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Original Article

Identification of an exposure risk to heavy metals from pharmaceutical-grade rubber stoppers

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ABSTRACT

Exposure to low concentrations of heavy metals and metalloids represents a well-documented risk to animal and human health. However, current standards (European Pharmacopeia [EP], United States Pharmacopoeia [USP], International Organization for Standardization [ISO], YBB concerned with rubber closures) only require testing for Zn in pharmaceutical-grade rubber stoppers and then using only pure water as a solvent. We extracted and quantified heavy metals and trace elements from pharmaceutical-grade rubber stoppers under conditions that might occur during the preparation of drugs. Pure water, saline, 10% glucose, 3% acetic acid (w/v), 0.1 mol/L hydrochloric acid, and diethylenetriaminepentaacetic acid (4 mg/mL, 0.4 mg/mL, and 0.04 mg/mL) were used as extraction agents. We quantified the extracted arsenic, lead, antimony, iron, magnesium, aluminum, and zinc using inductively coupled plasma mass spectrometry. The concentration of extracted metals varied depending on the different extraction solutions used and between the different rubber stopper manufacturers. Rubber stoppers are ubiquitously used in the pharmaceutical industry for the storage and preparation of drugs. Extraction of heavy metals during the manufacturing and preparation of drugs represents a significant risk, suggesting a need for industry standards to focus on heavy metal migration from rubber stoppers.

1. Introduction

Rubber products are regularly used as closures, gaskets, and joints in the production of pharmaceutical agents. The fabrication of rubber is very complex and typically requires the use of agents that may be contaminated with heavy metals such as MgO, ZnO [1], and kaolin [2]. Kaolin, in particular, is known to contain various kinds of heavy metals [2–4]. Therefore, there is a potential risk of contaminants, such as heavy metals, migrating from the rubber products to pharmaceutical agents during drug preparation and storage [5,6].

Studies examining pharmaceutical glass products have shown results similar to those reported here. In particular,

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significant amounts of silicate, borate, sodium, and aluminum were released from heated glass, and the use of acidic or glucose-containing solutions resulted in extraction of high levels of aluminum, copper, and lead [7]. Arsenic has also been detected in glass ampules containing intravenous nutrition formulas [8]. However, to our knowledge, there have been no studies examining the potential for heavy metals to be extracted from pharmaceutical-grade rubber stoppers. Here, we used an inductively coupled plasma-mass spectrometry (ICP-MS) method to evaluate if potentially toxic heavy metals were extractable from commonly used pharmaceutical-grade rubber stoppers under various conditions.

Exposure to heavy metals is known to result in serious adverse health effects. Although there is no full consensus on the definition of heavy metals, they are commonly defined as high-density chemicals that can be highly toxic [9]. The mechanisms of heavy metal toxicity are often due to a bond formation of the metals with thiol groups of proteins [10]. When heavy metals enter the cell, they can alter biochemical pathways that may ultimately lead to death or illness [11,12]. Recent data suggest that exposure to lead (Pb) resulting in blood concentrations under 10 μg/dL can have negative effects on children's cognition [13,14]. High Pb exposures in pregnant women can cause low infant birth weight, prematurity, miscarriage, or stillbirth [15]. Exposure to aluminum (Al) can damage various systems of the body including the hematopoietic, renal, and skeletal systems together with the central nervous system being its primary target. Al has also been proposed to be involved in the pathophysiology of neurodegenerative disorders (Parkinsonism dementia, Alzheimer's disease, etc.), although this is still controversial [16,17]. Similarly, arsenic (As) is a known toxic agent and carcinogen [18]. Although iron (Fe), zinc (Zn), and magnesium (Mg) are essential for living organisms at low concentrations, exposure to higher concentrations can lead to toxic effects [19], particularly in susceptible subpopulations (infants, individuals with altered renal function, elderly, etc.).

Use of ICP-MS was considered a most advantageous testing method for the determination of metals, with high precision, wide range, low disturbance, high accuracy, high speed, and the ability to quantify multiple metals simultaneously [20,21]. Therefore, ICP-MS was used to quantify the metals in all experiments.

2. Methods

2.1. *Sample preparation*

We used 10 batches of rubber stoppers from five different manufacturers (five groups were randomly selected from every batch and analyzed in duplicate). The whole, uncut stoppers were put into suitable polypropylene containers and macerated in the extraction solution using the conventional ratio (1 dm^2:100 mL) according to EN 1186-1:2002 and European Union (EU) 10-2011. The samples were then heated in an autoclave to reach a temperature of 121 ± 2°C within 20–30 minutes and maintained at this temperature for 60 minutes, which conforms to the test conditions of USP APPENDIX 660. Samples were allowed to cool to room temperature over a period of about 30 minutes, then mixed and decanted immediately. A blank solution was prepared in the same manner.

