Investigation of Discoloration of Furosemide Tablets in a Light-Shielded Environment

Shinji Katsura, Nobuo Yamada, Atsushi Nakashima, Sumihiro Shiraishi, Mihoko Gunji, Takayuki Furuishi, Tomohiro Endo, Haruhisa Ueda, and Etsuo Yonemochi

Abstract

We observed that uncoated furosemide tablets turned yellow in a light-shielded automatic packaging machine and discoloration of the furosemide tablets was heterogeneity and occurred on the surface of the tablets only. The machine was equipped with an internal blower to maintain a constant temperature. Therefore, we investigated the effect of air flow on the discoloration of the furosemide tablets using a blower in a dark environment. The color difference (ΔE) of the furosemide tablets increased linearly as the blowing time increased. We performed structural analysis of the yellow compound in the furosemide tablets by LC-MS and identified the compound as a hydrolysate of furosemide. This suggested that furosemide hydrolysis was accelerated by the air flow. The furosemide tablets were prepared with the most stable furosemide polymorph, form I. X-Ray powder diffractometry and IR spectroscopy showed that during tablet preparation, no crystal transition occurred to an unstable furosemide polymorph. Furthermore, IR spectroscopy showed that the crystal form of furosemide in the yellow portion of the tablets was form I. To elucidate the factors producing the discoloration, we investigated the effect of humidity and atmosphere (air, oxygen, and nitrogen) on the discoloration of the furosemide tablets. The results suggested that the discoloration of the furosemide tablets was accelerated by oxidation, although humidity did not affect the hydrolysis. Therefore, we concluded that the discoloration of the furosemide tablets in the automatic packing machine was caused by acceleration of oxidative degradation by air flow.

Key words furosemide; tablet; light-shielded environment; discoloration; hydrolysis; oxidation

Furosemide (4-chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid) is widely used as a diuretic in the treatment of edema. Although furosemide is available in liquid and solid dosage forms, most pharmacopeias mention that bulk furosemide and furosemide formulations should be protected from light because furosemide is photosensitive. Furthermore, the photostability of furosemide in solution and in the solid state is different. In solution, furosemide is easily hydrolyzed to saluamine (4-chloro-5-sulfamoylanthranilic acid; CSA) and furfuryl alcohol under light irradiation. In contrast, in the solid state, Matsuda and Tatsumi reported that furosemide has four polymorphs, two solvates, and one amorphous form, and that furosemide (form I) showed almost no discoloration, even when it was irradiated with intense light, compared with the other polymorphs and solvates. Furthermore, De Villiers et al. reported that the photostability of form I is higher than that of form II furosemide under air and nitrogen atmospheres.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline on photostability (ICH Topic Q1B) was published in 1996 and has been implemented in all three regions (U.S., EU, and Japan). The guideline shows a useful basic protocol for testing of new drug substances and associated drug products for manufacturing, storage, and distribution, but this guideline does not cover the photostability of drugs under conditions of patient use. Sometimes, the discoloration of not only drug substances but also tablets is occurred during storage after opening package. In usual, the color change of tablets is considered to change the physicochemical properties of drug substances on the surface of the tablet by light irradiation, changing temperature and absorption of humidity. Some reports show the discoloration of tablet such as carbamazepine and nifedipine. In these reports, the discoloration of these two drugs are induced by light irradiation. Moreover, automatic tablet counting and packaging machines becomes to be widely used for the one-dose packaging of tablets and capsules. However, discoloration of such tablets repackaged by one-dose packaging machines for dispensing has been sometimes observed in several kinds of tablets due to the light, humidity and temperature in the room.

We prepared uncoated furosemide tablets from form I and observed the tablets turned yellow in a light-shielded automatic packaging machine. Discoloration of the furosemide tablets was heterogeneity and occurred on the surface of the tablets only. We found that the machine was equipped with an internal blower to maintain a constant temperature, so, we hypothesized that the discoloration of the furosemide tablets

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in the automatic packaging machine was caused by air flow. Therefore, we investigated the effect of air flow on the discol-
oration of the furosemide tablets by using a blower in a dark environment. The results showed that the furosemide tablets were turned yellow by air flow.

