**Effects of Storage Conditions on Dissolution Rates of Indomethacin Capsules**

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Received December 2, 2017; accepted May 12, 2018

Using indomethacin (IND) capsules (Caps) before the date of expiration, we conducted the dissolution tests that are prescribed in the 17th Revision Japanese Pharmacopoeia (JP 17) and found a preparation within a serial number (Lot) that failed to satisfy the specified dissolution rate. Thus, we investigated factors that decrease dissolution rates of IND Caps during storage at room temperature (r.t.). In elution profiles of IND Caps samples, dissolution rates decreased as the expiration date approached. Moreover, after extended elution of formulations with unacceptable 20 min dissolution rates, dissolution rates remained in less of those specified in JP 17. Moreover, changes in dissolution profiles of preparations with pending expiry did not reflect changes in Caps shells, suggesting changes in the active contents. However, IND contents of the offending preparations decreased very little, even under accelerated conditions. Thus, in further experiments, we investigated preservation conditions of IND Caps, and found that humidity greatly decreases dissolution rates, and preservation of press-through package (PTP) sheets were also suggested to affect IND Caps. Finally, we found that the use of aluminum bag packaging prevents decreases in dissolution rates of IND Caps.

**Key words** indomethacin; dissolution rate; storage condition; aluminum bag; capsule

Stability examinations of pharmaceutical products are regularly conducted to maintain effectiveness and safety, and the ensuing data are used to set preservation methods and validity periods. Although analyses of preparations after storage under acceleration conditions (AC) indicate the influence of short-term deviations from storage methods and can be used to predict degrees of deterioration and circulation periods for which quality remains appropriate, but it may not be applicable for predicting physical changes.1) In medical institutions, the shelf life of medicines after opening the final packaging is often overestimated, and in certain cases the expiration date may become imminent. Hence, confirmation of the medicines long-term preservation after opening the final package or the medicines expiration dates was imminent is warranted to ensure the same quality as that immediately after manufacture. Accordingly, the dissolution test method is widely used to manage formulation design and quality, and to prevent significant bioinequivalence.2) Because the absorption and bioavailability of drugs greatly depends on the dissolution state of a drug, proper dissolution characteristics are considered predictive of drug efficacy. Physical properties of drugs can cause biological non-equivalence, and drug solubility can change with crystal polymorphisms, leading to fluctuations in elution rates between brands and lots.3) In in vitro dissolution tests, particles that are formed by disintegration of the preparation in the test solution are dispersed in the test solution, and the drug is eluted from the particle surface. Subsequently, apparent elution rates are determined, but vary between specific formulation conditions and in addition physicochemical properties that are peculiar to drugs.4) Indomethacin (IND), which is known as a poorly water-soluble drug, has a crystal polymorphism.5) There are several reports regarding the crystal polymorphism and dissolution behavior of IND,6–9) but there are few reports regarding changes in storage conditions and dissolution rate.

In this study, we performed the 17th Revision Japanese Pharmacopoeia (JP 17) dissolution tests and liquid chromatography analyses to confirm the quality and effectiveness of pharmaceutical products that were stored at room temperature (r.t.) and remained within the expiration period. Herein, we report assays and dissolution tests of IND capsules (Caps) that were stored at r.t., and show the presence of products that failed to meet the content specifications prescribed by JP 17. Because the dissolution rate of this preparation breached that prescribed by JP 17, we investigated factors that decrease dissolution rates.

**Experimental**

**Sample, Reagent** Experiments were conducted using commercially available 25-mg IND Caps purchased from a pharmaceutical wholesaler of manufacturing numbers 0121E4 (Lot. 1), 1C22D4 (Lot. 2), 2K01B2 (Lot. 3), 3F14B7 (Lot. 4), and 5H10G9 (Lot. 5). Lot. 1, Lot. 2, and Lot. 3 were about 2, 1 and 0.5 years since purchase, and other lots used the latest purchased items. Dissolution and HPLC tests were performed using the JP General Test Method, the standard products of JP, and reagents for the general test method of JP. All other reagents were of laboratory grade, and the test Caps were Japanese pharmacopoeia capsules (Matsuya; JP Caps).

