—Regular Article—

Effect of pH and Additives on the Compatibility between Vancomycin and Furosemide Injections

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(Received January 25, 2018; Accepted March 2, 2018)

The physicochemical compatibility between injections of different agents is very important. An injection of the antibiotic vancomycin (VCM) is acidic and its standard pH range is 2.5–4.5. In clinical treatments, VCM injections are often used with Lasix® (furosemide) injections. The Lasix® injection is alkaline and its standard pH range is 8.6–9.6. Therefore, mixing VCM injections with Lasix® injections may cause compatibility problems. We evaluated the effect of pH on the compatibility between VCM (original and two generic) and Lasix® injections. Compatibility was not observed in non-pH-adjusted VCM with Lasix® injections, but white crystals appeared when VCM injections adjusted to pH 2.5 experimentally were mixed with a Lasix® injection, suggesting that the acidic condition of VCM injections cause compatibility. However, the residual rates of VCM did not change after 24 h in all mixtures. We analyzed the crystals by mass spectrometry and ^{1}H-NMR, and identified them to comprise furosemide.

Key words——compatibility; vancomycin; furosemide; generic drug

INTRODUCTION

The physicochemical compatibility (hereafter termed ''compatibility'') between injections of different agents is very important because crystals may be produced by the interaction between the main components and/or additives.[1,2] This phenomenon can lead to closure of the administration route, reduce the effect of drugs, and damage tissue.

Several factors must be considered in the compatibility between more than two types of injections, such as pH change and decomposition of the main component of the injection. Only a few studies focusing on the compatibility have been reported. In general, information about the compatibility is provided by pharmaceutical companies, but the compatibility is usually evaluated for one production lot. In fact, when certain injections have been administered to patients, a different compatibility to that published by the pharmaceutical company has been reported.[3] In addition, there is no consensus for the evaluation of compatibility, and each pharmaceutical company uses its own conditions. Therefore, understanding the compatibility of different injections precisely and comparing the information provided by each pharmaceutical company can be difficult.

Regarding generic injections, a biologic equivalence study is not required[4] and different additives from original injections are available. However, different additives have been reported to affect the stability of injections between original injections and generic injections.[5,6] In addition, far less information is provided for generic injections than for original injections. In hospitals, therefore, clinical pharmacists often use the data from the original injections when using generic injections. Hence, it is necessary to organize information about the compatibility not only in original injections, but also in generic injections.

Vancomycin (VCM) is an important antibiotic.[7] It is the first choice for the treatment of methicillin-resistant *Staphylococcus aureus* infections in guidelines for the management of infectious diseases published by the Japanese Society of Chemotherapy and the Japanese Association for Infectious Diseases. The standard range of pH in VCM injections is 2.5 to 4.5.[8]

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In clinical treatments, VCM is often used with Lasix®. For critical patients, Lasix® is often used continuously to maintain moisture balance. When these patients are suspected of infection, VCM injection is used in combination for severe infection. For this reason, these two formulations are frequently mixed at the time of combination use. The main component of a Lasix® injection is furosemide. The Lasix® injection is alkaline, with a standard range of pH of 8.6 to 9.6 according to information (revised in March 2016) from the manufacturer (Nichi-Iko Pharmaceutical, Toyama).[9] A change in pH is one of main factors for the compatibility between injections, so the broad acidic pH of VCM injection may affect its compatibility with the alkaline Lasix® injection.

Currently, one original VCM injection and eight generic VCM injections are available in Japan. Among them, the original VCM injection (Shionogi, Osaka) and one generic VCM injection, VCM "TOWA" (Towa Pharmaceutical, Osaka), contain no additives. Another generic VCM injection, VCM "MEEK" (Meiji Seika Pharma, Tokyo), contains D-mannitol and macrogol as additives. Matsumoto *et al*. reported that these additives shorten the time needed to dissolve VCM.[10] Therefore, these additives may affect the compatibility of VCM with other injections. However, the influence of pH and additives on the compatibility between VCM injections and other injections is poorly understood.

We wished to clarify the effect of pH range and different additives in VCM injections on the compatibility with the Lasix® injection. Hence, we evaluated the compatibility of several VCM injections (original injection and two generic injections) and the Lasix® injection based on the method described in the compatibility chart of the original VCM.

