Drug Development and Industrial Pharmacy, 2011; 37(12): 1394–1401

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.580349

informa
healthcare

RESEARCH ARTICLE

A strategy for reducing particulate contamination on opening glass ampoules and development of evaluation methods for its application

Kyeong-Ryoon Lee[1], Yoon-Jee Chae[1], Sung-Eel Cho[2], and Suk-Jae Chung[1]

[1]*Department of Pharmaceutics, College of Pharmacy, Seoul National University, Gwanak-gu, Seoul, South Korea and* [2]*KT&G Central Research Institute, Yuseong-gu, Daejeon, South Korea*

Abstract

A single-dose glass ampoule was developed for ease of administration. When glass ampoules are opened, resulting in contamination by particulate matter. Reducing its contamination may minimize the risk in patients due to particulates. This study reports on an attempt to reduce insoluble particulate contamination by developing methods for the precise measurement of this. A vacuum machine (VM) was used to reduce the level of insoluble particulate contamination, and a microscopy, scanning electron microscopy-energy dispersive X-ray spectrometer (SEM-EDS) and inductively coupled plasma-atomic emission spectrometer (ICP-AES) were used to evaluate the level of reduction. The method permitted the insoluble particle content to be reduced by up to 87.8 and 89.3% after opening 1 and 2 mL-ampoules, respectively. The morphology of the glass particulate contaminants was very sharp and rough, a condition that can be harmful to human health. The total weight of glass particles in the opened ampoules was determined to be 104 ± 72.9 μg and 30.5 ± 1.00 μg after opening 1 and 2 mL-ampoules when the VM was operated at highest power. The total weights were reduced to 53.6 and 50.6%, respectively for 1 and 2 mL-ampoules, compared to opening by hand. The loss of ampoule contents on opening by the VM was 6.50 and 4.67% for 1 and 2 mL-ampoules, respectively. As a result, the VM efficiently reduced glass particulate contamination and the evaluation methods used were appropriate for quantifying these levels of contamination.

Keywords: Ampoule, glass, contamination, scanning electron micrograph-energy dispersive X-ray spectrometer (SEM-EDS), inductively coupled plasma–atomic emission spectrometer (ICP-AES)

Introduction

Single-dose glass ampoules have been developed for ease of administration, accurate measurement of dosage, sterility and use in prepackaged kits. Because the injections are administered directly into the body via non-oral routes, they must be completely protected from contamination by micro-organisms and inorganic particles in all the processes of manufacturing, dispensing and administration. Therefore, many companies now use improved techniques with strict environmental control. Nevertheless, workers in clinics still need to treat ampoules carefully on opening them, because glass contamination occurs on opening single-dose glass ampoules[1–7]. These insoluble particles have been reported to damage various organs such as lung, brain, kidney, liver and spleen[8–10]. The intravenous administration of drugs with glass particles may lead to complications, including pulmonary thrombi and micro-emboli[11], infusion phlebitis, end-organ granuloma formation and inflammation[12–15]. Intramuscular injections of solutions containing insoluble particles may also lead to complications including pain, bleeding or haematoma formation, acute inflammatory induration and the formation of transient nodules[14,16]. It is important to use a proper injection technique for reduction of injection site complications such as tissue injury, abscesses, nerve injury or bruising[17–20]. Therefore, various types of filters have been used recently such as in-line filters, membrane filters or

Address for Correspondence: Prof. Suk-Jae Chung, Department of Pharmaceutics, College of Pharmacy, Seoul National University, Gwanak 599, Gwanak-ro, Gwanak-gu, Seoul 151-742, South Korea. Tel.: +82-2-880–7865. Fax: +82-2-885-8317. E-mail address: sukjae@plaza.snu.ac.kr

(Received 18 January 2011; revised 31 March 2011; accepted 05 April 2011)

syringe filters to reduce the incidence of glass particle contamination[21–23]. In many hospitals, however, in-line filters or membrane filters are used only during total parenteral nutrition or preparation of anticancer drug because of cost and time-consuming[24]. Therefore, it is meaningful to reduce easily the extent of contamination by glass particles on opening in the field. To date, there are no reports of the use if a machine to reduce particulate contamination. In this study, we report the development of a vacuum machine (VM) that can be used easily to reduce particles in medications after opening glass ampoules.

In addition, to evaluate particulate contamination in medications contained in opened glass ampoules, microscopy, filtration-microscopy, electric zone-sensing, photo-dispersion and scanning electron micrograph methods have been used generally[25,26]. However, little information is available on the weight of glass contaminants that are produced during the opening such vials. Therefore, it is meaningful to develop various evaluation methods for its contamination as well as its reduction. In this study, the weight of the glass particles occurred from the glass ampoules on opening them was measured by quantifying components of the ampoules with inductively coupled plasma-atomic emission spectrometer (ICP-AES).