2.2. *Preparation of extraction solutions*

We used water purified with a Milli-Q system (Millipore, Billerica, MA, USA). Saline and 10% glucose (common transfusion preparations) were purchased from Baxter (China Shanghai). The 3% acetic acid (w/v) (as the acid simulation according to the EU 10-2011), 0.1 mol/L hydrochloric acid (HCl; as the extraction for plastic in EP APPENDIX 3.1.3), and diethylenetriaminepentaacetic acid (DTPA; 4 mg/mL, 0.4 mg/mL, and 0.04 mg/mL) (a metal ion chelating agent preparation) were diluted or dissolved in water using an ultrasonic water bath. All chemicals were at least of analytical grade.

2.3. *Quantification of heavy metals*

Measurements were made using an Agilent Technologies 7500ce ICP-MS system (Agilent Technologies, Wilmington, DE, USA) equipped with an octopole collision/reaction cell, Agilent 7500 ICP-MS ChemStation software, a Babington nebulizer, a Peltier cooled (2°C) quartz Scott-type double pass spray chamber, and an Agilent I-AS integrated autosampler. We also used a Metler-AE240 electronic balance and MLS-3780 pressure steam sterilizer for extraction purposes. Standard solutions of Pb, antimony (Sb), Fe, Mg, Al, and Zn (1000 μg/mL) were purchased from Shanghai Institute of Measurement and Testing Technology. Standard solutions of As (1000 pg/mL) were purchased from the National Standard Material Center. Once the proper mass isotope was selected, bismuth (Bi) was used as the internal standard for Pb, and scandium (Sc) was used as the internal standard for Fe, Mg, and Al.

2.4. *Statistical analysis*

All statistical analyses were done with SPSS 16.0 statistical software. Differences among groups were analyzed by variance tests. A p value < 0.05 was considered significant.

3. Results

3.1. *Extraction of heavy metals using different solvents*

Using pure water, saline, or 10% glucose, we measured very low or undetectable concentrations of As, Sb, Pb, Fe, and Al. However, under acidic conditions using acetic acid or HCl and in the presence of DPTA, we observed a significant increase in the extraction of most metals tested (see Tables 1–7).

Concentrations of As did not reach detectable levels when water and saline were used as solvents for most samples; however, detectable levels were measured using 10% glucose. We observed a significant increase in the detectable As concentrations when using 3% acetic acid (w/v), 0.1M HCl, and DTPA as solvents (Table 1).

We found detectable levels of Sb in the extract regardless of the solvent used (Table 2). However, when using DTPA as a solvent, the increase in Sb was significant.

Table 1 – Concentration of As in extracts (μg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	[a]	[a]	0.03 ± 0.1	3.65 ± 0.6*	4.68 ± 0.4*	4.94 ± 0.5*
2	[a]	[a]	0.08 ± 0.1	2.71 ± 0.4*	1.84 ± 0.4*	5.64 ± 0.5*
3	[a]	[a]	1.04 ± 0.6	2.04 ± 0.4*	2.37 ± 0.2*	4.61 ± 0.5*
4	[a]	[a]	0.04 ± 0.1	1.94 ± 0.5*	2.25 ± 0.5*	3.40 ± 0.5*
5	[a]	[a]	0.03 ± 0.1	2.05 ± 0.3*	3.09 ± 0.5*	4.66 ± 0.5*
6	[a]	[a]	0.02 ± 0.1	3.14 ± 0.5*	2.79 ± 0.6*	4.97 ± 0.5*
7	[a]	[a]	0.07 ± 0.1	2.58 ± 0.4*	1.86 ± 0.5*	5.51 ± 0.6*
8	[a]	[a]	0.67 ± 0.1	1.86 ± 0.5*	4.13 ± 0.4*	5.15 ± 0.5*
9	[a]	[a]	0.03 ± 0.1	1.88 ± 0.6*	2.07 ± 0.4*	3.72 ± 0.5*
10	[a]	[a]	0.04 ± 0.1	2.39 ± 0.2*	2.59 ± 0.3*	4.77 ± 0.5*

*$p < 0.05$ compared to pure water, saline, and 10% glucose.
DTPA = diethylenetriaminepentaacetic acid; HCl = hydrochloric acid.
[a] Undetected.

