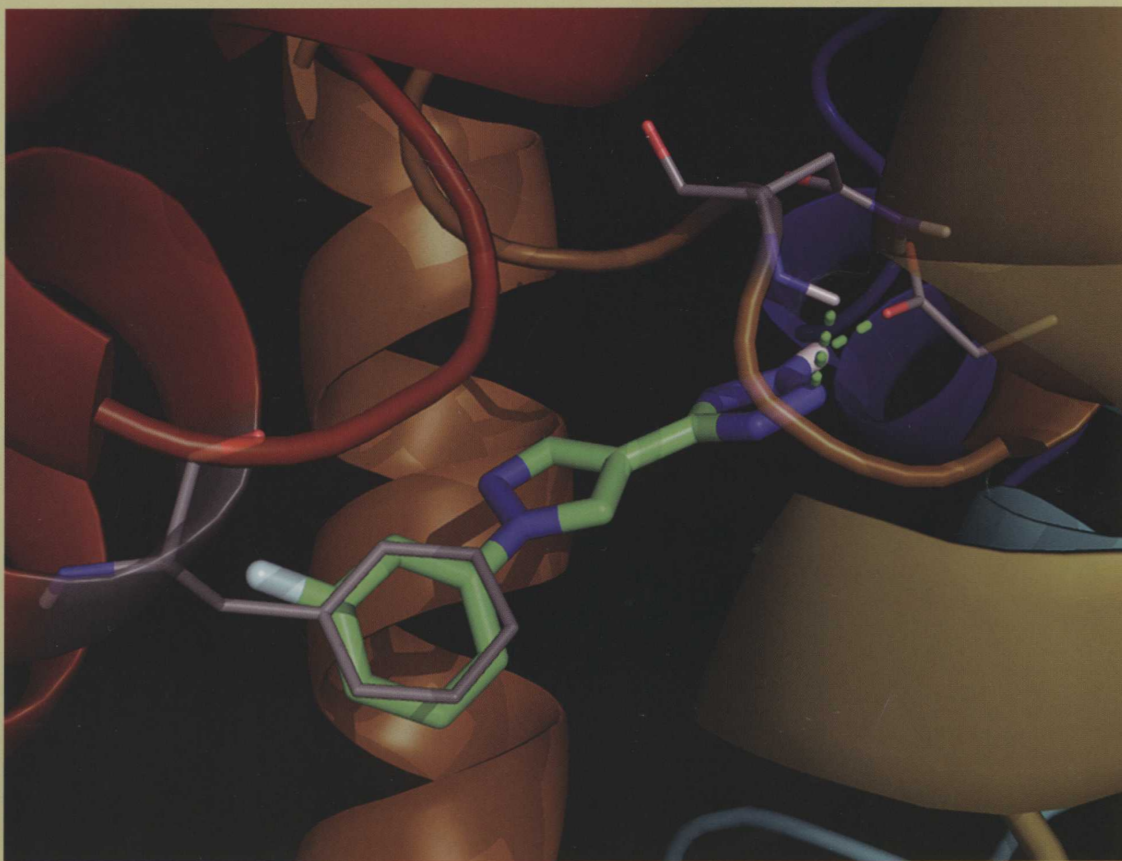


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Vol. 61 No. 5



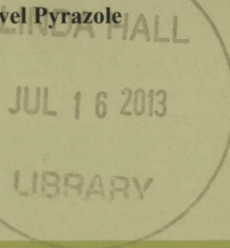
Synthesis, Docking Studies, Pharmacological Activity and Toxicity of a Novel Pyrazole Derivative (LQFM 021)—Possible Effects on Phosphodiesterase

pp. 524–531



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Journal History

The Pharmaceutical Society of Japan (PSJ) has been devoted to promoting the pharmaceutical sciences since its establishment in 1880. The PSJ launched its official journal, the *Yakugaku Zasshi*, in Japanese in 1881, and the English-language *Pharmaceutical Bulletin* in 1953. The *Pharmaceutical Bulletin* continued as the *Chemical & Pharmaceutical Bulletin* from 1958 through 2011. The *Journal of Pharmacobio-Dynamics*, which started publication in 1978, was succeeded by the *Biological & Pharmaceutical Bulletin* from 1993 through 2011. The *Journal of Health Science* (JHS) originated from the *Kousyuu-Eisei Nenpou* (Annual reports of the Public Health Division of the Pharmaceutical Society of Japan) in 1953 and was succeeded by the JHS (1999–2011) through *Eiseikagaku* (Journal of Hygienic Chemistry; Japanese Journal of Toxicology and Environmental Health) (1956–1998). The PSJ has decided to reorganize its journal systems to provide research-related information more efficiently and to showcase the latest achievements in the pharmaceutical sciences, which continue to expand rapidly in scope and scale. Under a unified editorial system, PSJ journals started to be published from January 2012 as the new *Chemical and Pharmaceutical Bulletin* (CPB) and *Biological and Pharmaceutical Bulletin* (BPB), both in English, with *Yakugaku Zasshi* as a Japanese-language journal. Most of the fields and subjects published previously in the JHS continue in the BPB, and some are taken over by the CPB.

Scope of the CPB and BPB

The CPB covers chemical fields in the pharmaceutical and health sciences, ranging from synthetic chemistry to medicinal chemistry. The BPB covers various biological topics in the pharmaceutical and health sciences, including basic and clinical applications. For details of their scope, please refer to the submission topic categories below.

Submission Topic Categories

1. Organic chemistry; 2. Medicinal chemistry; 3. Chemical biology; 4. *In silico* science; 5. Analytical chemistry; 6. Physical chemistry; 7. Inorganic chemistry; 8. Physical pharmacy; 9. Pharmaceutical engineering; 10. Pharmacognosy; 11. Natural product chemistry; 12. Epidemiology; 13. Health statistics; 14. Toxicology; 15. Hygiene; 16. Forensic science; 17. Environmental science; 18. Regulatory science; 19. Biochemistry; 20. Molecular and cellular biology; 21. Microbiology; 22. Pharmacology; 23. Biopharmacy and pharmacokinetics; 24. Biogenetic engineering; 25. Clinical pharmacy; 26. Clinical-bioanalytical chemistry; 27. Pharmaceutical care and science; 28. Pharmaceutical education; and 29. Miscellaneous.

The publication of this journal has been supported in part by a Grant-in-Aid for Publication of Scientific Research Results from the Japan Society for the Promotion of Science (JSPS), which is gratefully acknowledged.