Our objectives were not only to reduce glass particle contamination produced on opening single-dose ampoules by the VM but also to establish reliable analytical methods to evaluate glass contamination produced as a result of opening ampoules.

Materials

The VM, Cutting Apparatus of Ampule (IPC: B67B 7/46), used for opening glass ampoules was designed by Samsung Electronics, Gwangju, Inc. This machine contains a hole fitted with a cap and neck of ampoules, and withdraws particles by means of a vacuum. A waste box is available to collect broken caps that are produced on opening the ampoules. The ampoules (1 and 2 mL) were purchased from Shinil Co., Ltd, Seoul, Korea, and were made of Fiolax® amber glass produced by Schott AG (Mainz, Germany). Water for Injection (WFI) was supplied by the Pharmaceutical Plant for Education and Research (PPER) in Seoul National University, Korea. The WFI was produced and managed under United States Pharmacopeia (USP) requirements and used instead of medications in the ampoules. All used ampoules were washed with WFI and air using a Washing Machine for Ampoules (Jowon Tech. Co., Ltd., Korea). Both the WFI and air were filtered through a 0.22-μm pore size filter. Syringes (Korea Vaccine Co., Ltd., Korea) and filters (ISOPORE® Millipore Co., Ltd., Bedford, MA) were also used in this study. All experiments were performed in the PPER built under the regulation of Korean Good Manufacturing Practice (KGMP) for injections and always maintained in a clean state as shown in Table 1.

Methods

Filling and closing for ampoules

All ampoules were washed with WFI and then dried in an oven at 50°C for approximately 24 h. The washed 1 or 2 mL-ampoules were filled with 1 or 2 mL of WFI, which had been filtered through a 0.22-μm pore size membrane, and then closed by the Filling And Closing Machine for Ampoules (Jowon Tech. Co., Ltd., Korea). All the filled volume of the ampoules was verified.

Opening ampoules and sampling

The ampoules were opened by hand or using the VM. The opening method by hand was not different from that generally used for opening. When the VM was used to open ampoules, the power of the vacuum was set from 1st grade (i.e., the weakest) to the 5th grade (i.e., the strongest). The power of the vacuum (i.e., watts) of each grade had been evaluated previously (Table 2). All of the contents in the opened ampoules were aspirated by a syringe equipped with a needle of 23 G.

The number of the particles

The collected contents by the syringe were expelled onto an individual membrane filter. The samples were subjected to filtration through a membrane filter (0.08 μm pore size, Millipore® ISOPORE Disc filter 25 mm) under a vacuum. The opened ampoules were washed more than five times with filtrated WFI by the membrane filter (0.08 μm pore size), and the mixtures were then subjected to above membrane to ensure all particles were collected. Each membrane filter was isolated and then fixed on a slide glass for microscopic examination. After drying the membranes in semi-darkness for 24 h, they were examined by microscopy (BH2, Olympus, Japan) to count the number of glass particles. The number of the counted glass particles obtained for various vacuum

Table 1. Cleanness of the pharmaceutical plant for education and research (PPER).

Filling room	Washing room	Clean booth	Change of air	Temperature	Humidity
Class 10,000	Class 100,000	Class 100	20 times/h	20~25°C	50% RH

Table 2. Vacuum powers of the VM.

Grade	1st grade	2nd grade	3rd grade	4th grade	5th grade
Power (watt)*	64.6	86.9	104	120	133

*The data were obtained from the Samsung Electronics, Gwangju, Inc.

powers was compared with that obtained by hand-opening.

The morphologies of the glass particles

The filter that contained insoluble particles was observed by microscopy and was cut to a circle with a diameter of 10 mm. The piece was then coated with a gold film after attaching it to a copper grid. The morphologies of the particles on the membrane were observed by scanning electron micrograph-energy dispersive X-ray spectrometer (SEM-EDS, JSM 5410LV, JEOL, Japan) and were then photographed. All observed particles were identified as glass based on EDS data.