First, after mixing each VCM injection (actual pH range of the formulation dissolved in water for injection is 3.2–3.7) and the Lasix® injection directly, we observed the appearance of the mixture. Then, using an HPLC, we measured the pH of the mixture and residual rate of VCM 24 h after mixing. Subsequently, we evaluated the effect of pH range of the VCM injection on the compatibility between VCM and Lasix® injections. The pH of VCM injections (original and two generic injections) was experimentally adjusted to 2.5, and VCM injections at pH 2.5 were mixed with the Lasix® injection to clarify the effect of acidic condition of VCM injection on the compatibility between VCM and Lasix® injections. Then, the appearance, pH, and residual rate of VCM were evaluated. In addition, any crystals that appeared during the compatibility evaluation were collected after filtrated and analyzed by MS and ^{1}H-NMR to identify the precipitating substance. Furthermore, to evaluate the degree or pattern for precipitating crystals when testing the compatibility between all VCM and Lasix® injections, a light obscuration particle counter was used when changing the pH of the VCM injection (unchanged and pH values of 3.2, 2.8, and 2.5).

METHODS

Materials Three VCM formulations were selected (Table 1). The original VCM (formulation A) and two generic VCM (formulations B and C) injections were used. Each drug was prepared in two lots. VCM (bulk powder) and the Lasix® injection (Sanofi K.K., Tokyo) were used in the present study. Acetonitrile (Sigma-Aldrich Japan, Tokyo), methanol (Kanto Chemicals, Tokyo) and tetrahydrofuran (Nacalai Tesque, Kyoto) of HPLC grade were used. Phosphoric acid, trimethylamine and hydrochloric acid (all AR grade) were obtained by Wako Pure Chemical Industries (Osaka). *N*,*N*-Dimethylformamide and dimethyl sulfoxide (DMSO)-d_6 were purchased from Merck (Darmstadt) and Nacalai Tesque (Kyoto), respectively.

Preparation of a Mixture of VCM and the Lasix® Injection According to the method described by Shionogi for evaluation of the compatibility (method B),[11] VCM and Lasix® injections were mixed, and each mixture was collected 0, 3, 6 and 24 h after the mixing. The compatibility was evaluated according to the change of appearance, pH change, and residual rate of VCM. In addition, to evaluate the effect of the pH range of VCM injection on the compatibility with the Lasix® injection, the pH of VCM injections was adjusted to 2.5 (which is the most acidic condition in the range of standards using 0.1 mol/L hydrochloric acid) and the VCM injections at pH 2.5 were mixed with the Lasix® injection.

Appearance of the Mixture The color of the mixture of VCM and Lasix® injections was evaluated according to the method described in the Japanese Pharmacopoeia.[12]

pH Measurement All pH values were measured using a pH meter F-8L (Horiba, Kyoto) or pH meter F-72S (Horiba, Kyoto).

Table 1. Product Information of Vancomycin Injections

Formulation		Company	Type	Lot No.	Additives
A	Vancomycin	Shionogi & Co., Ltd.	Original	0164 0175	no additive
B	Vancomycin for i.v. infusion "MEEK"	Meiji Seika Pharma Co., Ltd.	Generic	I5BX27 I5BX59	D-mannitol and macrogol
C	Vancomycin for i.v. infusion "TOWA"	Towa Pharmaceutical, Co., Ltd.	Generic	D004 D010	no additive

Table 2. Observation of the pH of VCM and Appearance in the Mixture of VCM (Formulation A, B and C) and Lasix® Injections

Formulation	A			B			C		
Lot No.	0164	0175	Appearance	I5BX27	I5BX59	Appearance	D004	D010	Appearance
Time, h	pH			pH			pH		
0	4.07±0.05	4.02±0.03	Clear	4.02±0.02	4.44±0.03	Clear	3.67±0.05	3.94±0.01	Clear
3	4.00±0	3.99±0.03	Clear	4.01±0.05	4.41±0.01	Clear	3.66±0.04	3.94±0.01	Clear
6	4.00±0.01	3.98±0.03	Clear	3.99±0.08	4.44±0.02	Clear	3.64±0.06	3.94±0	Clear
24	4.01±0.01	3.96±0.06	Clear	4.02±0.06	4.46±0.02	Clear	3.66±0.04	3.96±0.02	Clear