**Preservation Conditions and Preparation of Sample**

Preservation at r.t

On the package insert the storage method of IND Caps is stated as r.t. preservation. Considering the preservation environment of medicines in medical institutions, unopened IND Caps in press-through packages (PTP) of Lot. 1, Lot. 2, Lot. 3, Lot. 4, and Lot. 5 were stored at r.t. (1–30°C) in the drawers in a room where humidity was not controlled after purchase. IND Caps of Lot. 1, Lot. 2, and Lot. 3 were removed and divided into contents and Caps shells, and JP Caps were filled with the contents from Lot. 1, Lot. 2, and Lot. 3. Lot. 1 and Lot. 2 Caps shells were filled with the contents from Lot. 3.
Table 1. Compositions of Contents and Capsules (Caps) Shells, and Numbers of Test Vessels

<table>
<thead>
<tr>
<th>Preparation Contents/capsules shells</th>
<th>Contents</th>
<th>Storage condition</th>
<th>Period of retention</th>
<th>Capsules shells</th>
<th>Storage condition</th>
<th>Period of retention</th>
<th>Number of vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot. 1/JP</td>
<td>Lot. 1</td>
<td>r.t.</td>
<td></td>
<td>JP Cap</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Lot. 2/JP</td>
<td>Lot. 2</td>
<td>r.t.</td>
<td></td>
<td>JP Cap</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Lot. 3/JP</td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>JP Cap</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Lot. 1/Lot. 3</td>
<td>Lot. 1</td>
<td>r.t.</td>
<td></td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Lot. 2/Lot. 3</td>
<td>Lot. 1</td>
<td>r.t.</td>
<td></td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Lot. 3/Lot. 1</td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>Lot. 1</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Lot. 3/Lot. 2</td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>Lot. 2</td>
<td>r.t.</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>AC 1/Lot. 3</td>
<td>Lot. 3</td>
<td>AC</td>
<td>1 month</td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Lot. 3/AC 1</td>
<td>Lot. 3</td>
<td>r.t.</td>
<td></td>
<td>Lot. 3</td>
<td>AC</td>
<td>1 month</td>
<td>3</td>
</tr>
</tbody>
</table>

the shells of Lot. 3 Caps were filled with the contents from Lot. 1 or Lot. 2 (Table 1).

Preservation under Acceleration Conditions (AC)
PTP sheets of Lot. 3 were stored in a constant climate cabinet (thermo-hygrostat PR-1ST (ESPEC CORP., Osaka, Japan)) at 40±2°C at a relative humidity (RH) of 75±5% for 2 weeks, and 1, 3, 6, and 9 months. In addition, Lot. 3 IND Caps were stored at r.t. or under AC for 1 month, IND Caps were removed and divided into contents and Caps shells. Subsequently, Caps shells of Lot. 3 were filled with the contents of IND Caps that were stored under AC for one month (AC 1), and IND Caps shells of AC 1 were filled with the contents of Caps of Lot. 3 (Table 1).

Preservation under Dry Conditions
PTP sheets of Lot. 4 were stored in a ventilated constant temperature dryer (ADVANTEC® DRM620TB (Toyo Sei-sakusho Kaisha, Ltd., Japan)) at 40°C for 1 and 2 weeks, and for 1, 3, 6, and 9 months.

Storage under AC in Different Packaging Forms
PTP sheets of Lot. 5 were stored in polyethylene bags with zippers (140×100×0.04 mm, SEISANIPPONSHA Ltd., Japan) (UNP) or aluminum bags with zippers of 160×100×0.114 mm with gussets (30 mm) (SEISANIPPONSHA Ltd.; ALP) and were stored in a constant climate cabinet (LHL-114; ESPEC CORP.) at 40±2°C and RH of 75±5% for 45 d.