The weight of the glass particles

The total weight of the glass particulate contaminants (i.e., inorganic materials) in medications was measured using an inductively coupled plasma-atomic emission spectrometer (ICP-AES, ICPS-1000IV, Shimadzu, Japan). The used ampoules were made of Schott Fiolax® amber, which is a composite of several components as Table 3. To dissolve the inorganic materials including glass particles, hydrofluoric acid (HF) was added to the samples followed by shaking for 5 min. Aqua regia (i.e., HNO_3:HCl, 1:3) was added, followed by a further shaking for 5 min. The amount of Fe, which has a high sensitivity and selectivity, was examined by the ICP-AES to quantify the amount of Fe_2O_3 in the medication after confirmation of complete dissolution using a membrane filter of 0.8 μm pore size. The total weight of inorganic contamination in the vial contents was calculated by ratio of components, as shown in Table 3.

The loss of contents

The loss of contents was calculated to evaluate the loss that occurs on opening ampoules by hand or using the VM. The loss volume (V_{LOSS}) on opening an ampoule was calculated by the difference between the volume of the contents under the Filling and Closing Machine for Ampoules setting (V_{SET}) and the volume of remaining contents after opening an ampoule (V_R); $V_{LOSS} = V_{SET} - V_R$. The measurement of all volumes was estimated by density of contents (i.e., WFI) after weighing them. In this study, the Filling and Closing Machine for Ampoules was validated to dispense a fixed volume.

Results

The number of particles observed in the contents by microscopy on opening ampoules by hand or using the VM was examined. The particles produced on opening by hand were found to be 84.1 ± 16.6 and 24.9 ± 6.27 in 1 and 2 mL-ampoules, respectively. The range of particles produced on opening using the VM were found to be 10.3 ± 6.19 (i.e., 5th grade) to 98.2 ± 23.6 (i.e., 1st grade) in 1 mL-ampoules and 24.9 ± 6.27 (5th grade) to 233 ± 42.5 (i.e., 1st grade) in 2 mL-ampoules (Table 4). Figure 1 shows the observed particles after opening ampoules by hand and the VM. The efficiencies of the VM, compared to the number of the particle produced after opening ampoules by hand (i.e., efficiency = $(N_{HAND} - N_{VM}) / N_{HAND} \times 100\%$, where the N_{HAND} and N_{VM} represent the mean number of particles observed after opening ampoules by hand and the VM, respectively), was calculated to be in the range from –16.8% (i.e., 1st grade) to 87.8% (i.e., 5th grade) and from –8.58% (i.e., 1st grade) to 89.3% (i.e., 5th grade) for 1 and 2 mL-ampoule, respectively (Figure 2).

Several large rough and/or sharp particles were produced in samples that were opened by hand, and these were detected on the membrane filter by SEM (Figure 3). All of the observed particles were identified as glass fragments that arose in used ampoules, as evidenced by the detection of Si by EDS (Figure 3).

The total weights of the glass particles after opening ampoules by hand or using the VM were evaluated by the ICP-AES. In the case of opening by hand, the total weights of glass particles were found to be 224 ± 26.3 μg and 61.7 ± 12.6 μg in 1 and 2 mL-ampoule, respectively. In the case of opening using the VM, the total weights of glass particles were calculated to be in the range from 104 ± 72.9 μg (5th grade) to 221 ± 27.2 μg (1st grade) and from 30.5 ± 1.00 μg (5th grade) to 61.7 ± 12.6 μg (1st grade) in 1 and 2 mL-ampoules, respectively (Table 5). Figure 4 shows the weights of glass particles after opening ampoules by hand and using the VM.

The loss volumes of contents after opening ampoules by hand were 0.20 and 0.17% of the filled volume in 1 and 2 mL-ampoules, respectively. In case of using the VM, the loss of volumes increased up to 6.50 and 4.67% in 1 and 2 mL-ampoules, respectively (Table 6). Figure 5 shows the loss volumes of contents after opening 1 and 2 mL-ampoules by hand or using the VM.

Table 3. Chemical composition[a] of Schott Fiolax® amber.

Component	SiO_2	B_2O_3	Al_2O_3	Na_2O	K_2O	BaO	CaO	TiO_2	Fe_2O_3
% of weight	70	7	6	7	1	2	< 1	5	1

[a]Technical data provided by Schott in Germany.

Table 4. The number of the particles produced after opening ampoules ($n = 10$).

	Vacuum power grade					
	H[a]	1st	2nd	3rd	4th	5th
1 mL	84.1 ± 16.6	98.2 ± 23.6	48.6 ± 23.6	33.0 ± 9.00	10.7 ± 2.01	10.3 ± 6.19
2 mL	233 ± 42.5	253 ± 31.0	131 ± 36.3	95.3 ± 6.26	75.8 ± 22.4	24.9 ± 6.27

[a]By hand without the VM.

