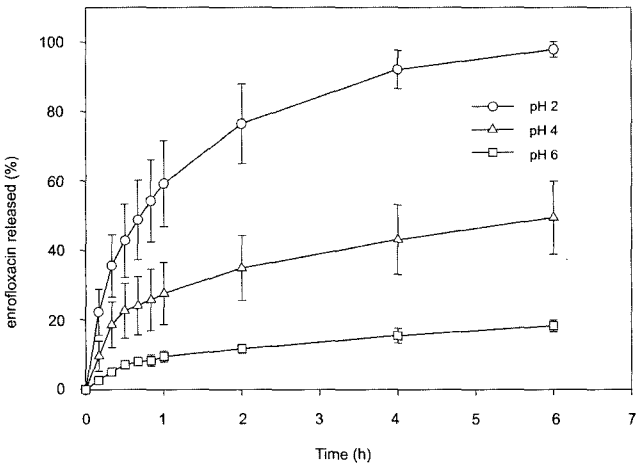

Fig. 6. Effect of pH on the release rate of Carbo-enrofloxacin complex I (n=3)

enrofloxacin release rate from the Carbo-enrofloxacin complex I was significantly lower than that of the enrofloxacin powder at all pH values tested. The complexation between the Carbopol and enrofloxacin effectively reduced the release rate of the enrofloxacin from the complex. In particular, the initial enrofloxacin release rate from the Carbo-enrofloxacin complex I was significantly lower at pH 6.0 than that of the enrofloxacin powder, indicating that complexation between Carbopol and enrofloxacin can be used to mask the bitter taste of enrofloxacin. It is interesting to note that the swelling property of Carbopol was not observed in the case of the Carbo-enrofloxacin complex I or II, which is another indication of complex formation. The enrofloxacin release rate from the Carbo-enrofloxacin complex II was lower than that of the enrofloxacin powder. However, it was higher than that of the Carbo-enrofloxacin complex I due to the uncomplexed enrofloxacin in the Carbo-enrofloxacin complex II.

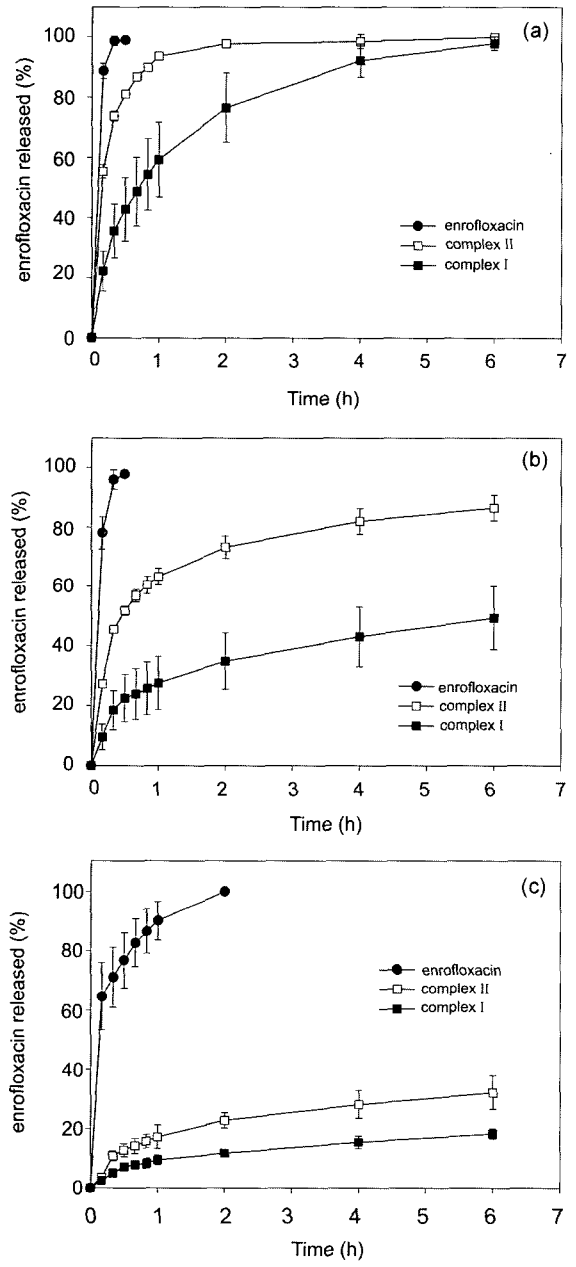

Fig. 7. Comparison of the release rates of enrofloxacin from Carbo-enrofloxacin complex I (■), Carbo-enrofloxacin complex II (□), and enrofloxacin powder (●) at the pH 2.0 (a), 4.0 (b) and 6.0 (c). (n=3)

In conclusion, the complexation of enrofloxacin with Carbopol can be used to mask the bitter taste and to delay the release rate of enrofloxacin. As is indicated in the release profile of the complex at pH 6.0, minimal amount of enrofloxacin was released and it may provide slight bitter taste. The time required to feel the bitter taste need to be investigated in the future study. Depending on the results of taste experiment, further formulation efforts may be required.

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