Quantitative determination of thallium binding to ferric hexacyanoferrate: Prussian blue[☆]

Yongsheng Yang[a], Patrick J. Faustino[a,*], Joseph J. Progar[b], Charles R. Brownell[a],
Nakissa Sadrieh[c], Joan C. May[b], Eldon Leutzinger[d], David A. Place[d],
Eric P. Duffy[d], Lawrence X. Yu[e], Mansoor A. Khan[a], Robbe C. Lyon[a]

[a] Food and Drug Administration, Center for Drug Evaluation and Research, Office of Testing and Research, Division of Product Quality Research,
Life Science Building-64, 10903 New Hampshire Avenue, Silver Spring, MD 20993, United States
[b] Food and Drug Administration, Center for Biologics Evaluation and Research, Office of Vaccines Research and Review,
Laboratory of Analytical Chemistry, 5516 Nicholson Lane, Kensington, MD 20850, United States
[c] Food and Drug Administration, Center for Drug Evaluation and Research, Office of Pharmaceutical Science, Building-21,
10903 New Hampshire Avenue, Silver Spring, MD 20993, United States
[d] Food and Drug Administration, Office of New Drug Quality Assessment, Division of Medical Imaging, Building-22,
10903 New Hampshire Avenue, Silver Spring, MD 20993, United States
[e] Food and Drug Administration, Center for Drug Evaluation and Research, Office of Generic Drugs,
7500 Standish Place, Rockville, MD 20855, United States

Abstract

Ferric hexacyanoferrate, $(Fe_4^{III}[Fe^{II}(CN)_6]_3)$, also known as insoluble Prussian blue (PB), is the active pharmaceutical ingredient (API) of Radiogardase which is the first approved drug product (DP) for treatment of thallium and radiocesium poisoning. The aim of this study is (1) to determine the *in vitro* thallium binding capacity and binding rates of insoluble PB; and (2) to evaluate the effect of physiological pH conditions, PB particle size and storage conditions on the binding to PB. Experimental pH levels from 1.0 to 7.5 were used to cover the range of pH levels that PB may encounter when traveling through the gastrointestinal (GI) tract in humans. Measurements of thallium binding were made between 1 and 24 h, to cover gastric and intestinal tract residence time. PB was found to have a binding capacity of approximately 1400 mg/g at pH 7.5. When the pH decreased, the binding decreased as well. The results indicated that the hydration state of PB influences the thallium binding process. It was also found that there exits a direct correlation between the moisture loss in PB and the thallium binding rate constant. The PB with 17 mol of water had a binding rate constant of 0.52, which was reduced to 0.32 when PB was dehydrated to 2.5 mol of water. Significant differences were observed in both binding capacity and binding rate constant among PB fractions with different particle size ranges. PB fraction with particle size of 220–1000 μm had a binding rate constant of 0.43, which increased to 0.64 when the particle size was reduced to 32–90 μm. Batch-to-batch variation in thallium binding was also observed among the APIs and the DPs and this was related to particle size and hydration state. These findings can be utilized to evaluate and predict drug product quality under certain manufacturing and dry storage conditions.
Published by Elsevier B.V.

Keywords: Prussian blue; Thallium binding; Particle size; Moisture loss; Hydration; Product quality

1. Introduction

Thallium salts have been used as medicinal agents, as key ingredients in a variety of manufacturing processes, and as a potent rodenticide. Moreover, thallium is a waste product of coal combustion and the manufacturing of cement. The emissions from coal plants alone are said to liberate approximately 600 tons of thallium per year, one-quarter of which is from

the US (Evers, 1988). Total emissions of thallium from all sources are estimated at more than 1500 tons per year (Heim et al., 2002). As a result of this contamination, concentrations of thallium in topsoil are reported to be as high as 0.5 mg/kg in some areas (Heim et al., 2002). Whereas the elemental form of thallium has essentially no toxicity, its univalent (thallous) and trivalent (thallic) salts are highly toxic. Thallium poisoning is frequent in many countries as a result of accidental exposures (Chakrabarti et al., 1985), contamination or adulteration of drugs of abuse (Insley et al., 1986; Meggs et al., 1994), attempted homicides (Rusyniak et al., 2002; Atsmon et al., 2000), contaminated herbal products (Schaumburg and Berger, 1992) and occupational exposure (Hirata et al., 1998). More recently, the potential for nuclear terrorism through the use of a 'dirty bomb' prepared with radioactive isotopes including cesium and thallium has become a significant concern world-wide, especially in the US, which has prompted FDA approval of PB (Ring, 2004; Elcock et al., 2004).

Treatment of thallium-exposed patients begins with gastrointestinal decontamination. Activated charcoal demonstrates substantial thallium adsorption *in vitro* (Lehmann and Favare, 1984; Hoffman et al., 1999), and may have particular clinical utility because thallium demonstrates extensive enterohepatic circulation (Thompson, 1981). Unfortunately, animal investigations of the use of activated charcoal for thallium poisoning are limited and contradictory (Leloux et al., 1990) and human data are lacking.

