Methods of Evaluation of Release of Carbon Dioxide from Effervescent Suppositories

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Two different test methods were studied for the evaluation of release profiles of carbon dioxide, CO2, from effervescent suppositories. Three lots of commercial suppositories containing sodium bicarbonate and anhydrous sodium dihydrogen phosphate were used. The volume of CO2 released from these suppositories in normal saline with or without polysorbate 80 as a medium was measured with a gas burette.

In the measurement performed using the apparatus without stirring, method 1, only 60% of CO2 was released from the suppositories in the medium without polysorbate 80. In this measurement, a native release profile was detected because the medium was not stirred. In the case with stirring, however, method 2, 100% was released from those suppositories in the medium containing 1% polysorbate 80 with comparatively low standard deviations.

These findings indicate that method 1 is most beneficial for a test comparing the effects of various factors such as additives and melting points on the release profiles of CO2 from the effervescent suppositories. However, this method is not practical for quality control of formulations because of the incomplete release of CO2; method 2, in contrast, is useful because of its complete release and low standard deviations.

These results suggest that methods 1 and 2 for release tests of CO2 are most applicable to the early and late formulation studies of effervescent suppositories, respectively.

Keywords effervescent suppository; carbon dioxide; release test; sodium bicarbonate; gas burette; laxative

Lecicarbon® suppositories, which contain sodium bicarbonate, NaHCO3, and anhydrous sodium dihydrogen phosphate, Na2HPO4, as active ingredients, are effervescent laxatives on the market. When the oleaginous bases have been melted after the rectal administration of these suppositories, carbon dioxide, CO2, is released from the suppositories by the neutralization of NaHCO3 and Na2HPO4. The released CO2 stimulates the peristalsis of the large intestine, causing smooth defecation.2)

CO2 has heretofore been widely used in gastric X-ray examination together with barium sulfate because its intestinal absorption is better than air. Since CO2 does not produce a sense of abdominal fullness, it has been similarly utilized in double contrast studies in the large intestine.3) Thus, CO2 released from Lecicarbon® suppositories is free from harmful side effects for the digestive system.4)

In most of the reported studies on the release of drugs from suppositories, the release profiles are evaluated by the assay of drugs dissolved in a test fluid. In the case of effervescent suppositories, it is difficult to determine the released drugs because of the rapid neutralization. The efficacy of Lecicarbon® suppositories is based on the CO2 those drugs produce, making it important to examine the in vitro release profiles of CO2 from the effervescent suppositories. Several analyses of NaHCO3 and potassium bicarbonate contents in certain effervescent formulations have been reported,5) however, little has been published on a means to evaluate the release rate of CO2 from such suppositories.

Two methods of determining CO2 release are described here as they relate to the rate and amount released from the effervescent suppositories and the quality of the formulations.

Experimental

Materials All ingredients and chemicals were JP XII grade, except NaH2PO4 (USPII grade), and were used without further purification.

Formulations Three lots of Lecicarbon® suppositories (lots No. A41, B42 and C43, Zeria Pharmaceutical Co.) containing 500 mg of NaHCO3 and 680 mg of Na2HPO4 were used in this study. These suppositories prepared by the fusion method also contain soybean lecithin and hydrogenated oil as an additive and a base, respectively. The weights, melting points, dropping points and penetration time of these suppositories are summarized in Table I.

Measurement of Dropping Points The dropping points of the suppositories are defined as the temperature at which a drop of the melted suppository becomes heavy enough to fall freely from a grease cup orifice. A piece of suppository was placed in the grease cup, and any excess was removed from the cup with a knife. The dropping points of the suppository were measured using a dropping point meter (models FP-5 and FP-5S, Mettler Instrument Co.) under the following conditions: starting temperature of 30°C; heating rate of 1°C/min.

Measurement of Penetration Time The penetration time of the suppositories was measured using a penetrometer49 (model PM-3, Erweka Co.) at 37°C to evaluate softening points. Method I for Release Test Figure 1 shows the apparatus employed.

Normal saline was used as a test fluid because phosphate buffer solution, which is the fluid generally used in a test for drug-release from a suppository, contained the same ion as NaH2PO4. To prepare normal saline containing saturated CO2, CO2 from NaHCO3, Na2HPO4 and water was bubbled into 100 ml of normal saline for 30 min with stirring by a magnetic stirrer (400 rpm). This normal saline containing saturated CO2 (6 ml) as a medium was poured into a test tube (18 mm i.d. x 200 mm), which was immersed in a thermostatic bath maintained at 37°C. A weighed

<table>
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<th>TABLE I. Physicochemical Properties of Suppositories</th>
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<td>Lot No.</td>
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<td>A41</td>
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Each value represents the mean±S.D. (n=3). a) The melting points were measured by an open capillary tube method (method 2) according to JP XII.
suppository was placed in 6 mL of the medium and the cork of the by-pass was immediately closed. The accumulated volume of CO₂ released from the suppository was measured with a 100-mL gas burette filled with light liquid paraffin at room temperature; the measurement was conducted under atmospheric pressure using a leveling bulb connected with the burette.

