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Failure of stability prediction for minodronic acid injectable by accelerated stability testing

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Abstract

A liquid formulation containing 0.5 mg/ml minodronic acid, 40 mM, pH 4.5, citrate, and sodium chloride added to adjust the osmolarity of the final formulation was stored in flint glass ampoules at 25, 40, 50, and 60 °C. At specified times, the drug potency and pH, and the tendency to generate particulate matter, were measured. Test samples stored at 40 °C for 6 months or at 50 and 60 °C for 3 months were stable with no potency loss and no particulate increase. However, despite the satisfactory stability at high temperatures, the amount of particulate matter increased when the formulation was stored at 25 °C. Scanning electron microscopy-energy dispersive X-ray analysis of the particulate matter revealed that it contains aluminum and phosphorus, the latter thought to be derived from minodronic acid. In contrast, the number of the particulate matter did not increase, when the formulation was stored in either plastic containers or in SiO_2-treated glass ampoules. The spike of minodronic acid solution with aluminum ions led to the particulate generation. These results demonstrate that the particulate matter is a complex of minodronic acid molecules and aluminum ions, which apparently leached from the glass of regular ampoules. Since the particulate generation could not be observed at higher temperatures, it was suggested that the complex formation was exothermic and accelerated testing did not predict the stability in terms of particulate generation.

Keywords: Minodronic acid; Parenteral formulation; Stability; Particulate matter; Complex; Aluminum ion

1. Introduction

Pharmaceutical scientists routinely conduct 'accelerated testing' or 'stress testing' for a candidate formulation under exaggerated storage conditions to predict the stability of the formulation. This testing is designed to increase the degradation rate of the drug product to obtain information more quickly, allowing for rapid screening of stable, safe formulations.

Minodronic acid hydrate, [1-hydroxy-2-(imidazo[1,2-*a*]pyridin-3-yl)ethylidene]bisphosphonic acid monohydrate, is a new bisphosphonate which

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is expected to be clinically useful in the treatment of osteoporosis and hypercalcemia. In animal studies, this compound inhibits bone resorption with 100-fold greater potency than pamidronate (Kudo et al., 1992), and has 10-times greater efficacy than incadronate disodium, disodium cycloheptylaminomethylenediphosphonate monohydrate (Kudo et al., 1990, 1992).

As previously reported (Nakamura et al., 2001), preliminary formulation studies for minodronic acid injectables indicated that 10 mM or higher citrate buffers with pHs ranging from 3 to 5 optimized the stability of minodronic acid in solution. In unstable formulations of the pH 6 and 7, white precipitates were visually observed after storage for 4 weeks at 60 °C, which were presumed to be a complex of minodronic acid and aluminum ions apparently leached from the glass of the ampoules.

In this study, the formulation, which contains 0.5 mg/ml minodronic acid, 40 mM, pH 4.5 citrate buffer, and sodium chloride to adjust the osmolarity suitable for human use, was chosen for further stability evaluation. Under stress conditions (40–60 °C), the formulation was stable with no drug loss and no particulate increase. When stored at 25 °C, however, the particulate increased unexpectedly at the 5 month time-point, which was detected by a particle counter, but not by visual inspection. The purpose of the present study is to investigate the cause-and-effect relationship between the particulate formation and aluminum ions, using plastic containers to minimize the aluminum existence in the formulation or deliberately spiking with aluminum ions. Although aluminum ions play key roles in the particulate formation obviously, its existence did not simply lead to the particulate formation. We also discuss the relationships between the particulate formation and the storage temperature. The unexpected results of the current study provide us with important suggestions on feasibility of accelerated tests routinely accepted for pharmaceutical scientists.

2. Materials and methods

2.1. Materials

Minodronic acid hydrate was synthesized by Yamanouchi Pharmaceutical Co., Ltd. Citric acid monohydrate (analytical grade) was purchased from Komatsuya Chemical Co., Ltd. (Wakayama, Japan); sodium chloride and sodium hydroxide (analytical grade) were from Iwai Chemicals Company (Tokyo, Japan). An atomic absorption standard for Al^{3+} (1000 ppm, $AlCl_3$ in 1N HCl) was obtained from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Hydrochloric acid for atomic absorption measurement was from Kanto Chemical Co., Inc. (Tokyo, Japan). All other reagents were analytical grade chemicals.