Data are the mean±S.D. ($n=3$). The appearance of the mixture was evaluated according to methods described in the Japanese Pharmacopoeia.[12]

Evaluation of the Residual Rate of VCM The VCM concentration was measured by HPLC. The residual rate of VCM was calculated using the titer of VCM immediately after mixing with Lasix® injection (100%). VCM was detected using an HPLC-UV system comprising an LC-10AD$_{VP}$ pump (Shimadzu, Kyoto), with a 20-μL sample loop, an Intersil ODS-4 column (250 mm×4.6 mm, i.d., 5 μm; GL Sciences, Tokyo) and an SPD-10AV ultraviolet detector (detection wavelength, 280 nm; Shimadzu). VCM was separated within the mixture of solution A and B (9 : 1, v/v) where A was triethylamine [adjusted to pH 3.2 by phosphoric acid : tetrahydrofuran=100 : 1 (v/v)] and B was acetonitrile : tetrahydrofuran [100 : 1 (v/v)] at a flow rate of 1.0 mL/min.

MS The crystals obtained at 24 h after mixing from the mixture of VCM (at pH 2.5) and Lasix® injections were filtered and dissolved by *N*,*N*-dimethylformamide. Then, they were analyzed using a mass spectrometer (JMS-700N; JEOL, Tokyo).

^{1}H-NMR The crystals obtained at 24 h after mixing from the mixture of VCM (at pH 2.5) and Lasix® injections were analyzed by a ^{1}H-NMR system at 500 MHz (500PS; Agilent Technologies, Santa Clara) using DMSO-d_6 solvent.

Counting the Number of Particles The pH of VCM injections was adjusted to 3.2, 2.8, and 2.5 with 0.1 mol/L hydrochloric acid. At 0, 3 and 24 h after mixing non-pH-adjusted or pH-adjusted VCM with Lasix® injections, the number of particles was measured with a light obscuration particle counter (KL-04; RION, Tokyo) as described by the Japanese Pharmacopoeia[13] or literatures.[14–17]

Statistical Analyses Data are the mean±S.D. Comparisons were made using ANOVA. $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

To clarify the effects of pH and different additives of VCM injections on the compatibility with Lasix® injections that have been used with VCM injections in clinical treatments, we evaluated the compatibility of several VCM (original and two generic injections) and Lasix® injections.

As shown in Table 2, there was no change in appearance. In addition, we calculated the residual rate of VCM for 24 h between all VCM and Lasix® injections in two lots. We found no significant differences between the residual rate at 0 h and that at 3, 6 and 24 h in any of the mixtures ($p>0.05$) (Fig. 1).

The pH would be an important factor in the compatibility of VCM with Lasix® injections. We expect-

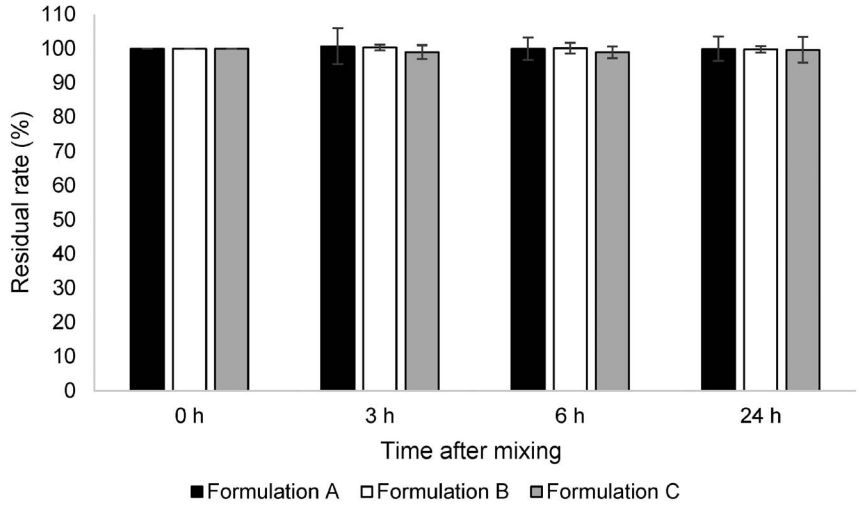

Fig. 1. The Residual Rate of VCM in a Mixture of VCM Injections (Formulation A, B and C) and Lasix® Injections 0, 3, 6 and 24 h after Mixing

Data are the mean±S.D. (n=6). There was no significant difference between the residual rate 0 h after mixing and that at other times in each formulation according to ANOVA.