To prepare the calibration curve for CO₂ in this method, prior to the measurement with the suppositories, gelatin capsules containing various amounts of a NaHCO₃-NaH₂PO₄ mixture (100:136, 200:272, 300:408, 400:544 and 500:680) were placed in the medium maintained at 37°C and vigorously stirred with a tube touch mixer for a few minutes. The maximum volume of CO₂ released from those capsules was similarly measured with the gas burette.

**Method 2 for Release Test** Figure 2 shows the apparatus employed in method 2. CO₂ was similarly bubbled into 100 mL of normal saline containing 1% polysorbate 80 for 30 min. This normal saline with polysorbate 80 (20 mL) was poured into a 300-mL three-necked round-bottomed flask, maintained at 37°C and stirred at 82 rpm with a Teflon® mixing wing. After a weighed suppository was placed in 20 mL of the medium, the accumulated volume of CO₂ released was measured by the method described above.

The maximum volume of CO₂ released from the same gelatin capsules was similarly measured by method 2 prior to the measurement with the suppositories, and the calibration curve for CO₂ in method 2 was also drawn.

**Calculation** The accumulated volume of CO₂ released from the suppository was determined every 2 min for 60 min at room temperature. Each observed accumulated volume of CO₂ released from the suppository was converted into a normalized accumulated volume according to Eq. 1 under the following constant conditions: at 25°C, per 2.6 g of the suppository.

\[
V = V_{obs} \times \frac{2.6 \times 273.15 + 25}{W \times 273.15 + T}
\]

Where \( V \) is the normalized accumulated volume of CO₂, \( V_{obs} \) is the observed accumulated volume of CO₂, \( W \) is the weight of the suppository, and \( T \) is the observed room temperature.

**Results and Discussion**

**pH of Normal Saline Containing Saturated CO₂** Normal saline with or without 1% polysorbate 80 (100 mL) was boiled for a few minutes, then cooled to room temperature and CO₂ was bubbled into each solution for 30 min. Figure 3 shows the relationships between the pH of those solutions and the bubbling time of CO₂. The pH of both solutions decreased with the bubbling time of CO₂ and reached a constant value after 10 min. The pH of the normal saline without polysorbate 80 was in good agreement with the literature value⁷) in the case of water. Therefore, CO₂ was considered to be completely saturated in 100 mL of normal saline by the 30 min bubbling. CO₂ was then no longer soluble in the medium, so that all of the CO₂ released from the suppositories was detected with the gas burette. On the other hand, the solubility of CO₂ in light liquid paraffin was negligible during the release test.

**Release of CO₂ from Gelatin Capsules** As shown in Eq. 2, CO₂ is generated by the neutralization of NaHCO₃ and NaH₂PO₄.

\[
\text{NaHCO}_3 + \text{NaH}_2\text{PO}_4 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{Na}_2\text{HPO}_4
\]

A suppository contains 500 mg (5.95 mmol) of NaHCO₃ and 680 mg (5.67 mmol) of NaH₂PO₄, so that 5.67 mmol of CO₂ is released from the suppository by the complete neutralization. From the equation for the gas state shown in Eq. 3, the volume of CO₂ released from a suppository

![Fig. 1. Schematic Illustration of Apparatus Employed in Method 1 for Release Test](image)

![Fig. 2. Schematic Illustration of Apparatus Employed in Method 2 for Release Test](image)

![Fig. 3. Relationship between pH of Medium and Bubbling Time of CO₂ Polysorbate 80: (●) without, (○) with.](image)
is theoretically 139 ml at 25°C at a pressure of 1 atm.

\[ V = \frac{nRT}{P} \]  

(3)

Where \( V \) is the volume, \( n \) is the molar quantity, \( R \) is the gas constant, \( T \) is the temperature, and \( P \) is the pressure.

In order to avoid the influence of the oleaginous bases of the suppositories, gelatin capsules containing the mixture of NaHCO₃ and NaH₂PO₄ were employed in this study. Figure 4 shows the relationships between the maximum volume of CO₂ released from those capsules and the amounts of the mixture. In Fig. 4, 100% of the mixture corresponds to 0.5 g of NaHCO₃ and 0.68 g of NaH₂PO₄. The difference in the release amount of CO₂ observed between the calibration curves results from the presence or absence of polysorbate 80. Therefore, polysorbate 80 is considered to enhance the reaction of NaHCO₃ with NaH₂PO₄. The recoveries (vs. the theoretical value) of CO₂ in methods 1 and 2 calculated from those plots were 72.5 and 76.0%, respectively. These results suggest that the concentrations of NaHCO₃ and NaH₂PO₄ in both test fluids are not enough to react completely in spite of the complete dissolution of these active ingredients by stirring. A large excess of NaHCO₃ or NaH₂PO₄ is required for the complete reaction. However, those plots gave linear relationships with good correlation coefficients: 0.9998 and 0.9957, respectively. Thus, these two different methods were found to be applicable to the evaluation of the in vitro release profiles of CO₂ from the effervescent suppositories.

