**SUPPLEMENTARY INFORMATION**

Unconventional Separation of Arsenic Trioxide from Unused Aqueous Chemotherapeutic Agents by Direct Evaporative Crystallization

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**INSTRUMENTATION**

*Powder X-Ray Diffraction (PXRD)*. The PXRD pattern of the powder sample was collected by a Bruker AXS D8 Advance PXRD (Karlsruhe, Germany). X-ray radiation Cu Kα1 (λ = 1.5405 Å) was set at 40 kV and 25 mA passing through a nickel filter. Samples were subjected to powder X-ray diffraction analysis with a sampling width of 0.1o in a continuous mode with a scanning rate of 5o/min over an angular range of 5o to 80o.

*Optical Microscopy (OM)*. OM characterization were performed by Olympus BX-51 (Tokyo, Japan) equipped with a digital camera made by Moticam 2000 (Hong Kong, China). The obtained images were transformed by Motic Image Plus (version 2.0) into a digital photograph. Analysis of the crystal size distribution was performed by ImageJ 1.51g software equipped with Microscope Measurement Tools v1 plugin.

*High-Performance Liquid Chromatography (HPLC)*. The HPLC method of Gonzalez-Contreras et al.[1] was followed with modifications. HPLC measurement of the samples was performed using Shimadzu Prominence-i LC-2030C 3D Plus. The column used was Phenomenex Rezex ROA-Organic Acid, 7.8 x 300 mm, 8 μm particle size. The mobile phase was 0.01 N sulfuric acid aqueous solution. For concentration measurement in the evaporation flask, the solution sample was diluted ten times with water prior to injection. 20 μL of sample was injected into the column with the mobile phase pumped in an isocratic flow at 1.0 mL/min with the column temperature set at 65oC. The sample was detected by its UV absorbance at 197 nm which was suitable for samples with a very dilute arsenic concentration.

For the assay measurement of As2O3 crystals recovered from the evaporative crystallization, about 5 mg of the powder samples were dissolved in 0.2 mL of 2 N NaOH (aq), followed by the addition of 0.2 mL of 2 N HCl (aq), which was later diluted with the mobile phase to a total volume of 10 mL. Detection was performed at 210 nm, which was suitable for the powder assay measurement. For the recovered As2O3 powder sample, the HPLC assay % was calculated based on the formula:

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| --- | --- | --- |
|  |  | () |

where *ASP* = average peak area of As2O3 in the sample solution, *ASTD* = average peak area of As2O3 in the standard solution, *WSP*= weight of As2O3 to be examined (mg), *WSTD* = weight of the As2O3 standard (mg), and *f* = factor value of As2O3 standard, which was 0.9999.

**SATURATION VOLUME**

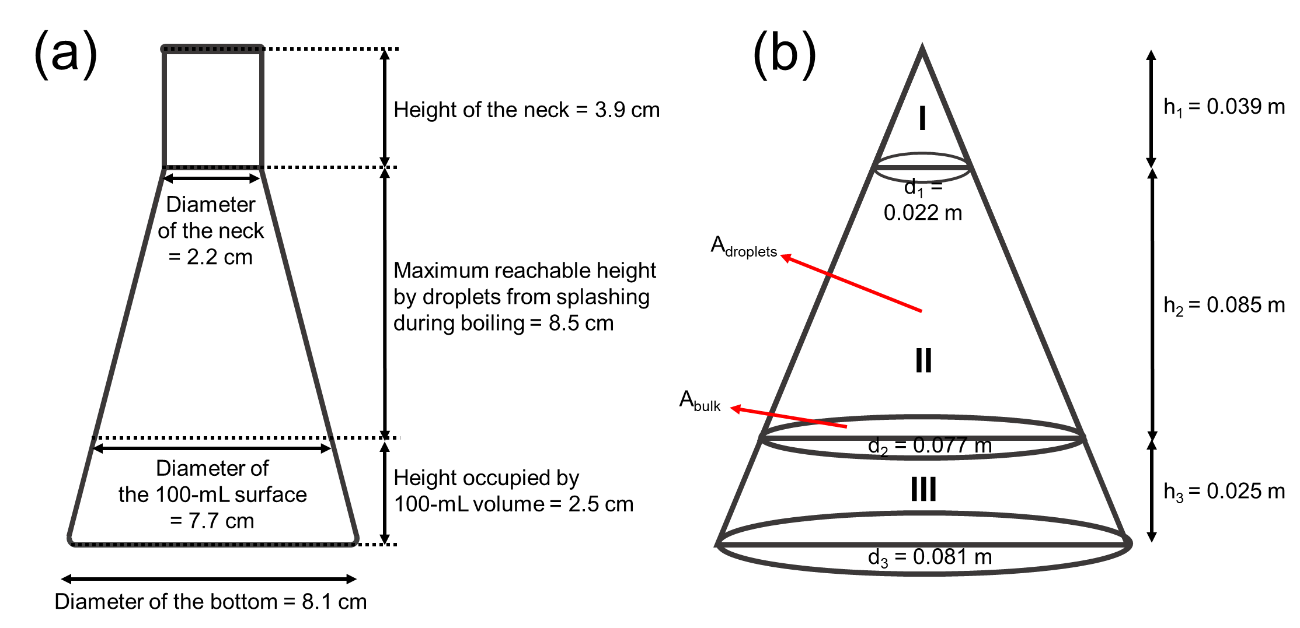
Assuming the number of mole of As2O3 was unchanged, therefore the volume of water at the saturation point is given by:

