Generation of Formaldehyde by Pharmaceutical Excipients and Its Absorption by Meglumine

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Formaldehyde is a well-known air impurity. The possibility was investigated in this study that pharmaceutical excipients commonly used in oral solid dosage forms might also be sources of formaldehyde. The results showed that formaldehyde is generated by the excipients lactose, D-mannitol, microcrystalline cellulose, low-substituted hydroxypropylcellulose, magnesium stearate and light anhydrous silicic acid. Since the quality and safety of pharmaceutical products can be significantly affected by the presence of formaldehyde, various amines were then investigated for their ability to decrease levels of formaldehyde using an aqueous solution system. Of the four amines investigated, only meglumine proved capable of reducing formaldehyde levels. The reaction product between formaldehyde and meglumine was obtained by fractionation using the preparative HPLC system and the structure was clarified by ^{1}H-, ^{13}C-NMR, various types of two-dimensional NMR and mass spectroscopy. The reaction product was determined to be a compound with a 1,3-oxazinane skeleton and containing one more carbon than meglumine. It was presumed that formaldehyde reacted with the secondary amino group in meglumine to form the reaction product *via* an iminium salt intermediate by cyclization. As meglumine is permitted to be used as a pharmaceutical excipient in both oral and parenteral dosage forms by regulations worldwide, the addition of meglumine to pharmaceutical products can be expected to contribute to the stabilization of many drug substances.

Key words　meglumine; formaldehyde; degradation; amine; excipient

Formaldehyde is a well-known air impurity. It is reported to be present as a trace impurity in materials containing polyoxyethylene chains, such as poly(ethylene terephtalate) for plastic bottles[1] and various surfactants such as polyethylene glycols 300[2] and 400[3] and polysorbate 80.[2] It is also reported that polyethylene glycol 300,[4] polysorbate 80[2,4] and certain other polyoxyethylene surfactants[4,5] can generate formaldehyde due to oxidation under various storage and handling conditions. However, there has been no study to investigate whether common pharmaceutical excipients in oral solid dosage forms such as fillers, disintegrants, binders and lubricants could be possible sources of formaldehyde. Neither has there been a study investigating the effect of storage on the amount of formaldehyde generated by these materials.

It is highly important that sufficient quality and safety for pharmaceutical products is assured, and an essential requirement for this is that degradation of drug substances is suppressed to meet regulatory criteria.[6] Degradation is often caused by various impurities in drug substances, pharmaceutical excipients and packaging materials amongst others and tends to occur in drug products with low dosage strengths. Several studies have demonstrated that drug substances are degraded due to formaldehyde present, for example, as an impurity in pharmaceutical excipients or as a material used for the synthesis of drug substances.[2,3,7—9] When formaldehyde contained in excipients causes degradation, then this can be avoided by using high purity excipients.[2,3] However, considering that trace formaldehyde is also present in air, selection of excipients alone is not a satisfactory solution. Formaldehyde in air and originating from other materials requires to be eliminated as well.

The objective of this study was to clarify whether formaldehyde is generated by pharmaceutical excipients commonly used for oral solid dosage forms and to find a material that can eliminate formaldehyde in drug products with the aim of suppressing degradation of drug substances.

Experimental

Materials　Pharmaceutical excipients: Japanese Pharmacopoeia (fifteenth edition) grade excipients were used. Lactose (Pharmatose® 200M) (DMV International, Veghel, The Netherlands), D-mannitol (Mitsubishi Shoji Foodtech Co., Ltd., Tokyo, Japan), low-substituted hydroxypropylcellulose (L-HPC, LH-21) (Shin-Etsu Chemical Industry Co., Ltd., Tokyo, Japan), hydroxypropylcellulose low-viscosity type (HPC L) (Nippon Soda Co., Ltd., Tokyo, Japan), microcrystalline cellulose (Ceolus® PH-101) (Asahi Kasei Corp., Tokyo, Japan), magnesium stearate (vegetable origin) (Taihei Chemical Industrial Co., Ltd., Osaka, Japan) and light anhydrous silicic acid (Aerosil® 200) (Nippon Aerosil Co., Ltd., Tokyo, Japan) were used. Meglumine was purchased from Merck KgaA, Darmstadt, Germany.

