Methods of Evaluation of Release of Carbon Dioxide from Effervescent Suppositories[1)]

Toru HAKATA,*[,a] Masao IIJIMA,[a] Toshio OKABE,[a] Shigeo KIMURA,[a] Hiroshi SATO,[a] Yoshiteru WATANABE[b] and Mitsuo MATSUMOTO[b]

Central Research Laboratories, Zeria Pharmaceutical Co., Ltd.,[a] 2512–1, Oshikiri, Konan-machi, Osato-gun, Saitama 360–01, Japan and Showa College of Pharmaceutical Sciences,[b] 3–3165, Higashitamagawagakuen, Machida, Tokyo 194, Japan. Received August 27, 1992

Two different test methods were studied for the evaluation of release profiles of carbon dioxide, CO_2, from effervescent suppositories. Three lots of commercial suppositories containing sodium bicarbonate and anhydrous sodium dihydrogen phosphate were used. The volume of CO_2 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 CO_2 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 CO_2 from the effervescent suppositories. However, this method is not practical for quality control of formulations because of the incomplete release of CO_2; 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 CO_2 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, $NaHCO_3$, and anhydrous sodium dihydrogen phosphate, NaH_2PO_4, 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, CO_2, is released from the suppositories by the neutralization of $NaHCO_3$ and NaH_2PO_4. The released CO_2 stimulates the peristalsis of the large intestine, causing smooth defecation.[2)]

CO_2 has heretofore been widely used in gastric X-ray examination together with barium sulfate because its intestinal absorption is better than air. Since CO_2 does not produce a sense of abdominal fullness, it has been similarly utilized in double contrast studies in the large intestine.[3)] Thus, CO_2 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 CO_2 those drugs produce, making it important to examine the *in vitro* release profiles of CO_2 from the effervescent suppositories. Several analyses of $NaHCO_3$ 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 CO_2 from such suppositories.

Two methods of determining CO_2 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 NaH_2PO_4 (JSPI 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 $NaHCO_3$ and 680 mg of NaH_2PO_4 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-53, 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 penetrometer[6)] (model PM-3, Erweka Co.) at 37 °C to evaluate softening points.

Method 1 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 NaH_2PO_4. To prepare normal saline containing saturated CO_2, CO_2 from $NaHCO_3$, NaH_2PO_4 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 CO_2 (6 ml) as a medium was poured into a test tube (18 mm i.d. × 200 mm), which was immersed in a thermostatic bath maintained at 37 °C. A weighed

TABLE I. Physicochemical Properties of Suppositories

Lot No.	Weight (g)	Melting point[a)] (°C)	Dropping point (°C)	Penetration time (min)
A41	2.60±0.01	34.8±0.05	36.7±0.23	4.8±0.20
B42	2.63±0.03	34.9±0.00	36.6±0.06	4.8±0.00
C43	2.63±0.02	34.4±0.00	36.3±0.15	4.9±0.12

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 cock of the by-pass was immediately closed. The accumulated volume of CO_2 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 CO_2 in this method, prior to the measurement with the suppositories, gelatin capsules containing various amounts of a $NaHCO_3$–NaH_2PO_4 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 CO_2 released from those capsules was similarly measured with the gas burette.

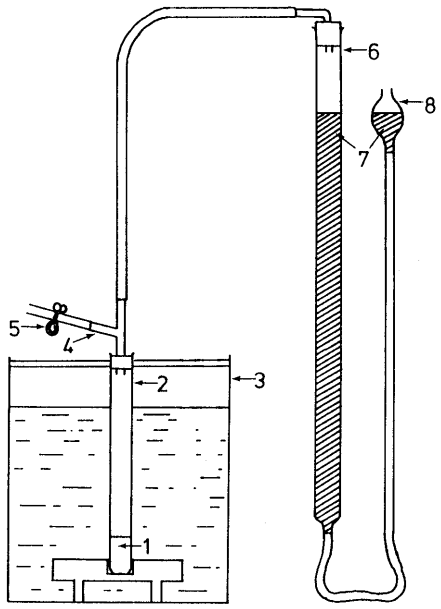

Fig. 1. Schematic Illustration of Apparatus Employed in Method 1 for Release Test

1, medium; 2, test tube; 3, thermostatic bath; 4, by-pass; 5, pinch cock; 6, gas burette; 7, light liquid paraffin; 8, leveling bulb.