Discussion

The number of particles produced after opening ampoules using the VM was less than those opened by hand in both 1 and 2 mL-ampoules, when the 2nd and 5th grade of vacuum power was used. In the case of the 1st grade of vacuum power, the particles produced on opening the ampoules increased by up to 17 and 9% in 1 and 2 mL-ampoules, respectively. This can be attributed to shock to the ampoules on opening by the VM, which is stronger than that produced when an ampoule is opened by hand. In the other cases, however, the power of the vacuum was sufficient to remove the particles on opening the ampoules. The most significant reduction of particulate contamination was found at 5th grade of vacuum power of the VM and was found to be 87.8 and 89.3% in 1 and 2 mL-ampoules, respectively. Thus, if the hole of the VM is modified so as to absorb the shock that occurs mechanically on opening the ampoules, particulate contamination would likely be reduced even more than what was observed in this study. In addition, it was found that the remaining particles after opening 1 mL-ampoules were consistently less than those on opening 2 mL-ampoules. When opening larger size ampoules, therefore, more attention should be paid in the process. As a result, the reduction efficiencies of the VM according to the vacuum powers can be estimated by equation; $N_p = N_0 \times \exp(-\lambda \cdot Pv)$, where N_p, N_0, λ and P_v represent the number of particles, estimated number of particles without a vacuum, the extent of reduction and the vacuum power, respectively. The estimated N_0 and λ were 786 and 0.0321 for 1 mL-ampoules, and 137 and 0.0262 for 2 mL-ampoules, respectively. The R^2s were 0.988 and 0.975 for 1 and 2 mL-ampoules, respectively. Figure 6 shows the exponential decline of the produced particles after opening 1 and 2 mL-ampoules using the VM.

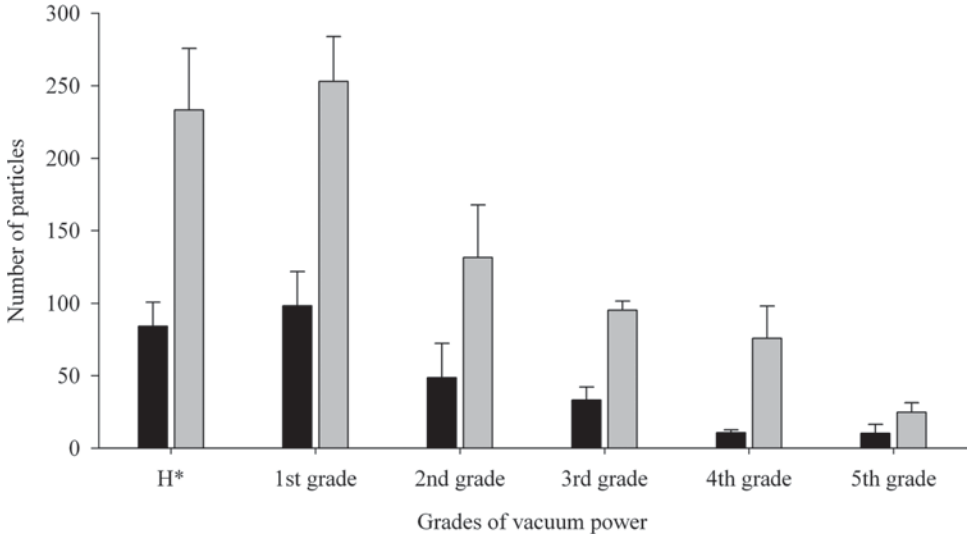

Figure 1. Number of observed particles after opening ampoules. Black and gray bars represent the number of particles in contents of 1 and 2 mL-ampoules, respectively. Each bar represents the mean ± SD ($n = 10$). *By hand without the VM.