For years, PB has been accepted as the antidote of choice in patients with thallium poisoning. This is based on substantial *in vitro* binding data (Lehmann and Favare, 1984; Hoffman et al., 1999), diverse animal investigations (Heydlauf, 1969; Kravzov et al., 1993; Meggs et al., 1997; Rios and Monroy-Noyola, 1992), and numerous human case reports and series demonstrating safety and efficacy (Atsmon et al., 2000; Malbrain et al., 1997; Meggs et al., 1994; Pai, 1987; Pedersen et al., 1978; Stevens et al., 1974; Kamerbeek et al., 1971). PB was first prepared in 1704 as a potential dye (Buser et al., 1977). It is also known as Iron blue, Chinese blue, Paris blue as well as a variety of less commonly known names, Brunswick blue and Turnbull's blue (Thompson and Callen, 2004). All of these names refer to the blue colored complex of ferric-hexacyanoferrate(II) (Fig. 1.) with the empirical formula of $Fe_4^{III}[Fe^{II}(CN)_6]_3$ (Monona, 1994). There are a number of related hexacyanoferrate compounds, inaccurately called PB, such as potassium hexacyanoferrate $KFe_3^{III}[Fe^{II}(CN)_6]_3$, $K_2Fe_2^{III}[Fe^{II}(CN)_6]_3$ and ammonium hexacyanoferrate $NH_4Fe_3^{III}[Fe^{II}(CN)_6]_3$ (Beck, 1987). The potassium form of hexacyanoferrate forms a colloidal suspension and is known as soluble PB. It is generally believed that chemical ion-exchange, physical adsorption and ion trapping may all be involved in the PB thallium or cesium binding process. For the ion-exchange mechanism, thallium may exchange with hydrogen from water (hydrodium ion) bound in the PB crystal lattice (Buser et al., 1977). Or, if monovalent cations are present as a result of different synthetic reagents and routes, ion exchange with alkali metal impurities such as sodium, potassium or ammonium may occur (Sharpe, 1976). The adsorption of ion onto the crystal lattice or trapping within

the cavities of the crystal lattice may also occur. Thus, when given orally, PB binds unabsorbed thallium in the gut thereby reversing the concentration gradient and resulting in a reduction in body burden. In addition, PB can interfere with thallium's enterohepatic circulation causing a further reduction in tissue stores. Based on the aforementioned mechanisms, both *in vitro* and *in vivo* binding of thallium to PB could be strongly influenced by its physiochemical properties such as particle size, moisture content and the pH levels of reaction medium. However, there is currently insufficient published data to address the related issues.

The majority of the *in vitro*, animal and human data have shown that soluble PB is effective in thallium poisoning. A recent review of thallium toxicity suggests that the soluble form may be the more effective form of PB in thallium poisoning (Hoffman, 2003). While the insoluble PB may ultimately be effective, there are few studies to support its use in thallium poisoning. Only additional well-designed *in vitro* studies or controlled clinical trials can provide useful evidence. Therefore, we have systematically and extensively studied the *in vitro* binding capacity of insoluble PB, ferric-hexacyanoferrate(II), $Fe_4^{III}[Fe^{II}(CN)_6]_3$, to thallium especially to determine the relationship between physicochemical properties of PB and its binding capacity. Meanwhile, by simulating the physiologically relevant conditions, we have compared the *in vitro* thallium binding capacity under various pH conditions over a wide range of incubation times. The comparison of the thallium binding from batch to batch among the APIs and DPs was also studied.

2. Materials and methods

2.1. Chemicals and reagents

Thallium certified standard solution (1000 ppm, 1 mL = 1 μg Tl) was purchased from High-Purity Standards (Charleston, SC). Thallium chloride was purchased from Aldrich (Milwaukee, WI). Fisher certified buffer solutions (pH 1.0–5.0) were purchased from Fisher Scientific (Fair Lawn, NJ). Dibasic potassium and monobasic potassium phosphate were purchased from J.T. Baker Inc. (Phillipsburg, NJ). PB APIs were purchased from Sigma Corporation (St. Louis, MO) and six different lots of APIs, API-1, API-2, API-3, API-4, API-5 and API-6, were provided by the Heyl Corporation. Five lots of DPs, DP-1, DP-2, DP-3, DP-4 and DP-5, (500 mg/capsule) were provided by the Heyl Corporation and the Oak Ridge Institute for Science and Education (ORISE). There was no apparent paring relationship between the APIs and APIs that the DP contained. Deionized water was supplied in house by a Millipore Milli-Q System (Bedford, MA). All other chemicals were of reagent grade.

2.2. Preparation of pH solutions

The solutions of pH 1, 2, and 5 were prepared by diluting corresponding certified buffer solutions five times with deionized water. The phosphate buffer solution of 40 mM with pH 7.5 was prepared by using dibasic potassium phosphate and monobasic potassium phosphate.
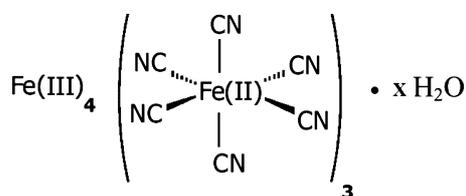
Fig. 1. The chemical structure of Prussian blue.

2.3. Preparation of calibration standards and quality control standards

Thallium standard (1000 ppm Tl, 1 mL = 1 μg Tl) was used as standard stock solution. Seven calibration standard solutions were prepared by transferring 1.5, 2.5, 5.0, 7.5, 10.0, 15.0 and 20.0 mL of stock solution to 50-mL volumetric flasks and bringing to the volume with the corresponding pH solution to obtain the final concentrations of 30, 50, 100, 150, 200, 300 and 400 ppm Tl, respectively. Three standard curves, which included standard blanks, were prepared daily.

The quality control (QC) Tl stock solution (2500 ppm Tl, 1 mL = 2.5 mg Tl) was prepared by dissolving 2.9344 g of thallium chloride in 1000 mL of deionized water. The lower limit of quantification (LLOQ) of 30 ppm, low QC of 50 ppm, intermediate QC of 200 ppm and high QC of 400 ppm were prepared by transferring 0.6, 1.0, 4.0 and 8.0 mL of stock solution to 50-mL volumetric flasks and then adding the corresponding pH solution to the volume. Five standards at each QC level were prepared daily.

2.4. Samples for determination of maximal binding capacity (MBC)

Five concentrations thallium ranging from 600 to 1500 ppm were prepared in pH 7.5 buffer. PB API (0.1 g) was added to 50 mL of thallium solution in a 100-mL flask. The flask was tightly stopped and incubated in a shaking water bath at 37 °C at 75 shakes/min for 0.5, 1, 2, 3, 4, and 24 h. Following incubation, sample was filtered through a 0.2 μm Acrodisc® filter (Gelman Laboratory, MI) to remove particulates. The clear-filtered solution was diluted by 5 times and 10 mL aliquot of sample was used for thallium analysis. All samples were prepared in triplicate (Fig. 1).