|  |  |  |
| --- | --- | --- |
|  |  | (S1) |

where Cinitial and Vinitial were the initial concentration of As2O3 and initial volume of the aqueous As2O3 solution, respectively. Csaturation and Vsaturation were the concentration and volume of the system at the saturation point. Three different pressures were applied for evaporative crystallization. Water would boil at different saturated temperatures. Therefore, there were three different saturation points. The calculations were listed in Table S1.

**DROPLET EVAPORATION RATE**

The dimensions of the 250 mL Erlenmeyer flask used for evaporative crystallization were shown in Figure S1. During boiling, it was assumed that the droplets from splashing could reach the inner wall region right below the neck of the flask. The surface area of the droplets, Adroplets, was calculated based on this assumption. The surface area of the bulk solution, Abulk, was calculated using the diameter of the 100-mL surface of 7.7 cm.



**Figure S1.** Dimensions of (a) the 250 mL Erlenmeyer flask used in the evaporative crystallization of As2O3 and (b) the cone impression of the 250 mL Erlenmeyer flask for surface area calculation.

As depicted in Figure S1, the surface area of the bulk surface, Abulk, is the area of the bottom part of cone II. For the sake of simplicity, the surface area change over time was assumed to be negligible. The equation is given by:

|  |  |  |
| --- | --- | --- |
|  |  | (S2) |

while the surface area of the wall surface covered by droplets splashed from the bulk solution, Adroplets, is equal to the lateral surface area of cone II minus cone I, as depicted in Figure S1:

|  |  |  |
| --- | --- | --- |
|  |  | (S3) |

Evaporation rate of the droplets, revaporation droplets, on the surface of the wall was calculated by the Hertz-Knudsen equation [2,3]:

|  |  |  |
| --- | --- | --- |
|  |  | (S4) |

From the Equation (S4), the equilibrium pressure of the vapor-liquid phase, *peq*, was unknown. Assuming that the partial pressure of the droplets due to evaporation was negligible, the vapor-liquid equilibrium pressure, *peq*, was approximated by re-arranging equation (S4) and turning it into equation (S5). *peq* was calculated by using the evaporation rate of from the bulk solution, revaporation bulk, obtained from the volume measurement of the condensated water collected over time. The value of *peq* is then used to calculate revaporation droplets in equation (S4).

|  |  |  |
| --- | --- | --- |
|  |  | (S5) |

The operating parameters and results were shown in Table S2. The results showed that the evaporation rate of the droplets were 2.6, 3.0, and 5.0 mL/min for 1.0, 0.7, and 0.4 atm variables, respectively, which was about three times faster than evaporation rate of the bulk solution.