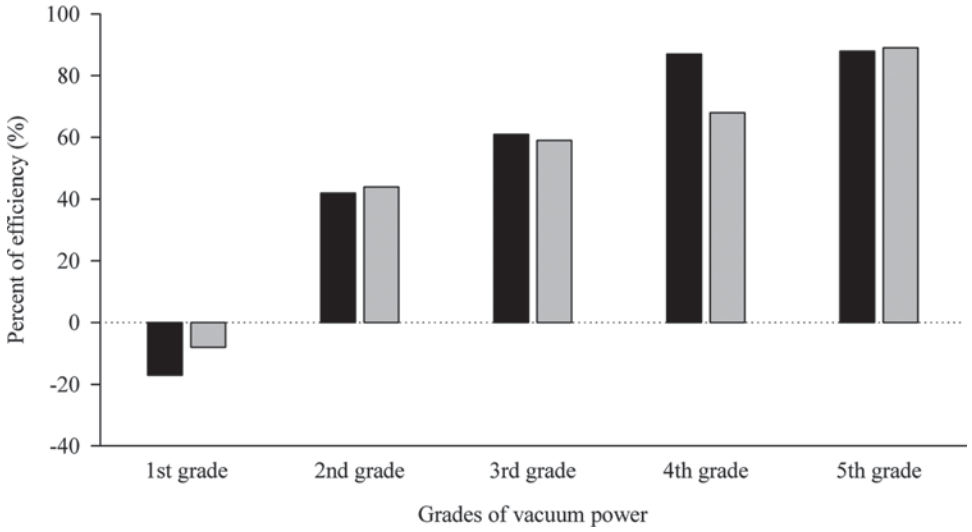

Figure 2. The efficiency of the VM. Black and gray bars represent the percentage of efficiency of the VM for 1 and 2 mL-ampoules, respectively. Dotted line represents the efficiency on opening by hand (i.e., 0%).

The morphologies of glass particles, as observed by SEM-EDS, were very rough and/or sharp, which could lead to several complications in humans because these glass particles are insoluble in the body. The safety of injections into muscles and blood vessels of humans must be considered. Therefore, it is important to develop instruments for reducing the numbers of these particles before injection or a direct infusion of medication into humans.

Especially, total weight of the glass particles after opening ampoules was analyzed by ICP-AES in this study, which has never been reported in previous studies. This quantitative evaluation is important along with the number and morphologies of particles in reducing damage caused by injections using ampoules. However, it was difficult to quantify the amount of SiO_2, a major component of glass ampoules (Table 3), because SiF_4, in the gas state in room temperature, is produced by a reaction between SiO_2 and HF, used as a solvent to dissolve fractions of the glass. Though Fe is a minor component of the ampoules, elemental Fe can function as a