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Vol. 61, No. 5 May 2013

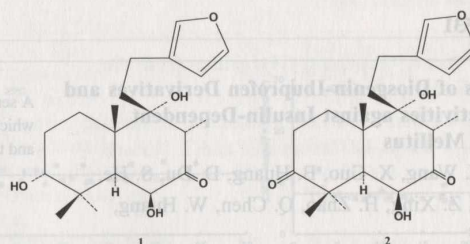
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Contents

Regular Articles

Two New Labdane Diterpenes from Fresh Leaves of *Leonurus japonicus* and Their Degradation during Drying

H. Fuchino, A. Daikonya, T. Kumagai, Y. Goda, Y. Takahashi, and N. Kawahara

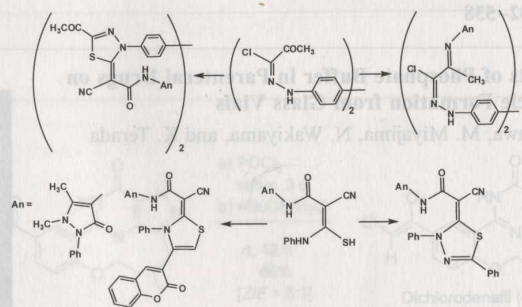


Compound 1 and 2 were isolated from *Leonurus* Herb. They immediately decomposed even at 40 °C in chloroform solution.

pp. 497–503

Azoles and bis-Azoles: Synthesis and Biological Evaluation as Antimicrobial and Anti-cancer Agents

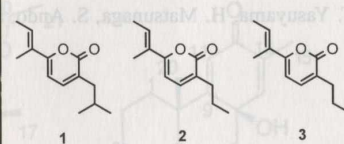
N. A. Kheder, S. M. Riyadh, and A. M. Asiry



pp. 504–510

Nocapyrones H–J, 3,6-Disubstituted α -Pyrones from the Marine Actinomycete *Nocardopsis* sp. KMF-001

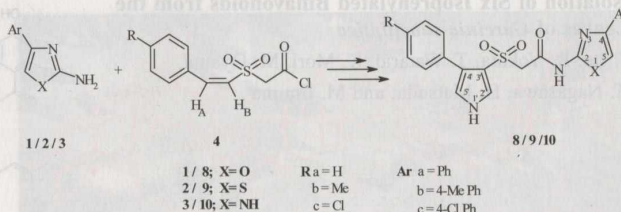
M. C. Kim, O.-W. Kwon, J.-S. Park, S. Y. Kim, and H. C. Kwon



pp. 511–515

Synthesis and Antimicrobial Activity of Azole Derivatives

T. Bhanu Prakash, L. Mallikarjuna Reddy, A. Padmaja, and V. Padmayathi

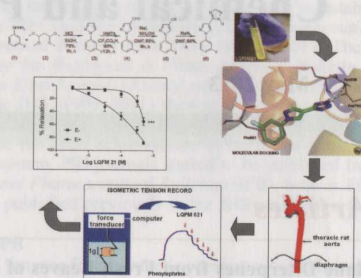


pp. 516–523

Synthesis, Docking Studies, Pharmacological Activity and Toxicity of a Novel Pyrazole Derivative (LQFM 021)—Possible Effects on Phosphodiesterase

D. Ramos Martins, F. Pazini, V. de Medeiros Alves, S. Santana de Moura, L. Morais Lião, M. Torquato Quezado de Magalhães, M. Campos Valadares, C. Horta Andrade, R. Menegatti, and M. Lavorenti Rocha

pp. 524–531

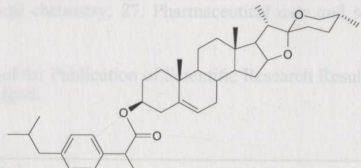


Synthesis of Diosgenin-Ibuprofen Derivatives and Their Activities against Insulin-Dependent Diabetes Mellitus

G. Xin, Y. Wang, X. Guo, B. Huang, D. Du, S. He, R. Zhang, Z. Xing, H. Zhao, Q. Chen, W. Huang, and Y. He

pp. 532–538

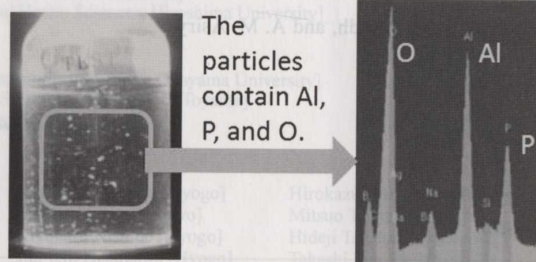
A series of new type of diosgenin-ibuprofen derivatives have been synthesized, one of which was found to have good anti-inflammatory effect on RAW264.7 cells *in vitro*, and to reduce the risk of NOD mice to develop type 1 diabetes significantly *in vivo*.



Effects of Phosphate Buffer in Parenteral Drugs on Particle Formation from Glass Vials

T. Ogawa, M. Miyajima, N. Wakiyama, and K. Terada

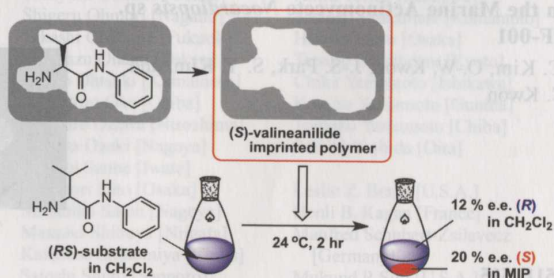
pp. 539–545



A Study of the Various Factors That Affect the Properties of Molecularly Imprinted Polymers

T. Yasuyama, H. Matsunaga, S. Ando, and T. Ishizuka

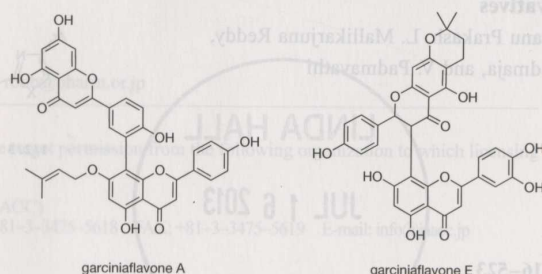
pp. 546–550



Isolation of Six Isoprenylated Biflavonoids from the Leaves of *Garcinia subelliptica*

T. Ito, R. Yokota, T. Watarai, K. Mori, M. Oyama, H. Nagasawa, H. Matsuda, and M. Iinuma

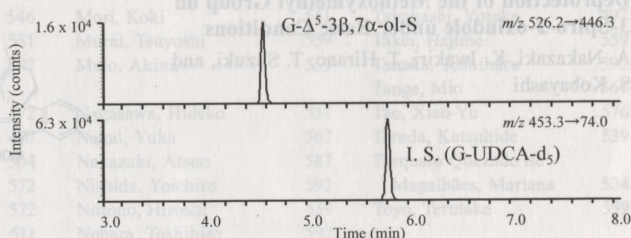
pp. 551–558



Measurement of Transport Activities of 3β -Hydroxy- Δ^5 -bile Acids in Bile Salt Export Pump and Multidrug Resistance-Associated Proteins Using LC-MS/MS

T. Murai, K. Oda, T. Toyo, H. Nittono, H. Takei, A. Muto, A. Kimura, and T. Kurosawa

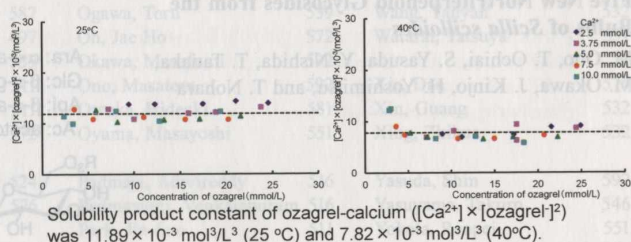
pp. 559–566



Prediction of Compatibility between Ozagrel Sodium Preparation for Injection and Calcium on the Basis of the Solubility Product

M. Tange, M. Yoshida, M. Hazekawa, T. Haraguchi, Y. Nakai, and T. Uchida

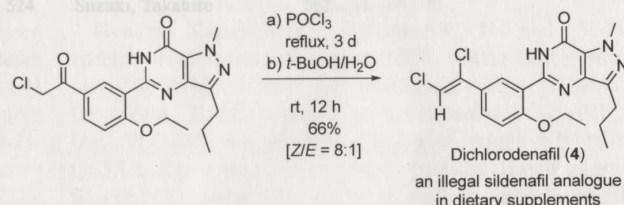
pp. 567–571



Notes

Efficient Synthesis of Dichlorodenafil, an Unapproved Sildenafil Analogue Appearing in Non-prescription Supplements