Table 2 – Concentration of Sb in extracts (μg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	0.10 ± 0.1	0.12 ± 0.1	0.19 ± 0.2	0.20 ± 0.1	0.19 ± 0.1	1.58 ± 0.2*
2	0.12 ± 0.1	0.11 ± 0.1	0.10 ± 0.1	0.16 ± 0.1	0.13 ± 0.1	1.80 ± 0.2*
3	0.15 ± 0.1	0.10 ± 0.1	0.16 ± 0.1	0.19 ± 0.1	0.07 ± 0.1	1.60 ± 0.2*
4	0.16 ± 0.1	0.09 ± 0.1	0.15 ± 0.1	0.21 ± 0.1	0.13 ± 0.1	1.62 ± 0.2*
5	0.07 ± 0.1	0.05 ± 0.1	0.19 ± 0.2	0.18 ± 0.1	0.18 ± 0.1	1.46 ± 0.1*
6	0.07 ± 0.1	0.11 ± 0.1	0.14 ± 0.1	0.20 ± 0.1	0.11 ± 0.1	1.47 ± 0.2*
7	0.08 ± 0.1	0.10 ± 0.1	0.10 ± 0.1	0.10 ± 0.1	0.07 ± 0.1	1.87 ± 0.2*
8	0.06 ± 0.1	0.15 ± 0.1	0.17 ± 0.1	0.12 ± 0.1	0.11 ± 0.1	2.53 ± 0.2*
9	0.11 ± 0.1	0.16 ± 0.1	0.09 ± 0.1	0.30 ± 0.1	0.13 ± 0.1	1.55 ± 0.2*
10	0.41 ± 0.1	0.10 ± 0.1	0.17 ± 0.1	0.08 ± 0.1	0.18 ± 0.1	1.51 ± 0.2*

*$p < 0.05$ compared to pure water, saline, and 10% glucose.
DTPA = diethylenetriaminepentaacetic acid; HCl = hydrochloric acid; Sb = antimony.

Table 3 – Concentration of Pb in extracts (μg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	[a]	[a]	[a]	1.54 ± 0.6*	0.88 ± 0.6*	215.6 ± 9.4**
2	0.69 ± 0.6	0.67 ± 0.5	0.67 ± 0.5	3.91 ± 0.7*	2.50 ± 0.6*	259.2 ± 12.0**
3	[a]	[a]	[a]	0.81 ± 0.4*	0.92 ± 0.5*	212.2 ± 13.0**
4	[a]	[a]	[a]	1.57 ± 0.7*	0.86 ± 0.5*	145.1 ± 11.4**
5	[a]	[a]	[a]	1.54 ± 0.5*	10.6 ± 0.8*	215.2 ± 10.4**
6	[a]	[a]	[a]	1.59 ± 0.6*	0.92 ± 0.6*	231.3 ± 11.4**
7	0.58 ± 0.6	0.62 ± 0.6	0.66 ± 0.6	4.06 ± 0.4*	3.04 ± 0.6*	263.0 ± 14.3**
8	[a]	[a]	[a]	0.70 ± 0.5*	0.95 ± 0.5*	218.3 ± 10.1**
9	[a]	[a]	0.58 ± 0.5	1.54 ± 0.5	0.81 ± 0.5	140.4 ± 12.8**
10	[a]	[a]	[a]	1.52 ± 0.6*	10.46 ± 0.9*	223.7 ± 11.3**

*$p < 0.05$ compared to pure water, saline, and 10% glucose.
**$p < 0.01$ compared to all other solutions.
DTPA = diethylenetriaminepentaacetic acid; HCl = hydrochloric acid; Pb = lead.
[a] Undetected.

Pb was undetectable in most samples extracted with pure water, saline, and 10% glucose (Table 3). However, significant concentrations of Pb were detected under acid conditions. Of particular note, Pb was found in all 10 samples in the presence of DTPA, reaching levels greater than 260 μg/L in one instance. Very low concentrations of lead were detected in two out of 10 samples extracted with water and saline and three out of 10 samples extracted with 10% glucose.

Fe was detectable in all samples. However, we detected significant concentrations of Fe under acidic extraction conditions—an effect that was increased further in the presence of DTPA, reaching concentrations as high as 1393 μg/L in one sample (Table 4).