Table 3. Observation of pH of VCM and Appearance in a Mixture of VCM Injection at pH 2.5 (Formulation A, B, C) and Lasix® Injections

Formulation	A		B		C	
Lot No.	0175	Appearance	I5BX57	Appearance	D004	Appearance
Time, h	pH		pH		pH	
0	3.32±0.01	Clear	3.26±0.01	Clear	3.94±0.01	Clear
3	3.34±0.05	White crystal	3.26±0.01	White crystal	3.94±0.04	White crystal
6	3.40±0.04	White crystal	3.28±0.05	White crystal	3.94±0.01	White crystal
24	3.49±0.02	White crystal	3.39±0.10	White crystal	3.96±0.03	White crystal

Data are the mean±S.D. (n=3). The appearance of the mixture was evaluated according to methods described in the Japanese Pharmacopoeia.[12]

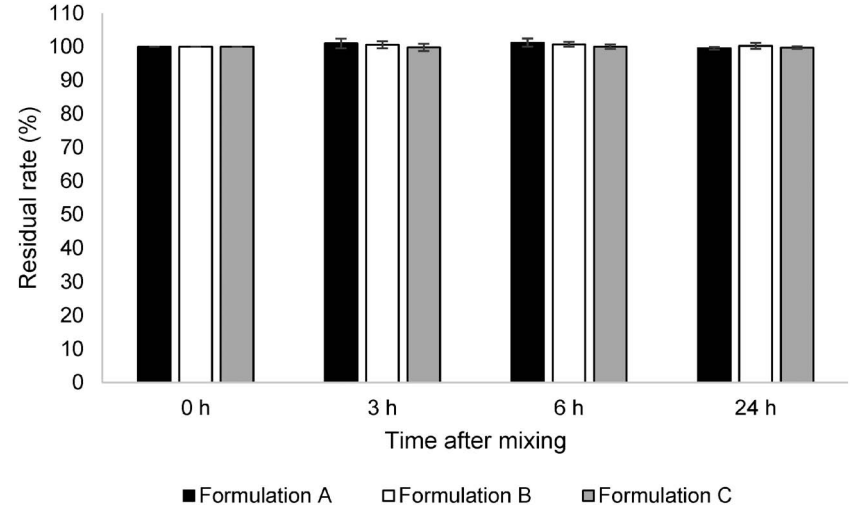

Fig. 2. The Residual Rate of VCM and Appearance of a Mixture of VCM Injections at pH 2.5 (Formulation A, B and C) and Lasix® Injections 0, 3, 6 and 24 h after Mixing

Data are the mean±S.D. (n=3). There was no significant difference between the residual rate 0 h after mixing and that at other times in each formulation according to ANOVA.