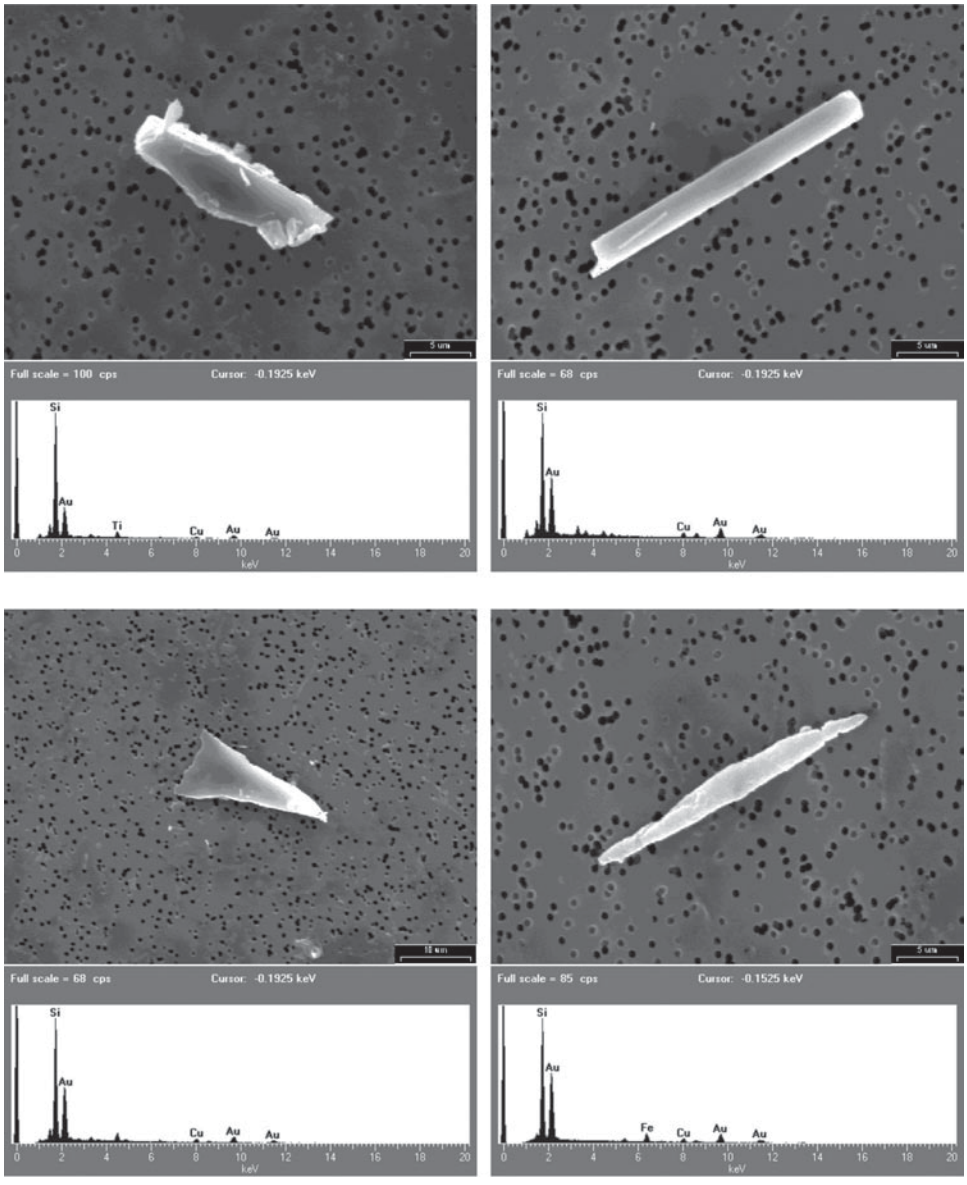

Figure 3. Identification of particle contaminants by SEM-EDS.

Table 5. Weights (μg) of glass particles after opening ampoules ($n = 10$).

	Vacuum power grade					
	H[a]	1st	2nd	3rd	4th	5th
1 mL	224 ± 26.3	221 ± 27.2	133 ± 36.1	146 ± 42.5	124 ± 68.5	104 ± 72.9
2 mL	61.7 ± 12.6	47.2 ± 1.42	42.5 ± 8.44	32.4 ± 6.05	32.6 ± 5.16	30.5 ± 1.00

[a]By hand without the VM.

Table 6. Loss volumes (μL) of contents in opened ampoules ($n = 10$).

	Vacuum power grade					
	H[a]	1st	2nd	3rd	4th	5th
1 mL	2.11 ± 1.09	14.3 ± 10.4	26.7 ± 10.8	53.3 ± 13.7	56.7 ± 21.7	65.0 ± 14.2
2 mL	2.33 ± 2.31	19.3 ± 6.11	55.0 ± 2.65	87.3 ± 18.0	90.3 ± 15.4	93.3 ± 28.6

[a]By hand without the VM.

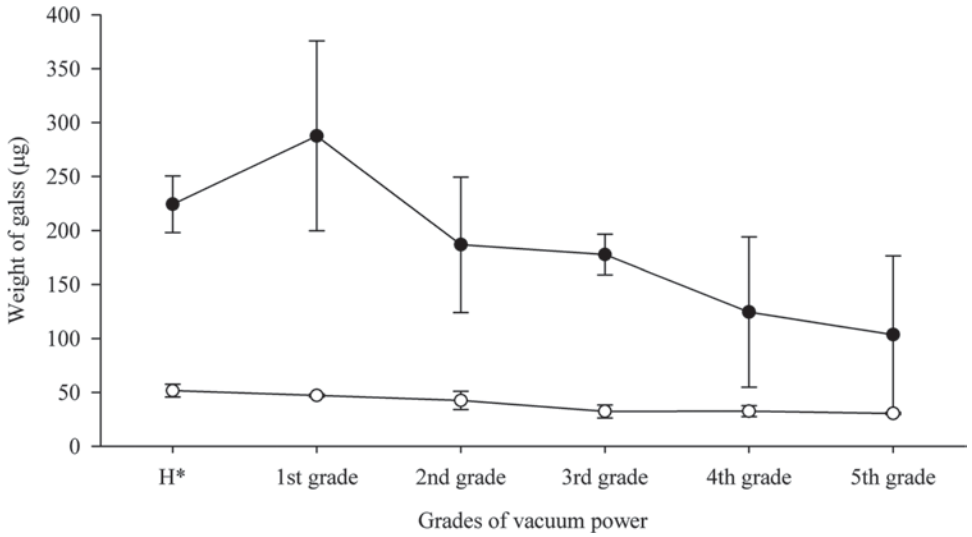

Figure 4. The total weight of glass particles after opening ampoules. Black and white circles represent the total weight of glass particles at 1 and 2 mL-ampoules, respectively. Each circle represents the mean ± SD ($n = 10$). *By hand without the VM.