2.5. Calculation of MBC

The MBC was calculated from the slope of the Langmuir isotherm (Gessner and Hasan, 1987Gessner et al., 1987) using least squares linear regression. The Langmuir isotherm for chemical adsorption was generated by plotting the ratio of the free thallium to PB bound thallium versus the free thallium using the 24-h binding data. Thallium binding data (shown in Fig. 2) was extended to 48 h with API-1 and no significant binding difference was observed between 24 and 48 h (270.5 versus 271.1 mg/g, respectively), which suggests that the thallium binding approaches equilibrium at 24 h. Therefore, the binding data

at 24 h was used for plotting the Langmuir isotherm according to the Langmuir equation.

The Langmuir equation is:

$$\frac{C}{x/m} = \frac{1}{k_1 k_2} + \frac{C}{k_2}$$

where C equals the free thallium concentration in mg/L, x is the amount of thallium bound in mg, m is the weight of PB in grams, k_1 is affinity binding constant, and k_2 is capacity constant (maximal binding capacity in mg of thallium per g of PB).

2.6. Samples for pH and time-dependent thallium binding profile

One concentration of thallium (600 ppm) was prepared at pH levels 1, 2, 3, 5 and 7.5. API-1 or DP-1 (0.1 g) was added to 50 mL of thallium solution in a 100-mL flask. The flask was tightly stopped and incubated in a shaking water bath at 37 °C at 75 shakes/min for 1, 4, and 24 h. The samples were filtered as described above in Section 2.4.

2.7. Samples for determining thallium binding following API exposure to low pH solutions sequentially

To mimic physiological conditions that would be experienced during the transition process in GI tract, API-1 was pre-exposed to low pH and then followed by the thallium binding test at higher pH media (from pH 2.0 to 7.5). For control purpose, 0.1 g API-1 was mixed with 50 mL of pH 1.0 solution containing 600 ppm Tl in a 100-mL glass flask. It was incubated in a shaking water bath at 37 °C with 75 shakes/min for 1 h and 4 h. The samples were filtered as described above in Section 2.4. For pH 2.0 condition, 0.1 g API-1 was mixed with 10 mL of pH 1.0 buffer solution and incubated in a shaking water bath at 37 °C with 75 shakes/min for 1 h. Then the sample was centrifuged and solution was removed. The PB pellet was transfer to a 100-mL glass flask, 50 mL of pH 2.0 solution containing 600 ppm Tl was added. The aforementioned procedure was followed to prepare the sample for thallium analysis. For pH 5.0 condition, PB was sequentially exposed to pH 1.0 and 2.0 for 1 h each and then the thallium binding test was conducted in pH 5.0 buffer. For pH 7.5 condition, PB was exposure to pH 1.0, 2.0, and 5.0 for 1 h each progressively and then the binding test was conducted in pH 7.5 buffer. To ensure that the whole procedure of sample preparation did not cause meaningful loss of PB, a control experiment was conducted. In the control experiment, PB was sequentially exposure to deionized water for three cycles of 1 h each and then the binding test was conducted in pH 7.5 buffer.

2.8. Samples for effect of moisture loss and PB particle size on thallium binding

One concentration of thallium (1200 ppm) was prepared at pH 7.5. API-1 (0.1 g) was added to 50 mL of thallium solution in a 100-mL flask. The flask was tightly stopped and incubated in a shaking water bath at 37 °C at 75 shakes/min for 0.5, 1, 2, 3,

and 4 h. The samples were filtered as described above in Section 2.4.

2.9. Drying PB at 105 °C

API-1 was selected and dried in an oven for 2 and 24 h at 105 °C, respectively. Samples were removed from the oven and immediately placed in desiccator with calcium sulfate desiccant until use.

2.10. Preparation of API particle size fractions

API-1 was selected and fractioned using an ATM Sonic Sifter (ATM Corporation, Milwaukee, WI). Four particle size fractions: 32–90 μm, 90–150 μm, 150–212 μm, and 212–1000 μm representing the major particle size distribution fractions were collected. The fractions of 32–90 μm and 212–1000 μm were tested for thallium binding.

2.11. Calculation of binding rate constant

Each binding rate constant (k) was calculated based on the equation: $C = C_o \, e^{-kt}$, where C equals the free thallium concentration (ppm) at any time point, C_o equals the initial free thallium concentration (1200 ppm), t equals time point (h). Alternatively, natural logarithm of free thallium concentration versus time point was plotted. The binding rate constant (k) equals the slope of the best straight line calculated by linear regression using the least squares method. The binding rate constants were compared using Student t-test. A p value < 0.05 was considered statistically significant for all results.

2.12. Analytical method for thallium

All standards and samples were analyzed by an OPTIMA 3300DV Inductively Coupled Plasma Spectrometer (PerkinElmer, Shelton, CT) under the following conditions: wavelength, 351.92 nm; plasma aerosol type, wet; nebulizer start-up, instant; source equilibration delay, 15 s; read delay time, 60 s; sample flow rate, 1.5 mL/min; plasma, 15.0 L/min; background correction, 2-point. Each sample was analyzed in triplicate. Thallium concentrations were determined by comparison to a linear standard curve.

