Evaluation of Aminophylline Suppositories Prepared in a Hospital Pharmacy

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Physical and chemical stability of suppositories containing aminophylline and various bases prepared in our hospital pharmacy was investigated. Ethylenediamine in aminophylline decreased, the melting points of the suppositories rose, and the disintegration and liquefaction times were prolonged in suppositories with Witepsol base when stored at room temperature.

Thin-layer chromatography produced evidence supporting the assumption that ethylenediamine may react with some Witepsol constituents to form an acid amidc linkage. However, the suppositories were stable at lower temperatures. Suppositories prepared with PEG base were found to be satisfactorily stable at room temperature.

(Key Words: Aminophylline Suppositories, Witepsol Base, PEG Base, Physical and Chemical stability)

INTRODUCTION

Aminophylline or theophylline-ethylenediamine with a strong bronchiectatic effect and relatively infrequent side effects has been extensively used as therapeutic agent for asthma. Suppositories of aminophylline are being applied more frequently in place of injections and oral administration. In our hospital suppositories containing 100mg of aminophylline are prepared using an equivalent mixture of Witepsol H-15 and E-75 (hereafter abbreviated as H-15 + E-75 base) and are used clinically. However, since ethylenediamine in aminophylline is sensitive to heat, light, and air, special attention should be paid to temperature, time and other factors in the preparation of such suppositories. Many reports (1, 2, 6, 7) have been published on the stability of aminophylline in various bases in Europe and the USA. In this work we investigated the stability of aminophylline at the time of preparation of suppositories and during a definite time of preservation in order to ensure the quality of suppository preparations made in the hospital pharmacy.

MATERIALS AND METHODS

Suppositories containing 100mg of aminophylline were prepared with various bases. They were examined concerning alternation of their external appearance, changes in quantity and disintegration of their constituents,

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and their physical properties (1) when prepared, (2) after preservation at room temperature (24±2°C), and (3) after preservation in a cold place (5-10°C). (These conditions for preservation were used throughout the experiment unless otherwise specified.) In addition, suppositories containing bases only were prepared in the same manner and were examined concerning their physical properties under the above three conditions.

1. Preparation of suppositories

The suppositories used in the experiment were prepared as described in Table 1.

Table 1 Formula of aminophylline suppositories

| Formula 1. | Aminophylline 74.0g | Witepsol H-15 1,000.0g | for 740 suppositories |
| Formula 2. | Aminophylline 74.0g | Witepsol E-75 1,000.0g | for 740 suppositories |
| Formula 3. | Aminophylline 74.0g | Witepsol W-35 1,000.0g | for 740 suppositories |
| Formula 4. | Aminophylline 74.0g | Witepsol H-15 500.0g | for 740 suppositories |
| Formula 5. | Aminophylline 100.0g | P.E.G. 6000 850.0g | for 1,000 suppositories |
| | | P.E.G. 1540 510.0g | |
| | | Water 260.0ml | |

1. Aminophylline suppositories with Witepsol base (Table 1, Formula 1-4)

Various bases were melted at 70°C and left to be cooled. When they were cooled to 45°C, they were mixed with aminophylline previously passed through a 100-mesh sieve in a mortar, transferred to a suppository plunger, kept at 32-36°C and squeezed into 1.5ml containers. Thereafter, the upper part of the container was covered with cellophane and subsequently sealed with vinyl tape. The preparation time from the start of base melting to completion of packing was about 2 hours and 20 minutes (about an hour and 15 minutes after mixing with aminophylline). Suppositories containing bases only were prepared separately using the same procedure under the same conditions (Table 1, formula 1-4; however, the active agent was excluded).
2. Aminophylline suppositories with P.E.G. base (Table 1, Formula 5)

P.E.G. 6000 and P.E.G. 1540 were melted at 80°C. Aminophylline was dissolved separately in distilled water at 80°C. Both solutions were mixed at 70°C. The mixed solution was cooled under stirring. When it was cooled to 55°C, it was transferred to a suppository plunger and squeezed into containers under stirring at 47°C. The upper part of the container was covered with cellophane, and subsequently sealed and packed with vinyl tape. It took 3 hours from start of base melting to completion of packing (2 hours and 25 minutes after mixing with aminophylline). In addition, a suppository containing P.E.G. base only was prepared by the same procedure under the same conditions (Table 1, Formula 5). The suppository plunger used was Elwaker AR 400 and the containers used had a net volume of 1.5 ml and were made of white opaque P.V.C. by Nichii Packing Co., Ltd.