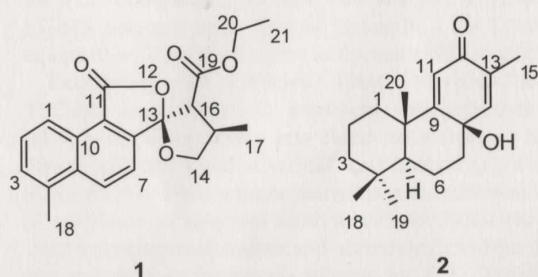
J. Y. Kim, I. G. Hwang, J. H. Oh, I. H. Kang, S. W. Kwon, and D. Kim



pp. 572–575

Two New Diterpenoids from Cell Cultures of *Salvia miltiorrhiza*

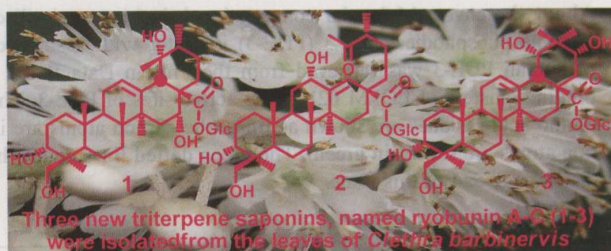
D.-W. Zhang, X. Liu, D. Xie, R. Chen, X.-Y. Tao, J.-H. Zou, and J. Dai



pp. 576–580

Structures of Ryobunins A–C from the Leaves of *Clethra barbinervis*

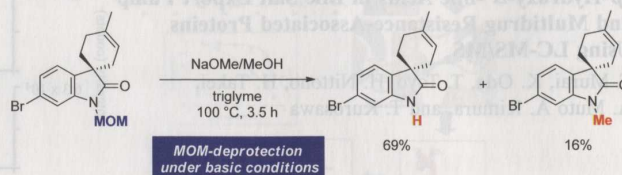
S. Sugimoto, K. Matsunami, and H. Otsuka



pp. 581–586

Deprotection of the Methoxymethyl Group on 3-Spiro-2-oxindole under Basic Conditions

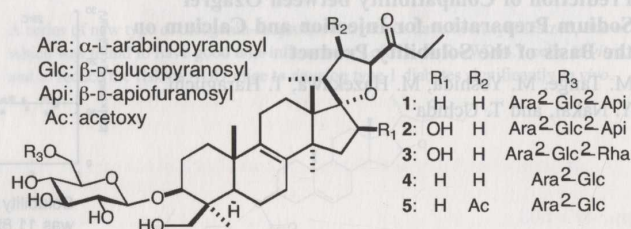
A. Nakazaki, K. Iwakiri, T. Hirano, T. Suzuki, and S. Kobayashi



pp. 587–591

Five New Nortriterpenoid Glycosides from the Bulbs of *Scilla scilloides*

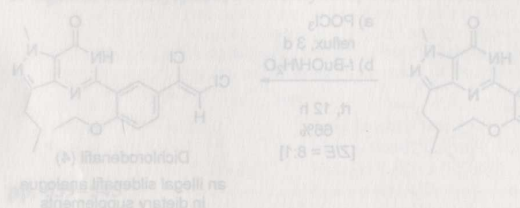
M. Ono, T. Ochiai, S. Yasuda, Y. Nishida, T. Tanaka, M. Okawa, J. Kinjo, H. Yoshimitsu, and T. Nohara



pp. 592–598

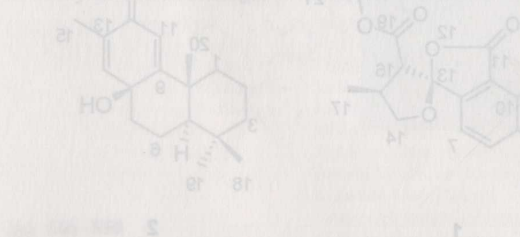
Effects of Phosphate Buffer in Parenteral Drugs on Particle Formation from Glass Vials

T. Ogawa, M. Miyajima, N. Wakiyama, and K. Torada



A Study of the Various Factors That Affect the Properties of Molecularly Imprinted Polymers

T. Yasuyama, T. Kishida, S. Agata, and T. Shimizu



About the cover: Molecular computational docking study-discovery of a new pyrazole derivative (LQFM 021), a possible phosphodiesterase-3 (PDE-3) inhibitor with vasorelaxant activity and low toxicity. The crystal structure of human PDE-3 was retrieved from the Protein Data Bank. This Figure shows the major energetically favored binding clusters of LQFM 021 in the active sites of PDE-3. The 3D representation of PDE-3 structures with select amino acid residues are shown as lines (the carbon atoms are in gray). The LQFM 021 is shown as sticks (the carbon atoms are shown in green). The green dotted lines denote hydrogen bonds. See the article by Ramos Martins *et al.* on page 524 of this issue.

Ando, Shin	546	Ishizuka, Tadao	546	Mori, Koki	551	Takahashi, Yutaka	497
Asiry, Ahlam Maade	504	Ito, Tetsuro	551	Murai, Tsuyoshi	559	Takei, Hajime	559
Bhanu Prakash, Talapur	516	Iwakiri, Kanako	587	Muto, Akina	559	Tanaka, Toshiharu	592
Campos Valadares, Marize	524	Kang, Il Hyun	572	Nagasawa, Hideko	551	Tange, Mio	567
Chen, Qianming	532	Kawahara, Nobuo	497	Nakai, Yuka	567	Tao, Xiao-Yu	576
Chen, Ridao	576	Kheder, Nabila Abdelshafy	504	Nakazaki, Atsuo	587	Terada, Katsuhide	539
Dai, Jungui	576	Kim, Deukjoon	572	Nishida, Yoichiro	592	Torquato Quezado de Magalhães, Mariana	524
Daikonya, Akihiro	497	Kim, Jong Yup	572	Nittono, Hiroshi	559	Toyo, Terutake	559
de Medeiros Alves, Vinicius	524	Kim, Min Cheol	511	Nohara, Toshihiro	592	Uchida, Takahiro	567
Du, Dan	532	Kim, Sun Yeou	511	Ochiai, Tetsuya	592	Wakiyama, Naoki	539
Fuchino, Hiroyuki	497	Kimura, Akihiko	559	Oda, Kana	559	Wang, Yanyan	532
Goda, Yukihiko	497	Kinjo, Junei	592	Ogawa, Toru	539	Watarai, Tatsuya	551
Guo, Xiurong	532	Kobayashi, Susumu	587	Oh, Jae Ho	572	Xie, Dan	576
Haraguchi, Tamami	567	Kumagai, Takeo	497	Okawa, Masafumi	592	Xin, Guang	532
Hazekawa, Mai	567	Kurosawa, Takao	559	Ono, Masateru	592	Xing, Zhihua	532
He, Shiliang	532	Kwon, Hak Cheol	511	Otsuka, Hideaki	581		
He, Yang	532	Kwon, Oh-Wook	511	Oyama, Masayoshi	551		
Hirano, Tomohiro	587	Kwon, Sung Won	572				
Horta Andrade, Carolina	524	Lavorenti Rocha, Matheus	524	Padmaja, Adivireddy	516	Yasuda, Shin	592
Huang, Baozhan	532	Liu, Xiao	576	Padmavathi, Venkatapuram	516	Yasuyama, Takuro	546
Huang, Wen	532	Mallikarjuna Reddy, Lingaladinne	516	Park, Jin-Soo	511	Yokota, Renpei	551
Hwang, In Gyun	572	Matsuda, Hideaki	551	Pazini, Francine	524	Yoshida, Miyako	567
Iinuma, Munekazu	551	Matsunaga, Hirofumi	546	Ramos Martins, Daniella	524	Yoshimitsu, Hitoshi	592
		Matsunami, Katsuyoshi	581	Riyadh, Sayed Mohamed	504	Zhang, De-Wu	576
		Menegatti, Ricardo	524	Santana de Moura, Soraya	524	Zhang, Rui	532
		Miyajima, Makoto	539	Sugimoto, Sachiko	581	Zhao, Hang	532
		Morais Lião, Luciano	524	Suzuki, Takahiro	587	Zou, Jian-Hua	576