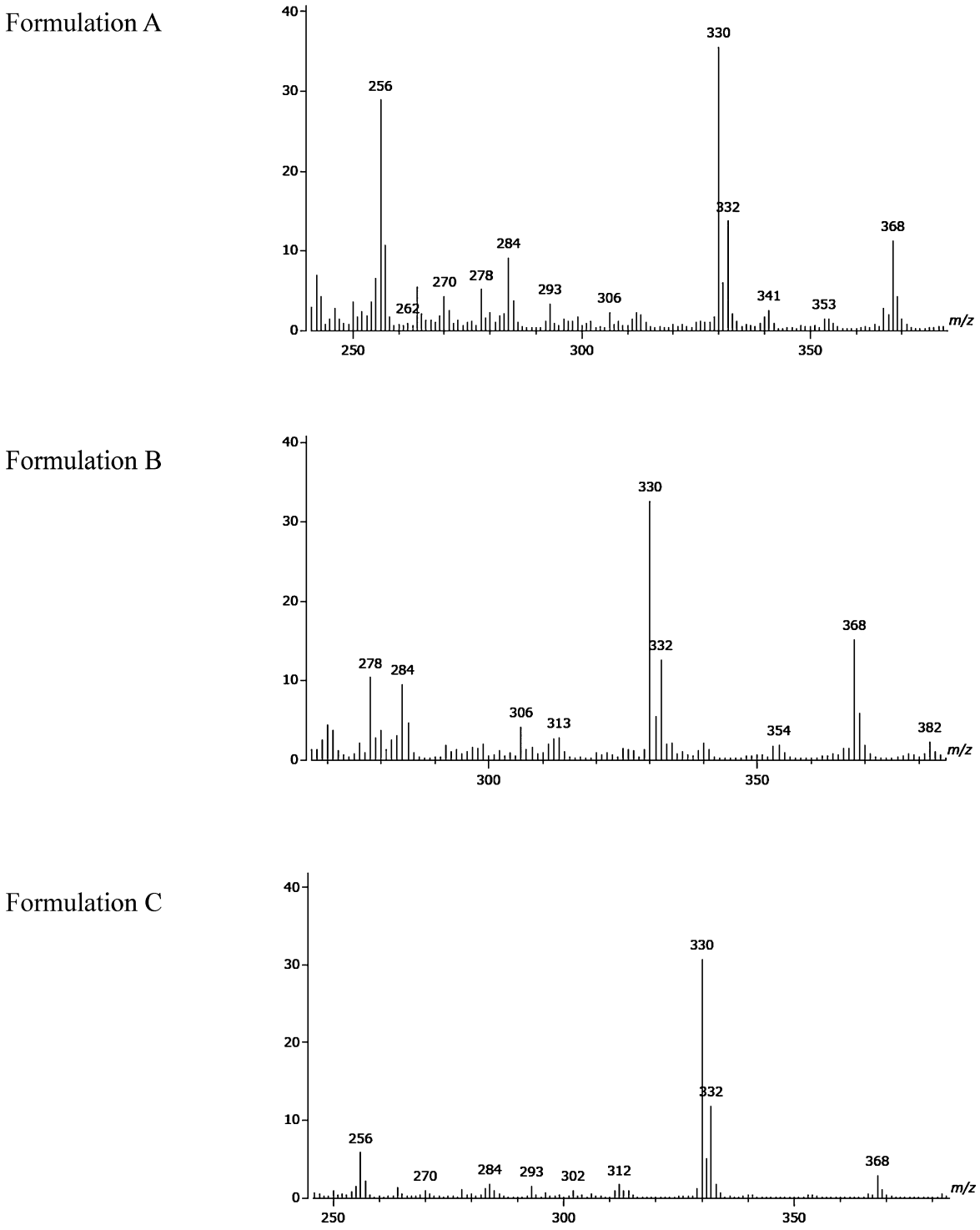

Fig. 3. Mass Spectra of the Crystals Obtained at 24 h after Mixing from a Mixture of VCM (at pH 2.5) and Lasix® Injections

ed the compatibility between VCM and Lasix® injections to cause at the more acidic condition of VCM injection. To investigate if the acidic condition of VCM injection affected the compatibility between VCM and Lasix® injections, we experimentally adjusted the pH of VCM injection to 2.5 (the most acidic pH in the standard range of VCM injection) and mixed the VCM injection at pH 2.5 with the Lasix® injection. In all mixtures, white crystals appeared 3 h after mixing (Table 3 and Supplementary Material). However, the residual rate of VCM did not change for 24 h compared with the residual rate at 0 h after