Regular 2-ml flint glass ampoules were obtained from Japan Glass Industry Co., Ltd. (Tokyo, Japan). Two-milliliter flint glass ampoules, whose inner surface had been coated with SiO_2, were purchased from Fuji Glass Co., Ltd. (Tokyo, Japan). Daikyo Resin CZ vials and Formulation No. 777 rubber closures, which have extremely low amounts of extractable metal ions, were obtained as plastic containers from Daikyo Seiko, Ltd. (Tokyo, Japan).

2.2. Preparation of the minodronic acid solution

The formulation contained 0.5 mg/ml minodronic acid, 40 mM, citrate as a pH 4.5 buffer, and sodium chloride added to adjust the osmolarity of the final formulation. The necessary amount of each ingredient was dissolved in water for injection at approximately 80% of the final volume. The pH value of the formulation was adjusted to 4.5 with a 1N sodium hydroxide solution. The minodronic acid solution was brought to the final volume with water and filter sterilized through a 0.2 μm filter. The formulation was filled into clean containers (an ampoule or a vial); these containers were sealed, and sterilized in an autoclave at 115 °C for 30 min.

2.3. Stability evaluation

The samples were stored in a temperature-controlled incubator. At appropriate time intervals, the samples were withdrawn and cooled, if necessary, to ambient temperature to test the pH and remaining minodronic acid content of, and particulate matter in, each sample.

2.4. pH measurement

The pH of minodronic acid solutions was measured using an HM-26S pH meter (TOA Electronics Ltd.; Tokyo, Japan) or the equivalent.

2.5. High performance liquid chromatography

High performance liquid chromatography (HPLC) analysis was performed to measure minodronic acid stability. A Shimazu chromatographic system was used; it comprised an LC 9A pump, an SIL 6A autosampler, an SPD UV detector, and a C-R4A integrator (Shimazu; Kyoto, Japan) or the equivalent. Chromatography was conducted using a Develosil ODS-5 column (150×4.6 I.D. mm, 5 μm; Nomura Chemical; Tokyo, Japan). The column temperature was maintained at 25 °C. The sample volume was 10 μl, and minodronic acid was detected by its absorbance at 226 nm. The flow rate was 1.2 ml/min.

The mobile phase consisted of a 5:95 (v/v) methanol–10 mM sodium pyrophosphate solution containing 1 mM tetra-*n*-butylammonium phosphate, adjusted to pH 7.6 with orthophosphoric acid. The mobile phase was filtered and degassed before use.

2.6. Visual inspection and particle counting

Visual inspection of samples was made according to Method 1, Foreign Insoluble Matter Test for Injections, stipulated in the Pharmacopoeia of Japan.

The number of particulate matter in minodronic acid solutions was determined with a HIAC/ROYCO Model 4100 particle counter, a Model 3000 syringe controller, an HRLD-150 sensor (Pacific Scientific; Menlo Park, CA) and a DPU-411 Type II thermal printer (Seiko Instruments Inc.; Chiba, Japan). A sample collection probe was inserted directly into each open container, and the solution within was drawn into the HIAC system and analyzed.

2.7. Scanning electron microscopy-energy dispersive X-ray analysis

The sample solution was passed through a 0.2 μm filter to isolate the particulate. The filter that captured the particulate was dried in a desiccator. The particulate and the filter supporting it were carbon-film-coated with a JEC-550 twin coater in preparation for scanning electron microscopy–energy dispersive X-ray (SEM/EDX) analysis which was performed on a JEOL analytical system (JEOL Ltd.; Tokyo, Japan). The preparations were examined with a JSM-5400 scanning microscope to select a suitable object for EDX analysis. The conditions for SEM were as follows: 15 kV electron beam potential, a 20 mm working distance, and a back-scattered electron (BSE) detector. EDX analysis was performed with a JED-2001 EDX microanalyzer to identify the partial elemental content of the particulate. A 20 kV electron beam was used to probe the sample, and a SiLi detector was used to detect the X-rays generated by the particulate.