In evaluations of the quality of "Yakumoso," it has been considered that it is better to choose a sample with a bluish color and to let it dry in the shade after harvesting.⁹ Several compounds have been reported to be constituents of Leonurus Herb. In particular, there have been many reports on the isolation of labdane-type diterpenes possessing furan and diepoxy moieties. According to recent reports, the furan-type labdane diterpenes are artifacts of diepoxy-type compounds.¹⁰ In addition to diterpenes, rutin,¹¹ a flavone glycoside, leonurine,¹² a bitter alkaloid, leonuridine¹³ and stachydrine¹⁴ have also been reported.

TLC-MS is a method for measuring MS directly after a sample is obtained on TLC. While the Direct Analysis in Real Time (DART) method¹⁵ is well known for TLC-MS, a new method that uses "TLC-MS interface" equipment,¹⁷ which is attached to an existing LC-MS system, has recently been described. This latter method is quite different from the former in principle. The TLC-MS interface is attached to LC-MS, and then the head is placed on a target spot on TLC. An eluent is then passed through the head and the substance absorbed on silica-gel is eluted from the silica-gel. Finally, the eluted compounds are sent to the MS instrument. Although each method has both advantages and disadvantages, TLC-MS is especially effective for measuring an MS spectrum directly without purification for target compounds that are unstable and readily decompose during purification under column chromatography.

In this paper, we report the structure elucidation of two new compounds found in fresh leaves of *L. japonicus* using TLC-MS and their degradation during drying.

General Experimental Procedures: ¹H- and ¹³C-NMR spectra were measured with a JEOL Alpha 300 spectrometer (300 MHz, ¹³C 75 MHz) was performed with a SHIMADZU QP 5050A. HPLC was run on a Shimadzu LC-10AVP system. TLC-MS was performed using an Applied Biosystems QSTAR XL with an atmospheric pressure chemical ionization (APCI) source, attached to an Agilent Technology HPLC system (Agilent 1260 Binary Pump system) and a CAMAG TLC-MS interface. A silica TLC plate (Art. 5715) was used for TLC comparison and HPLC-MS analysis. High-resolution LC-MS was run on a Thermo Scientific LTQ Orbitrap XL equipped with an electrospray ionization (ESI) source.

Extraction and Isolation: Plant Extraction for TLC, TLC-MS and GC-MS: *L. japonicus* was harvested in July 2009, washed, separated into three parts (leaves, stem and flower) and then dried at various temperatures (15, 40, 50, 70, 90°C) for 4 d. These sample materials were pulverized and 1 g of powdered sample was shaken with methanol (10 mL) for 10 min at room temperature and centrifuged, and the supernatant was used as the sample solution for TLC, TLC-MS, and GC-MS. These sample solutions were stored in a freezer when not in use.

Comparison of Components under Various Drying Conditions: Methanol extracts of each part under various drying temperatures were compared by TLC with the following conditions: developing solvent system: chloroform-methanol (5:1); detection: spraying with 10% sulfuric acid, then heating.

GC-MS was carried out under the following conditions: Column: SLB-5ms (0.32 mm i.d.×30 m) (SUPRECO); Column temperature: 90°C (15 min)→120°C (30 min)→150°C (35 min)→220°C (40 min)→275°C (40 min); Injection volume: 5 µL; Interface temperature: 280°C; Electron ionization (EI)-

The authors declare no conflict of interest.

vi

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Effects of Phosphate Buffer in Parenteral Drugs on Particle Formation from Glass Vials

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^aFormulation Technology Research Laboratories, Daiichi Sankyo Co., Ltd.; 1–12–1 Shinomiya, Hiratsuka, Kanagawa 254–0014, Japan; and ^bFaculty of Pharmaceutical Sciences, Toho University; 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan.

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The characteristics of inorganic particles generated in glass vials filled with phosphate buffer solutions were investigated. During storage, particles were visually detected in the phosphate buffer solution in particular glass vials which pass compendial tests of containers for injectable drugs. These particles were considered to be different from ordinal glass delamination, which has been reported in a number of papers because the particles were mainly composed of Al, P and O, but not Si. The formation of the particles accelerated at higher storage temperatures. Among the surface treatments tested for the glass vials, sulfur treatment showed a protective effect on the particle formation in the vials, whereas the SiO₂ coating did not have any protective effects. It was found that the elution ratio of Al and Si in the solution stored in the glass vials after the heating was similar to the ratio of Al and Si in borosilicate glass. However, the Al concentration decreased during storage (5°C, 6 months), and consequently, particle formation was observed in the solution. Adding citrate, which is a chelating agent for Al, effectively suppressed the particle formation in the heated solution. When 50 ppb and higher concentrations of Al ion were added to the phosphate buffer solution, the formation of white particles containing Al, P and O was detected. It is suggested that a phosphate buffer solution in a borosilicate glass vial has the ability to form particles due to interactions with the Al that is eluted from the glass during storage.

Key words aluminum; borosilicate glass; tubing vial; aluminum phosphate complex

The primary packaging system for a sterile drug product should provide adequate protection against any contamination from the external environment, and this protection should be assured during the entire shelf life of the product. In contrast, particularly for liquid, injectable drugs, components of the drugs are always in contact with the surface of the packaging component during storage. Therefore, during the course of formulation development of injectable drugs, it is essential to evaluate the physicochemical compatibility between the drug formulation and the packaging components to select appropriate primary packaging systems.^{1,2)}

Among the packaging materials that are compatible with injectable drug solutions, glass containers, such as vials or ampoules, have been widely chosen and used. For parenteral drug products, borosilicate glasses (Type I) composed principally of silicon dioxide and boric oxides and soda-lime glasses composed of relatively high levels of sodium oxide and calcium oxide are used. Borosilicate glasses in particular are known to have a good chemical durability and are used commonly in the pharmaceutical industry as the primary container. However, these glass containers, even when made from borosilicate glasses, will unavoidably suffer some undesirable events from being in contact with the drug solutions in long-term storage. In certain cases, insoluble particle formation occurs in glass containers when drug products are stored in glass containers. The formation of insoluble particles is one of the most critical incidents for pharmaceutical companies, resulting in recalls of their drug products from the market. To avoid these incidents, many researchers have investigated the causes of particle formation in glass containers, and glass vial manufacturers have developed several types of glass surface

treatment techniques. For example, sulfur treatment is known to prevent some particle formations caused by increasing pH of the drug solution. Otherwise, SiO₂ coating can suppress the contact between solution and glass surface containing some cations richly. However, these techniques are not enough to avoid particle formations thoroughly, because the mechanism of the particle formation is not completely understood.³⁾

Some researchers have reported that water can collapse the composition of glass under extreme conditions, and borosilicate glass is no exception.^{4–9)} In addition to water, other elements in drug solutions are known to induce the collapse of glass surfaces. Ennis *et al.*¹⁰⁾ evaluated the tendencies of delamination from borosilicate glass vials based on the fact that the cidofovir injectable drug product formed glass delamination, and they concluded that the high pH of the solution enhanced the corrosive attack on the inner surface of the glass container, resulting in delamination. Iacocca *et al.*^{11,12)} also investigated causes of glass delamination using some drug products with borosilicate glass vials, suggesting a scheme of corrosive attacks by the drug solution on the glass surface, which relates to the delamination. From these reports, the corrosive attacks by drug solutions on glass surfaces have induced delamination, and their study results are expected to eliminate the delamination potential of injectable drugs during future drug development. However, there are some reports of particle formation involving a mechanism other than delamination.¹³⁾ In addition to delamination, it is necessary to address the other particles that are formed as the result of interactions between the inner surface of the glass and the drug products.