Mg was found in all solutions. Mg concentrations approaching 2 mg/mL were measured in Samples 2 and 7 even under physiological conditions using water, saline, or 10%

Table 4 – Concentration of Fe in various extractions (μg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	2.99 ± 1.5	2.48 ± 1.3	1.84 ± 1.1	40.12 ± 1.4*	362.12 ± 13.2**	112.1 ± 14.2*
2	1.01 ± 0.5	0.95 ± 0.6	11.53 ± 5.3	90.85 ± 6.1*	154.52 ± 13.9*	161.7 ± 18.2*
3	13.42 ± 7.6	9.78 ± 5.4	4.56 ± 1.4	90.85 ± 6.4*	71.03 ± 13.6*	1393.0 ± 23.2**
4	0.87 ± 0.4	1.42 ± 1.0	8.52 ± 4.2	6.80 ± 5.3*	141.22 ± 7.7**	80.81 ± 10.1*
5	10.01 ± 4.8	9.01 ± 4.5	6.07 ± 3.6	92.65 ± 4.8*	98.02 ± 10.4*	122.8 ± 19.4**
6	12.55 ± 5.5	12.05 ± 6.7	5.44 ± 2.2	57.93 ± 2.9*	41.48 ± 14.7*	115.4 ± 14.3**
7	0.40 ± 0.2	0.33 ± 0.3	0.49 ± 0.2	141.35 ± 3.1*	37.86 ± 16.3*	142.58 ± 12.8*
8	1.53 ± 0.8	2.36 ± 1.6	0.56 ± 0.5	172.15 ± 4.3*	20.86 ± 11.4*	1357.0 ± 23.0**
9	3.12 ± 1.6	3.67 ± 1.2	3.70 ± 1.8	47.46 ± 7.2*	38.23 ± 7.9*	82.54 ± 12.7**
10	0.17 ± 0.1	0.37 ± 0.2	11.59 ± 5.2	89.85 ± 4.6*	47.97 ± 10.8*	131.78 ± 11.3**

$^{*}p < 0.05$ compared to pure water, saline, 10% glucose.

$^{**}p < 0.01$ compared to all other solutions.

DTPA = diethylenetriaminepentaacetic acid; Fe = iron; HCl = hydrochloric acid.

Table 5 – Concentration of Mg in extracts (mg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	0.25 ± 0.2	0.27 ± 0.1	0.26 ± 0.1	12.82 ± 4.7**	5.80 ± 1.4*	2.23 ± 0.9*
2	1.91 ± 0.3	1.50 ± 0.3	2.00 ± 0.6	67.01 ± 4.7**	4.4 ± 1.2*	1.53 ± 0.7
3	0.36 ± 0.1	0.35 ± 0.2	0.40 ± 0.2	7.83 ± 3.1**	0.12 ± 0.1	1.47 ± 0.7*
4	0.11 ± 0.1	0.13 ± 0.1	0.12 ± 0.1	0.13 ± 0.1	0.34 ± 0.2	3.41 ± 0.8*
5	0.29 ± 0.1	0.36 ± 0.1	0.33 ± 0.1	40.32 ± 5.4**	7.28 ± 1.6*	2.59 ± 0. 3*
6	0.29 ± 0.2	0.31 ± 0.1	0.34 ± 0.2	11.35 ± 4.5**	5.32 ± 1.6*	2.35 ± 0.8*
7	1.92 ± 0.4	1.20 ± 0.2	2.13 ± 0.4	68.18 ± 5.3**	4.86 ± 1.9*	1.67 ± 0.8
8	0.43 ± 0.2	0.35 ± 0.1	0.43 ± 0.1	7.69 ± 3.6**	0.11 ± 0.1	1.54 ± 0.7*
9	0.12 ± 0.1	0.14 ± 0.1	0.14 ± 0.1	0.14 ± 0.1	0.34 ± 0.1	1.33 ± 0.9*
10	0.32 ± 0.1	0.32 ± 0.2	0.29 ± 0.2	38.33 ± 7.3**	7.06 ± 1.6*	2.54 ± 0.8*

$^{*}p < 0.05$ compared to pure water, saline, and 10% glucose.

$^{**}p < 0.01$ compared to all other solutions.

DTPA = diethylenetriaminepentaacetic acid; HCl = hydrochloric acid; Mg = magnesium.

Table 6 – Concentration of Zn in various extractions (μg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	0.04 ± 0.10	40 ± 18.1	48 ± 18.7	10,730 ± 18.9**	2868 ± 17.7*	783 ± 17.8*
2	^{a}	^{a}	^{a}	75 ± 17.7*	436 ± 17.9**	167 ± 16.5*
3	2000 ± 17.4	1999 ± 16.7	1994 ± 18.6	16,220 ± 17.6**	6963 ± 18.8*	5823 ± 18.7*
4	2913 ± 18.1	2932 ± 16.6	2939 ± 16.2	8016 ± 17.3*	10,550 ± 20.2**	9523 ± 19.3*
5	^{a}	^{a}	^{a}	167 ± 19.0*	277 ± 17.9*	368 ± 17.4*
6	31 ± 1 6.7	34 ± 16.4	36 ± 15.2	10,747 ± 17.2**	2859 ± 18.8*	762 ± 19.9*
7	^{a}	^{a}	^{a}	70 ± 18.5*	446 ± 16.9*	156 ± 19.1*
8	1993 ± 18.2	2003 ± 16.1	2003 ± 17.5	16,307 ± 18.4**	6953 ± 18.3*	5861 ± 19.8*
9	2939 ± 15.2	2946 ± 17.2	2949 ± 18.6	8039 ± 17.7*	10,661 ± 20.9**	9651 ± 19.5*
10	^{a}	^{a}	^{a}	154 ± 17.9*	263 ± 19.6*	360 ± 17.9*