3. Results

3.1. Determination of maximal binding capacity (MBC)

The binding of PB API-1 to different concentrations of thallium at pH 7.5 is shown in Fig. 2A. The data suggests that the thallium binding was approaching equilibrium at 24 h. Therefore, the binding data at 24 h was used for plotting Langmuir isotherm as shown in Fig. 2B was considered to be near equilibrium. The MBC was found to be approximately 1400 mg/g. It is important to note that the MBC is an approximate value since the 24 h *in vitro* data used to generate the MBC was considered
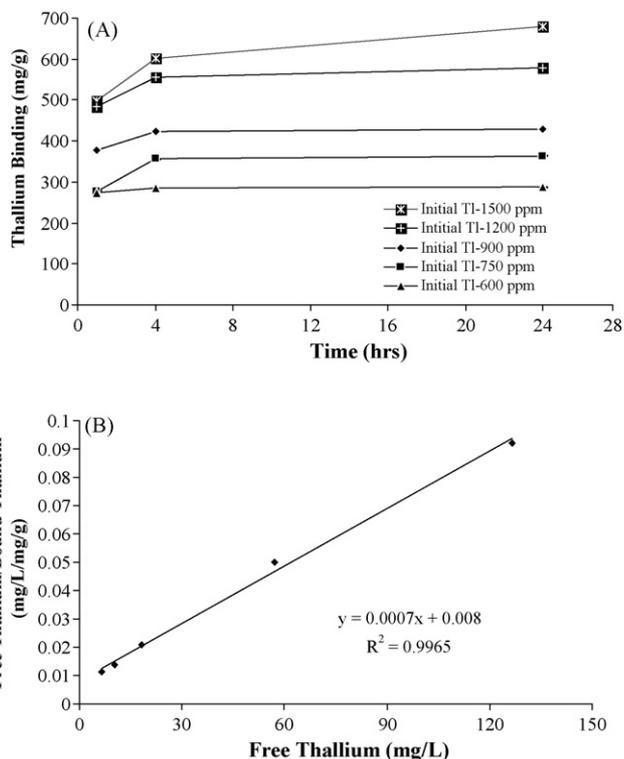

Fig. 2. The concentration-dependent thallium binding profile of API-1 at pH 7.5 (A). The Langmuir isotherm was plotted based on 24-h binding data with best straight line calculated by linear regression using the least squares method. The *X* axis represents the concentration of free thallium (mg/L) in the solution near equilibrium. The *Y* axis represents the ratio of the free thallium concentration (mg/L) versus the bound thallium (mg/g) near equilibrium (B). The data represent the mean and standard deviation of triplicate samples.

to be approaching or near equilibrium. For the purpose of comparison, the MBC of cesium to PB API-1 was determined to be 700 mg/g (data not shown) following the same procedure.

3.2. The pH- and time-dependent thallium binding profile

Fig. 3 illustrates the pH-dependent profile for thallium (600 ppm) binding to API-1 and DP-1. The lowest amount of thallium binding occurred at pH 1.0 at 1 h. The binding gradually increased as pH increased. The thallium binding was found to be 287 and 286 mg/g for API-1 and DP-1 at pH 7.5 for 24 h incubation period, respectively. Both API-1 and DP-1 followed a similar thallium binding pH-profile with a comparable amount of thallium binding.

3.3. Thallium binding profile following API exposure to low pH solutions sequentially

To further simulate the physiologic conditions experienced in GI tract and to determine whether the binding capacity change is the consequence of PB chemical changes caused by cyanide release in low pH or competitive exchange of hydrogen ions, the thallium (600 ppm initial concentration) binding of API-1 was examined following exposure to pH gradients from 1.0 to 7.5 sequentially. The results in Fig. 4 show that pretreatment
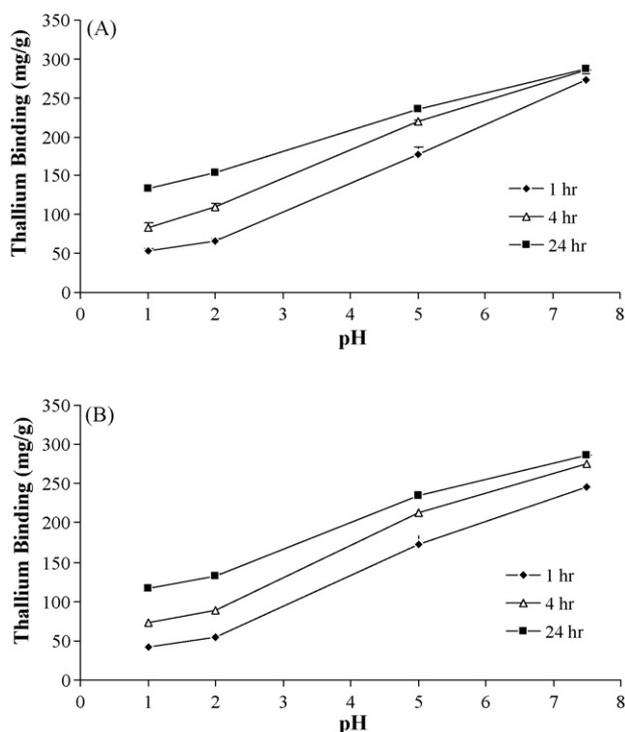
Fig. 3. The pH-dependent thallium binding profiles of API-1 (A) and DP-1(B) using an initial thallium concentration of 600 ppm. The data represents the mean and standard deviation of triplicate samples.

(initial exposure to pH 1.0 for 1 h) had no effect on the thallium binding capacities of API-1 at pH 2.0. Even after sequential exposure to pH of 1.0, 2.0, 3.0, and 5.0 for 1 h each, the thallium binding capacities of PB at pH 7.5 were similar to those without pretreatment. The control experiment data shown indicated that there was negligible PB loss during the whole sample procedure since no binding difference was found between treatment and non-treatment PB (see Fig. 4). The fact that thallium binding capacity was able to recover when the higher GI pH is introduced indicated that lower pH conditions might affect the PB thallium binding process, but did not affect the PB thallium binding capacity.

3.4. Effect of moisture loss and PB particle size on the thallium binding

As shown in Fig. 5A, the thallium binding capacity for API-1 was systematically reduced by drying. The original API-1 bound 17 mol of water (26.2% weight loss by thermogravimetric analysis). After drying at 105 °C for 2 h, the API-1 dehydrated to 9 mol of water and after 24 h the API-1 dehydrated to 2.5 mol of water. The thallium binding for sieved fractions of API-1 is shown in Fig. 5B. The smaller PB particles had greater binding capacity for thallium, as expected based on the increase in surface area with volume.