The weight of the droplets, mdroplets, across the inner wall surface above the 100-mL water level, mdroplets, was approximated by the weight of the flask and the weight of the wetted inner wall surface below the neck, substracting the weight of the flask and the weight of the wetted inner wall surface below the 100-mL water level mark which was 2.5 cm away from the flask bottom. The inner surface above the 100-mL water level mark was wiped dry by a piece of tissue. This weight of droplets and the rate of evaporation of droplets could then be used to approximate the time needed to evaporate all of the droplets by Equation (S6). The parameters used and the results were listed in Table S3.

|  |  |  |
| --- | --- | --- |
|  |  | (S6) |

**SUBLIMATION OF As2O3 CRYSTALS**

Since As2O3 immediately crystallized out via aqueous As2O3 solution droplet deposition on the wall, the total pressure inside the vessel should be equal to the sum of the vapor pressure of water and As2O3 solids, as in Equation (S7). The solute effect can be neglected.

|  |  |  |
| --- | --- | --- |
|  |  | (S7) |

Assuming an ideal gas state,, the ratio of vapor pressure for As2O3 solids and the one for water vapor should be equal to the ratio of the number of moles of As2O3 and the number of moles of water in the vapor phase. The equation was given by:

|  |  |  |
| --- | --- | --- |
|  |  | (S8) |

Rearranging Equation (S8) and changing the terms into mass and volume, the equation becomes:

|  |  |  |
| --- | --- | --- |
|  |  | (S9) |

where MWAs2O3 was 197.8 g/mol, MWH2O was 18 g/mol, VH2O was 100 mL, and ρH2O was 1.0 g/mL.

According to Behrens and Rosenblatt [4], the partial pressure of As2O3 arsenolite form between 94oC to 156oC is given by:

|  |  |  |
| --- | --- | --- |
|  |  | (S10) |

where T is the temperature of the solids in K, which should be equal to the temperature of the wall surface of 120oC. Table S4 lists out all of the parameters and results for the calculation. A comparison of the calculated mass of As2O3 sublimed with the mass of the As2O3 determined by HPLC showed a slight difference, possibly due to some As2O3 vapor deposition on the condenser wall or the connectors.

**ENERGY CALCULATION**

Energy calculation for evaporative crystallization of As2O3 was calculated. There were five kinds of heat that were involved in the process: (1) Sensible heat to raise water temperature to boiling temperature, E1, (2) Latent heat of vaporization of water due to boiling, E2, (3) Latent heat of condensation due to water vapor recondensed back in the condenser, E3, (4) Sensible heat when temperature of the condensate in the condenser decreased to the cooling temperature, E4, and (5) Heat of crystallization of As2O3.

(1) Sensible heat from initial temperature to boiling temperature (E1) was given by:

|  |  |  |
| --- | --- | --- |
|  |  | (S11) |

where ρH2O was 1.0 g/mL, and cpH2O was 4.182 J/(g.K).

(2) Latent heat of vaporization (E2) was given by:

|  |  |  |
| --- | --- | --- |
|  |  | (S12) |

where ρH2O was 1.0 g/mL, and ΔHvaporization was 2257.06 J/g.

(3) Latent heat of condensation (E3) is given as

|  |  |  |
| --- | --- | --- |
|  |  | (S13) |

where ρH2O was 1.0 g/mL, and ΔHcondensation was -2257.06 J/g.

(4) Sensible heat (E4) from boiling temperature to the condenser temperature is given as

|  |  |  |
| --- | --- | --- |
|  |  | (S14) |

where ρH2O was 1.0 g/mL, and cpH2O was 4.182 J/(g.K).

(5) Heat of crystallization of As2O3 (E5) is given as

|  |  |  |
| --- | --- | --- |
|  |  | (S15) |

where ΔHcrystallization was 7480.76 J/g.

Assuming that: (1) the effect of solute towards physical properties of water, and (2) the effect of temperature towards density and heat capacity of water, were negligible, the total energy needed for evaporative crystallization of As2O3 was:

|  |  |  |
| --- | --- | --- |
|  |  | (S16) |

Note that because the system releases heat in E3 and E4. Therefore, the energy required to provide to the condenser was given by the absolute value.

Finally, the theoretical energy consumption could be calculated by substituting all the unknown terms. Calculations were performed at p = 1.0, 0.7, and 0.4 atm; initial temperature of 25oC, condenser temperature of 25oC, and initial volume of 100 mL. Table S5 listed the parameters used.

