For nitroglycerin (NTG) sublingual route of administration enables rapid absorption and fast pharmacological effect. However, chemical instability of NTG is a technological problem, both in solid and liquid formulations. Moreover, the substance is volatile, explosive and absorbs into plastic containers. Many nonvolatile excipients are used as carriers for NTG to improve its stability, for example povidone, glyceryl monostearate, propylene glycol or microcrystalline cellulose. Stabilization of NTG can be also achieved by formation of an inclusion complex with cyclodextrins (1-3).

Cyclodextrins have been extensively studied in aim to improve the solubility, stability and bioavailability of a number of drug compounds (4-8). Fifty years after CD became available for pharmaceutical applications about 30ñ40 different products present on the worldwide market contain drug-CD complexes and among them some are intended for sublingual application (9). On the European market sublingual tablets with nicotine complexed with \( \beta \)-cyclodextrin (Nicorette MicrotabÆ) are available, which offer fast absorption, without unpleasant taste and local mucose irritation (8). These products are not available in Europe.

The aim of this study was to develop sublingual tablets with NTG-CD and to compare stability of NTG in these tablets with tablets containing NTG titrated with crosspovidone (NTG-CP).

EXPERIMENTAL

Materials
Nitroglycerin (NTG) titration with cross-linked polyvinylpyrrolidone (NTG-CP) containing 25% (w/w) of NTG and \( \beta \)-cyclodextrin inclusion complex with NTG (NTG-CD) containing 2.0% (w/w) of NTG – manufactured by Dynamit Nobel Special Chemistry (Leverkusen, Germany); co-processed mixture of maize starch with lactose 15:85 – StarLac (Meggle, Wusserburg, Germany); partially pregelatinized maize starch – Starch 1500 (Colorcon, Dartford, England); sodium starch glycolate – Ultraamylopectin, Vivastar (J. Rottenmaier, Rosenberg, Germany); croscarmellose sodium – Ac-Di-Sol (FMC BioPolymer, Philadelphia, USA), lactose – Tabletose 80 (Meggle, Wusserburg, Germany); \( \beta \)-cyclodextrin (\( \beta \)-CD) from Fluka Chemie (Neu-Ulm, Switzerland); cross-linked polyvinylpyrrolidone (CP) – Kollidon CL (BASF, Ludwigshafen, Germany).
Tablets preparation and characterization

Tablets (total mass 100 mg) were prepared by direct compression of the powders (Table 1) using 6 mm diameter flat punches (tablet press EKO, Korsch, Berlin, Germany).

The NTG-CD and NTG-CP content in tablets was 25 mg and 2 mg, respectively, what corresponds to 0.5 mg of NTG. Placebo tablets were prepared with the same procedure using β-CD and CP.

Tablet hardness was measured with a hardness tester (Erweka TBH 20, Heusenstamm, Germany). Disintegration time \textit{in vitro} was determined in a Ph. Eur. apparatus (Pharma Test Haimburg, Germany) using water at 37 ± 0.5°C and discs.

In the preliminary studies disintegration time \textit{in vivo} was evaluated. Placebo tablets were given to 12 volunteers who estimated disintegration time of the tablet in the mouth. Additionally the organoleptic properties, namely sand sensation and taste, for each formulation were defined.

Stability studies

Tablets with NTG-CD and NTG-CP were placed in amber glass vials (National Scientific, Switzerland) with PTFE lined full caps tightly closed and protected with Parafilm (American National Can, USA). Five tablets were stored in each vial. The vials were stored at 25°C/60% RH and 40°C/75% RH (Binder storage cabinet, Tuttingen, Germany). NTG assay was performed after 1, 6 and 12 months for long term stability condition and after 1, 3 and 6 months for accelerated condition.

Analysis of NTG content

NTG extraction from tablets

Extraction method described in USP 30 for nitroglycerin tablet in the section “uniformity of dosage units” indicates 50% methanol as an extraction solvent, however, the procedure was not appropriate for sublingual tablets with NTG-CD since CD is only slightly soluble in 50% methanol (2) and the tablets with NTG-CD did not disintegrate entirely in this medium. Thus disintegration in water was performed before methanol was added.

Powdered tablets were placed in Chromacol vials (Trumbull, USA), 2.5 mL of water was added, and the suspension was sonicated for 5 min. Then, 2.5 mL of methanol was added, samples were agitated in Vortex and placed in an ultrasound bath for another 5 min. The resulting suspension was centrifuged (2000 × g, 15 min), the supernatant was filtered (Rundifilter MN 615, Macherey-Nagel, Germany) and diluted 1:1 with the mobile HPLC phase. The recovery of NTG was in the range 96-103%. Samples were prepared in triplicate.