Dissolution Test Tests were conducted according to JP 17 using the rotating basket method with a dissolution tester (NTR-6200 A Toyama Sangyo Co., Ltd., Japan). The test was conducted with 900 mL of a 4:1 solution of water and phosphate buffer (pH 7.2) at a rotation speed of 100 rpm and a temperature of 37±0.5°C. In the eluates, the amount of one sample was 25 mL, it were filtered through membrane filters (Millex-AA, Merck Millipore Corporation, U.S.A.) with a pore size of 0.8 µm, and were quantitated using spectrophotometers (Ultrospec1100pro Amersham Company (2-1, 2-2) and JASCO V-730 JASCO Corporation (2-3, 2-4)) at 320 nm. JP 17 specifies not less than 75% of the dissolution rate of IND Caps in 20 min.

JP 17 Dissolution Tests
A total of 6 IND Caps each from Lot. 1, Lot. 2, and Lot. 3 were stored at r.t. and eluates were collected at 20 min after the start of the test.

Dissolution Tests of Samples over Time
The eluate was sampled 6 times in total, at 5, 10, 15, 20, 30, and 60 min after the start of the test, and the same volume of test solution was replenished immediately after collection.

Influences of Storage Conditions
IND Caps that were stored at r.t., or under 40°C or under AC for 1 (only at 40°C drying conditions) or 2 weeks or 1, 3, 6, and 9 months, were eluted and examined. For IND Caps stored at AC for 3, 6, and 9 months, in addition to 6 samples of the eluate, eluates were collected every 1 h from 60 min until 6 h later, and were collected 8, 10, and 24 h later. Six Caps were tested for each condition.

Influences of Storage Conditions on Caps Shells and Contents
Dissolution tests were performed on IND Caps samples (Table 1) that were stored at r.t. or AC 1, and were compared with JP Caps. The combined preparations Lot. 1/JP, Lot. 2/JP, Lot. 3/JP, Lot. 1/Lot. 3, Lot. 2/Lot. 3, Lot. 3/Lot. 1, and Lot. 3/Lot. 2 were tested with 6 Caps each, and those of AC 1/Lot. 3 and Lot. 3/AC 1 were tested with 3 Caps each.

Influences of Packaging Forms
IND Caps in PTP sheets were stored at r.t. (PTP), in a polyethylene bags with zippers (UNP), or in an aluminum bag with a zipper (ALP) in a constant climate cabinet at 40±2°C under RH of 75±5% for 45 d. Tests were performed with 3 bags per packaging material, 6 Caps per bag, and 6 Caps per PTP sheet.

Quantitative Test
Liquid Chromatography (HPLC)
Quantitative determinations were performed according to the internal standard method of JP 17 using HPLC (LC-2000 Plus series JASCO). The mobile phase was a mixed solution (7:3) of methanol and phosphate (1 in 1000), the column was an Inertsil ODS-3, the detector was a JASCO UV-2075 Plus (254 nm), the column temperature was set at 25°C, and the flow rate was set at 1.6 mL/min and was varied only within the range of system suitability.

Preparation of the Sample Powder
The contents of 30 Caps from Lot. 1, Lot. 2, and Lot. 3 were removed and mixed, and sample powders were adjusted and quantified. In addition, after storage under AC for 9 months, each contents of 3 Caps from Lot. 3 were removed and used as sample powder for quantification.

Evaluations of Similarities of Dissolution Profiles
According to the Guideline for Bioequivalence Studies of Generic Products (Guideline of Generic Products), the acceptance criteria for similarity of dissolution profiles involves comparison of the average dissolution rate of the test product with that...
of the reference product. When the average dissolution of the reference product reaches 85% within 15 min, if the average dissolution of the test product reaches 85% within 15 min, or is within that of the reference product ±15% at 15 min, the dissolution profile of the test product is considered to be similar to that of the reference product.10) Similarly, when the average dissolution rate of the standard preparation exceeded 85% after 15 min from the start of elution, an error bar with an average dissolution rate ±15% at each elution time was displayed, and the similarity was evaluated.