**Table S1.** Parameters Used in Calculation of Equation (S1).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Pressure (atm) | a (oC) | (mL) | (mg.mL-1) | b (mg.mL-1) | (mL) |  |
| 1.0 | 100.0 | 100.0 | 2 | 83.5 | 2.4 | 2.4% |
| 0.7 | 90.3 | 100.0 | 2 | 72.3 | 2.8 | 2.8% |
| 0.4 | 76.1 | 100.0 | 2 | 57.5 | 3.5 | 3.5% |
| a Boiling temperatures of water are interpolation from pp 857 of Ref. [5]. The effect of solute was  assumed to be negligible  b Solubility values at Tboiling were obtained from Ref. [6]. Solubility at 76.1 and 90.3oC  were interpolation values, while solubility at 100oC was extrapolated value | | | | | | |

**Table S2.** Parameters Used and the Results of Equation (S4) and (S5).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  | |  | |  |  |  |  |  |  | |
| (atm) | (Pa) | (mL.min-1) | (mol.s-1) | (oC) | (K) | (m2) | (Pa) | (m2) | (kg.mol-1) | (J.K-1.mol-1) | (mol.s-1) | (mL.min-1) |
| 1.0 | 101325 | 0.83 | 7.7 x 10-4 | 100 | 373 | 5.15 x 10-3 | 91266.7 | 1.4 x 10-2 | 0.018 | 8.314 | 2.4 x 10-3 | 2.6 |
| 0.7 | 70927.5 | 0.97 | 9.0 x 10-4 | 90.3 | 363.3 | 5.15 x 10-3 | 59326.5 | 1.4 x 10-2 | 0.018 | 8.314 | 2.8 x 10-3 | 3.0 |
| 0.4 | 40530 | 1.62 | 1.5 x 10-3 | 76.1 | 349.1 | 5.15 x 10-3 | 21537.5 | 1.4 x 10-2 | 0.018 | 8.314 | 4.8 x 10-3 | 5.0 |

**Table S3.** Parameters and Results Calculated Based on Equation (S6).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pressure (atm) | (g) | (mol.s-1) | (g.mol-1) | teva droplets (s) |
| 1.0 | 0.13 | 2.4 x 10-3 | 18 | 3.0 |
| 0.7 | 0.13 | 2.8 x 10-3 | 18 | 2.6 |
| 0.4 | 0.13 | 4.8 x 10-3 | 18 | 1.6 |

**Table S4.** Parameters Used in Calculation of Equation (S9) and (S10).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pressure | a | b | c |  |  | d |  |  | e |
| (atm) | (atm) | (K) | (atm) | (g.mol-1) | (mL) | (g.mL-1) | (g.mol-1) | (g) | (g) |
| 1.0 | 1.0 | 393.0 | 2.9 x 10-6 | 197.8 | 100 | 1.0 | 18 | 3.2 x 10-3 | 1.5 x 10-3 |
| 0.7 | 0.7 | 393.0 | 2.9 x 10-6 | 197.8 | 100 | 1.0 | 18 | 4.6 x 10-3 | 2.4 x 10-3 |
| 0.4 | 0.4 | 393.0 | 2.9 x 10-6 | 197.8 | 100 | 1.0 | 18 | 8.0 x 10-3 | 3.7 x 10-3 |
| a Assumed to be equal to the total pressure due to the very small amount of poAs2O3  b Assumed to be equal to the temperature of the inner wall surface, which was 120oC  c Calculated by Equation (S10)  d Assumed to be constant for the sake of simplicity  e Obtained by multiplying As2O3 concentration in the condensed water with the volume of water collected in the condensate receiver | | | | | | | | | |