$^{*}p < 0.05$ compared to pure water, saline, 10% glucose.

$^{**}p < 0.01$ compared to all other solutions.

DTPA = diethylenetriaminepentaacetic acid; HCl = hydrochloric acid; Zn = zinc.

[a] Undetected.

glucose. However, a significant increase in the concentration of Mg was observed in acid and DTPA solutions (Table 5). Similarly, the concentration of Zn ions increased in acid and DTPA solutions (Table 6). In particular, it must be pointed out that in the presence of 3% acetic acid (w/v), levels of Mg and Zn reached 68 mg/mL and 16 mg/mL, respectively.

Al reached concentrations of more than 100 μg/L in three out of 10 samples tested using 10% glucose (Table 7). A significant increase in the concentration of Al was observed in acid and DTPA solutions. In particular, It must be pointed that in the presence of HCl, Al levels reached nearly 2 mg/mL.

3.2. *Effect of increasing concentrations of DTPA on heavy metal extraction*

Increasing the concentration of DTPA from 0.04 mg/mL to 4.0 mg/mL resulted in increased amounts of heavy metals extracted from the pharmaceutical-grade rubber stoppers for

Table 7 – Concentration of Al in extracts (μg/L), $n = 5$.

Sample #	Pure water	Saline	10% Glucose	3% Acetic acid (w/v)	0.1M HCl	DTPA (0.4 mg/mL)
1	4.75 ± 2.1	3.62 ± 1.5	15.58 ± 2.1	193 ± 15.1*	432 ± 20.1***	150 ± 16.7*
2	6.56 ± 4.2	6.78 ± 1.8	114.7 ± 15.4	391 ± 18.3**	1464 ± 18.9***	188 ± 18.5*
3	13.85 ± 6.7	13.70 ± 1.9	112 ± 18.3	370 ± 18.8**	731 ± 17.6***	100 ± 18.9*
4	4.77 ± 3.0	2.57 ± 1.1	5.22 ± 2.0	153 ± 18.5**	215 ± 17.5***	47 ± 15.7*
5	12.58 ± 5.8	15.23 ± 6.0	14.23 ± 2.0	547 ± 18.8**	1821 ± 19.4***	376 ± 17.7*
6	8.64 ± 6.6	6.87 ± 0.9	22.3 ± 19.4	187 ± 17.7*	440 ± 21.1***	146 ± 16.5*
7	2.39 ± 1.5	3.12 ± 1.7	2.12 ± 1.2	404 ± 17.8**	1410 ± 21.6***	177 ± 20.1*
8	4.43 ± 2.9	5.57 ± 1.2	68.9 ± 17.5	377 ± 18.2**	748 ± 19.6***	106 ± 17.8*
9	5.07 ± 2.4	6.69 ± 0.8	3.69 ± 2.9	146 ± 17.6**	210 ± 16.8***	42 ± 15.8*
10	2.21 ± 1.2	3.77 ± 1.2	113 ± 18.7*	557 ± 18.1**	1813 ± 18.1***	361 ± 18.4*

$^{*}p < 0.05$ compared to pure water, saline, and 10% glucose.
$^{**}p < 0.05$ compared to DTPA solutions.
$^{***}p < 0.05$ compared to all other solutions.
Al = aluminum; DTPA = diethylenetriaminepentaacetic acid; HCl = hydrochloric acid.

all metals tested (Figures 1–7). In particular, there were variable amounts of Fe (Figure 4), Mg (Figure 5), Al (Figure 6), and Zn (Figure 7) extracted from the diverse rubber stoppers tested, suggesting wide differences in the leachable heavy metal content in stoppers from different manufacturers.

3.3. *Effect of temperature on heavy metal extraction in pharmaceutical grade rubber stoppers*

The extraction temperature (50°C) was based on recommendations of the European Pharmacopoeia [22]. In order to determine the effect of temperature on heavy metal extraction from pharmaceutical-grade rubber stoppers, we increased the temperature in five samples during extraction with 0.1M HCl. When the extraction temperature was increased from 50°C to 98°C, the concentration of heavy metals in the extract also increased (Table 8).