HPLC analysis

La Chrom chromatograph (Merck – Hitachi, Tokyo, Japan) equipped with UV detector was used. Determinations were carried out on a column 125 × 4 mm in size, packed with C18 (5 µm) stationary phase (LiChrospher, Merck, Darmstadt, Germany). The mobile phase was 50% (v/v) methanol with flow rate 1 mL/min and the detection was performed at 210 nm (10). The retention time (t_R) for NTG was 6.2 min. Concentrations of nitroglycerin were calculated using calibration curves. HPLC conditions allow for determination of the acid hydrolysis products: 2,3-dihydroxypropyl nitrate and 2-hydroxy-1-(hydroxy-methyl)ethyl nitrate (t_R = 2.2 min) as well as 3-hydroxypropane-1,2-diyl dinitrate (t_R = 2.4 min) and 2-hydroxypropane-1,3-diyl dinitrate (t_R = 2.8 min) (10).

RESULTS AND DISCUSSION

Sublingual tablets – preparation and evaluation

The preliminary studies were performed for placebo tablets.

Short disintegration time, acceptable taste and mouth sensation were considered as critical parame-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Formulation & CD/A & CP/A & CD/B & CP/B \\
\hline
NTG-CD & 25.0 & - & 25 & - \\
\hline
NTG-CP & - & 2.0 & - & 2.0 \\
\hline
StarLac & 74.0 & 97.0 & - & - \\
\hline
Starch 1500 & - & - & 10.0 & 10.0 \\
\hline
Tabletose 80 & - & - & 64.0 & 87.0 \\
\hline
Magnesium stearate & 1.0 & 1.0 & 1.0 & 1.0 \\
\hline
\end{tabular}
\caption{Composition of nitroglycerin (NTG) sublingual tablets [mg/tablet].}
\end{table}
ters for sublingual tablets. Fast disintegration and drug release is the basic requirement for sublingual NTG tablets which are used with the aim of immediate intervention in the angina pectoris attack. Tablets with β-CD (25% w/w) or CP (2% w/w) were prepared. Four different disintegrants were used (% w/w): Ultramoylepectin (5% – 15%), Ac-Di-Sol (2% – 8%), Starch 1500 (2% and 10%) and StarLac (74% and 97%). Only tablets prepared with Starch 1500 or StarLac exhibited both small sand sensation and appropriate in vitro disintegration time. β-CD, despite of the large content in tablet, in comparison to CP, did not affect disintegration time of tablets prepared with Starch 1500 (44 – 42 s), but in the case of tablets prepared with StarLac in vitro disintegration time was longer than observed for tablets with CP (34 s vs. 24 s) and the difference was statistically significant. This did not correlate to hardness which was in the range 30-40 N for both types of tablets.

Placebo tablets were given to 12 volunteers sublingually and in vivo disintegration time was measured. Tablets prepared with StarLac and CD or CP disintegrated within 32 ± 17 s and 30 ± 15 s, respectively. Disintegration of tablets prepared with Starch 1500 was slower, but still the tablets disintegrated in less than 2 min, namely the time 91 ± 13 s and 109 ± 28 s was observed for CD and CP containing tablets, respectively. Similarly like in vitro, the in vivo results demonstrate that CD does not have significant effect on disintegration process.

Generally, placebo prepared with StarLac was preferred by subjects due to shorter disintegration time, moderately sweet taste and smaller sand sensation.

Table 1 presents composition of the investigated tablets prepared with NTG-CD complex or NTG-CP titration. The NTG content in each tablet was 0.5 mg what corresponded to 25 mg and 2 mg of NTG-CD and NTG-CP per tablet, respectively. The hardness in the range 30-40 N was achieved by applying compression force between 3 and 9 kN, depending on formulation (CD/A < CP/A < CD/B < CP/B). For all formulations but CD/A in vitro disintegration time was less than 2 min (Table 2). Longer disintegration time observed for CD/A tablets than for respective placebo tablets may result from the presence of NTG. Irrespective of the disintegrant used the disintegration time in vitro was longer and less reproducible for tablets containing NTG-CD than for those with NTG-CP, although this effect was not observed in placebo tablets prepared with CD, as indicated above.

Stability of NTG in sublingual tablets

The results of measurements of disintegration time and hardness of the tablets stored for 6 or 12 months are shown in Table 2. Tablet hardness after 12 months was unchanged at 25°C, but after 6 months at 40°C it was noticeably lower. In vitro disintegration time was reduced in formulations prepared with StarLac and NTG-CD (CD/A) and with Starch 1500 and NTG-CP (CP/B), with the change more remarkable at 40°C. However, no clear correlation between changes in hardness and disintegration time was observed. Under both storage conditions tablet mass remained unchanged.

Chemical stability of NTG in tablets was examined. As presented in Fig. 1a, in accelerated storage test the loss of NTG content was significant. After 3 months, only one of the four formulations, namely CD/B did not show significant change of the initial content. After 6 months, however, the content of NTG in all investigated tablets was less than 80% of the initial dose, with only 48% in CP/B formulation. Results from the accelerated storage conditions are in good agreement with those obtained from the long term stability studies (Fig. 1b). After 12 months at 25°C the highest NTG content (88%) was determined in CD/B formulation, while from tablets CP/B only 68% of NTG was recovered.