Reagents: Acetonitrile of HPLC grade was obtained from Kanto Chemical Co., Inc., Tokyo, Japan. All other reagents were of analytical grade and purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Storage of Pharmaceutical Excipients　One hundred milligrams (due to its bulkiness) of light anhydrous silicic acid and 500 mg of the other excipients shown in Table 1 were stored individually in glass test tubes with glass closures at 80 °C for 14 d. Any formaldehyde present in those excipients before and after storage was extracted with purified water and quantified according to the HPLC method described below.

Storage of Formaldehyde Solutions with Amines　Three milliliters of purified water containing 1 μmol of formaldehyde were stored with 10 mmol of each amine powder in cups in glass test tubes with glass closures at 25 °C for 3 d. A diagram of this method is shown in Fig. 1. For meglumine, 3 ml of a saturated salt solution containing 1 μmol of formaldehyde was also stored with 10 mmol of meglumine in the same way as employed above. Saturated salt solutions of potassium acetate and potassium iodide were used to keep humidity in test tubes to 22% and 69% relative humidity (RH) respectively for the further studies on meglumine only.[10]

Assay of Formaldehyde by HPLC　Formaldehyde in the sample solution was quantified by modifying the method reported by Benassi *et al.*[11] A 0.1% 2,4-dinitrophenylhydrazine (DNPH) reagent was prepared by dissolv-
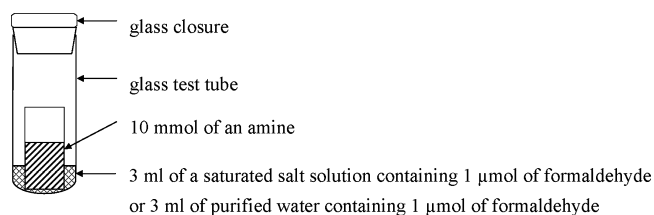
Fig. 1. Apparatus for Examining Absorption of Formaldehyde by Amines

Saturated solutions of potassium acetate and potassium iodide were used to keep humidity in test tubes to 22% RH and 69% RH, respectively.[11] Formaldehyde in those solutions was quantified after storage.

ing 50 mg of DNPH in 18 ml of 35% hydrochloric acid and then diluting with 32 ml of purified water. For the standard solution, formalin was appropriately diluted with purified water. The sample or standard solution was then further diluted with purified water and 0.5 ml of the solution was mixed with 0.5 ml of acetonitrile. It was derivatized by adding 0.1 ml of 0.1% DNPH reagent, shaking by hand for 30 s and leaving at room temperature for 15 min. The solution was analyzed by HPLC after stabilization with 0.2 ml of 1 mol/l phosphate buffer (pH 6.8) and 0.1 ml of 4 mol/l sodium hydroxide solution.

The assay by HPLC employed an HPLC system consisting of an LC-10AS pump, a CTO-10A column oven, an SPD-10A UV–visible detector, an SCL-10AVP system controller, an SIL-10AXL auto injector and a CLASS-VP data system (Shimadzu Corp., Kyoto, Japan). The method used a 150 mm×4.6 mm internal diameter column packed with octadecyl silica (ODS) of 5 μm particle size, L-column ODS (Chemicals Evaluation and Research Institute, Tokyo, Japan) at 40 °C. The mobile phase of 50% purified water and 50% acetonitrile was delivered at a flow rate of 1.0 ml/min. Injection volume was 20 μl. The detector was set at a wavelength of 345 nm. The amount of formaldehyde in the sample was calculated from the peak area ratio to the standard solution and expressed as a percentage to a theoretical amount in an initial solution.

Fractionation of the Reaction Product between Formaldehyde and Meglumine A solution containing 1 mol/l of meglumine and formaldehyde was prepared to fractionate the reaction product. The fractionation employed a preparative HPLC system similar to that for the assay of formaldehyde mentioned above. The method used a 250 mm×20 mm internal diameter column packed with ODS of 5 μm particle size, YMC-Pack Polyamine II (YMC Co., Ltd., Kyoto, Japan) at 40 °C. The mobile phase of 65% acetonitrile and 35% purified water was delivered at a flow rate of 9.9 ml/min. The detector was set at a wavelength of 210 nm and each injection volume was 100 μl. The fractions where retention time was approximately between 8.2 and 9.2 min were collected and freeze-dried by an FD-1 freeze dryer (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) to obtain around 15 mg of the semisolid.