Results and Discussion

Dissolution Test

Elution Examinations as Prescribed in JP 17

JP 17 specifies that the dissolution rate in 20 min of IND Caps is not less than 75%. In the present experiments, the average dissolution rate of Lot. 1 was 37.5% (maximum 64.9%, minimum 24.9%), that of Lot. 2 was 45.4% (maximum 57.6%, minimum 26.0%), and that of Lot. 3 was 93.9% (maximum 101.7%, minimum 84.2%). The number of Caps in which the 20 min dissolution rate exceeded 75% was 0 in both Lot. 1 and Lot. 2 whereas all six Caps from Lot. 3 met the requirements. Lot. 1 and Lot. 2 were before respectively from their expiration dates, and did not meet the specified dissolution rates. Because decreases in dissolution rates with storage time after manufacture or purchase date were greater in these Lots, environmental conditions during the storage period may have affected dissolution rates even in intact PTP sheets.

Dissolution Tests of Samples over Time

Influence of Preservation Conditions

The dissolution profiles of Lot. 1, Lot. 2, and Lot. 3 are shown in Fig. 1 and average dissolution rates at 60 min after the start of the testing were 35.4, 54.6, and 99.0%, respectively. Moreover, Lot. 1 and Lot. 2 had reduced average dissolution rates of less than 75% even after 60 min. The 60 min dissolution profile of Lot. 4, which was stored at r.t. or under dry conditions, is shown in Fig. 2. Average dissolution rates over 20 min of IND Caps that were stored at r.t. or for 1 or 2 weeks or 1, 3, 6, and 9 months under drying conditions were similar, at 95.0, 90.2, 90.7, 87.6, 89.4, 92.4, and 90.5%, respectively, and met the requirements of JP 17 regardless of storage temperature and period. In addition, the average dissolution rate of Caps from Lot. 4 over 15 min after storage at r.t. was 88.8%, and for 1 or 2 weeks or 1, 3, 6, and 9 months under drying conditions were 83.2, 83.9, 81.7, 80.6, 84.2, and 83.4%, respectively, and the average dissolution rate in each storage period dry conditions exceeded 80%. These data was within the range of ±15% (73.8–103.8%) of the Lot. 4 average dissolution rate; the dissolution behavior was similar.

Table 2 shows the average dissolution rate of Lot. 3 that were stored at r.t. or under AC for 2 weeks and AC 1. Average dissolution rates over 20 min of IND Caps that were stored at r.t. or for 1 or 2 weeks or 1, 3, 6, and 9 months under drying conditions were similar, at 95.0, 90.2, 90.7, 87.6, 89.4, 92.4, and 90.5%, respectively, and met the requirements of JP 17 regardless of storage temperature and period. In addition, the average dissolution rate of Caps from Lot. 4 over 15 min after storage at r.t. was 88.8%, and for 1 or 2 weeks or 1, 3, 6, and 9 months under drying conditions were 83.2, 83.9, 81.7, 80.6, 84.2, and 83.4%, respectively, and the average dissolution rate in each storage period dry conditions exceeded 80%. These data was within the range of ±15% (73.8–103.8%) of the Lot. 4 average dissolution rate; the dissolution behavior was similar.

Table 2. Average Dissolution Rates (%) of Indomethacin (IND) Capsules (Caps) from Lot. 3 after Storage at Room Temperature (r.t.) or under Acceleration Conditions (AC) for 2 Weeks or 1 Month (AC 1)

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Time (min)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>r.t.</td>
<td>14.9±8.7</td>
<td>58.2±17.0</td>
<td>88.2±7.8</td>
<td>95.5±6.2</td>
<td>97.9±5.3</td>
<td>99.0±4.8</td>
<td></td>
</tr>
<tr>
<td>2 weeks (AC)</td>
<td>3.6±7.1</td>
<td>28.3±33.8</td>
<td>42.8±39.4</td>
<td>48.1±38.2</td>
<td>53.6±37.3</td>
<td>57.9±35.9</td>
<td></td>
</tr>
<tr>
<td>AC 1</td>
<td>−0.3±0.5</td>
<td>0.2±0.6</td>
<td>1.3±0.9</td>
<td>2.6±1.6</td>
<td>5.1±1.3</td>
<td>10.3±1.4</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means±standard deviations (S.D.).
tion rates of Lot. 3 IND Caps that were stored for 3, 6, and 9 months under AC were 55.3, 50.9, and 35.3%, respectively (Fig. 3). These results indicate that dissolution rates of IND Caps are not influenced by drying conditions but decrease markedly under AC, suggesting that humidity greatly decreases dissolution rates of IND Caps.