In this study, we elucidate the factors contributing to the discoloration of the furosemide tablets in a light-shielded environment. We confirmed the crystal form of furosemide after tablet preparation, analyzed the structure of the yellow compound in the furosemide tablets, and examined the effects of humidity and atmosphere (air, oxygen, and nitrogen) on the discoloration of the furosemide tablets.

Experimental

Materials Bulk furosemide was purchased from a commercial source. Uncoated tablets of furosemide (20 mg) and placebo tablets were prepared by the Teva Pharma Japan (Nagoya, Japan). The placebo tablets were prepared with the same pharmaceutical formulation as the furosemide tablets. The Karl Fischer titration reagents, hydralan-composite 5 and aquamicon solvent ML, were purchased from Mitsubishi Chemical Analytech Co., Ltd. (Kanagawa, Japan). Aceto-
nitrile, tetrahydrofuran, acetic acid and formic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Kanto Chemical Co., Inc. (Tokyo, Japan). All other reagents were commercial products of analytical grade.

Effect of Air Flow on the Discoloration of the Furose-
mide Tablets The furosemide tablets were exposed to an air flow of 5.4 m/s using the blower (H-30P20, Toshiba, Tokyo, Japan) in a dark environment at ambient temperature (17–20°C). The color difference (ΔE) of the furosemide tablets was measured by spectrophotometer (SE-2000, Nippon Den-
shoku, Tokyo, Japan) at room temperature, and was calculated as an average value for five tablets (the measurement per tab-
et was obtained for N=3).

Elution Time of the Yellow Compound in a Chromato-
graphic Purity Test for Substances Related to Furosemide in the Japanese Pharmacopoeia Bulk furosemide (25 mg) was dissolved in water–acetonitrile (1:1, 25 mL) containing 2.2% acetic acid. The yellow portion of the furosemide tablets (20 mg) were cut off and collected then dissolved in water–
acetonitrile (1:1, 5 mL) containing 2.2% acetic acid, respect-
ively. The three solutions (20 µL) were injected into a HPLC system consisting of a separation module (Alliance W2695; Waters, Milford, MA, U.S.A.) and a photodiode array detector (W2998; Waters). The purity of these solutions was calculated by area percentage method. The HPLC method conformed to the chromatographic purity test for substances related to furosemide in the Japanese Pharmacopoeia, 16th Edition. A Mightsil RP-18 GP column (4.6 i.d.×250 mm, 5 µm particle size, Kanto Chemical Co., Inc.) was used. The photodiode array detector was set at 210–400 nm. The temperature of the column oven and the flow rate were 25°C and 1.2 mL/min, re-
spectively. The mobile phase was water–tetrahydrofuran–ace-
tic acid (70:30:1) and the run time was 50 min.

Structural Analysis of the Yellow Compound The yel-
low portion of the furosemide tablets (20 mg) was dissolved in water–acetonitrile (1:1, 5 mL). This solution (2 µL) was injected into a LC-MS system consisting of an LC system (Alli-
ance W2690; Waters) and a mass spectrometer (TSQ-7000, Thermo Fisher Scientific, Waltham, MA, U.S.A.). An Inertsil

ODS-3 column (2.1 i.d.×150 mm, 5 µm particle size, GL Sci-
ence, Tokyo, Japan) was used. The temperature of the column oven was set at 30°C. The mobile phases were 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The flow rate was set at 0.2 mL/min. The gradient program was as follows: 0 to 10 min, 80% A and 20% B; 10 to 18 min, linear gradient from 80 to 65% A; 18 to 50 min, 65% A and 35% B; 50 to 51 min, linear gradient from 65 to 80% A; 51 to 75 min, 80% A and 20% B. Electrospray ionization (ESI) was used in negative ion mode. The spray voltage was set at 3.5 kV. The capillary temperature was set at 350°C. The MS² measurement was performed by collision-induced dissociation (CID) with a collision energy of 30–40 V.