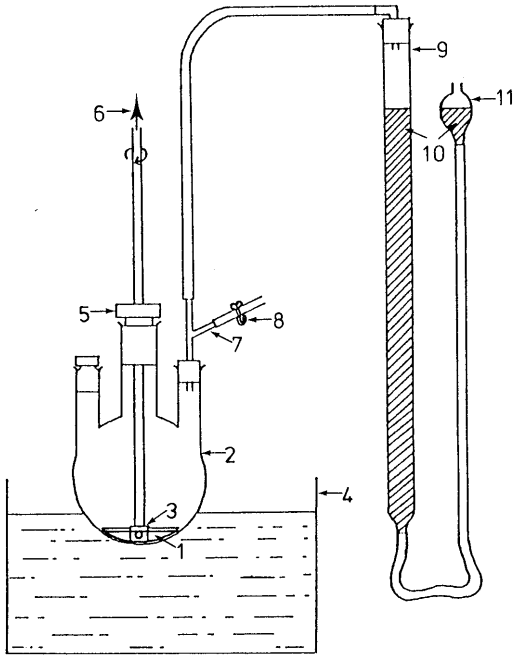

Fig. 2. Schematic Illustration of Apparatus Employed in Method 2 for Release Test

1, medium; 2, three-necked round-bottomed flask; 3, Teflon® mixing wing; 4, thermostatic bath; 5, Teflon® seal; 6, stirrer; 7, by-pass; 8, pinch cock; 9, gas burette; 10, light liquid paraffin; 11, leveling bulb.

Method 2 for Release Test Figure 2 shows the apparatus employed in method 2. CO_2 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 CO_2 released was measured by the method described above.

The maximum volume of CO_2 released from the same gelatin capsules was similarly measured by method 2 prior to the measurement with the suppositories, and the calibration curve for CO_2 in method 2 was also drawn.

Calculation The accumulated volume of CO_2 released from the suppository was determined every 2 min for 60 min at room temperature. Each observed accumulated volume of CO_2 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.

$$V = V_{obs} \times \frac{2.6}{W} \times \frac{273.15+25}{273.15+T} \quad (1)$$

Where V is the normalized accumulated volume of CO_2, V_{obs} is the observed accumulated volume of CO_2, W is the weight of the suppository, and T is the observed room temperature.

Results and Discussion

pH of Normal Saline Containing Saturated CO_2 Normal saline with or without 1% polysorbate 80 (100 ml) was boiled for a few minutes, then cooled to room temperature and CO_2 was bubbled into each solution for 30 min. Figure 3 shows the relationships between the pH of those solutions and the bubbling time of CO_2. The pH of both solutions decreased with the bubbling time of CO_2 and reached a constant value after 10 min. The pH of the normal saline without polysorbate 80 was in good agreement with the literature value[7] in the case of water. Therefore, CO_2 was considered to be completely saturated in 100 ml of noramal saline by the 30 min bubbling. CO_2 was then no longer soluble in the medium, so that all of the CO_2 released from the suppositories was detected with the gas burette. On the other hand, the solubility of CO_2 in light liquid paraffin was negligible during the release test.

Release of CO_2 from Gelatin Capsules As shown in Eq. 2, CO_2 is generated by the neutralization of $NaHCO_3$ and NaH_2PO_4.

$$NaHCO_3 + NaH_2PO_4 \rightarrow CO_2 + H_2O + Na_2HPO_4 \quad (2)$$

A suppository contains 500 mg (5.95 mmol) of $NaHCO_3$ and 680 mg (5.67 mmol) of NaH_2PO_4, so that 5.67 mmol of CO_2 is released from the suppository by the complete neutralization. From the equation for the gas state shown in Eq. 3, the volume of CO_2 released from a suppository

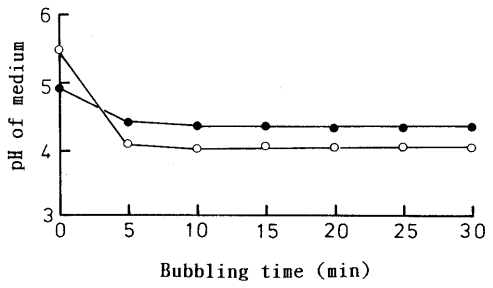

Fig. 3. Relationship between pH of Medium and Bubbling Time of CO_2

Polysorbate 80: (○) without, (●) with.