The thallium binding rate constants were calculated from the data shown in Fig. 5. The effect of PB moisture loss and PB particle size on the rate constants is specified in Table 1. The longer API-1 was dried, the smaller the binding rate constant. The binding rate constant for fully hydrated API-1 was 0.52, compared
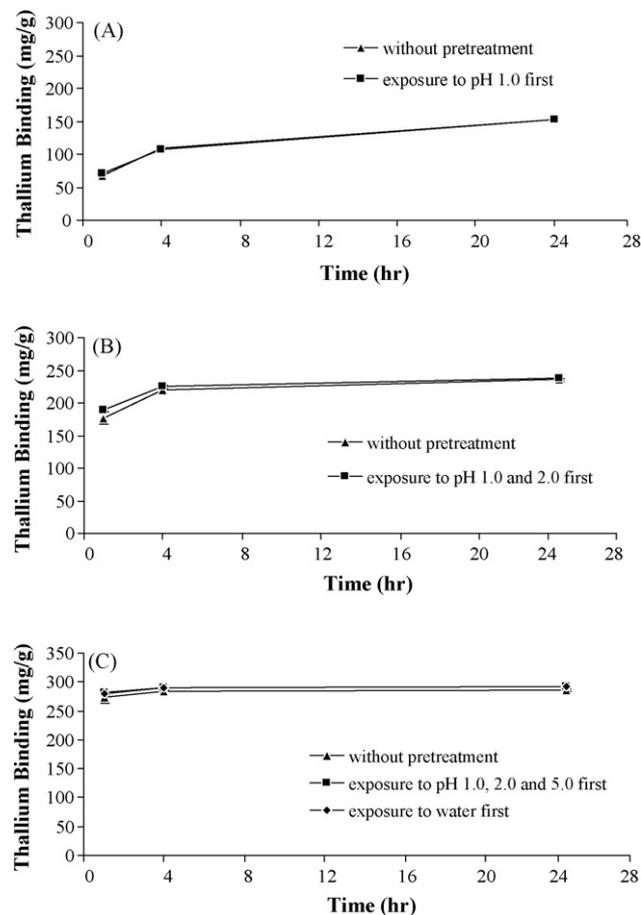

Fig. 4. The thallium binding profile following API-1 exposure to previous pH solutions sequentially. Initial thallium concentration was 600 ppm. API-1 was exposured to pH 1.0 for 1 h and the binding test was conducted at pH 2.0 (A). API-1 was exposured to pH 1.0 and 2.0 for 1 h each and the binding test was conducted at pH 5.0 (B). API-1 was exposured to pH 1.0, 2.0 and 5.0 progressively for 1 h each and the binding test was conducted at pH 7.5 (C). The non-treated API was used as a control for each condition. The data represents the mean and standard deviation of triplicate samples.

to 0.32 for API-1 dried for 24 h. The smaller the particle size, the higher the binding rate constant. The binding rate constant of fraction of 32–90 μm ($k = 0.64$) was significantly higher that of the fraction of 212–1000 μm ($k = 0.43$).

3.5. Comparison of thallium binding among batch to batch for both PB APIs and DPs

The six batches of APIs, API-1, API-2, API-3, API-4, API-5 and API-6 were tested. API-2 is unique in that it was manu-

Table 1
Thallium binding rate constant

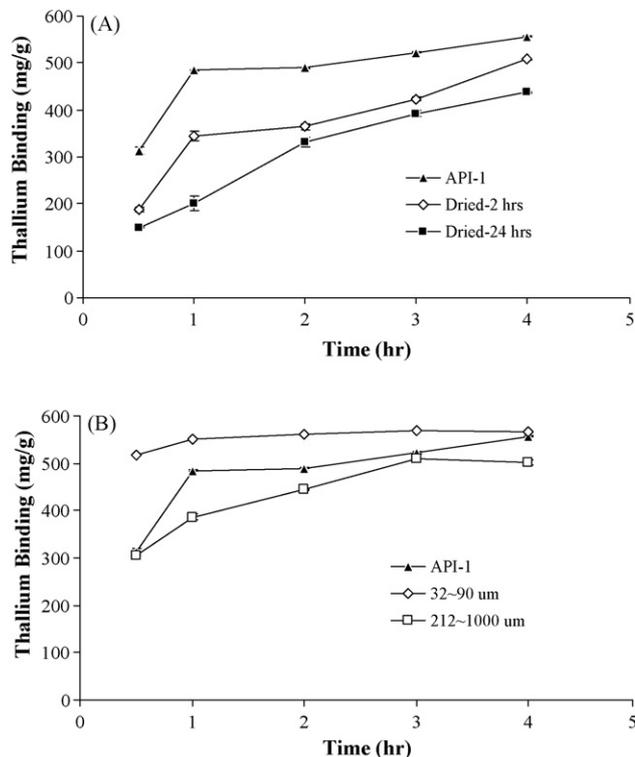| PB	Binding rate constant (k)	p value
API-1	0.52	–
API-1 dried for 2 h at 105 °C	0.43	0.016
API-1 dried for 24 h at 105 °C	0.32	0.006
API-1 fraction of 32–90 μm	0.64	0.017
API-1 fraction of 212–1000 μm	0.43	0.019

Fig. 5. Effect of moisture loss and PB particle size on the thallium binding. Initial thallium concentration was 1200 ppm. API–1 was dried for 2 and 24 h at 105 °C and the binding test was conducted in the pH 7.5 solution at 37 °C at times ranging from 0.5 to 4 h (A). API–1 was fractioned and the binding test was conducted in the pH 7.5 solution at 37 °C at times ranging from 0.5 to 4 h (B). The non-treated API was used as a control for each condition. The data represents the mean and standard deviation of triplicate samples.

factured in 1987 and utilized clinically as a medical treatment in the Goiânia radiocesium incident in Brazil in 1987. The five batches of DPs tested were DP-1, DP-2, DP-3, DP-4 and DP-5. Except for API-2, all other APIs and DPs were manufactured in 2002 and 2003. The results are shown in Fig. 6 for APIs and DPs. For all APIs, except API-2, the thallium binding was nearly constant (ranging from 262 to 287 mg/g) over 1–24 h. Similarly for all DPs, except DP-2, the thallium binding only ranged from 245 to 287 mg/g. The thallium binding was exceptionally low at 1 h for API-2 (120 mg/g) and for DP-2 (142 mg/g).