II. Observation of external appearance

Coloration and crystallization were observed macroscopically.

III. Examination of contents

1. Quantification of theophylline and ethylenediamine in the suppositories

Five suppositories were weighed accurately, melted at a temperature as low as possible, solidified under stirring at room temperature, and used as a sample. For quantification of ethylenediamine in aminophylline, an amount of sample equivalent to 0.25 g of aminophylline was weighed out accurately, 20 ml of distilled water was added and the sample was dissolved by heating. Subsequently, the resulting solution was processed according to BP 1973 and the content of ethylenediamine was estimated from the amount of 0.1 N sulfuric acid consumed. For quantification of theophylline, an amount of sample equivalent to 0.2 g of aminophylline was weighed out accurately 50 ml of distilled water and 8 ml of ammonium reagent were added, and the mixture was processed according to the Japanese Pharmacopoeia 9: Quantification of theophylline in aminophylline powder. The content of theophylline was estimated from the amount of 0.1 N ammonium thiocyanate consumed. The recovery rate of aminophylline obtained by the quantification method varied from 99.2 to 100.1% (n = 8 ~ 12) for all bases. Therefore, the estimated values were adopted without being corrected.

2. Examination of the uniformity of contents in the preparation

To examine the uniformity of contents throughout the preparation, 15 suppositories were selected at the start, in the middle, and at the completion of filling of the container by a plunger. Five suppositories were combined and used as an assay sample. Therefore, three samples were assayed for theophylline and ethylenediamine content at each stage of preparation.

3. Change of contents with time

Samples of five suppositories each were kept in a cold place or at room temperature and assayed for theophylline and ethylenediamine content at four indicated times.
The examination of disintegration products by thin-layer chromatography

One suppository was dissolved in 50 ml of chloroform and used as a sample. The sample was spotted on a thin-layer plate (adsorbent: Silica gel G, Wako gel B-5F, 0.25μm in thickness) in a volume of 15μl. The plate was developed with a mixture of chloroform and methanol (4:1, 19.5:0.5) and a mixture of ethanol and acetone (4:1) and subsequently examined with UV lamps, I2, and ninhydrin reagent.

V. Physical properties of suppositories

a) Melting point:

The melting point was determined by Method II for measurement of melting points in the Japanese Pharmacopoeia 9: General Methods of Examination.

b) Disintegration time:

The time required for a suppository to disintegrate and to become like paste or a solution was determined by the method of Fuwa et al. (5) in which an apparatus for the disintegration test prescribed in the Japanese Pharmacopoeia 9 and water were used to produce reciprocating motion at a frequency of 29-30 per minute and up-and-down motion with amplitude of 50 mm at 37 ± 0.5°C.

c) Liquefaction time:

The test tube method of Miyazaki et al. (8) was used. A suppository was let stand at 37 ± 0.1°C to determine the liquefaction time.

d) Solidity:

A suppository was cut to a thickness of 0.8 ± 0.1 cm in the center and its solidity was determined by a tablet solidity testing machine (Kiya-type) (4).

Four determinations were carried out in each case for a) to d).

EXPERIMENTAL RESULTS AND DISCUSSION

I. Physical properties of suppository bases

The physical and chemical properties of Witepsol bases are shown in Table 2. They all met the criteria specified in the Japanese Pharmacopoeia 9. Table 3 shows the physical properties of the suppositories consisting of bases at the time of preparation and after 12 months of preservation.
Table 3  Alternation of bases with the lapse of time

<table>
<thead>
<tr>
<th></th>
<th>At the time of preparation</th>
<th>Preserved for 12 months</th>
<th>at low temperature (5-10°)</th>
<th>at room temperature (24 ± 2°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>34.5</td>
<td>35.2</td>
<td>37.3</td>
<td>35.4</td>
</tr>
<tr>
<td>Disintegration time (min)</td>
<td>11</td>
<td>17</td>
<td>11.11</td>
<td>18</td>
</tr>
<tr>
<td>Liquefaction time (min)</td>
<td>12</td>
<td>11</td>
<td>16.07</td>
<td>16</td>
</tr>
<tr>
<td>Solidity (kg)</td>
<td>3.8</td>
<td>4.8</td>
<td>5.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

a) Changed after one month.

b) Changed after 2 months

c) Measured at 40 ± 0.5°C

*( ) : The melting point in parentheses was determined after preservation in a capillary.