Influence of Caps Shells and Contents

(1) Storage at r.t.

Dissolution profiles of combination samples comprising test Caps using JP Caps shells (Lot. 1/JP, Lot. 2/JP, and Lot. 3/JP) are shown in Fig. 4. At 20 min after the start of elution of Lot. 1/JP and at 15 min after the start of elution of Lot. 2/JP, average dissolution rates were within the range of the standard deviation of the corresponding Lot. (Lot. 1, 2), and dissolution profiles were approximately equal. However, the 15-min average dissolution rate of Lot. 3/JP was 87.0%, and was within the range of 88.2 ± 15% of the average dissolution rate of Lot. 3, indicating similar. Furthermore, the average dissolution rates of the samples Lot. 1/Lot. 3 and Lot. 2/Lot. 3, which comprised Lot. 3 Caps shells filled with the contents of Lot. 1 and Lot. 2, respectively, were in the range of the standard deviation of the average dissolution rates of Lot. 1 and Lot. 2, and were approximately equal (Fig. 5).

In contrast with Lot. 3 contents filled in Lot. 1 or Lot. 2 Caps shells, average 15-min dissolution rates of Lot. 3/Lot. 1 and Lot. 3/Lot. 2 were 86.4 and 83.6% respectively, and were equivalent to that of Lot. 3 (Fig. 6). Hence, changes in elution profiles of preparations that are approaching the expiration date do not remarkably reflect the properties of Caps shells, suggesting that changes in contents contributed predominantly to poor dissolution rates.

(2) Preservation under AC

In Lot. 3, the average 60-min dissolution rate for samples AC 1/Lot. 3 and Lot. 3/AC 1, combination samples comprised of Lot. 3 and AC 1, were 30.8 and 58.8%, respectively. The average dissolution rate for samples of AC 1/Lot. 3, Lot. 3/AC 1, and AC 1, Lot. 3 are shown at Table 3. The average dissolution rates of the samples AC 1/Lot. 3 and Lot. 3/AC 1 always exceeded those of samples AC 1, but none exceeded the dissolution rate of Lot. 3. Moreover, samples of the AC 1/Lot. 3 exceeded the average dissolution rate of the Lot. 3/AC 1 until 10 min after the start of the test, but had smaller dissolution rates after 15 min. The average dissolution rate of samples of AC 1/Lot. 3 exceeded 5% after 5 min, whereas Lot. 3/AC 1 was 0.6% after 5 min and 10.1% after 10 min. Lag time was observed in sample Lot. 3/AC 1. According to the Guideline
of Generic Products, lag time is defined as the time when 5% of the labeled claim of the active ingredient dissolves from the product.\textsuperscript{10} It reflects the time taken by the drug from the formulation to be released. In experiments with samples of the Lot. 3/AC 1, the Caps shells dissolved slowly, potentially delayed elution of IND into the test solution. These observations suggest that AC greatly affects dissolution rates of IND Caps and affects both Caps shells and contents.

In the present drug preparations, the capsules are mainly made of gelatin. Gelatin-based capsules have low oxygen permeability but are easily softened by moisture, resulting in rapid in vitro disintegration with little variation in disintegration time.\textsuperscript{11} However, due to reactions of gelatin lysine residues with aldehydes and ketoses, or oxidation of lysine residues to aldehydes, aqueous solubility of these capsules decreases with time.\textsuperscript{12} Sakaeda \textit{et al.} compared elution properties of hard gelatin capsules and showed greatly decreased dissolution rates under conditions of 40°C and 75% RH for 6 months, and 60°C for 1 week. Hence, the solubility of gelatin hard capsules was greatly influenced by the storage environment depending on the product. Moreover, the contents of these capsules can react with gelatin lysine residues and decrease gelatin solubility. Finally, lactose was previously considered causative of undesirable changes in gelatin capsules\textsuperscript{12}, and is present as an additive in IND Caps, likely influencing solubility along with humidity.