^{1}H- and ^{13}C-NMR Spectroscopy Samples were dissolved in deuterated dimethylsulfoxide before measurement. Proton and ^{13}C-NMR spectra were acquired using a Mercury vx-400 Spectrometer (Varian, Inc., CA, U.S.A.) at 40 °C with an operating frequency of 400 and 100 MHz, respectively. Proton and ^{13}C chemical shift assignments were referenced internally to the tetramethylsilane peak. The two-dimensional NMR experiments of H–H COSY (correlated spectroscopy), HSQC (heteronuclear single quantum correlation spectroscopy) and HMBC (heteronuclear multiple bond correlation spectroscopy) were also conducted for structure determination.

Results and Discussion

Generation of Formaldehyde by Pharmaceutical Excipients The possibility was investigated that pharmaceutical excipients commonly used for oral solid dosage forms might be sources of formaldehyde. After storage at 80 °C for 14 d, formaldehyde was detected in each of the excipients in concentrations varying from 0.3 to 1.3 μg per gram of excipient. Formaldehyde was detected only in L-HPC and HPC L before storage (Table 1). The results indicate that formaldehyde was generated by these excipients during storage. Since these materials are commonly used for oral solid dosage forms, drug substances in pharmaceutical products containing these materials will be exposed to formaldehyde and may degrade

Table 1. Amounts of Formaldehyde Generated in Various Pharmaceutical Excipients during Storage

Excipient	Amount of formaldehyde (μg/g excipient)	
	Initial	After 14 d at 80 °C
Lactose	0.0±0.0	0.5±0.0
D-Mannitol	0.0±0.0	0.5±0.1
Microcrystalline cellulose	0.0±0.0	1.0±0.1
L-HPC	0.5±0.1	1.1±0.4
HPC L	1.4±0.2	0.8±0.0
Magnesium stearate	0.0±0.0	0.3±0.2
Light anhydrous silicic acid	0.0±0.0	1.3±0.9

The experiment was conducted with each excipient separately. The data are expressed as means±standard deviations, $n=3$.
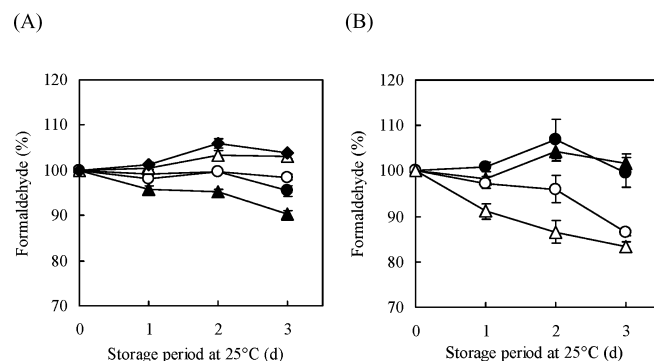

Fig. 2. (A) Percentages of Formaldehyde Absorbed by Amines

●, control (without amines); ▲, meglumine; ◆, proline; ○, alanine; △, arginine.
(B) Percentages of Formaldehyde Absorbed by Meglumine

●, 22% RH (without meglumine); ▲, 69% RH (without meglumine); ○, 22% RH; △, 69% RH. The data are expressed as means±standard deviations, $n=3$.

during storage. For instance, assuming that the drug substance constitutes 1% of total weight of a pharmaceutical product, has ten times the molecular weight of formaldehyde and reacts with it in the mole ratio of one to one, 0.1% of the drug substance will be degraded by 1 μg of formaldehyde per gram of excipient mixture. Considering that pharmaceutical products are required to be chemically stable for long periods such as 6 months at 40 °C/75% RH and 3 years at 25 °C/60% RH, it can be anticipated that more formaldehyde will be generated and consequently more drug substance will be degraded during storage.