Determination of Polymorph by X-Ray Powder Diffraction and IR Spectroscopy X-Ray powder diffraction (XRD) profiles were recorded on an X-ray diffractometer (RINT Ultima III, Rigaku, Tokyo, Japan) using Ni-filtered Cu-Kα radiation at room temperature. The XRD profiles of bulk fu-
rosemide, the furosemide tablets, and the placebo tablets were measured under the following conditions: voltage of 40 kV, current of 40 mA, divergence slit of 1 mm, scattering and receiving slits open, scan speed of 2°/min, scan axis of 2θ, scan range of 3–40°.

IR spectra were recorded on an IR spectrophotometer (Ni-
colet iS10, Thermo Fisher Scientific) in Nujol mull at room temperature. The IR spectra of bulk furosemide, the furo-
semide tablets, and the placebo tablets were measured under the following conditions: 32 scans, resolution of 4, scan range of 4000–450 cm⁻¹.

Effect of Humidity on the Discoloration of the Furose-
mide Tablets Water adsorption and desorption profiles were recorded on a vapor sorption analyzer (VTI/SGA-100; TA Instruments, Newcastle, DE, U.S.A.). The water adsorption and desorption profiles of bulk furosemide and the furosemide tablets were measured under the following conditions: sample weight of 15–25 mg, temperature of 25°C, relative humidity (RH) step of 5–95%, equilibrium criterion of 0.01% (wt) over 5 min, maximum equilibrium time of 180 min.

Isothermal calorimetric profiles were recorded on a microcalorimeter (TAM III, TA Instruments). The isothermal calorimetric profiles of bulk furosemide were measured under the following conditions: sample weight of 95–105 mg, tem-
perature of 30°C, RH of 43, 56, 75, 84, and 97% RH. The RH conditions were controlled by saturated salt solutions.

Determination of Water Content of the Furosemide Tablets Thermogravimetry (TG) and differential thermal analysis (DTA) thermograms were recorded on a TG-DTA thermal analyzer (EXSTAR 6000, SEI; Chiba, Japan). The TG and DTA thermograms of bulk furosemide and the furosemide tablets were measured under the following conditions: sample weight of 10–20 mg, heating rate of 10°C/min, scan range of 40–250°C.

The water content of the furosemide tablets was measured by a Karl Fischer volumetric titrator (AQV-2100, Hiranuma, Ibaraki, Japan) and was calculated as an average value for two tablets. The measurement was performed with hydralan-
composite 5 as a titrant and aquamicon solvent ML.

Effect of Temperature and Humidity on the Discolor-
ation of the Furosemide Tablets The furosemide tablets were kept in an incubator (15, 25, 40, 50, 60°C) and a thermo-
hygrostat (25°C/75% RH, 40°C/75% RH). The ΔE of the
furosemide tablets was measured with a spectrophotometer at room temperature as an average value for five tablets. The measurement for one tablet was performed with \( N = 3 \).

**Effect of Atmosphere (Air, Oxygen, and Nitrogen) on the Discoloration of the Furosemide Tablets**

The furosemide tablets were kept in an atmosphere of air, 99.9% oxygen, or 99.9% nitrogen at room temperature, respectively. The \( \Delta E \) of the furosemide tablets was measured with a spectrophotometer at room temperature as an average value for one tablet \( (N = 2) \).

Stress studies were performed by HPLC with the chromatographic purity test for substances related to furosemide in the Japanese Pharmacopoeia. The sample solutions for stress studies were prepared as follows.

- **Non-stress conditions**: Bulk furosemide (10 mg) was dissolved in water–acetonitrile (1:1, 10 mL) containing 2.2% acetic acid.
- **Thermal conditions**: Bulk furosemide (10 mg) was dissolved in water–acetonitrile (1:1, 10 mL) containing 2.2% acetic acid and heated at 80°C for 2 h.
- **Acidic conditions**: Bulk furosemide (10 mg) was added to 0.01 N HCl (10 mL) and the supernatant was used as the sample solution.
- **Basic conditions**: Bulk furosemide (10 mg) was dissolved in 0.01 N NaOH (10 mL).
- **Oxidation conditions**: Bulk furosemide (10 mg) was added to 0.1% H2O2 (10 mL) and the supernatant was used as the sample solution.