**Table S5.** Parameters Used for Calculating Equation (S11) to (S16).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pressure |  |  | a |  | b | b | c | d | e |  |
| (atm) | (mL) | (K) | (K) | (K) | (g.mL-1) | (J.g-1.K-1) | (J.g-1) | (g) | (J.g-1) | (kJ) |
| 1.0 | 100 | 298 | 373 | 278 | 1.0 | 4.182 | 2257.06 | 0.2 | 7480.76 | 500.1 |
| 0.7 | 100 | 298 | 363.3 | 278 | 1.0 | 4.182 | 2282.40 | 0.2 | 7480.76 | 500.2 |
| 0.4 | 100 | 298 | 349.1 | 278 | 1.0 | 4.182 | 2318.60 | 0.2 | 7480.76 | 500.1 |
| a Boiling temperature of water are interpolation from Ref. [5] pp 857. The effect of solute was assumed to be negligible  b Assumed to be constant throughout the process for the sake of simplicity  c Enthalpy of vaporization at Tboiling obtained from Ref. [5] pp 857 with interpolation. The effect of solute was assumed to be negligible  d Mass of As2O3 dissolved in the aqueous solution  e Enthalpy of crystallization is two times the minus sign of enthalpy of dissolution of As2O3; ΔHAs2O3(cr) = 2 ΔHH3AsO3(aq)  The value of ΔHH3AsO3(aq) is -740 kJ.mol-1, obtained from Ref. [7] | | | | | | | | | | |

**LIST OF NOMENCLATURES**

|  |  |  |
| --- | --- | --- |
| **Symbol** | **Description** | Unit |
|  | Surface area of bulk As2O3 aqueous solution | m2 |
|  | Surface area of wall surface of the flask covered by droplets | m2 |
|  | Initial concentration of As2O3 in the aqueous solution before evaporation | mg.mL-1 |
|  | Concentration of As2O3 in the aqueous solution at saturation condition | mg.mL-1 |
|  | Heat capacity of water | J.g-1.K-1 |
|  | Diameter of cone I (see Figure S1) | m |
|  | Diameter of cone II (see Figure S1) | m |
|  | Energy change involved when the system undergoes change, see description in Equation (S11) to (S15) | J |
|  | Total energy required to perform evaporative crystallization of As2O3 | J |
|  | Height of cone I (see Figure S1) | m |
|  | Height of cone II minus cone I (see Figure S1) | m |
|  | Molecular weight of As2O3 | g.mol-1 |
|  | Molecular weight of water | kg.mol-1 (Eq. (S4), (S5))  g.mol-1 (Eq. (S6),(S9)) |
|  | Mass of As2O3 underwent sublimation | g |
|  | Mass of As2O3 underwent crystallization | g |
|  | Mass of droplets covering wall of the flask above the solution level, see Figure S1 | g |
|  | Mole of As2O3 | mole |
|  | Mole of H2O | mole |
|  | Total pressure inside the evaporation flask | atm |
|  | Vapor pressure of As2O3 arsenolite form | atm (Eq. (S9), (S10)) |
|  | Vapor pressure of water | Pa (Eq. (S4), (S5))  atm (Eq. (S9)) |
|  | Equilibrium pressure of the vapor-liquid phase | Pa |
|  | Gas constant, 8.314 J.K-1.mol-1 | J.K-1.mol-1 |
|  | Rate of evaporation of bulk solution of As2O3 aqueous solution | mol.s-1 |
|  | Rate of evaporation of droplet of As2O3 aqueous solution | mol.s-1 |
|  | Temperature of As2O3 solids | K |
|  | Boiling temperature of water at a given pressure | K |
|  | Condenser temperature | K |
|  | Initial temperature of the As2O3 aqueous solution | K |
|  | Time required for As2O3 aqueous solution droplets to evaporate | s |
|  | Volume of the As2O3 aqueous solution before evaporation | mL |
|  | Volume of the As2O3 aqueous solution when the system reached saturation point | mL |
| *Greek letters* |  |  |
| ΔHAs2O3(cr) | Enthalpy of crystallization of As2O3 from dissolved H3AsO3 | J.g-1 |
| ΔHH3AsO3(aq) | Enthalpy of dissolution of As2O3 into H3AsO3 | J.g-1 |
|  | Enthalpy of condensation of water | J.g-1 |
|  | Enthalpy of crystallization of As2O3 | J.g-1 |
|  | Enthalpy of vaporization of water | J.g-1 |
|  | Density of air | g.mL-1 |
|  | Density of water | g.mL-1 |

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