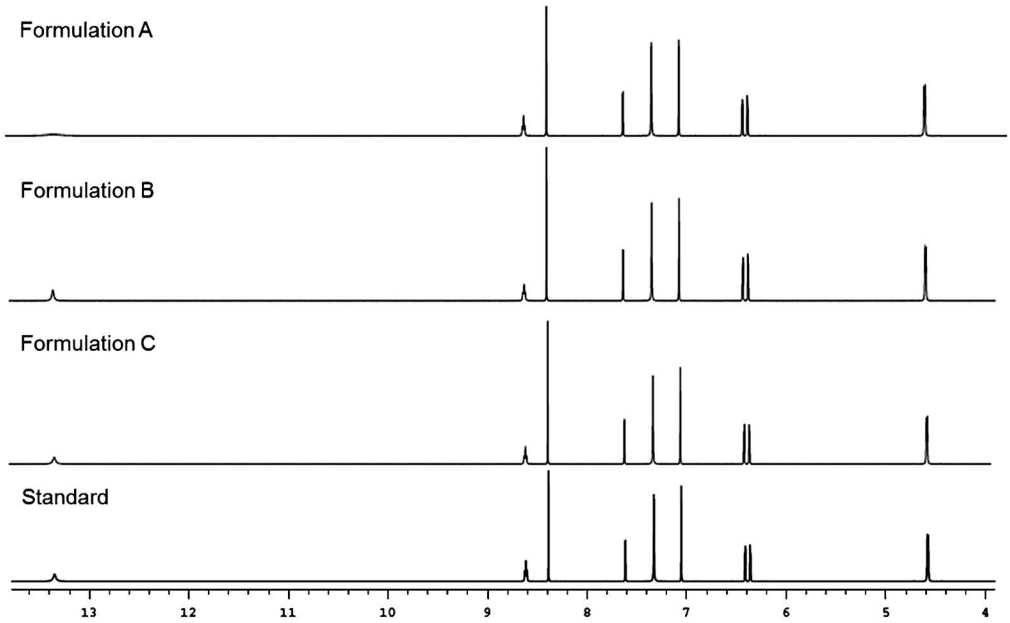

Fig. 4. [1]H-NMR Analysis of the Crystals Obtained at 24 h after Mixing from a Mixture of VCM (at pH 2.5) and Lasix® Injections