Absorption of Formaldehyde by Amines Several studies have demonstrated that drug substances are degraded due to formaldehyde present as an impurity in excipients and drug substances.[2,3,7—9] Therefore, to reduce the amount of formaldehyde found in pharmaceutical excipients, drug substances and air, formaldehyde absorbing materials were investigated by storing candidate materials with formaldehyde solutions. Primary and secondary amines are well known to react with aldehydes to form imines and enamines, respectively.[12] In this study, L-alanine, meglumine, L(+)-arginine and L(−)-proline were selected as a primary amine, a secondary amine, an amine with both primary and secondary amine groups and a cyclic amine, respectively. The results showed that only with meglumine did levels of formaldehyde decrease after storage at 25 °C for 3 d and that the decrease was approximately 10% of the initial amount (Fig. 2A). As for the other three amines, no decrease in formaldehyde was

observed compared with the control that contained no amine. This result proves that only meglumine is effective in reducing levels of formaldehyde.

Drug products may possibly be exposed to a wide range of humidity. In order to examine the effect of humidity on absorption of formaldehyde by meglumine, formaldehyde solution and meglumine powder were stored separately under two different levels of humidity. Around 15% of the initial amount of formaldehyde decreased after storage for 3 d both under conditions of 25 °C/22% RH and 25 °C/69% RH (Fig. 2B). These results suggest that meglumine can reduce levels of formaldehyde regardless of humidity.

Structure Determination of the Reaction Product between Formaldehyde and Meglumine Figure 3 shows the ^{1}H-NMR spectrum of the reaction product between formaldehyde and meglumine obtained by fractionation using the preparative HPLC system. The ^{13}C-NMR and DEPT (distortionless enhancement by polarization transfer) spectra are presented in Fig. 4. The molecular formula and weight of meglumine are $C_7H_{17}NO_5$ and 195, respectively. In the ^{1}H-NMR spectrum presented in Fig. 3 signals were observed from 2.2 to 5.0 ppm of the chemical shift and from their inte-

grated values it was considered that the number of proton atoms is a multiple of 17 (the integral curve is not shown). The four circled signals between 4.2 ppm and 5.0 ppm were considered to be protons from hydroxyl groups because they disappeared in a deuterated-water addition test. From the ^{13}C-NMR spectrum it was found that the reaction product has eight carbon atoms which were identified as one methyl, three methylene and four methine carbons based on the DEPT spectra (Fig. 4). The mass spectrum showed that the molecular weight is 207 which is 12 larger than that of meglumine. Considering the molecular formula of meglumine and the nitrogen rule, the formula for the reaction product was presumed to be $C_8H_{17}NO_5$ which has one more carbon than meglumine. The reaction product was considered to have one aliphatic ring because it has an unsaturation degree of one from the formula and the ^{1}H-NMR spectrum showed

Table 2. ^{1}H- and ^{13}C-NMR Assignments of the Reaction Product between Formaldehyde and Meglumine
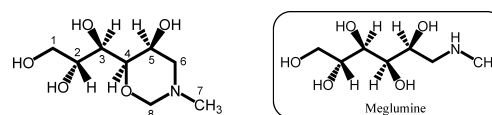
Position	Chemical shifts (δ_C, ppm)	Chemical shifts (δ_H, ppm), multiplicity, J (Hz), integration
1a	62.52	3.54, m, 1H
1b		3.36, m, 1H
2	70.03	3.52, m, 1H
3	73.27	3.56, m, 1H
4	76.49	3.48, dd, 3.9, 1.5, 1H
5	66.57	3.65, td, 2.2, 1.5, 1H
6a	58.01	2.81, dt, 12.9, 2.2, 1H
6b		2.53, dd, 12.9, 2.2, 1H
7	40.78	2.34, s, 3H
8a	85.27	4.36, dd, 8.7, 2.2, 1H
8b		3.83, d, 8.7, 1H
1-OH	—	4.27, t, 5.0, 1H
2-OH	—	4.52, d, 5.1, 1H
3-OH	—	4.71, d, 3.7, 1H
5-OH	—	4.97, d, 4.6, 1H