4. Discussion

Pharmaceutical drug preparations are performed under a variety of conditions, using various solvents, temperatures, and methods. Acetic acid is commonly used in the pharmaceutical industry for preparing the cervix prior to colposcopy-directed biopsies [23] and in the treatment of simple renal cysts [24], as well as being a common acid food stimulant in the EU 10-2011. DTPA is a metal ion chelator used to treat metal poisonings such as Pb and in the treatment of idiopathic pulmonary hemosiderosis [25]. It has also been used in the development

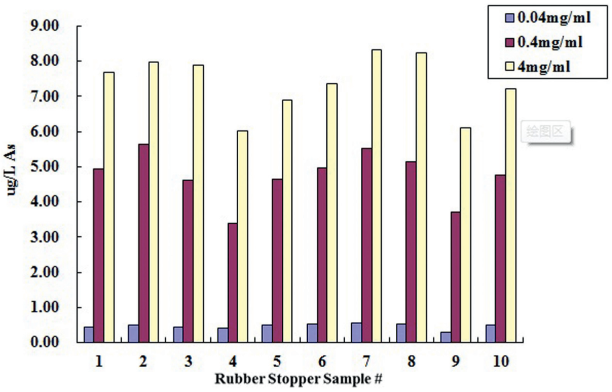

Figure 1 – Arsenic (As) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

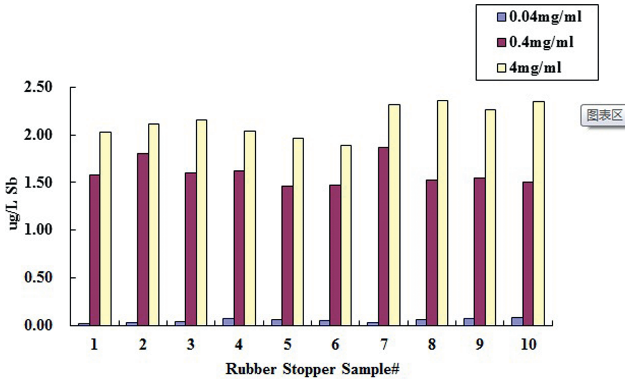

Figure 2 – Antimony (Sb) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

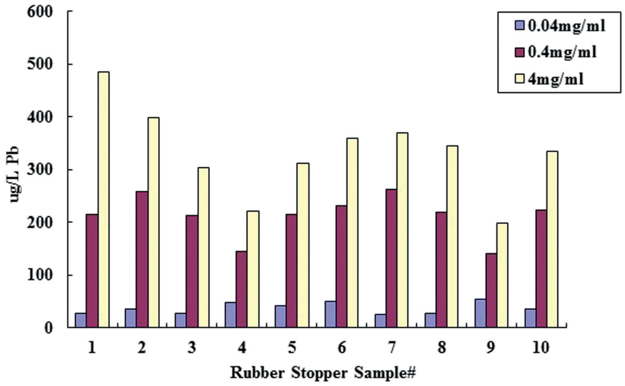

Figure 3 – Lead (Pb) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

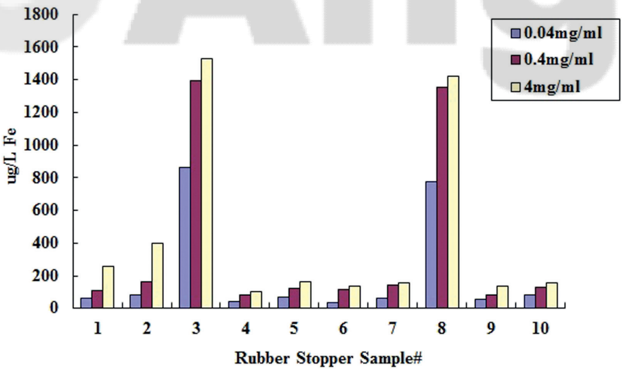

Figure 4 – Iron (Fe) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

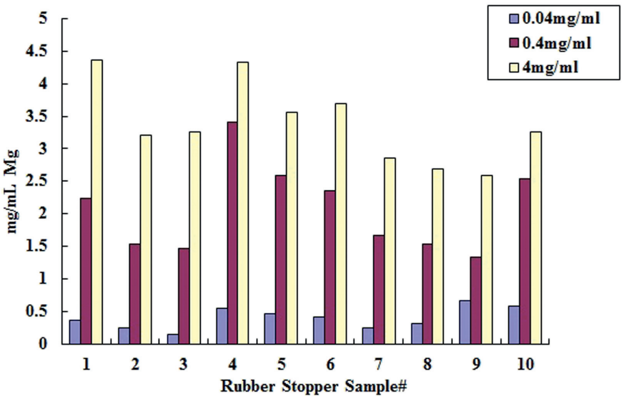

Figure 5 – Magnesium (Mg) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

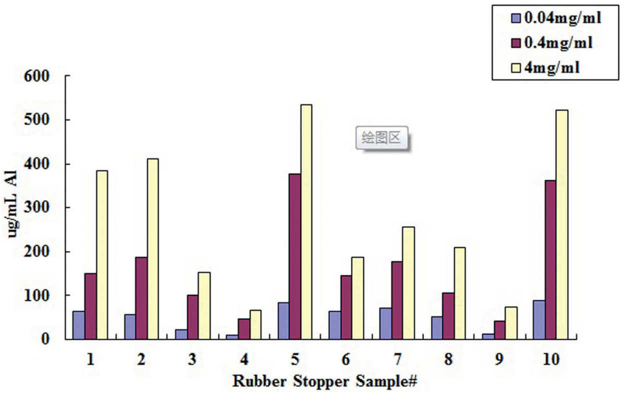

Figure 6 – Aluminum (Al) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

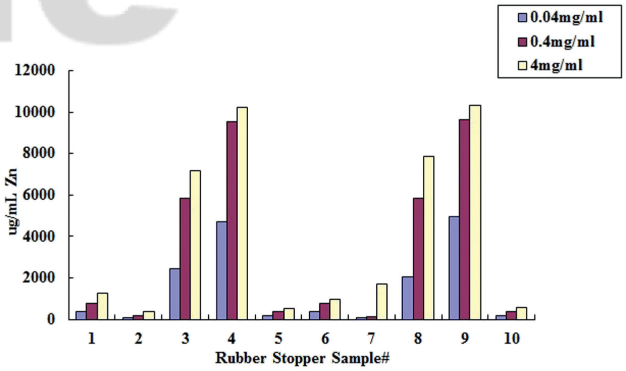

Figure 7 – Zinc (Zn) extracted from pharmaceutical grade rubber stoppers with increasing concentrations of diethylenetriaminepentaacetic acid (DTPA).

Table 8 – Effect of temperature on heavy metal extraction from pharmaceutical grade rubber stoppers, $n = 3$.

Heavy metal (μg/L)	50°C	98°C
As	[a]	0.499 ± 0.4
Sb	[a]	0.332 ± 0.2
Pb	2.562 ± 0.8	16.84 ± 0.9
Fe	6.05 ± 1.1	228.1 ± 40.3
Mg	2437 ± 123.3	5445 ± 163.3
Al	247 ± 24.2	1695 ± 113.3
Zn	142 ± 13.7	290 ± 17.9

Al = aluminum; As = arsenic; Fe = iron; Mg = magnesium; Pb = lead; Sb = antimony; Zn = zinc.

[a] Undetected.