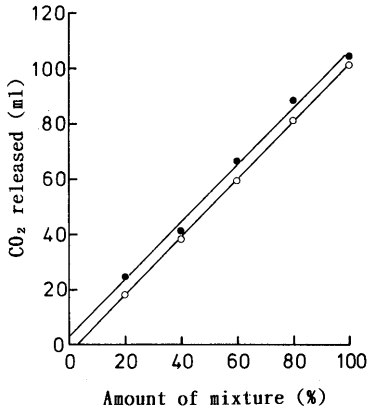

Fig. 4. Relationship between CO_2 Released from Gelatin Capsules and Percentage of Labeled Amount of Mixture

(○) method 1; (●) method 2.

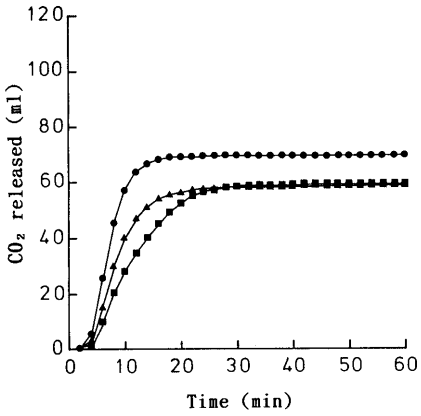

Fig. 5. Release Profile of CO_2 from Three Lots of Suppositories Determined by Method 1

Lot No.: (●) A41, (■) B42, (▲) C43.

is theoretically 139 ml at 25 °C at a pressure of 1 atm.

$$V = nRT/P \quad (3)$$

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 $NaHCO_3$ and NaH_2PO_4 were employed in this study. Figure 4 shows the relationships between the maximum volume of CO_2 released from those capsules and the amounts of the mixture. In Fig. 4, 100% of the mixture corresponds to 0.5 g of $NaHCO_3$ and 0.68 g of NaH_2PO_4. The difference in the release amount of CO_2 observed between the calibration curves results from the presence or absence of polysorbate 80. Therefore, polysorbate 80 is considered to enhance the reaction of $NaHCO_3$ with NaH_2PO_4. The recoveries (*vs.* the theoretical value) of CO_2 in methods 1 and 2 calculated from those plots were 72.5 and 76.0%, respectively. These results suggest that the concentrations of $NaHCO_3$ and NaH_2PO_4 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 $NaHCO_3$ or NaH_2PO_4 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 CO_2 from the effervescent suppositories.

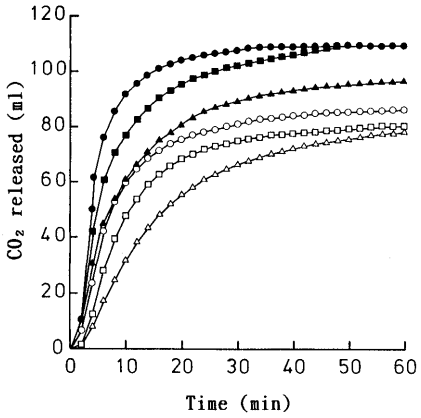

Fig. 6. Effect of Medium Volume and Polysorbate 80 on Release Profile of CO_2 from Suppository (Lot No. C43) Determined by Method 2

Medium volume without polysorbate 80: (○) 10 ml, (□) 20 ml, (△) 30 ml. Medium volume with polysorbate 80: (●) 10 ml, (■) 20 ml, (▲) 30 ml.