4. Discussion

Since PB was introduced as a countermeasure for thallium poisoning in 1960s, substantial *in vitro* binding tests (Lehmann and Favare, 1984; Hoffman et al., 1999), extensive animal investigations (Heydlauf, 1969; Kravzov et al., 1993; Meggs et al., 1997; Rios and Monroy-Noyola, 1992), and many human case reports (Atsmon et al., 2000; Malbrain et al., 1997; Meggs et al., 1994; Pai, 1987; Pedersen et al., 1978; Stevens et al., 1974; Kamerbeek et al., 1971) have been published. The majority of human cases have been treated with soluble rather than insoluble PB. Animal and *in vitro* studies have also largely utilized soluble rather insoluble PB. A recent review of thallium toxicity concluded that the soluble product seems to be the more effec-
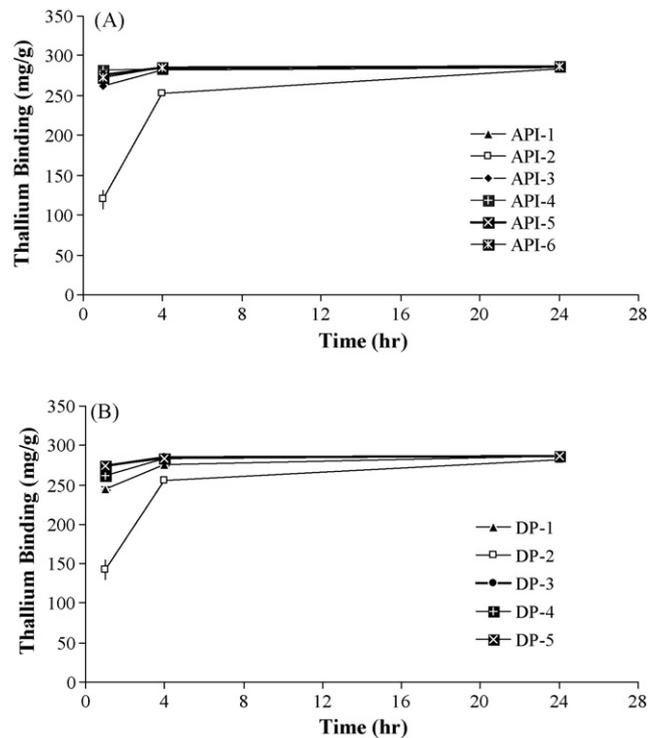

Fig. 6. Comparison of thallium binding for several batches of PB APIs (A) and DPs (B). The data represent the mean and standard deviation of triplicate samples.

tive form of PB in thallium poisoning (Hoffman, 2003). So far, little evidence is available to support whether insoluble PB is an effective treatment for thallium poisoning. Only one animal study showed that the insoluble PB decreased the half-life of radioactive thallium-204 from 3.8 to 2.2 days in rat (Thompson and Callen, 2004). In the present study, our data suggested that insoluble PB had a MBC of 1428 mg/g for thallium which was even higher than that of cesium (700 mg/g).

An *in vitro* study compared the cesium binding characteristics of soluble and insoluble PB (Verzijl et al., 1992) at 37 °C at pH 1.0, 5.6, and 7.5. Insoluble PB was found to have 3 times the binding capacity of the soluble formulation at pH 7.5. Moreover, insoluble PB has been the most widely used form in treating radiocesium poisoning, and the majority of clinical evidence involves the insoluble form (Melo et al., 1994; Lipsztein et al., 1991; Farina et al., 1991; Oliveira et al., 1991). With the above facts it has been shown that insoluble PB is very effective in binding thallium, and it therefore can be used as an antidote in thallium poisoning.

In a previous study we found that the cesium binding to PB APIs and DPs were significantly affected by media pH levels (Faustino et al., 2007). Verzijl et al. (1992) also reported that binding of radioactive cesium-137 to PB was pH dependent. Whether pH has any effect on the binding of thallium to PB was unknown. Normally, under fasting conditions, the pH ranges in the gastrointestinal tract vary from 1.0 to 2.5 in the stomach, 4.9 to 6.4 in the duodenum, 4.4 to 6.4 in the jejunum, 6.5 to 7.4 in the ileum and 7.4 in colon (Fleisher et al., 1999). For normal subjects, average gastric emptying time (80% of content)

is approximately 30 min for a low-calorie bland meal, and 3.5 h for a high-fat liquid meal (Houghton et al., 1990). The intestinal residence time in normal subjects varies from 20 to 30 h due to the influence by many factors (LeRoy et al., 1966). To simulate the physiologically relevant circumstance encountered by PB when traveling through the GI tract after oral administration, we have investigated the thallium binding to PB under the pH conditions of 1.0 to 7.5 and incubation times ranged from 1 to 24 h. For both API-1 and DP-1, the thallium binding to PB displayed a pH-dependent profile with binding gradually increasing with increasing pH. The maximum binding was found at pH 7.5 which should be an optimum pH condition for binding.

It is generally believed that chemical ion-exchange, physical adsorption and ion trapping may all be involved in the PB thallium or cesium binding process. Our data indicates that pH affects the binding rate. In the process of thallium binding to PB, the chemical ion-exchange reaction may be affected more profoundly. The physical adsorption and ion trapping process may be affected as well, but possibly to a lesser extent. We have found cyanide release from PB was also pH dependent: the lower pH, the higher cyanide release (Yang et al., 2007b). Similar results were reported by other authors (Verzijl et al., 1993). Whether the binding capacity change is the consequence of cyanide release caused by pH or competitive exchange of hydrogen ions is unknown. Experimentally, the cyanide released from PB was less than 0.05% of its weight under extremely low pH condition (Yang et al., 2007a), which is unlikely to cause any significant change in PB structure and thereby further to alter the binding capacity. To investigate this, the thallium binding of API-1 was examined following exposure to pH gradients from 1.0 to 7.5 sequentially in order to mimic the physiological conditions. After PB was pretreated in lower pH media, progressive pretreatment of PB with low pH media did not affect its thallium binding capacity. The data further suggests that the lower pH conditions might affect the PB thallium binding reaction, but do not affect its intrinsic ability to bind thallium. Since these results suggest that thallium binding by PB will not be impacted by gastric low pH and the binding of thallium by PB occurs primarily in the small intestine, it is not likely that the overall therapeutic efficacy of PB will be compromised.