of experimental drug delivery systems [26]. Unsurprisingly, in the presence of DTPA, we observed an increase in most of the heavy metals tested, particularly Pb and Fe. HCl (0.1 mol/L) is used for extraction preparations listed in the European Pharmacopoeia. The manufacturing process for producing rubber requires the use of agents such as ZnO, MgO, and kaolin, a silicate mineral with the chemical formula $Al_2O_3 \bullet 2SiO_2 \bullet 2H_2O$ [27,28] that is used as a filling agent. Kaolin has also been found to contain Fe_2O_3, TiO_2, and trace amounts of K_2O, Na_2O, CaO, and MgO [4]. Studies have shown that under acidic conditions, Al ions are easily extracted from kaolin [29,30]. As a result, there is a significant potential for rubber stoppers to contain heavy metals and minerals, which can then migrate to pharmaceutics during drug preparations. The results of the present study demonstrate that heavy metals may be released from kaolin used during the manufacturing of rubber stoppers under acidic conditions, especially Al. The increased Mg and Zn may come from the additives (MgO and ZnO) during the manufacturing process. Differences in the concentration between heavy metals extracted with HCl and acetic acid may be explained by the hard–soft-acid–base theory (HSAB), which suggests that hard acids react easily with hard bases, and there are different methods to calculate the scale for soft and hard [31–33]. Overall, central atoms of small size, with a large positive charge and low polarizability are termed hard acids, including Al^{3+}, Fe^{3+}, and Mg^{2+}. Large central atoms with a small positive charge and high polarizability are soft acids, including Zn^{2+} and Pb^{2+}. Ligating atoms with high electronegativity, low polarizability, and low oxidation potential are called hard bases. For example, CL^- is "harder" than CH_3COO^-. The Coulomb force is the main force for hard acids combined with hard bases; interactions between soft acids and soft bases are primarily covalent. Therefore, HCl is used in the extraction of aluminum ore [34], whereas Pb and Zn are extracted with acetic acid [35]. This study also confirmed that the concentration of Zn^{2+} and Pb^{2+} extracted with acetic acid is higher than that extracted with HCl, whereas the concentration of Al^{3+} and Fe^{3+} is higher when extracted with HCl.