Abbreviations: d=doublet; dd=double doublet; dt=double triplet; m=multiplet; s=singlet; t=triplet; td=triple doublet. The assignments for 'a' and 'b' on positions 1, 6 and 8 are interchangeable, respectively.
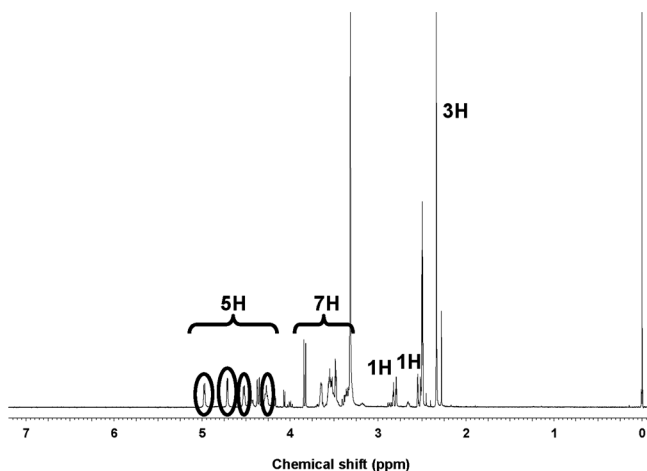

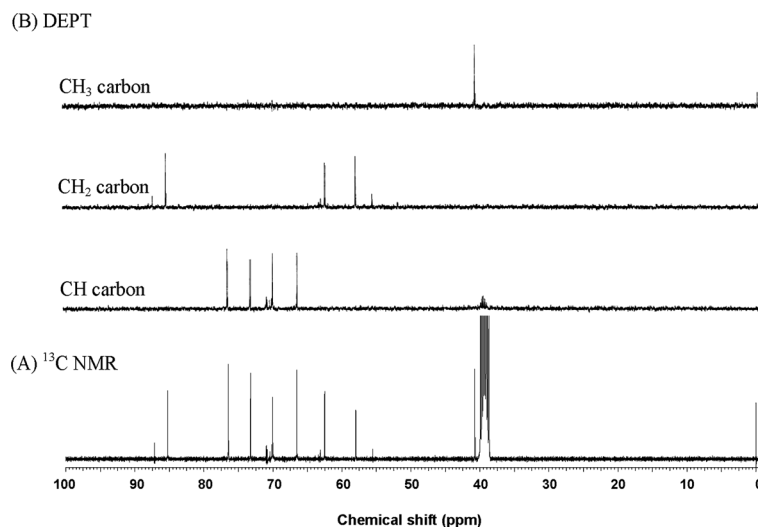Fig. 3. ^{1}H-NMR Spectrum of the Reaction Product between Formaldehyde and Meglumine

Fig. 4. (A) ^{13}C-NMR and (B) DEPT Spectra of the Reaction Product between Formaldehyde and Meglumine

neither presence of aromatic nor olefin hydrogen that are usually found between about 4.5 ppm and 8.0 ppm. Based on the two-dimensional NMR experiments of H–H COSY, HSQC and HMBC spectra (data are not shown), ^{1}H- and ^{13}C-NMR signals were assigned and the structure of the reaction product was determined to be a compound having a 1,3-oxazinane skeleton as presented in Table 2. It was presumed that formaldehyde reacted with secondary amino group in meglumine to form the reaction product *via* an iminium salt intermediate by cyclization.

Conclusions

Formaldehyde has been proved to be generated by various pharmaceutical excipients commonly used for oral solid dosage forms such as lactose, D-mannitol, microcrystalline cellulose, L-HPC, magnesium stearate and light anhydrous silicic acid during the storage of these materials. After investigating the formaldehyde absorbing ability of various amines, only meglumine proved to be able to reduce formaldehyde levels. The structure of the reaction product between formaldehyde and meglumine was clarified by ^{1}H-, ^{13}C-NMR, various kinds of two-dimensional NMR and mass spectroscopy. The reaction product was determined to be a compound with a 1,3-oxazinane skeleton and containing one more carbon than meglumine. It was presumed that formaldehyde reacted with secondary amino group in meglumine to form the reaction product *via* an iminium salt intermediate by cyclization. As meglumine is permitted to be used as a pharmaceutical excipient in both oral and parenteral dosage forms by regulations worldwide, the addition of meglumine to pharmaceutical products can be expected to contribute to the stabilization of many drug substances.

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