Phosphate buffers are often present in injectable drug products, especially biological drugs, because the components of phosphate buffer are abundantly present in the human body, and phosphate ions have a wider range of buffering ability

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than other buffer solutions. However, it is empirically known that phosphate buffer is incompatible with glass vials and that particles are formed. However, no research papers have been found that thoroughly investigated this incompatibility of phosphate buffer with glass vials. Therefore, evaluating the suitability of phosphate buffer and glass containers is expected to provide useful information for future drug manufacturing.

In this study, we investigated the characteristics of the insoluble particles that formed from the interactions of phosphate buffer solution and borosilicate glass.

Experimental

Materials Dibasic sodium phosphate, monobasic sodium phosphate, sodium chloride, citric acid and sodium citrate were all special-grade reagents and were purchased from Kanto Chemical (Tokyo, Japan) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polysorbate 80 was JP, USP-NF and Ph. Eur compendium quality and was purchased from CRODA International (East Yorkshire, U.K.). The other chemicals were all commercially available, reagent-grade chemicals. All water used in this study was ultrapure water supplied by an ultrapure water system (Millipore, Billerica, MA, U.S.A.).

Borosilicate (Type I) tubing glass vials (colorless, 10mL) were obtained from three manufacturers (suppliers A, B and C) of glass vials. The glass vials are manufactured from glass tubes supplied by Nippon Electric Glass (Shiga, Japan). Plastic vials (Daikyo Crystal Zenith (CZ) resin, colorless, 10mL) were obtained from Daikyo Seiko (Tokyo, Japan).

For rubber closures, S10-F6, D777-1 and RB2-40 closures were obtained from Daikyo Seiko.

Phosphate Buffer Preparation All phosphate buffer solutions (16 or 20mm) were prepared by dissolving dibasic sodium phosphate and monobasic sodium phosphate in water and adjusting the pH to 7 with sodium hydroxide solution (1N or 0.1N). All of the buffer solutions contained 0.9% sodium chloride, which served as a tonicity agent. If necessary, polysorbate 80 (0.02%) was added to the solution.

Preparing Phosphate Buffer with Aluminum (Al) Ions The given concentration of Al ions was prepared by diluting an Al standard solution for inductively coupled plasma (ICP) 1000mg/L (Kanto Chemical) or an aqueous solution of aluminum chloride hexahydrate (0.895g/L; 100ppm as Al) with the buffer solution. To eliminate the amount of Al eluted from a glass vial, these solutions were stored in CZ plastic vials.

Preparing Phosphate Buffer with Various Concentrations of Citrate Mixtures of phosphate buffer and citrate buffer were prepared from 16mm phosphate buffer solution and 16mm citrate buffer solution. Phosphate buffer solution containing 0.9% sodium chloride and 0.02% polysorbate 80 was prepared as described above. Citrate buffer solution was also prepared by dissolving citric acid, sodium citrate, sodium chloride (0.9%) and polysorbate 80 (0.02%) in water and adjusting the pH to 7 with sodium hydroxide (1N or 0.1N). The two buffer solutions were mixed to make citrate buffer concentrations of 0.16, 0.33, 0.66 and 3.3mm.

Filling and Storage of the Sample Solution Rubber closures and plastic vials were washed with water and then autoclaved in a steam sterilizer (HV-25, Hirayama, Saitama, Japan) at 121°C for more than 30min. Glass vials were washed by water and then heat sterilized in a clean oven (DRC633FA,

Advantec, Tokyo, Japan). Sample solutions were filtered by a 0.22µm Millex GV Durapore membrane filter (Millipore); then, 10mL was added to each vial in a clean bench, and the vial was immediately stoppered and capped. Each filled sample was stored at the given conditions or heated to given conditions in the steam sterilizer.

Treating Particles with Citric Acid To confirm whether the particle contained Al, a 1M citric acid aqueous solution was added to the vial that contained the particles, and the vial was stored at 40°C for 2 days. The final citrate concentration in the vial was 20mm.

Visual Inspection Each sample vial was inspected for insoluble particulate matter under a 5000lux light condition.

Inductively Coupled Plasma (ICP) Analysis Al and Si concentrations were measured using ICP optical emission spectrometry (ICP-OES) (VISTA-PRO, Seiko Instruments, Chiba, Japan). To avoid effects of precipitating particles on measurement, each sample solution was filtered through a 0.45µm Millex HV Durapore membrane filter (Millipore) prior to the measurement. The filtrates were transferred into a polypropylene 15mL plastic tube (Becton Dickinson, Franklin Lakes, NJ, U.S.A.). Standard solutions (0.02–0.50ppm for Al and 0.2–10ppm for Si, respectively) were prepared by serial dilution of an ICP standard 1000mg/L of multi-element IV (including Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Mg, Mn, Na, Ni, Pb, Sr, Tl and Zn) and an ICP standard 1000mg/L of Si (Merck, Darmstadt, Germany) with 0.9% sodium chloride solution. The nominal detection wavelength for analysis was 396.152nm for Al and 251.611nm for Si.

Microscopic Observation Each sample solution that contained the particles was filtered by a 0.45µm nitrocellulose filter (HABG045, Millipore). After the filter was washed twice with equal volumes of water and sample solution and dried in ambient air, it was then observed with a digital microscope (VH-Z100UW and VHX-1000, Keyence, Osaka, Japan) at 200-fold magnification.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectrometry (SEM-EDX) Observation Each sample solution that contained the particles was also filtered by a 0.45µm silver filter (AG4502550, Millipore). The filter was washed twice with equal volumes of water and the sample solution and dried in ambient air and was observed and analyzed with SEM-EDX (JSM-5600LV JED-2140, JOEL, Tokyo, Japan).

Results and Discussion

The Observation of Particles in Phosphate Buffer in Glass Vials during Storage and an EDX Analysis of the Particles Table 1 shows the results of a visual inspection of the vials filled with phosphate buffer solution after storage at 40°C. The phosphate buffer solution (16mm, containing 0.02% polysorbate 80) simulated the placebo solution of a biological drug, and it was stored in glass vials manufactured by supplier A. Particles were detected in one of 10 vials after storage for 3 months, in 8 of 10 vials after 4 months and finally in all 10 vials after 6 months. These particles were transparent and reflected light irregularly. They were also observed as flakes in some of the vials because of their thinness (Fig. 1). With digital microscopic observation, numerous particles were found on the filter captured from one vial (Fig. 2).