2.8. Quantification of aluminum in the minodronic acid solution

The aluminum content of the samples was assessed with a SpectrAA 300 (Varian, Inc.; Victoria, Australia) atomic absorption spectrometer. The polypropylene flasks and pipettes used to prepare test and standard solutions were rinsed with 0.1N hydrochloric acid before use to prevent aluminum contamination. The sample injection volume was 10 μl. Aluminum line at 309.3 nm with a slit width of 0.5 nm was monitored. The lamp current was 10 mA. The graphite furnace method conditions are given in Table 1. Argon was used as the inert gas to remove the sample components from the atomizer at each stage of the analysis and to protect the graphite tube from oxidation.

Table 1
The graphite furnace method conditions for aluminum measurement by atomic absorption

Step no.	Temperature (°C)	Time (s)	Internal gas flow (l/min)
1	100	15	3
2	120	45	3
3	200	5	3
4	700	5	3
5	1000	5	3
6	1400	5	3
7	1400	5	3
8	2600	1	0
9	2600	2	0
10	2700	2	3

2.9. Addition of aluminum ions to minodronic acid solution

It was reported that bisphosphonates form complexes with metal ions (Claessens and van der Linden, 1984; Lamson et al., 1984). In this study, to evaluate the effect of particulate formation of minodronic acid solution, Al^{3+} was deliberately added to the formulation. Two drug solutions of 300 ml each were prepared. Three milliliters of the 1000 ppm Al^{3+} standard solution was added to one of the drug solutions to yield a final metal ion concentration of about 10 ppm. The control solution containing no metal ion was constructed by adding 3 ml of water to the other 300 ml volume of the formulation. The formulations were sterilized through a 0.2 μm filter and were filled into glass ampoules, which were sealed, and sterilized in an autoclave at 115 °C for 30 min. The samples were then analyzed for the remaining drug concentration and the amount of the particulate matter present after storage for 1 week at ambient temperature.

3. Results and discussion

3.1. Stability of the minodronic acid formulation

The stability of the formulation, which was filled in a regular 2-ml flint glass ampoule, was evaluated under accelerated conditions. The stability of the formulation is shown in Table 2. Under all temperature conditions selected for this study, each test item such as pH, remaining drug content, or particulate count, remained unchanged for designated periods, compared with the initial values. These results led to the prediction that the formulation would also be stable at lower temperatures with no change in tested items.

However, the number of particulate matter in the formulation increased when it was stored at 25 °C, which is generally accepted for a standard storage temperature of pharmaceutical products. This increase was detected by a particle counter, but not by visual inspection under white light with an illuminance of 1000 lx. Particulate generation

Table 2
Stability of the minodronic acid solution under high temperature conditions

Temperature (°C)	Items tested	Time		
		Initial	3 months	6 months
40	pH	4.5	4.5	4.5
	Minodronic acid remaining (%)	100	100.4	100.1
	Particulate (number/ml)	<50	<50	<50
50	pH	4.5	4.5	nt
	Minodronic acid remaining (%)	100	100.3	nt
	Particulate (number/ml)	<50	<50	nt
60	pH	4.5	4.5	nt
	Minodronic acid remaining (%)	100	99.0	nt
	Particulate (number/ml)	<50	<50	nt

Minodronic acid remaining is expressed as the mean of two determinations. nt, not tested.

Table 3
Particulate (≥ 2 μm) generation in various containers during storage at 25 °C

Period (months)	0	1	2	3	4	5	6	7	8	9	10	11	12
Regular ampoule	−	−	−	−	−	++	+++	++	+++	++	+++	++	+++
SiO_2-treated ampoule	−	nt	nt	−	nt	nt	−	nt	nt	−	nt	nt	−
Plastic vial	−	nt	nt	−	nt	nt	−	nt	nt	−	nt	nt	−

The symbols indicate particulate counts per milliliter as follows: − (0–49), + (50–149), ++ (150–299), +++ (≥ 300). nt, not tested.

in regular flint ampoules during storage at 25 °C is shown in Table 3. The table indicates a sudden increase in particulate count at the 5 month timepoint. The rest of test items were stable even at the 12 month timepoint; the remaining amount of minodronic acid was 99.3% and the pH value was 4.5. Apparently, based on results that the drug content loss was not observed under high temperatures, 40, 50, and 60 °C up to 3 months (Table 2), it is suggested that minodronic acid is thermally stable. However, a very small amount of the degraded product of minodronic acid might initiate the particle formation.