**Release of CO₂ from Suppositories (Method 1)**

Figure 5 shows the release profiles of CO₂ from three lots of the suppositories determined by method 1. CO₂ was rapidly released from each suppository, and the release reached a plateau after 20—30 min in either case. The release rate and the extent of the plateau were in the orders of lot Nos. B42 < C43 < A41 and lot Nos. C43 < B42 < A41, respectively. Each amount of CO₂ released from the suppository was considerably lower than that from the gelatin capsules as described above. After the measurement by method 1, the medium containing a melted suppository was vigorously stirred with the tube touch mixer. The amount of CO₂ released from the suppository was equal to that from the corresponding capsule. In the measurement by method 1, the neutralization was found to be prevented because the particles of NaHCO₃ and NaH₂PO₄ were covered with the melted oleaginous bases. Therefore, these findings suggest that the release of CO₂ determined by this method is considerably influenced by preparation conditions, storage temperature, content uniformity, the melting points and so on. This measurement method, however, does allow detection of a native release profile because the medium was not stirred. Thus, the method is useful for evaluation of the effects of various factors on the release of CO₂ from the suppositories.

**Effects of Medium Volume and Polysorbate 80 on Release of CO₂ from Suppositories (Method 2)**

Complete neutralization of the active ingredients was not observed in the measurement by method 1, so that it is not suitable for the evaluation of the quality of the formulations. Method 2, the measurement using the apparatus with stirring, was therefore used in subsequent experiments. Figure 6 shows the effects of the medium volume and polysorbate 80 on the release profiles of CO₂ at a constant stirring rate (82 rpm). The release rate of CO₂ from the suppository (lot No. C43) increased with decreasing volume of the medium in both cases in the presence and absence of polysorbate 80. Each amount of CO₂ released increased with the addition of 1% polysorbate 80. CO₂ was gradually and completely released from the suppository in 20 ml of normal saline.
containing 1% polysorbate 80, making this medium the most appropriate for method 2.

Effect of Stirring Rate on Release of CO₂ from Suppositories (Method 2) Figure 7 shows the effect of the stirring rate on the release profile of CO₂ from the suppository (lot No. C43). The release rate and amount of CO₂ increased with faster stirring rate. At 310 rpm, the release rate of CO₂ was so rapid that the release profile could not be well evaluated. The preferable stirring rate to evaluate the quality of the formulations was found to be 82 rpm.

Release of CO₂ from Suppositories (Method 2) Figure 8 shows the release profiles of CO₂ from three lots of the suppositories determined by method 2. Unlike method 1, complete release of CO₂ was observed in the measurement by method 2. The released amount of CO₂ after 60 min was in the order of lot Nos. A41 < B42 < C43. This order was equal to that of the freshness of the suppositories. The dropping points of the suppositories were in the order of lot Nos. C43 < B42 < A41 (Table I). The decrease in the release amount of CO₂ was therefore considered to be closely related to a rise in the dropping points of the suppositories. However, such a relationship was not observed between the release amount of CO₂ determined by method 1 (Fig. 5) and the dropping points. With no stirring of the test fluid (method 1), the amount of CO₂ released seems to be more significantly influenced by other factors such as content uniformity than by the dropping points of the suppositories. These findings suggest that a change caused by standing can be evaluated by this method 2. Furthermore, the differences in the released amount of CO₂ between the three lots of the suppositories were less than those determined by method 1 (Fig. 5).

For comparative evaluation of the release profiles of CO₂, each volume of CO₂ released was converted into a percentage of that according to the calibration curves shown in Fig. 4. Figure 9 shows the converted release profiles of three lots of the suppositories determined by the two different methods. These results indicate that in the evaluation based on the results of the recovery tests only 60% of CO₂ is released from the suppositories by method 1, whereas 100% is released by method 2.

In conclusion, method 1 was found to be useful for a comparative test of the effects of factors such as additives and melting points of the suppositories on the release profiles of CO₂ from the effervescent suppositories. The native release of CO₂ can be detected by method 1 with high sensitivity. However, this method is not practical for evaluation of the quality of formulations since its release of CO₂ is incomplete. We found that method 2 was useful for the quality control of the suppositories because of its complete CO₂ release and comparatively low standard deviations. These findings suggest that methods 1 and 2 to determine release of CO₂ are most applicable to early and late formulation studies of effervescent suppositories, respectively.

References and Notes
1) This paper is part I of "Formulation Studies of Effervescent Suppositories."