\textbf{(3) Influence of the packaging form}

In samples of Lot. 5, average 20-min dissolution rates of PTP that were stored at r.t. (PTP–r.t.) or AC (PTP–AC), and UNP and ALP that were stored for 45 d under AC, were 100.1, 53.3, 62.7, and 98.6% (Fig. 7), respectively. In addition, average 15-min dissolution rates for PTP–r.t. and ALP were 92.5 and 90.4%, and the corresponding dissolution profiles were considered similar. In contrast, dissolution rates of PTP–AC and UNP decreased and did not meet the requirements of JP 17. The average dissolution rate of UNP was within the range of the standard deviation of the average dissolution rate of PTP–AC and did not differ with it, suggesting that the water molecules in the storage space pass through a polyethylene bags with zippers. Moreover, the average dissolution rates of ALP were similar to those of PTP–r.t., indicating that the aluminum bag with the zipper preserves the dissolution rate of IND Caps.

\textbf{Quantitative Tests} We investigated the influence of the IND contents on dissolution rates of the preparations. The JP 17 stipulate that the main drug is present in IND Caps at ±10.0% of the displayed amount. In the present study, average IND contents of Caps from Lot. 1, Lot. 2, and Lot. 3, which were stored at r.t., were 98.1, 99.6, and 99.2%, respectively, thus meeting the requirements of JP 17 (Table 4). In addition, the average IND contents of 3 Lot. 3 IND Caps that were stored for 9 months under AC was (96.8%) also within ±10.0% of the indicated amount (Table 5). Thus, we conclude that the IND contents contributed little to the present reductions in dissolution rates \textit{per se}, even after storage at r.t. or under AC.

\textbf{Conclusion} The present experiments show that IND Caps that remain within the expiration date can have dissolution rates that fail to satisfy JP 17 criteria, and that these instances reflect storage conditions. Our data show that dissolution rates of IND Caps preparations decreased with the approach of expiration dates and suggest that changes in the contents may contribute to poor solubility. However, IND contents decreased very little in the present Lot that had unacceptable dissolution rates. In addition, when stored under dry conditions, dissolution...
rates decreased very little with storage time. We also found that AC caused only slight decreases in IND contents, even after 9 months, although average dissolution rates decreased markedly after 2 weeks of AC storage and failed to meet JP 17 requirements. Hence, changes in both Caps shells and contents likely contribute to decreases in dissolution rates under AC. The present data suggest that humidity greatly decreases dissolution rates of IND Caps, even when stored in PTP sheets. However, even under AC that cause average elution rates to decrease in PTP sheets, storage of PTP sheets in an aluminum bag with a zipper greatly protected against loss of solubility. Bufferin 81-mg tablets containing aspirin and Limaprost preparations, which are known to decompose over time because of moisture, are recommended to be stored with desiccant in aluminum pack as stringent humidity control is needed.\textsuperscript{13,14} IND content in IND Caps slightly decreased after 9 months of AC storage, and on comparing the dissolution rates obtained after preservation in PTP sheets and storage in an aluminum bag with a zipper, it was suggested that the aluminum bag is more effective.

In summary, when moisture proof pillow packaging is unavailable or during transfer, we recommend storage of humidity-sensitive medicines, such as IND Caps, in aluminum bags with zippers.

**Acknowledgments** I would like to express my gratitude to Mr. Toshirou Fukami (Meiji Pharmaceutical University), Mr. Yoshihisa Yamamoto (Teikyo Heisei University) and Mr. Tsuguchika Yoshida (former Teikyo Heisei University) for their cooperation in this research.

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**References**