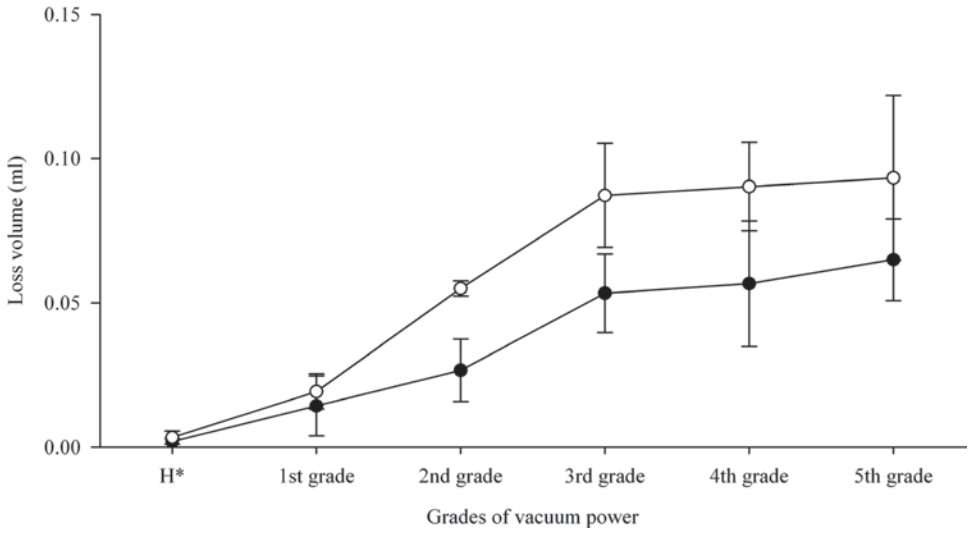

Figure 5. Volume loss in opened ampoules. Black and white circles represent the loss volume of contents in 1 and 2 mL-ampoules, respectively. Each circle represents the mean ± SD ($n = 10$). *By hand without the VM.

marker in such systems because of high selectivity and sensitivity in ICP-AES. The analytical method was validated and it was acceptable to understand the relative amount according to the vacuum power in this study. Therefore, it was possible to quantify the amount of Fe_2O_3 and to calculate the total weight of the glass particles. The determined weights of remaining particles in opened ampoules were approximately 0.007 and 0.001% of the weight of used 1 and 2 mL-ampoules, respectively, at 5th power grade of vacuum power. The percent of reduction, based on that opened by hand, in total weight were approximately 50% in both 1 and 2 mL ampoules. Nevertheless, the observed numbers of particles were reduced by approximately 90% in both 1 and 2 mL-ampoules in separate study. It is likely that the VM removed small-sized particles on opening the ampoules. Additionally, although the number of observed particles in 1 mL-ampoules, as evidenced by microscopy, was less than the corresponding value for 2 mL-ampoules, the total weight of the particles in 1

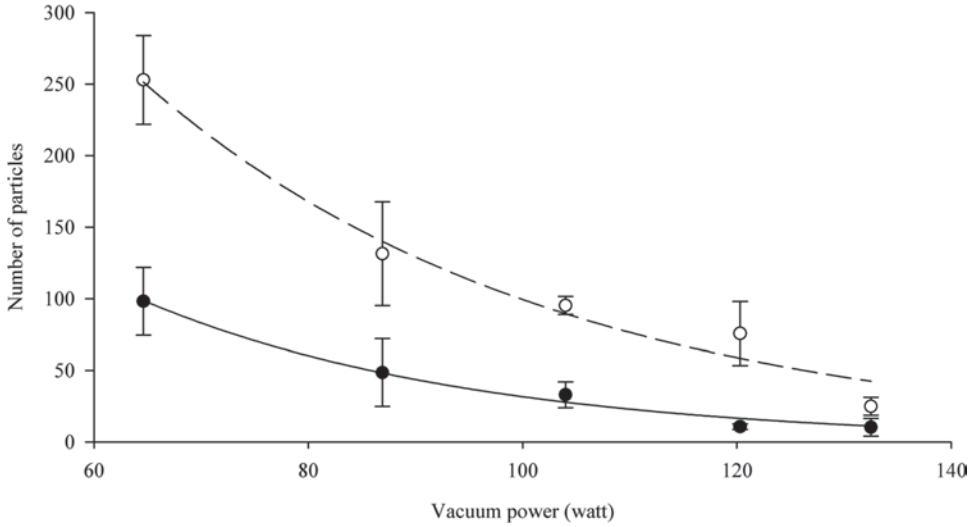

Figure 6. Number of particles produced after opening ampoules using the VM. Black and white circles represent the number of the particle at 1 and 2 mL-ampoules, respectively. Each circle represents the mean ± SD ($n = 10$). Black line and dotted line represents the estimated exponential decline at 1 and 2 mL-ampoules, respectively.

mL-ampoules was more than that in 2 mL-ampoules. These results show that larger particles were produced on opening 1 mL-ampoules than for 2 mL-ampoules.

The loss of volumes (i.e., medication) after opening ampoules by hand or using the VM were found to be less than approximately 6.50 and 4.67% in 1 and 2 mL-ampoules, respectively.