**( ) : Base consisting of H-15 and E-75 in equal volumes.
The disintegration time of W-35 base was prolonged after the one month of preservation and its liquefaction time was also prolonged after the second month of preservation. In the case of the H-15 + E-75 base, the disintegration and liquefaction times were prolonged after 12 months of preservation. The prolongation was clearly explained by the rise in the melting point. In addition, the melting point rose after preservation at room temperature, but it dropped again after melting, suggesting that multiform crystals might exist in small quantities and might be transformed into a stable form with a higher melting point during preservation at room temperature. In particular, the W-35 base was more marked in this trend than the E-75 and H-15 bases because it contains 5-10% monoglyceride.

II. Observation of external appearance and theophylline and ethylenediamine contents

Progressive coloration occurred after one month of preservation at room temperature. Coloration was most marked in the suppositories prepared with W-35 base, followed by those prepared with E-75, H-15 + E-75, H-15, and P.E.G. bases in that order. They were colored a dark yellow to light yellow. Crystallization was not observed on the surface of the bases. The results of the test of uniformity of contents at the time of preparation are shown in Fig. 1. The mean of three determinations obtained at the start, in the middle, and at the completion of squeezing into containers is expressed as a percentage of that for the original powder. Both theophylline and ethylenediamine contents were uniform with an average deviation smaller
than 5% for all bases. However, ethylenediamine content was approximately 10% lower than theophylline content for W-35 and E-75 bases, suggesting that those bases may disintegrate ethylenediamine to a higher degree than H-15, H-15 + E-75, and P.E.G. bases. Special attention should be paid to this point in the preparation of suppositories.

Alternation of theophylline and ethylenediamine contents is shown as a function of time for 12 months of preservation in Fig. 2. The theophylline and ethylenediamine contents at the time of preparation are expressed as 100%. The theophylline content showed practically no variation for any of the bases for 12 months of preservation at room temperature or in a cold place. In contrast, the ethylenediamine content decreased gradually in Witepsol base when preserved at room temperature, and was nearly 0% after 12 months. However, when preserved in a cold place, the decrease in ethylenediamine content remained about 10% for all bases after 6 months and reached 13% in H-15, 23% in H-15 + E-75, 34% in E-75, and 24% in W-35 after 12 months. The ethylenediamine content in P.E.G. base did not

![Graph showing theophylline and ethylenediamine contents over time]

<table>
<thead>
<tr>
<th>12 months of preparation</th>
<th>Cold room (°C)</th>
<th>Room temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-15</td>
<td>99.1</td>
<td>101.6</td>
</tr>
<tr>
<td>W-35</td>
<td>100.8</td>
<td>99.3</td>
</tr>
<tr>
<td>E-75</td>
<td>102.2</td>
<td>99.9</td>
</tr>
<tr>
<td>H + E</td>
<td>100.6</td>
<td>101.0</td>
</tr>
<tr>
<td>P.E.G.</td>
<td>99.0</td>
<td>100.5</td>
</tr>
</tbody>
</table>

Fig. 2: Ethylenediamine and theophylline contents of aminophylline suppositories
- ○ H-15 base ▲ W-35 base □ E-75 base ● H + E base × P.E.G. base
- Preserved at room temperature (24 ± 2°C)
- Preserved in a cold place (5 – 10°C)
vary at all when preserved in a cold place, and remained as high as 90% and 83% even when preserved at room temperature for 6 and 12 months, respectively. Coloration of suppositories appeared to be related to the decrease in ethylenediamine content: coloration was stronger in suppositories with a greater decrease in ethylenediamine content.