The sample solutions were analyzed by HPLC. The HPLC system consisted of a separation module (Alliance W2695; Waters) and a photodiode array detector (W2998; Waters). The purity of the sample solutions was calculated by the area percentage method. A Mightysil RP-18 GP column (4.6 i.d.\( \times 250 \text{mm}, 5 \mu \text{m} \) particle size, Kanto Chemical) was used. The UV detector was set at 272 nm. The temperature of the column oven was set at 25°C. The mobile phase was water–tetrahydrofuran–acetic acid (70:30:1) and the flow rate was 1.26 mL/min. The sample solutions (20 \( \mu \text{L} \)) were injected into the HPLC system and the run time was 50 min.

**Results and Discussion**

**Effect of Air Flow on the Discoloration of the Furosemide Tablets**

Figure 1 shows the change in \( \Delta E \) of the furosemide tablets with blowing time in the dark at ambient temperature (17–20°C). The furosemide tablets were exposed to an air flow of 5.4 m/s and the \( \Delta E \) was calculated as an average value for five tablets \( (N = 3) \).

![Fig. 1. Change in \( \Delta E \) of the Furosemide Tablets with Blowing Time in the Dark at Ambient Temperature (17–20°C)](image)

The samples were analyzed by the chromatographic purity test for substances related to furosemide in the Japanese Pharmacopoeia. The content at retention time of 5.9 min for the yellow portion of the furosemide tablets was increased compared with that of furosemide tablets (Fig. 2(c)). The retention time of the yellow compound was 5.9 min, and we performed LC-MS to identify its chemical structure.

**Structural Analysis of the Yellow Compound**

Figure 3 shows the HPLC chromatogram of the yellow portion of the furosemide tablets under the LC-MS conditions. To elucidate where the yellow compound eluted, we performed HPLC analysis with a photodiode array detector and obtained the
UV spectrum of the yellow compound (Fig. 4). The peak for the yellow compound was 5.9 min under LC-MS conditions, because the UV spectrum at 5.9 min under the LC-MS conditions matched that at 5.9 min during the chromatographic purity test for substances related to furosemide. Therefore, we performed structural analysis for this peak by LC-MS. Figure 5 shows the negative ion mode ESI-MS1 and ESI-MS2 spectra of the yellow compound. The MS spectra showed a deprotonated molecular ion ([M−H]−). These results suggested that the yellow compound was CSA, which is a hydrolysate of furosemide. Figure 6 shows the fragmentation pattern of CSA by CID (Negative Ion Mode ESI-MS2) NMR.12) These results also suggested that the yellow compound was CSA and hydrolysis of furosemide was accelerated by exposure to air flow.

Determination of Polymorph by XRD and IR Spectroscopy Figure 7 shows the XRD profiles of bulk furosemide, the furosemide tablets and the placebo tablets. The XRD profile of furosemide (form I) has three characteristic peaks at 2θ of 21.3°, 22.9°, and 24.8°.13) The XRD profile of bulk furosemide had the three characteristic peaks for form I and was similar to that of the form I polymorph, suggesting that bulk furosemide was form I. The furosemide tablets were prepared using form I, which is the most stable furosemide polymorph. Therefore, we examined whether a crystal transition from
form I to a different, unstable polymorph occurred during the preparation. The XRD profile of the furosemide tablets contained the three characteristic form I peaks (Fig. 7(b)). However, the profile was not identified as form I, because the peaks in the placebo tablets overlapped the three form I peaks (Fig. 7(c)). Therefore, we confirmed the crystal form of furosemide in the tablets by IR spectroscopy.