Release of CO_2 from Suppositories (Method 1) Figure 5 shows the release profiles of CO_2 from three lots of the suppositories determined by method 1. CO_2 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 CO_2 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 CO_2 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 $NaHCO_3$ and NaH_2PO_4 were covered with the melted oleaginous bases. Therefore, these findings suggest that the release of CO_2 determined by this method is considerably influenced by preparation conditions, storage temperature,[8] 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 CO_2 from the suppositories.

Effects of Medium Volume and Polysorbate 80 on Release of CO_2 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 CO_2 at a constant stirring rate (82 rpm). The release rate of CO_2 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 CO_2 released increased with the addition of 1% polysorbate 80. CO_2 was gradually and completely released from the suppository in 20 ml of normal saline

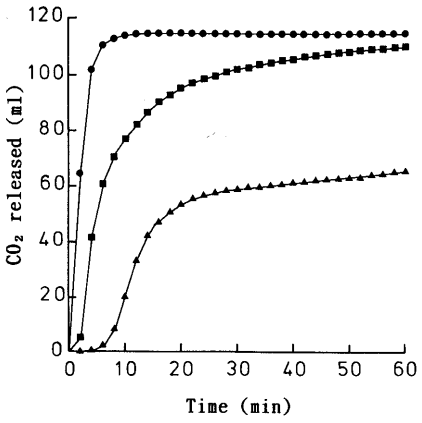

Fig. 7. Effect of Stirring Rate on Release Profile of CO_2 from Suppository (Lot No. C43) Determined by Method 2

Stirring rate: (●) 310 rpm, (■) 82 rpm, (▲) 0 rpm.

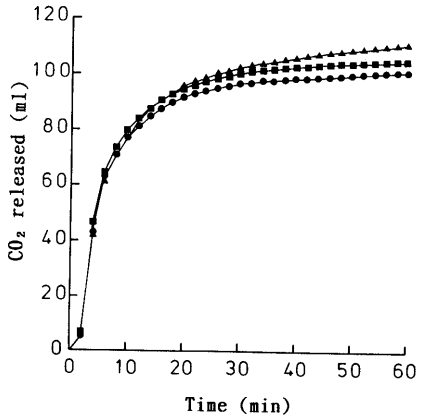

Fig. 8. Release Profile of CO_2 from Three Lots of Suppositories Determined by Method 2

Lot No.: (●) A41, (■) B42, (▲) C43.

containing 1% polysorbate 80, making this medium the most appropriate for method 2.

Effect of Stirring Rate on Release of CO_2 from Suppositories (Method 2) Figure 7 shows the effect of the stirring rate on the release profile of CO_2 from the suppository (lot No. C43). The release rate and amount of CO_2 increased with faster stirring rate. At 310 rpm, the release rate of CO_2 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 CO_2 from Suppositories (Method 2) Figure 8 shows the release profiles of CO_2 from three lots of the suppositories determined by method 2. Unlike method 1, complete release of CO_2 was observed in the measurement by method 2. The released amount of CO_2 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 CO_2 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 CO_2 determined by method 1 (Fig. 5) and the dropping points. With no

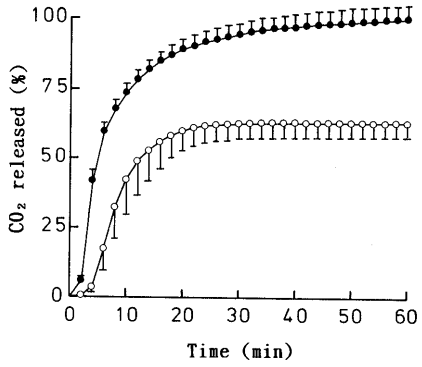

Fig. 9. Release Profile of CO_2 from Suppository Determined by Two Different Methods

(○) method 1, (●) method 2. Each value represents the mean ± S.D. ($n=9$).

stirring of the test fluid (method 1), the amount of CO_2 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 CO_2 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 CO_2, each volume of CO_2 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 CO_2 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 CO_2 from the effervescent suppositories. The native release of CO_2 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 CO_2 is incomplete. We found that method 2 was useful for the quality control of the suppositories because of its complete CO_2 release and comparatively low standard deviations. These findings suggest that methods 1 and 2 to determine release of CO_2 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."
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