The physical properties of PB (e.g. particle size, moisture content) may have a significant effect on its clinical efficacy. The variations in the product preparation may result in significant variability in the particle size distribution. On study (Kravzov et al., 1993; Rios et al., 1991) compared synthesized PB with a crystal size of 176.8 Å with a commercial preparation of crystal size measuring 311.9 Å. The synthesized compound, which because of its small size and larger surface area, bound more thallium *in vitro* and had higher antidotal efficacy in rat. Unfortunately, little can be concluded from the study because of the small number of animals involved and soluble PB used. In the present study, it was found that PB with smaller particle size had the higher binding capacity and the faster binding rate. The loss of moisture resulted in reduced binding rates and reduced binding capacity, indicating that the water molecule in PB is involved in thallium and PB interaction, or more likely thallium may exchange with hydrogen from water (hydrodium ion, H_3O+) bound in the PB crystal lattice.

A comparative study of six PB APIs and five DPs indicated little difference in the thallium binding capacities, with the exception of API-2 and DP-2. API-2 which was manufactured in 1987 had a larger particle size with d_{50} of 252 μm (98 μm for API-1) and lower water content (12 mol water versus 16 mol water for API-1), possibly due to long term storage. Compared to the other drug product lots, DP-2 differed in physical appearance, had a significantly larger particle size (d_{50} of 223 μm) and lower water content (12.0 mol water). There was no apparent paring relationship between the APIs and the APIs that the DP contained.

5. Conclusions

Insoluble PB has a high binding capacity for thallium with an *in vitro* MBC of approximately 1400 mg/g, indicating it is a useful antidote in thallium poisoning. Low pH conditions may affect the interacting process between PB and thallium, but may not alter its intrinsic ability to bind thallium. This suggests that the gastric low pH condition may not have a significant influence on the overall therapeutic outcome for PB, since the *in vivo* binding of thallium by PB occurs primarily in the small intestine. Since the particle size affects both thallium binding rate and binding capacity to PB, it is recommended that this attribute be well controlled during the manufacturing process to ensure the product quality. The fact that the moisture loss in PB has a profound impact on its binding capacity suggests that the interaction between water molecule or hydrodium form in PB and thallium ion may be a very important mechanism of action. Meanwhile, the proper storage condition for PB is required to ensure optimal effectiveness.

References

Atsmon, J., Taliansky, E., Landau, M., Neufeld, M.Y., 2000. Thallium poisoning in Israel. Am. J. Med. Sci. 320, 327–330.

Beck, M.T., 1987. Critical survey of stability constants of cyano complexes. Pure Appl. Chem. 12, 1703–1720.

Buser, H.J., Schwarzenbach, D., Pette, W., Ludi, A., 1977. The crystal structure of Prussian blue: $Fe_4[Fe(CN)_6]_3·H_2O$. Inorg. Chem. 16, 2704–2709.

Chakrabarti, A.K., Ghosh, K., Chaudhuri, A.K., 1985. Thallium poisoning: a case report. J. Trop. Med. Hyg. 88, 291–293.

Elcock, D., Klemic, G.A., Taboas, A.L., 2004. Establishing remediation levels in response to a radiological dispersal event (or "dirty bomb"). Environ. Sci. Technol. 38, 2505–2512.

Evers, U., 1988. Environmental exposure to thallium. Sci. Total Environ. 71, 285–292.

Farina, R., Brandao-Mello, C.E., Oliveira, A.R., 1991. Medical aspects of 137Cs decorporation: the Goiania radiological accident. Health Phys. 60, 63–66.

Faustino, P.J., Yang, Y.S., Progar, J.J., Brownell, C.R., Sadrieh, N., May, J.C., Leutzinger, E., Place, D.A., Duffy, E.P., Houn, F., Loewke, S.A., Mecozzi, V.J., Ellison, C.D., Khan, M.A., Hussain, A.S., Lyon, R.C., 2007. Quantitative determination of cesium binding to ferric hexacyanoferrate: prussian blue. J. of Pharm. Biomed Anal., in press, doi:10.1016/j.jpba.2007.11.049.

Fleisher, D., Li, C., Zhou, Y.J., Pao, L.H., Karim, A., 1999. Drug, meal and formulation interactions influencing drug absorption after oral administration—Clinical implications. Clin. Pharmacokinet. 36, 233–254.

Gessner, P.K., Hasan, M.M., 1987. Freundlich and Langmuir isotherms as models for the adsorption of toxicants on activated charcoal. J. Pharm. Sci. 76, 319–327.

Heim, M., Wappelhorst, O., Markert, B., 2002. Thallium in terrestrial environments: occurrence and effects. Ecotoxicology 11, 369–377.

Heydlauf, H., 1969. Ferric-cyanoferrate(II): an effective antidote in thallium poisoning. Eur. J. Pharmacol. 6, 340–344.

Hirata, M., Taoda, K., Ono-Ogasawara, M., Takaya, M., Hisanaga, N., 1998. A probable case of chronic occupational thallium poisoning in a glass factory. Ind. Health 36, 300–303.

Hoffman, R.S., 2003. Thallium toxicity and the role of Prussian blue in therapy. Toxicol. Rev. 22, 29–40.

Hoffman, R.S., Stringer, J.A., Feinberg, R.S., Goldfrank, L.R., 1999. Comparative efficacy of thallium adsorption by activated charcoal, Prussian blue, and sodium polystyrene sulfonate. Clin. Toxicol. 37, 833–837.

Houghton, L.A., Mangnall, Y.F., Read, N.W., 1990. Effect of incorporating fat into a liquid test meal on the relation between intragastric distribution and gastric emptying in human volunteers. Gut 31, 1226–1229.