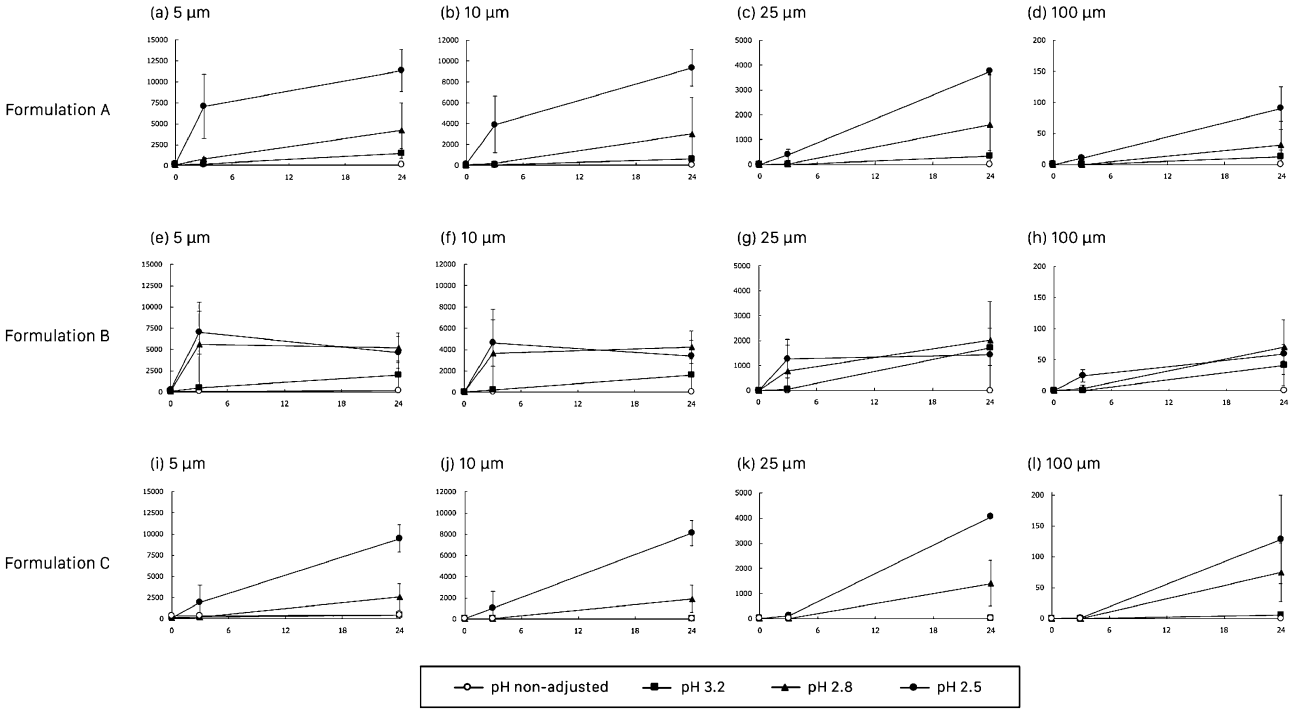

Fig. 5. The Number of Insoluble Particles of Diameter 5, 10, 25 and 100 μm from a Mixture of VCM at Various pH Values (pH non-adjusted as well as pH values of 3.2, 2.8 and 2.5) and Lasix® Injections

Formulation A is (a) to (d); formulation B is (e) to (h); formulation C is (i) to (l). Data are the mean±S.D. ($n=3$).

mixing in all mixtures ($p>0.05$) (Fig. 2), suggesting that the crystals are not VCM.

To identify the composition of the white crystals, we collected them after filtrating from the mixture 24 h after mixing and undertook MS and [1]H-NMR. Figure 3 shows that the main peak of the substance in the crystals to be at m/z 330, approximately the same as that for furosemide. In addition, the [1]H-NMR spectra of the crystals corresponded to those of the furosemide reference standard (Fig. 4). These results strongly suggested that the main compound of the crystals is furosemide. The standard range of pH in

the Lasix® injection was 8.6–9.6. It has been reported by Nichi-Iko Pharmaceutical (in March 2016) that cloudiness and precipitations appear at acidic pH under pH 6.3 in Lasix® injection:[9] our results are in accordance with that report. Therefore, VCM and Lasix® injections may cause compatibility in an acidic condition even within the standard range of pH for VCM injections. Taking this information into consideration, the standard pH range of VCM injection could be narrowed when used with Lasix® injections in the clinical use.

The number of insoluble particles of diameter 5, 10, 25 and 100 μm from the mixture was measured to evaluate the degree or pattern of precipitating crystals between all VCM and Lasix® injections. Consequently, the number of particles of diameter 5, 10, 25 and 100 μm in formulations A and C increased as the VCM injection became acidic. The highest number of insoluble particles was observed in a mixture of VCM injection at pH 2.5 and the Lasix® injection in formulation A [Figs. 5(a)–(d)] and formulation C [Figs. 5(i)–(l)]. In contrast, in formulation B, the number of particles of diameter 5, 10, 25 and 100 μm increased in the mixture of VCM injection at pH 2.8 and the Lasix® injection in a similar way to that in the mixture of VCM injection at pH 2.5 and the Lasix® injection [Figs. 5(e)–(h)]. The number of particles of diameter 25 and 100 μm in the mixture of VCM injection at pH 3.2 and the Lasix® injection at 24 h after mixing was also similar to that in the mixture of VCM injection at pH 2.5 and the Lasix® injection [Figs. 5(g) and (h)]. Formulation B contained D-mannitol and macrogol as additives, but formulations A and C contained no additives. These results suggest that crystals of furosemide may appear when VCM injections in the acidic pH range of the formulation (2.5–3.2) is mixed with Lasix®. There is a possibility that the production rate and particle diameter of crystals are different between products or between lots. For another possibility, the additives such as D-mannitol and/or macrogol may be the influencer for the compatibility between VCM and Lasix® injections.

CONCLUSIONS

A mixture of non-pH-adjusted VCM with Lasix® injections did not cause compatibility. However, white crystals appeared when VCM injections experimentally adjusted at pH 2.5 were mixed with the Lasix® injection, suggesting that the acidic condition of VCM injections cause the compatibility. However, the residual rates of VCM were unchanged for 24 h in all mixture. Thus, we analyzed these crystals by MS and ^{1}H-NMR and identified the crystals to comprise furosemide. We hope that these findings are useful for regulation of the pH range and/or additives in VCM injections to avoid the compatibility with Lasix® injections for clinical use.

Acknowledgment We thank Arshad Makhdum, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflict of Interest This study was partly funded by Towa Pharmaceutical. Mariko Yamaoka, Satomi Sasahara, Katsutomo Hata and Hidehisa Tachiki are company members of Towa Pharmaceutical.

Supplementary Material The online version of this article contains supplementary material.

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