Heavy metals are known to cause adverse health effects; therefore, limits have been set to define the allowable concentrations of metal ions in drinking water (Table 9). The results of this study demonstrate a wide range in extractable metal ions from pharmaceutical-grade rubber stoppers, likely because of the different manufacturing processes used to produce these materials. In particular, results showing that Pb, Sb, and As were detectable in some samples using water, saline, and/or 10% glucose are of significance to human health. Detectable Pb levels were extracted from three out of 10 rubber stoppers using only water, saline, and 10% glucose. Higher Pb levels were measured using the more potent extraction mediums. Lead is a particularly dangerous neurotoxicant, with significant ramifications for the normal development of children. Permissible Pb blood levels are generally between 0.8 μg/L and 2.5 μg/L [36]; Pb is also known to bioaccumulate [37]. Consequently, detection of Pb levels, even at low concentrations, has serious ramifications for the safety of pharmaceuticals that may become contaminated as a result, particularly using relatively inert solvents such as water. Detectable levels of As were also measured in all samples extracted with 10% glucose, as well as with the more stringent solvents. Consumption of As through contaminated pharmaceuticals in addition to food sources represents a significant human health cancer concern [38]. In addition to Pb and As, detectable levels of Sb were measured in all samples tested, with all solvents tested. Although the health consequences of Sb exposure are not as well defined as those for Pb and As, there remains a cause for concern [39].

Although there is a wide range of standards that address the safety of pharmaceutical-grade rubber stoppers, only testing for the Zn ion using water as a solvent is commonly recommended [22,40–46]. Our study shows that extraction of metal ions is generally very low in pure water, but it significantly increased with the use of acid or chelating solutions. However, it is important to note that there were differences in the amount of metals extracted depending on the manufacturer, suggesting that some rubber stoppers are more likely to have leachable metals than others. These data suggest that testing standards should include metals other than just Zn and conditions other than just water, particularly for pharmaceutical-grade stoppers used during the sterilization process or for long-term storage of drugs.

Table 9 – Safety guidelines for heavy metals in drinking water.

Heavy metal (mg/L)	GB5749-2006 (China)	98/83/EC (EU)	WHO Guidelines for drinking water	USA EPA regulations
As	0.01	0.01	0.01	0.01[a]
Sb	0.005	0.005	0.02	0.006[a]
Pb	0.01	0.01	0.01	0.015[a]
Fe	0.3	0.2	0.3	0.3[b]
Mg	—	—	—	—
Al	0.2	0.2	0.2	0.05–0.2[b]
Zn	1.0	[a]	1.0	5[b]

— no requirement.

Al = aluminum; As = arsenic; EPA = Environmental Protection Agency; Fe = iron; EU = European Union; Mg = magnesium; NPDWRs = National Primary Drinking Water Regulations; NSDWRs = National Secondary Drinking Water Regulations; PB = lead; Sb = antimony; WHO = World Health Organization; Zn = zinc.

[a] NPDWRs.

[b] NSDWRs.

5. Conclusions

Exposure to heavy metals represents a well-known health risk, from neurotoxic effects to dementia and cancer, particularly in susceptible individuals such as children, immune compromised patients, individuals with uremia, and the elderly. Rubber stoppers are primarily used for infusion products and constitute high-risk packaging materials. Intake of heavy metals extracted from rubber stoppers can directly enter the human circulatory system. Our results demonstrate the potential for heavy metal contamination of therapeutic agents prepared in containers using rubber stoppers with a variety of extraction mediums. In particular, detectable levels of Pb, As, and Sb were measured in some of the samples extracted with water, saline, and/or 10% glucose, as well as with HCl, acetic acid, and DTPA. These heavy metals are of particular concern because of their high toxicity profiles and relatively low safety thresholds. Furthermore, differences in the amount and type of extracted heavy metals from the various samples indicate that manufacturing processes may affect the safety of pharmaceutical-grade rubber stoppers. Therefore, strengthening the control of heavy metal migration from rubber stoppers may be warranted.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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