Insley, B.M., Grufferman, S., Ayliffe, H.E., 1986. Thallium poisoning in cocaine abusers. Am. J. Emerg. Med. 4, 545–548.

Kamerbeek, H.H., Rauws, A.G., ten Ham, M., van Heijst, A.N., 1971. Prussian blue in therapy of thallotoxicosis. An experimental and clinical investigation. Acta Med. Scand. 189, 321–324.

Kravzov, J., Rios, C., Altagracia, M., Monroy-Noyola, A., Lopez, F., 1993. Relationship between physicochemical properties of Prussian blue and its efficacy as antidote against thallium poisoning. J. Appl. Toxicol. 13, 213–216.

Lehmann, P.A., Favare, L., 1984. Parameters for the adsorption of thallium ions by activated charcoal and Prussian blue. J. Toxicol. Clin. Toxicol. 22, 331–339.

Leloux, M.S., Lich, N.P., Claude, J.R., 1990. Experimental studies on thallium toxicity in rats. J. Toxicol. Clin. Exp. 10, 147–156.

LeRoy, G.V., Rust, J.H., Hasterlik, R.J., 1966. The consequences of ingestion by man of real and simulated fallout. Health Phys. 12, 449–473.

Lipsztein, J.L., Bertelli, L., Oliveira, C.A.N., Dantas, B.M., 1991. Studies of cesium retention in the human body related to body parameters and Prussian blue administration. Health Phys. 60, 57–61.

Malbrain, M.L., Lambrecht, G.L., Zandijk, E., Demedts, P.A., Neels, H.M., Lambert, W., De Leenheer, A.P., Lins, R.L., Daelemans, R., 1997. Treatment of severe thallium intoxication. J. Toxicol. Clin. Toxicol. 35, 97–100.

Meggs, W.J., Cahill-Morasco, R., Shih, R.D., Goldfrank, L.R., Hoffman, R.S., 1997. Effects of Prussian blue and *N*-acetylcysteine on thallium toxicity in mice. J. Toxicol. Clin. Toxicol. 35, 163–166.

Meggs, W.J., Hoffman, R.S., Shih, R.D., et al., 1994. Thallium poisoning from maliciously contaminated food. J. Toxicol. Clin. Toxicol. 32, 723–730.

Melo, D.R., Lipsztein, J.L., de Oliveira, C.A.N., Bertelli, L., 1994. 137CS internal contamination involving a Brazilian accident, and the efficacy of Prussian blue treatment. Health Phys. 66, 245–252.

Monona, R., 1994. The Artist's Handbook, second ed. Allworth Press, New York, p. 107.

Oliveira, A.R., Hunt, J.G., Valverde, N.J., Brandao-Mello, C.E., Farina, R., 1991. Medical and related aspects of the Goiania accident: an overview. Health Phys. 60, 17–24.

Pai, V., 1987. Acute thallium poisoning. Prussian blue therapy in 9 cases. West Indian Med. J. 36, 256–258.

Pedersen, R.S., Olesen, A.S., Freund, L.G., Solgaard, P., Larsen, E., 1978. Thallium intoxication treated with long-term hemodialysis, forced diuresis and Prussian blue. Acta Med. Scand. 204, 429–432.

Ring, J.P., 2004. Radiation risks and dirty bombs. Health Phys. 86, S42–S47.

Rios, C., Kravzov, J., Altagracia, M., Lopez-Naranjo, F., Monroy A, 1991. Efficacy of Prussian blue against thallium poisoning: effect of particle size. Proc. West Pharmcol. 34, 61–63.

Rios, C., Monroy-Noyola, A., 1992. D-penicillamine and Prussian blue as antidotes against thallium intoxication in rats. Toxicology 74, 69–76.

Rusyniak, D.E., Furbee, R.B., Kirk, M.A., 2002. Thallium and arsenic poisoning in a small midwestern town. Ann. Emerg. Med. 39, 307–311.

Schaumburg, H.H., Berger, A., 1992. Alopecia and sensory polyneuropathy from thallium in a Chinese herbal medication. JAMA 268, 3430–3431.

Sharpe, A.G., 1976. The Chemistry of Cyano Complexes of Transition Metals. Academic Press, New York, p. 99.

Stevens, W., van Peteghem, C., Heyndrickx, A., Barbier, F., 1974. Eleven cases of thallium intoxication treated with Prussian blue. Int. J. Clin. Pharmacol. 10, 1–22.

Thompson, D.F., 1981. Management of thallium poisoning. Clin. Toxicol. 18, 979–990.

Thompson, D.F., Callen, E.D., 2004. Soluble or insoluble Prussian blue for radiocesium and thallium poisoning? Ann. Pharmacother. 38, 1509–1514.

Verzijl, J.M., Joore, J.C., van Dijk, A., Glerum, J.H., Savelkoul, T.J., Sangster, B., van het Schip, A.D., 1992. In vitro binding characteristics for cesium of two qualities of Prussian blue, activated charcoal and Resonium-A. J. Toxicol. Clin. Toxicol. 30, 215–222.

Verzijl, J.M., Joore, J.C., van Dijk, A., Wierckx, F.C., Savelkoul, T.J., Glerum, J.H., 1993. In vitro cyanide release of four Prussian blue salts used for the treatment of cesium contaminated persons. J. Toxicol. Clin. Toxicol. 31, 553–562.

Yang, Y.S., Brownell, C.R., Sadrieh, N., May, J.C., Del Grosso, Lyon, R.C., Faustino, P.J., 2007a. Validation of an in vitro method for the determination of cyanide release from ferric-hexacyanoferrate: Prussian blue. J. Pharm. Biomed. Anal. 43, 1358–1363.

Yang, Y.S., Brownell, C.R., Sadrieh, N., May, J.C., Del Grosso, A., Place, D.A., Leutzinger, E., Duffy, E.P., He, R., Houn, F., Lyon, R.C., Faustino, P.J., 2007b. Quantitative measurement of cyanide released from prussian blue. Clin. Tox. 45 (7), 776–781.