Doherty and York reported that the IR spectrum in the region of 3400–3200 cm$^{-1}$ (secondary amine N–H stretching vibration) is characteristic for each polymorph. Figure 8 shows the IR spectra of bulk furosemide, the furosemide tablets, and the placebo tablets in Nujol mull. The IR spectrum of the furosemide tablets in the region of 3400–3200 cm$^{-1}$ was similar to that of bulk furosemide (form I) and there was no interference from the other tablet components. This suggested that the crystal form of furosemide in the tablets was form I.
and no crystal transition to an unstable furosemide polymorph occurred during preparation. Furthermore, we investigated the furosemide polymorph in the white and yellow portion of the tablets by IR and the results suggested that no crystal transition occurred by air flow (Fig. 9).

Effect of Humidity on the Discoloration of the Furosemide Tablets  Humidity may accelerate the hydrolysis of furosemide exposed to air flow. We examined the hygroscopicity of bulk furosemide and the furosemide tablets with a vapor sorption analyzer. Figure 10 shows the water adsorption and desorption profiles of bulk furosemide and the furosemide tablets. The amount of adsorbed water in bulk furosemide was not more than 2% (wt) at 95% RH. It was suggested that the bulk furosemide was no hygroscopicity. However, the amount of adsorbed water in furosemide tablets was about 14% (wt) at 95% RH and the water adsorption and desorption profiles of furosemide tablets were different from those of bulk furosemide. Because the amount of furosemide in the tablets was approximately 20% (wt) to the weight of the tablets and bulk furosemide was no hygroscopicity, we estimated the adsorption and desorption profiles of the furosemide tablets would be mainly derived from the additives.

To investigate the effect of the humidity on the hydrolysis of furosemide, we performed isothermal calorimetric measurements of bulk furosemide under various humidity conditions. Figure 11 shows the isothermal calorimetric profiles of bulk furosemide under various RHs. The calorimetric profile of bulk furosemide at 56% RH gradually increased with the measuring time. We assumed that the critical relative humidity (CRH) of bulk furosemide was around 56% RH based on the results in Fig. 10(a), and the difference in the calorimetric profiles of bulk furosemide at 56% RH was caused by the water adsorption at the CRH. In addition, the normalized heat profiles of bulk furosemide were not shifted to the exothermic side except for the calorimetric profile at 56% RH. Thus, the humidity did not affect the hydrolysis of bulk furosemide.

Determination of Water Content  Figure 12 shows the TG-DTA thermograms of bulk furosemide and the furosemide tablets. The DTA thermogram of bulk furosemide showed one endothermic peak at 220°C, and the weight loss (%) of bulk furosemide from 40 to 220°C was about 0.1%, suggesting that the water content of bulk furosemide was about 0.1%. The water content of furosemide tablets could not be calculated from the TG weight loss because the DTA thermogram showed two endothermic peaks at 150 and 250°C. Karl Fischer titration showed that the water content of the furosemide tablets was 3.8%. The content of furosemide in the tablets was approximately 20% (wt) to the weight of the tablets and weight loss (water content) of bulk furosemide was about 0.1%. Therefore, we estimated that the water content of the furosemide tablets by Karl Fischer titration was mainly derived from the additives. The water adsorption and desorption measurements (Fig. 10(b)) indicated that the amount of adsorbed water in the furosemide tablets at 40–60% RH was 1.8–3.6% (wt). Therefore, we assumed that the water content of the furosemide tablets was mainly adsorbed water from the additives.