The employed HPLC method allows detection of the degradation products as described in the experimental section. After 12 months at 25°C the area of the main peak from degradation products (2,3-dihydroxypropyl nitrate and 2-hydroxy-1-(hydroxy-methyl)ethyl nitrate) was 1.4 – 2.0% of the area of NTG peak, while after 6 months at 40°C the values were in the range 3.8 – 5.7%. However, no correlation with tablet composition was demonstrated.

The results indicate that tablets prepared with Starch 1500 exhibit better stability of NTG complexed with β-CD than those made with StarLac. On the other hand, when NTG titrated with CP was used, better stability was observed in tablets prepared with StarLac. Although combination of NTG-CD and Starch 1500 was the most favorable for both physical and chemical stability of tablets (formulation CD/B), but it must be concluded that additional protection of the tablets would be necessary to achieve at least 1 year stability with NTG loss less than 10%. Thus, CD complex alone does not guarantee the required stability of the tablets.

CONCLUSIONS

Sublingual tablets containing 0.5 mg of NTG were prepared using NTG-CP titration or NTG-CD
complex which comprised 2% (w/w) and 25% (w/w) of the tablet mass, respectively. The advantage of the use of larger amount of NTG-CD than NTG-CP is better uniformity of content (data not shown). High CD content did not affect in vitro disintegration time of tablets prepared with Starch 1500 but prolonged disintegration of tablets with StarLac. However, this effect was not observed for placebo tablets in vivo. In comparison to placebo, tablets containing NTG, both in a form of NTG-CD and NTG-CP, exhibited longer in vitro disintegration times, however, not exceeding 2 min. It was demonstrated that Starch 1500 is a better disintegrant than StarLac for sublingual tablets with NTG-CD and this was also confirmed during long time storage.

Regarding the assayed active substance, the best stability was observed for formulation containing NTG-CD complex and Starch 1500 as an excipient (CD/B formulation). In contrast, for NTG-CP better stability was achieved in tablets prepared with StarLac (CP/A formulation). In spite of the smaller loss of NTG-CD than NTG-CP in the stored tablets, the required stability of NTG, i.e. more than 90% of the initial content remained for at least 1 year, was not achieved. Developed NTG-CD sublingual tablets comply with the requirement of the active substance content not less than 85% during 1 year storage at controlled room temperature.

Table 2. Physical properties (x±sd, n=6) of NTG sublingual tablets in stability studies: at 40°C/60%RH for 6 months and at 25°C/75%RH for 12 months.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temp./time (months)</th>
<th>CD/A</th>
<th>CP/A</th>
<th>CD/B</th>
<th>CP/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass [mg]</td>
<td>t = 0</td>
<td>101.2 ± 1.5</td>
<td>98.4 ± 2.0</td>
<td>100.9 ± 1.4</td>
<td>99.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>25°C t = 12</td>
<td>101.1 ± 1.3</td>
<td>98.1 ± 1.1</td>
<td>102.0 ± 2.7</td>
<td>98.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>40°C t = 6</td>
<td>103.0 ± 2.6</td>
<td>99.0 ± 2.6</td>
<td>100.5 ± 3.7</td>
<td>100.0 ± 1.6</td>
</tr>
<tr>
<td>Hardness [N]</td>
<td>t = 0</td>
<td>40 ± 7</td>
<td>32 ± 0.6</td>
<td>41 ± 2</td>
<td>32 ± 5</td>
</tr>
<tr>
<td></td>
<td>25°C t = 12</td>
<td>37 ± 7</td>
<td>37 ± 2</td>
<td>44 ± 2</td>
<td>28 ± 4</td>
</tr>
<tr>
<td></td>
<td>40°C t = 6</td>
<td>23 ± 1</td>
<td>25 ± 2</td>
<td>24 ± 2</td>
<td>21 ± 0.6</td>
</tr>
<tr>
<td>Disintegration</td>
<td>t = 0</td>
<td>215 ± 65</td>
<td>43 ± 8</td>
<td>43 ± 31</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>time [s]</td>
<td>25°C t = 12</td>
<td>139 ± 42</td>
<td>43 ± 10</td>
<td>72 ± 22</td>
<td>23 ± 5</td>
</tr>
<tr>
<td></td>
<td>40°C t = 6</td>
<td>59 ± 30</td>
<td>31 ± 9</td>
<td>47 ± 15</td>
<td>8 ± 5</td>
</tr>
</tbody>
</table>

Figure 1. Stability of nitroglycerin [% of the initial dose] in two types (A and B) of sublingual tablets containing NTG complexed with β-cyclodextrin (CD) or titrated with cross-povidone (CP). Storage conditions: 40°C/75% RH (a) and 25°C/60% RH (b). The average values (n = 6) are presented.
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tablets, depending whether NTG-CP or NTG-CD was used.

REFERENCES


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