III. Examination of disintegration products by thin-layer chromatography

As shown in Fig. 3, two spots were demonstrated at different places from those of Witepsol base and aminophylline, indicating the presence of two disintegration products (hereafter called substance ① and substance ②, respectively). Substance ① was detected in W-35 and E-75 at the time of preparation, and appeared in H-15+E-75 base after a month and, although in very small quantities, in H-15 base after 4 months when they were preserved at room temperature or in a cold place. Substance ② was undetectable in any of the bases when preserved in a cold place. However,
when preserved at room temperature, it was detected in W-35 base after 2 months, in H-15 + E-75 and E-75 after 4 months, and in H-15 after 12 months. To examine substance \(^1\), the base was reacted with ethylenediamine for 2 hours at 90°C, isolated by T.L.C., and subjected to IR spectrum analysis and melting point determination. Witepsol is a glyceride obtained by esterification of OH bases of saturated C\(_{12}-C_{18}\) fatty acids. After the reaction, however, ester peaks of \(v_C=0\), 1740 cm\(^{-1}\) and \(v_C=0\), 1165 cm\(^{-1}\) disappeared, but amide absorption I of 1630 cm\(^{-1}\), amide absorption II of 1560 cm\(^{-1}\) and an absorption band of \(v_{\text{NH}}\) at 3300 cm\(^{-1}\) appeared in the IR spectral chart (Fig. 8). This suggests that substance \(^1\) may be an acid-amide body produced by reaction of ethylenediamine with components of the base. Reaction of P.E.G. base with aminophylline at 90°C for 7 hours produced a small amount of substance \(^3\) although it was not detected in fresh and old suppositories. Substances \(^2\) and \(^3\) could not be isolated because they were produced in very small quantities.

IV. Physical properties of suppositories

The solidity, melting point, disintegration time, and liquefaction time of aminophylline suppositories are shown as a function of preservation time in Figs. 4-7. Addition of aminophylline did not lower the melting point of any base. The solidity of a suppository was widely distributed with no special trends in relation to preservation time. The melting point, disintegration time and liquefaction time did not vary in any of the bases when they were preserved in a cold place. When preserved at room temperature, the melting point and disintegration time remained unchanged in P.E.G. base, but the disintegration and liquefaction times were markedly prolonged after 2 to 4 months in W-35, E-75, and H-15 + E-75 bases.

The melting point of the suppository prepared with Witepsol base rose gradually and reached a markedly high level after 12 months when the suppository was preserved at room temperature. The rise of melting point appears to be related to crystal polymorphism of the base itself. However, the melting point rose sharply after 12 months when the ethylenediamine content decreased to nearly 0%, suggesting that this phenomenon might be primarily due to formation of acid-amide bodies with a high melting point (108-112°C). In addition, aminophylline suppositories were prepared with Witepsol H-15:E-75 (50:50) base with 5% hydroxyamine hydrochloride added as a stabilizer as recommended by Kasem et al. (7) and with Witepsol H-15:W-35 (80:20) which was reported to be very stable with respect to disintegration time by Gyarmati Laszlo et al. (6), and were used for comparison without obtaining favorable results.

The above results indicate that aminophylline suppositories prepared with P.E.G. base are physicochemically the most stable. However, since their absorption by the rectum in vivo has not been examined, it is unreasonable to place one suppository above the others based only on the physicochemical data. Therefore, aminophylline suppositories prepared with Witepsol base consisting of equal amounts of H-15 and E-75 are stored in a cold place and used since this base is routinely available in our hospital and is relatively stable for at least 6 months at low temperatures.
Fig. 4 Solidity of aminophylline suppositories
○ H-15 base ▲ W-35 base □ E-75 base ● H+E base
× P.E.G. base
— Preserved at room temperature
--- Preserved in a cold place

Fig. 5 Melting point of aminophylline suppositories
○ H-15 base ▲ W-35 base □ E-75 base ● H+E base
× P.E.G. base
— Preserved at room temperature
--- Preserved in a cold place
Fig. 6 Disintegration time of aminophylline suppositories
○ H-15 base ▲ W-35 base □ E-75 base ● H + E base
× P.E.G. base
- Preserved at room temperature
--- Preserved in a cold place

Fig. 7 Liquefaction time of aminophylline suppositories
○ H-15 base ▲ W-35 base □ E-75 base ● H + E base
× P.E.G. base
- Preserved at room temperature
--- Preserved in a cold place
Measured at 40 ± 0.1° in E-75
Fig. 8 IR spectral chart.
REFERENCES


3) Dynamit Nobel Chemicals Index, p6.