These particles were also analyzed using EDX. The spectra

Table 1. Results of a Visual Inspection of Phosphate Buffer Solution in the Glass Vials Stored at 40°C

Storage period	Vials detected particles/vials inspected
2 months	0/10
3 months	1/10
4 months	8/10
6 months	10/10



Fig. 1. The Flake-Like Particles of Phosphate Buffer Solution in a Glass Vial

Phosphate buffer was 16mm, pH 7 and was contained 0.9% sodium chloride and 0.02% polysorbate 80. This photograph was taken after storage at 40°C for 6 months.

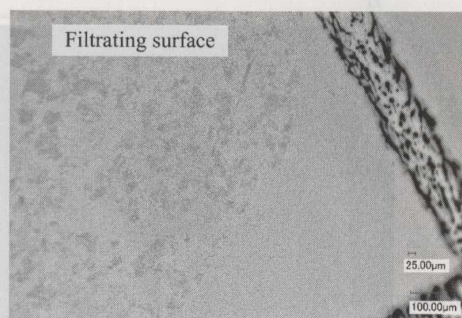
showed that the particles were mainly composed of aluminum (Al), phosphorus (P), and oxygen (O) (Fig. 3a). Because the rubber closure used in this study was Al-free, the source of Al should be the borosilicate glass vial used as a container for the sample. It is suggested that the particles were formed as a result of interactions between eluted Al ions from the surface of the glass vials and the phosphate ions in the solution.

A number of research articles regarding flake-like particles from drug solutions stored in glass vials have reported that the particles were generated by delamination of glass and that the delaminated particles are rich in silicon (Si) and O.^{10,11} However, the particles detected in this study mainly contained Al and P, suggesting that these particles were different from the ordinal delaminated particles reported in previous articles.

We have observed the interior of the vial which contained the particles (stored at 40°C for 6 months) using SEM, as reported by Iacocca and Allgeier¹¹ and Wen *et al.*¹⁴ However, no pitting was observed at the vial sidewall at 0 to 10mm from bottom (data not shown). These data also suggest that the particles formation in this study has little correlation with delamination due to corrosive attack at glass surface.

Boddapati *et al.*¹³ reported the phenomenon that barium (Ba) from borosilicate glass and sulfate ions from a drug formulation interacted, resulting in barium sulfate (BaSO₄) generation. The formation of Al-phosphate complexes proposed

a



b

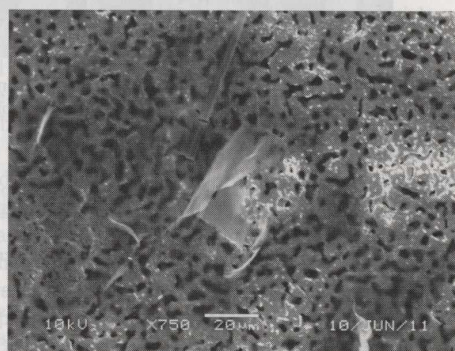


Fig. 2. The Flake-Like Particles from Phosphate Buffer (16mm) Solution Stored at 40°C in a Glass Vial, Captured by Filters

(a) The particles captured and observed with digital microscope, and (b) the particles captured and observed with SEM.

in this study is considered to be a similar phenomenon to the barium sulfate generation process.

Phosphate salts with some cations including aluminum were known to be very insoluble. For example, solubility products of aluminum phosphate, magnesium phosphate and calcium phosphate are 9.84×10^{-21} , 1.04×10^{-24} and 2.07×10^{-33} , respectively.¹⁵ Besides, Al-phosphate complexes have been known to be complexes of phosphate ions, Al ions and water with various ratios^{16,17} and some of Al-phosphate complexes are thought to be remarkably insoluble in neutral solution as well as aluminum phosphate.

For therapeutic use, the precipitations of Al-phosphate complexes are commonly manufactured by mixing phosphate buffer and aluminum chloride for adjuvants in vaccines.¹⁸⁻²¹ However, the Al-phosphate complex formation in other injectable drugs is unacceptable because these insoluble particles have the ability to harm the patient's veins. In particular, for development of antibody drugs, particles are a concern for biopharmaceutical manufacturers and regulatory officers²² based on reports that particles in protein drugs may serve as nuclei triggering the aggregation of the protein, increasing the risk of immunogenicity,²³⁻²⁵ and that particles may also enhance the immunogenicity of the protein drug by themselves.²⁶ Therefore, the phenomenon of particle formation from the storage of phosphate buffer solution in glass vials induced by interactions of the phosphate ions with Al eluted from the vial should be completely prevented in the field of injectable drug products.

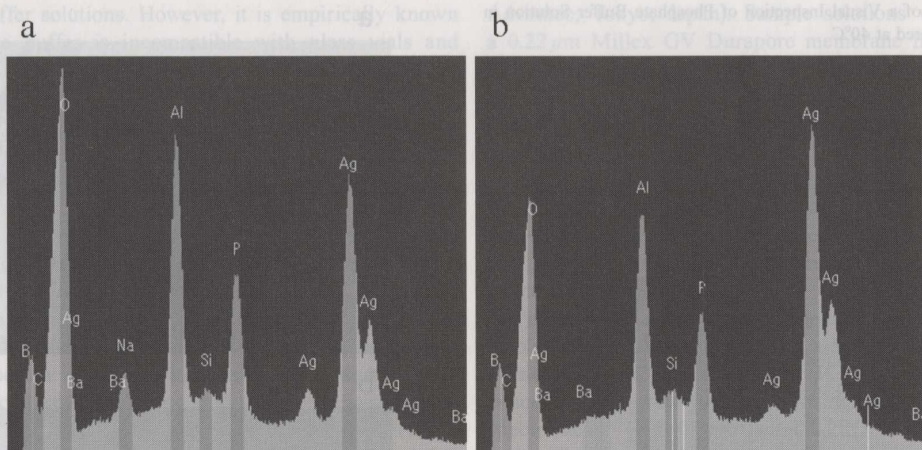


Fig. 3. EDX Chart Spectrum of (a) the Flake-Like Particles from Phosphate Buffer (16mM) Solution Stored at 40°C in a Glass Vial, and (b) White Particles from Phosphate Buffer Solution (16mM) with the Addition of 100ppb of Al Ions

Ag (silver) was both detected due to silver filters as background.

Table 2. Results of Visual Inspection of Phosphate Buffer Solution in Various Treatments of Glass Vials

Solution	Storage temp.	Storage period	Vials detected particles/vials inspected		
			Nontreated vial	Sulfur-treated vial	SiO ₂ -coated vial
Phosphate buffer with polysorbate 80	40°C	3 weeks	0/10	0/10	0/10
		6 weeks	0/10	0/10	0/10
		8 weeks	0/10	0/10	0/10
	60°C	3 weeks	0/10	0/10	0/10
		6 weeks	5/10	0/10	7/10
		8 weeks	8/10	0/10	10/10
	80°C	3 weeks	0/3	0/3	0/3
		6 weeks	3/3	2/3	3/3
		8 weeks	3/3	2/3	3/3
Phosphate buffer without polysorbate 80	40°C	3 weeks	0/5	0/5	0/5
		6 weeks	0/5	0/5	0/5
		8 weeks	0/5	0/5	0/5
	60°C	3 weeks	0/5	0/5	0/5
		6 weeks	2/5	0/5	2/5
		8 weeks	3/5	0/5	3/5
	80°C	3 weeks	0/5	0/5	0/5
		6 weeks	5/5	0/5	3/5
		8 weeks	5/5	3/5	5/5

Effect of Temperature, Surface Treatment and Polysorbate 80 on Particle Formation in Phosphate Buffer As described in a previous section, Al-phosphate complexes form during the storage of phosphate buffer solution in glass vials. In this section, the effects of the temperature, surface treatment and addition of polysorbate 80 on the formation of Al-phosphate complexes were evaluated. To compare the differences of surface treatment, we used sulfur-treated vial, SiO₂-coated vial, and nontreated vials which are treated and supplied by the same supplier (supplier B).