Effect of Temperature and Humidity on the Discoloration of the Furosemide Tablets  The change in $\Delta E$ of the furosemide tablets under various storage conditions is shown in Table 1. The yellow compound was the hydrolysate of furosemide and the results suggested that the hydrolysis of furosemide was not accelerated by humidity, consistent with the results of isothermal calorimetric measurements (Fig. 11). In contrast, the $\Delta E$ values for storage at 50 and 60°C after 2 weeks were about twice as large as the $\Delta E$ after 1 week, indicating that the hydrolysis of furosemide was accelerated by temperature.
Effect of Air, Oxygen, and Nitrogen on the Discoloration of Furosemide Tablets  

The change in $\Delta E$ of the furosemide tablets under air, 99.9% oxygen, and 99.9% nitrogen storage conditions are shown in Table 2. The furosemide tablets stored under oxygen showed the highest $\Delta E$ value. Therefore, we assumed that furosemide was hydrolyzed to CSA by oxidation and the hydrolysis was accelerated by oxygen. To investigate the acceleration of hydrolysis of furosemide by oxidation, we carried out several stress tests (under thermal, acidic, basic, and oxidative conditions). The content of CSA (%) under each stress testing was determined by the chromatographic purity tests for substances of furosemide analogue in the Japanese Pharmacopoeia. Kovar et al. and Rowbotham et al. reported that furosemide yields CSA upon oxidation with hydrogen peroxide.$^{1,14}$ Hence, we used hydrogen peroxide as oxidant.

Figure 13 shows the HPLC chromatograms of bulk furosemide under various stress tests conditions. The largest content of CSA (retention time of 5.7 min) was obtained under oxidative conditions and the CSA content under the oxidative condition was largest in the substances of furosemide analogue. Therefore, we estimated that the oxidative degradation of furosemide by air flow would be similar to that of furosemide by hydrogen peroxide so, the discoloration of the furosemide tablets in the automatic packaging machine was caused by increasing air flow.

**Conclusion**

The discoloration of the furosemide tablets in a light-shielded environment was induced by air flow. The yellow compound was identified by LC-MS as CSA, which is a hydrolysate of furosemide. The furosemide tablets were prepared using the most stable furosemide polymorph, form I. The XRD and IR measurements showed that no crystal transition occurred during tablet preparation. Furthermore, IR measurement of the yellow portion of furosemide tablets showed that

<table>
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<th>Storage conditions</th>
<th>$\Delta E$ 6 days</th>
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<tr>
<td>Air</td>
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<tr>
<td>Oxygen</td>
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<tr>
<td>Nitrogen</td>
<td>0.5</td>
</tr>
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Table 1. The Color Difference $\Delta E$ of the Furosemide Tablets under Various Storage Conditions

<table>
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<th>Storage conditions</th>
<th>$\Delta E$ 1 week</th>
<th>$\Delta E$ 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>15°C/40–60% RH</td>
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<td>0.2</td>
</tr>
<tr>
<td>25°C/40–60% RH</td>
<td>0.2</td>
<td>0.6</td>
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<tr>
<td>25°C/75% RH</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>40°C/40–60% RH</td>
<td>0.3</td>
<td>0.5</td>
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<td>0.5</td>
<td>0.4</td>
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<tr>
<td>50°C/40–60% RH</td>
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<td>1.1</td>
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<tr>
<td>60°C/40–60% RH</td>
<td>0.6</td>
<td>1.5</td>
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</tbody>
</table>

Table 2. The Color Difference $\Delta E$ of the Furosemide Tablets Stored under Air, 99.9% Oxygen, and 99.9% Nitrogen

*Fig. 13. HPLC Chromatograms of Bulk Furosemide under Various Stress Testing Conditions*  
(a) Non-stress conditions, (b) thermal conditions, (c) acidic conditions, (d) basic conditions, and (e) oxidative conditions.
no crystal transition occurred by air. To identify the factors producing the discoloration, we investigated the effect of humidity and atmosphere (air, oxygen, and nitrogen) on the discoloration of the furosemide tablets. The discoloration was accelerated by oxidation, although humidity did not affect the hydrolysis. We concluded that the cause of the discoloration in the packing machine was the acceleration of oxidative degradation by the air flow.

Acknowledgment The authors thank Mr. Tomomichi Saitou (Teva Pharma Japan) for preparing the furosemide tablets.

Conflict of Interest Shinji Katsura and Sumihiro Shiraiishi were, and Nobuo Yamada and Atsushi Nakashima are currently employees of Teva Pharma Japan Inc., respectively.

References