3.2. Chemical composition of the particulate detected in the formulation

The results of previous stress stability studies conducted at 60 °C revealed that the optimum pH range for minodronic acid stability in citrate buffer is from 3 to 5 (Nakamura et al., 2001). However, minodronic acid is unstable in solutions with pH values of 6 to 7; storage of these solutions in glass ampoules yields a readily visible white precipitate (Nakamura et al., 2001). The results of SEM/EDX analysis indicated the precipitate is a complex of minodronic acid molecules and aluminum ions, the latter thought to leach from the glass of the ampoules (Hoiberg, 1989; Pavanetto et al., 1989). Therefore, the particulate generated in the pH 4.5 citrate formulation used in this study is also assumed to be a complex of minodronic acid molecules and aluminum ions. This hypothesis was supported by the elemental composition determined by SEM/EDX analysis. The results revealed that in addition to C, O, Na, and P, which are obviously derived from the ingredients of the formulation, the particulate also contains aluminum, which is the same as the results for precipitates analyzed during previous studies (Nakamura et al., 2001). Therefore, it is suggested that the white precipitate and the particulate detected in this study are chemically the same as those described in previous studies, even though the particulate in this study was unable to be detected by visual inspection.

Two different containers were tested to confirm the cause-and-effect relationship between aluminum leaching from the glass and particulate formation; the first container was a plastic vial, which has an extremely small amount of metal ions, and the other container was a glass ampoule with SiO_2-coated inner surfaces to reduce the leaching of metal ions. As expected, an increase in the amount of particulate matter was not observed in either kind of container; the number of ≥ 2 μm particulate remained unchanged even at the 12 month timepoint after storage at 25 °C (Table 3). This result supports an assumption of the key role aluminum leaching from glass ampoules plays in particulate formation.

The aluminum concentration of formulation samples, which were stored in regular flint glass ampoules at 25 °C, was measured at some timepoints (Fig. 1). The concentration in the formulation increased almost linearly over time. Judging from this diagram, the concentration of aluminum at the 5 month timepoint, when the sudden increase of ≥ 2 μm particulate was first recognized, was approximately 0.5 ppm. This concentration was thought to be a threshold for the detection of the particulate. Therefore, it is thought that reducing aluminum ion concentration to less than 0.5 ppm is the first step in preventing particulate formation.

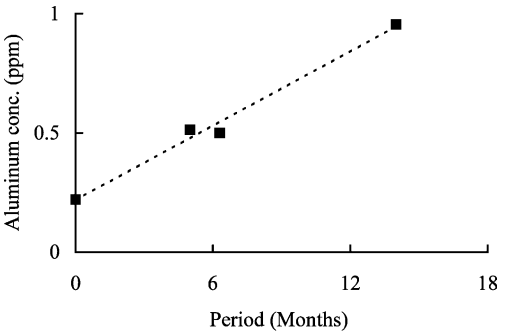

Fig. 1. The aluminum concentration in a minodronic acid–40 mM, pH 4.5, citrate solution during storage at 25 °C in regular flints glass ampoules. Each point represents the mean of two determinations.

As part of the investigation over the cause-and-effect relationship between the particulate formation and aluminum ions, excessive amounts of aluminum ions were deliberately added to minodronic acid solution to see if the particulate will generate and how much the drug loss will be observed. The sample solution spiked with Al^{3+} showed almost the same drug concentration as the samples treated with water (Table 4). However, the number of ≥ 2 μm particulate in the Al^{3+}-treated solution was almost 100 times greater than in the solution treated with water (Table 4). The results indicate that only a tiny amount of minodronic acid takes part in the particulate formation, even though the number of particulate matter produced appears large.

Table 4
The effect of aluminum ions on the stability of minodronic acid in solution after storage for 1 week at ambient temperature

Metal ion added	Particulate (number/ml)	Minodronic acid concentration (mg/ml)
Control (nothing added)	35 ± 10	0.50
Al^{3+}	3622 ± 972	0.49

The amount of particulate is expressed as the mean ± S.E. of, at least, seven determinations. Minodronic acid concentration is expressed as the mean of two determinations.