Phosphate buffer solutions (16mM, with or without 0.02% polysorbate 80) were placed in the three types of vials and then stored at 40, 60 or 80°C for up to 8 weeks. After storage, the particle formation was investigated visually. These results are shown in Table 2. Under the storage condition at 40°C for up to 8 weeks, no particles were detected. At 60°C, particles were detected in the nontreated vial and the SiO₂-coated vial for 6 weeks or longer. At 80°C, most samples contained

particles. The results strongly indicate that the tendency for particles to form depends on the temperature. In addition, the results indicated that sulfur-treated vials did not produce any particles at 60°C, in contrast with nontreated vials and SiO₂-coated vials. This suggests that sulfur treatment might have some effect on suppressing the particle formation. Sulfur treatment has been known to have two effects: it can restrain the alkali elution from the surface of a glass vial due to dealcalization,^{27,28)} but it can also pit the surface of the vial and thus enhance delamination.^{10,29)} Considering the fact that sulfur treatment suppressed the particle formation, dealcalization might have an effect on the suppression of particle formation. In contrast, SiO₂ coating is also known to protect the surface from alkali elution,^{2,12)} however, no suppression effects of the SiO₂ coating on the particle formation were shown in this study.

The ratios of the particle detection with and without the addition of 0.02% polysorbate 80 were only slightly different. In

Table 3. The Al and Si Concentrations of the Phosphate Buffer Solution in the Glass Vials

Vial	(A) After terminal sterilization ($n=10$)		(B) Stored at 5°C for 6 months after the terminal sterilization (A) ($n=2$) ^{a)}		(C) Added citrate (20 mM) into the sample after the storage (B) and then stored at 40°C for 2 days ($n=2$)	
	Al (ppm)	Si (ppm)	Al (ppm)	Si (ppm)	Al (ppm)	Si (ppm)
Supplier A (Lot 1)	0.23±0.02	2.2±0.1	0.02±0.00	2.3±0.2	0.23±0.02	2.2±0.1
Supplier A (Lot 2)	0.17±0.02	1.8±0.2	0.02±0.00	1.7±0.2	0.10±0.04	1.8±0.2
Supplier C	0.33±0.01	3.3±0.1	0.02±0.00	3.3±0.1	n.t.	n.t.

Each number represents average±standard deviation. a) Particles were detected in all the samples stored for 6 months after the terminal sterilization. n.t.: not tested.

this study, we used 0.02% polysorbate 80, which is considered to be the standard amount used as a surfactant for biological drugs. From these results, polysorbate 80 appears to have little influence on the particle formation.

The Correlation of the Particle Formation and Al and Si Concentration during Storage after Extraction To confirm if the particle formation has a correlation with Al elution, we measured concentration of Al in phosphate buffer in glass vials after an intentional elution (*i.e.* extraction). At that time, we also measured the concentration of Si, which is known as indicator of delamination.¹²⁾ This extraction was conducted at a high temperature for short time (121±1°C, 10 min) to keep the concentrations from any undesirable effects during extracting.

Phosphate buffer solutions (16 mM, with 0.02% polysorbate 80) were placed in nontreated vials manufactured by suppliers A and C. The filled vials were heated (121±1°C, 10 min) in a steam sterilizer, and then the vials were stored at 5°C for 6 months. The results of a visual inspection of the sample after storage showed that all of the vials obviously contained particles. The Al and Si concentrations in the samples before and after storage evaluated using the ICP method are shown in Table 3. The ratio of Si to Al of the sample just after the heating was approximately 10:1 in all tested samples (Table 3A). Both glass vials used in this study were made of borosilicate glass tubing manufactured by Nippon Electric Glass. The amount of SiO₂ and Al₂O₃ in the glass from Nippon Electric Glass is 70–75% and 5–10%, respectively, and this formulation means that the ratio of Si to Al as an atomic element is estimated to be approximately 6 to 13. The ratio of approximately 10 is quite similar to the ratio of the Si/Al concentration just after the heating, suggesting that both Si and Al were eluted from the glass vials in ratios similar to composition of the borosilicate glass, regardless of supplier. In contrast, for the sample stored at 5°C for 6 months (Table 3B), although the Si concentration did not change before and after the storage, the Al concentration decreased during the storage. The results suggest that the Al eluted from the glass vial after the heating would precipitate during storage, thus decreasing the Al concentration.

To confirm whether the decrease in the Al concentration was induced by precipitation, a 20 mM citric acid aqueous solution was added to the vials containing the particles (Table 3B), and these vials were stored at 40°C for 2 days. As a consequence, the Al concentration increased from adding citric acid, although the Si concentration did not increase (Table 3C). The results confirmed that the particles that contained Al and that were formed in the phosphate buffer after the heating were dissolved again, fully or partially, by adding citric acid. Although the reason for the dissolution of the particles was

not revealed, it is speculated that citric acid might work as a solubilizer due to its chelating ability, as described in the following section.

These results strongly suggest that the particles detected during the storage after the heating were precipitates containing Al ions that were eluted from the glass vial.

The Effect of Citrate Buffer on Particle Formation As described above, the particles formed in phosphate buffer solution stored in glass vials were considered to be complexes formed by interactions between Al ions that were eluted from the glass vial and phosphate in the buffer. In this section, an evaluation of the suppression of particle formation in the phosphate buffer was performed using various concentrations of citrate, which is known to chelate Al ions.³⁰⁾ The solutions were placed in nontreated glass vials manufactured by supplier B, and heating was conducted at 105 or 121°C for 10 or 240 min, and the vials were then stored at 5°C for 2 months. The results showed that all phosphate buffer solutions without citrate contained particles, except under the heating condition of 105°C for 10 min, which was the mildest of all of the heating conditions. In contrast, no particles were found in any of the phosphate buffer solutions containing citrate. It is shown that the mixing of citrate buffer, even at the low concentration of 0.16 mM, which was merely 1% of the phosphate buffer concentration, was effective to suppress the particle formation in the phosphate buffer.

Figure 4 shows the Al and Si concentrations of the samples that were stored at 5°C for 2 months. For the Al concentration, there appeared to be a significant difference between the samples with and without citrate buffer addition in contrast to the Si concentration, which did not change. Considering the results of the previous section in which the Al concentration just after the heating was approximately one-tenth that of the Si concentration and decreased during storage, the Al concentration in the phosphate buffer solution including citrate did not change during storage. The strong effect of citrate on suppressing both the decrease of the Al concentration and the formation of particles in the phosphate solution is thought to be due to the chelating affinity of citrate for Al.³⁰⁾

The Effect of Adding Al Ions to the Phosphate Buffer on the Particle Formation To confirm that the formation of particles resulted from the interaction of eluted Al ions and phosphate buffer, a given amount of Al ions was added to phosphate buffer stored in CZ plastic vials. In addition, visual inspection and Al concentration measurements of the phosphate buffer solution were performed.