Conclusion

Glass or inorganic particulate contaminants could be reduced on opening glass ampoules using the VM with a vacuum. The VM appears to be a viable instrument reducing contamination, especially small-sized particles which could not be removed with membrane filters. To evaluate the extent of particulate contamination, quantification of particulate weight by ICP-AES was carried out and this was found to be acceptable as well the use of microscopy and SEM-EDS.

Declaration of interest

The authors report no declarations of interest.

References

1. Ernerot L, Dahlinder LE. (1969). The contamination of ampoules in connection with opening. Acta Pharm Suec, 6:401–406.
2. Turco S, Davis NM. (1972). Glass particles in intravenous injections. N Engl J Med, 287:1204–1205.
3. Katz H, Borden H, Hirscher D. (1973). Glass-particle contamination of color-break ampules. Anesthesiology, 39:354.
4. Dorris GG, Bivins BA, Rapp RP, Weiss DL, DeLuca PP, Ravin MB. (1977). Inflammatory potential of foreign particulates in parenteral drugs. Anesth Analg, 56:422–428.
5. Carbone-Traber KB, Shanks CA. (1986). Glass particle contamination in single-dose ampules. Anesth Analg, 65:1361–1363.
6. Gillies IR, Thiel WJ, Oppenheim RC. (1986). Particulate contamination of Australian ampoules. J Pharm Pharmacol, 38:87–92.
7. Sabon RL Jr, Cheng EY, Stommel KA, Hennen CR. (1989). Glass particle contamination: Influence of aspiration methods and ampule types. Anesthesiology, 70:859–862.
8. Turco SJ, Davis NM. (1971). Detrimental effects of particulate matter on the pulmonary circulation. JAMA, 217:81–82.
9. DeLuca PP, Rapp RP, Bivins B, McKean HE, Griffen WO. (1975). Filtration and infusion phlebitis: A double-blind prospective clinical study. Am J Hosp Pharm, 32:1001–1007.
10. Schroeder HG, DeLuca PP. (1976). Particulate matter assessment of a clinical investigation on filtration and infusion phlebitis. Am J Hosp Pharm, 33:543–546.
11. Stehbens WE, Florey HW. (1960). The behavior of intravenously injected particles observed in chambers in rabbits' ears. Q J Exp Physiol Cogn Med Sci, 45:252–264.
12. Garvan JM, Gunner BW. (1964). The harmful effects of particles in intravenous fluids. Med J Aust, 2:1–6.
13. Michaels L, Poole RW. (1970). Injection granuloma of the buttock. Can Med Assoc J, 102:626–628.
14. Bélanger-Annable M.C. (1985). Long-acting neuroleptics: Technique for intramuscular injection. Can Nurse, 81:41–43.
15. Shaw NJ, Lyall EG. (1985). Hazards of glass ampoules. Br Med J (Clin Res Ed), 291:1390.
16. Hay J. (1995). Complications at site of injection of depot neuroleptics. BMJ, 311:421.
17. Cockshott WP, Thompson GT, Howlett LJ, Seeley ET. (1982). Intramuscular or intralipomatous injections? N Engl J Med, 307:356–358.
18. Beyea SC, Nicoll LH. (1995). Administration of medications via the intramuscular route: an integrative review of the literature and research-based protocol for the procedure. Appl Nurs Res, 8:23–33.
19. Day J, Henderson B, Butterworth T. (1995). Shaping up the depot. Nurs Times, 91:51–54.
20. Beyea SC, Nicoll LH. (1996). Back to basics. Administering i.m. injections the right way. Am J Nurs, 96:34–35.
21. Furgang FA. (1974). Letter: Glass particles in ampules. Anesthesiology, 41:525.
22. Katz H, Borden H, Hirscher D. (1974). Glass particle contamination of solutions. JAMA-J Am Med Assoc, 229:1169.
23. Zabir AF, Choy CY, Rushdan R. (2008). Glass particle contamination of parenteral preparations of intravenous drugs in anaesthetic

practice. Southern African Journal of Anaesthesia and Analgesia, 14:17–19.
24. Yorioka K, Oie S, Kamiya A. (2009). Comparison of particulate contamination in glass and plastic ampoules of glycyrrhizin injections after ampoule cutting. J Food Drug Anal, 17:225–228.
25. Oie S, Kamiya A. (2005). Particulate and microbial contamination in in-use admixed parenteral nutrition solutions. Biol Pharm Bull, 28:2268–2270.
26. Preston ST, Hegadoren K. (2004). Glass contamination in parenterally administered medication. J Adv Nurs, 48:266–270.