One question remains: why the aluminum–minodronic acid complex appeared as the detectable particulate when samples were stored at 25 °C for 5 months, even though no particulate increase was observed when samples were stored at 40 °C for 6 months. This observation is counter to expectation since the amount of aluminum leaching from glass ampoules is larger at higher storage temperatures. In this study, the aluminum concentration in solution after storage at 40 °C for 6 months was approximately 1.6 ppm, which was approximately three times greater than 0.5 ppm, the concentration in samples stored at 25 °C for 5 months. This demonstrates that a sufficient number of aluminum ions was present in the samples stored at 40 °C to form the complex. One of the possible answers is that the complex was once formed at higher temperatures following the endothermal reaction, but was partially cleaved due to high temperatures. As a result, the particulate became soluble. However, this hypothesis is denied by a previous report that the complex is relatively resistant to heat and the chemical structure of minodronic acid is maintained in the complex (Nakamura et al., 2001). The other is that since the reaction of the complex formation is exothermal, it does not proceed at higher temperatures. The similar reaction reported is that the complexation of Ca(II) with citric acid etc. is dependent on temperature and the complexation became weak with temperature (Hasegawa et al., 1982). Therefore, it is speculated that the particulate generation did not occur under these stress conditions, even though aluminum ions sufficiently leached after exposure to higher temperatures. In this sense, 25 °C may be a good temperature to form the particulate because it is high enough to leach aluminum from glass surfaces but is a low enough temperature to allow the complex to form and to produce the particulate. Aluminum concentration and storage temperatures may be important factors for the particle generation. Although more study is needed to elucidate the mechanism of this process, this study certainly raises a question on the

conventional stability prediction in terms of particulate generation.

4. Conclusions

The formulation containing 0.5 mg/ml minodronic acid, 40 mM, pH 4.5, citrate as a buffer, and sodium chloride added to adjust the osmolarity of the formulation was stable in regular 2-ml flint glass ampoules after storage for 6 months at 40 °C or, for 3 months at 50 and 60 °C. However, the increase of ≥ 2 μm particulate was detected by a particle counter after storage for 5 months at 25 °C. The SEM/EDX analysis revealed that the particulate contains aluminum and phosphorus: the latter was apparently derived from minodronic acid. The number of particulate matter did not increase, when the formulation was stored in either plastic containers, which have extremely low amounts of extractable metal ions, or in SiO_2-treated glass ampoules. The spike of minodronic acid solution with aluminum ions led to the particulate generation. It was demonstrated that the particulate was a complex of minodronic acid molecules and aluminum ions, which leached from the glass of untreated ampoules. It is suggested that the complex formation is exothermal and accelerated testing does not predict the particulate generation.

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References

Claessens, R.A.M.J., van der Linden, J.G.M., 1984. Stability constants of tin(II) and calcium diphosphonate complexes. J. Inorg. Biochem. 21, 73–82.

Hasegawa, K., Hashi, K., Okada, R., 1982. Physicochemical stability of pharmaceutical phosphate buffer solutions. I. Complexation behavior of Ca(II) with additives in phosphate buffer solutions. J. Parenter. Sci. Technol. 36, 128–133.

Hoiberg, C.P., 1989. Aluminum in parenteral products: overview of chemistry concerns and regulatory actions. J. Parenter. Sci. Technol. 43, 127–131.

Kudo, M., Abe, T., Kawamuki, K., Yamaoka, E., Isomura, Y., Takeuchi, M., Kawashima, H., 1990. Effect of YM175 on experimental hypercalcemia and tumor-induced osteolysis in rats. J. Bone Mineral Res. 5, S166.

Kudo, M., Abe, T., Motoie, H., Nagao, Y., Kawamuki, K., Ouchi, N., Kawashima, H., 1992. Pharmacological profile of new bisphosphonate, 1-hydroxy-2-(imidazo[1,2-*a*]pyridin-3-yl)ethane-1,1-bis(phosphonic acid). Bone Mineral 17, 170.

Lamson, M.L., Fox, J.L., Higuchi, W.I., 1984. Calcium and 1-hydroxyethylidene-1,1-bisphosphonic acid: polynuclear complex formation in the physiological range of pH. Int. J. Pharm. 21, 143–154.

Nakamura, K., Tanaka, T., Saito, K., Yokohama, S., Sonobe, T., 2001. Stabilization of minodronic acid in aqueous solution for parenteral formulation. Int. J. Pharm. 222, 91–99.

Pavanetto, F., Genta, I., Conti, B., Modena, T., Montanari, L., 1989. Aluminum, cadmium and lead in large volume parenterals: contamination levels and sources. Int. J. Pharm. 54, 143–148.