For the preliminary study, 25–1000 ppb of Al ions, which was prepared from ICP standard solution, was added to the phosphate buffer solution (16 mM, with 0.02% polysorbate 80), and each solution was placed in a CZ plastic vial and then

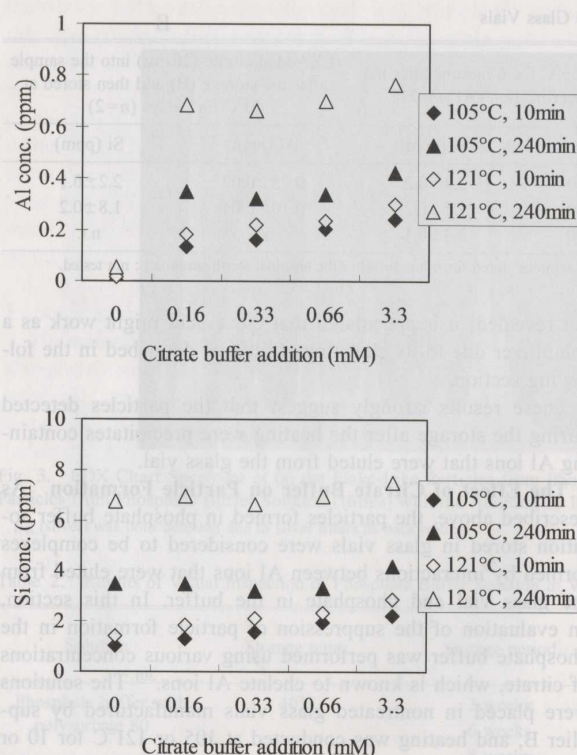


Fig. 4. The Concentrations of Al (Top) and Si (Bottom) in the Phosphate Buffer Solution with/without Citrate Buffer in Nontreated Vial Manufactured by Supplier B, Terminal Sterilized at Various Conditions and Then Stored at 5°C for 2 Months

stored at 5°C. As shown in Table 4, both the phosphate buffer without additional Al ions and the phosphate buffer containing 25 ppb of added Al ions did not form particles, even after 25 weeks in storage. In contrast, the addition of 50 ppb or more of Al produced white particles. The white particles were not recognized as flakes, however (data not shown). The EDX analysis of the particles that were acquired from the phosphate buffer with 100 ppb of Al ions (Fig. 3b) showed Al, P and O, as well as the result shown in Fig. 3a. The particles from this Al spiking study did not have flakes-like morphology which was observed in the glass vial containing phosphate buffer solution during storage (Fig. 1), however, the result that compositions of these two types of particles had the similar composition (Al, P and O) suggests Al-phosphate particles are formed in phosphate buffer with aluminum ion regardless the materials of vial container. In addition, these results also suggest that the addition of 50 ppb of Al ions in phosphate buffer solution has the ability to form a detectable amount of particles for this study condition. The reason why these particles have different morphologies is thought to be due to difference in increasing rate of Al ion in the solution.

Particle formation by Al addition into the phosphate buffer solution was evaluated further. Al ions, 25–500 ppb prepared from aluminum chloride, were added to the phosphate buffer (20 mM, without polysorbate 80) solution, and the solutions in CZ plastic vials were then stored at 5°C for a month. The results of visual inspection are shown in Table 5 (first row). White particles were detected in all samples with the addition of 100–500 ppb of Al ion and in 1 of 3 samples containing

Table 4. Interaction of Al Ions (0–1000 ppb) with Phosphate Buffer Solution in CZ Plastic Vials Stored at 5°C

Amount of Al ions added	Storage period	Particle (white particle) detection
0	2 weeks	No
	3 weeks	No
	4 weeks	No
	25 weeks	No
	2 weeks	No
25	3 weeks	No
	4 weeks	No
	25 weeks	No
	2 weeks	No
50	3 weeks	No
	4 weeks	Detected
	2 weeks	No
	3 weeks	No
100	4 weeks	Detected
	2 weeks	No
	3 weeks	No
200	4 weeks	Detected
	2 weeks	No
	3 weeks	No
1000	4 weeks	Detected
	2 weeks	No
	3 weeks	No
	4 weeks	Detected

50 ppb of Al ions. With the addition of 25 ppb of Al ions, particles were not detected.

Each sample of the tested vials was filtered through a 0.45 μ m filter, and the Al concentration of the filtrate was measured. The results of the measurement are shown in Table 5 (second row). The concentration of each sample was in the range of 11–29 ppb, regardless of the amount of added Al.

The concentrations are speculated to represent the solubility of the Al-phosphate complexes at 5°C, and the difference between the measured Al concentration in the sample and the added Al concentration is considered to represent the amount of precipitate present as Al-phosphate complexes. Each differential value subtracting the amount of Al added from the amount of Al measured is shown in Table 5 (third row). Since it was confirmed that neither the plastic vials nor the rubber closures elute Al under this study conditions (data not shown), the amount of spiking of Al ion must be a total content of Al in each sample. When 50 ppb of Al was added to phosphate buffer solution, only 1 of 3 samples contained detectable particles (Table 5 first row) and the differential value at that time was 33 ppb. On the other hand, when 100 ppb of Al was added, all of 3 samples contained detectable particles and the differential value was 81 ppb. From these results, this differential value suggests to indicate detectable insoluble particles and also suggests to have a limit for visual-detectable particles in between 14 ppb and 81 ppb, in the case of this study.

From these results, it is strongly suggested that the Al-containing particles in the phosphate buffer result from the precipitation of Al-phosphate complexes, which have a very low solubility. We have not encountered the formation of particle containing Al, P and O in glass vials during the storage at 5°C which is usual storage condition for biological drugs, however, it is thought the particles would be formed during the storage at even 5°C as long as there are certain amounts of Al in the phosphate buffer solution.

Table 5. Interaction of Al Ion (25–500 ppb) with Phosphate Buffer Solution (20 mm) in CZ Plastic Vials Stored at 5°C for a Month

	Amount of Al ions added				
	25 ppb	50 ppb	100 ppb	250 ppb	500 ppb
Vials detected particles (white particle)/vials inspected	0/3	1/3	3/3	3/3	3/3
Al concentration of the filtrate	11 ppb	17 ppb	19 ppb	20 ppb	29 ppb
Differential value subtracting the amount of Al added from the amount of Al measured	14 ppb	33 ppb	81 ppb	230 ppb	471 ppb

Conclusion

In this study, the characteristics of the generated inorganic particles in borosilicate glass vials filled with phosphate buffer solutions were investigated.

After storage at 40°C for 3 months or longer, flake-like particles were detected by visual inspection in the phosphate buffer solution held in the glass vials during storage. These particles were considered to be different from glass delamination because they were mainly composed of Al, P and O, but not Si. The formation of the particles accelerated at the higher storage temperature. Among the surface treatments investigated on the glass vials, sulfur-treated vials showed a protective effect on the particle formation; the SiO₂ coating did not. It is found that the elution ratio of Al and Si in the solution stored in the glass vial after the heating was similar to the ratio of Al and Si in borosilicate glass. However, the Al concentration decreased during storage at 40°C for 6 months, and consequently, particle formation was observed in the solution. Adding citrate, which is one of the chelating agents for Al, suppressed the particle formation in the heated solution effectively. When 50 ppb and higher concentrations of Al ions were added to the phosphate buffer solution, the formation of white particles containing Al, P and O was detected.

In conclusion, it is suggested that phosphate buffer solution stored in borosilicate glass vials has the ability to form particles due to interactions with Al, which is eluted from the glass vials during storage. To understand such phenomena absolutely, it is necessary to evaluate the particle formations quantitatively and eluting ability of phosphate buffer from glass vials. We are currently performing this work. The results from this study will provide a number of pharmaceutical researchers working on drug formulation with additional information about whether to use a phosphate buffer from the perspective of particulate matter formation.

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