Physicochemical Characterisation of Commercially Available Prussian Blue Insoluble Samples and Its Comparison with Radiogardase®-Cs

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ABSTRACT

The physicochemical properties of insoluble Prussian blue (PB) play an important role in its thallium binding ability. Therefore, the present study aimed to characterise various physicochemical parameters of PB available commercially and compare them with the USFDA-approved Radiogardase®-Cs. In addition, PB was synthesised by indirect and direct methods. PB samples and Radiogardase®-Cs were analysed for various parameters like particle size, moisture content, and thermogravimetric analysis (TGA) and correlated with its Maximum Binding Capacity (MBC) for thallium. Radiogardase®-Cs showed the highest MBC of 238 mg/g for thallium with D$_{90}$ of 785 µm and moisture content of 23.24 %. The MBC of other PB samples was found to be significantly lower than Radiogardase®-Cs which was found to be directly proportional to the moisture content. However, other parameters like particle size, and iron content vary significantly but no correlation was observed with MBC for thallium. This finding suggests that moisture content and MBC are extremely important parameters for optimising the PB to achieve desirable pharmacological efficacy for removing thallium in vivo.

Keywords: Prussian blue; Radiogardase®-Cs; Synthesis; Characterisation; Thallium

NOMENCLATURE

Cs : Cesium
TI : Thallium
USFDA : The United States Food and Drug Administration
MBC : Maximum binding capacity
APIs : Active pharmaceutical ingridients
PB : Prussian blue
AAS : Atomic absorption spectrophotometry
TGA : Thermogravimetric analysis
HNO$_3$ : Nitric acid
LOD : Limit of detection
LOQ : Limit of quantification
ICH : International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

1. INTRODUCTION

Prussian blue is known as ferric (III) hexacyanoferrate (II). As a result of iron ferrocyanide salts oxidation, it forms a deep blue pigment. The use of PB for thallium (TI) poisoning began in the 1970s and thereafter its use has been reported in many clinical and preclinical studies as an antidote for internal contamination of radioactive or nonradioactive isotopes of TI and cesium (Cs). However, a well systematic study of PB was reported in 1987 during the Goiania tragedy in Brazil. Around 249 patients internally contaminated with radioactive Cs were treated with PB which validated the efficacy of PB against Cs contamination. Later in 2003, USFDA approved a 500 mg hard capsule containing Ferric(III) hexacyanoferrate(II) (PB) under the tradename of Radiogardase®-Cs, which is indicated for decontamination of individuals who are internally contaminated with Cs or TI.

The mechanism of PB involves both adsorption and ion exchange of Cs$^+$/TI$^+$ ion with the hydrogen ion (H$^+$) or water-bound hydronium ion (H$_3$O$^+$). In addition to serving as an ion exchanger, Prussian blue has a crystal lattice that makes it suitable for working with univalent cations. Due to the fact that its affinity increases with increasing cation ionic radius, as it prefers to bind TI (ionic radius 0.147 nm) over potassium (ionic radius 0.133 nm). Thus, Prussian blue binds unabsorbed thallium in the gut, thus reversing the concentration gradient and reducing the burden on the body. Additionally, Prussian blue interferes with thallium’s enterohepatic circulation,
causing further tissue depletion.\textsuperscript{7,8} Still it is difficult to explain the mechanism by which thallium binds to Prussian blue due to the variety of structures formed by hexacyanoferrates(II, III). Because of these reasons, thallium removal methods remain speculative rather than understood in full.\textsuperscript{9}

Considering the local action of PB in intestines and no absorption in the systemic circulation, USFDA in a guidance document recommended that “the results of in vitro studies showing that a product’s binding to Tl or Cs is comparable to that of Radiogardase\textsuperscript{®}-Cs” is sufficient to submit a 505 (b) (2) application (guidance document).\textsuperscript{10} Considering this fact, the commercially available PB samples were compared with Radiogardase\textsuperscript{®}-Cs for various in vitro parameters along with MBC to Tl.

It is important to mention here that even though it’s an essential radionuclide decoration agent, no country has included its monograph in any official pharmacopoeias. The systematic literature has insufficient information on in vitro studies and in vivo efficacy of Cs/Tl binding efficiency to PB under various physicochemical conditions. Furthermore, PB available in the market is usually contaminated with various impurities that significantly affect its Cs/Tl binding efficacy. In addition, several in vitro and in vivo studies have used a variety of hexacyanoferrates coming from different precursors, synthesis methods, and manufacturing conditions that are known to affect binding efficiency. The present study was focused on the chemical as well as physical characteristics of PB. PB was synthesised by direct and indirect methods and various physicochemical parameters such as particle size, moisture content, iron content and thermal decomposition were determined. Further MBC of PB samples including commercially available PB (API-1, API-2, and API-3) and Radiogardase\textsuperscript{®}-Cs for Tl was determined using Langmuir adsorption isotherm models.

2. METHODOLOGY

2.1 Chemicals and Reagents

Different APIs of PB (Ferric(III) hexacyanoferrate(II)) were purchased from Tokyo Industries Co. Ltd. Japan (API-1), Senova Technology Co. Ltd, China (API-2) and Avra Synthesis Pvt. Ltd. India (API-3). Radiogardase\textsuperscript{®}-Cs was provided by Heyl Chem.-pharm. Fabrik GmbH & Co. KG, Berlin, Germany. High Purity Laboratory Chemicals Pvt. provided the atomic absorption spectrophotometry (AAS) standard solution of iron and Tl., it contains a metal ion concentration of 1000±10 mg/L. In addition to these chemicals, A high-quality analytical grade chemicals were used for the experiments.

2.2 Synthesis of Ferric(III) Hexacyanoferrate(II)

2.2.1 Direct Method (PB-1)

The direct method for synthesizing PB-1 involves a one-step reaction between potassium ferrocyanide and ferric chloride. Solutions of 40 Mm ferric chloride and potassium ferrocyanide were prepared in water separately. After preparation of both the solutions, potassium ferrocyanide solution over ferric chloride solution was added to yield Ferric(III) hexacyanoferrate(II). The resulting mixture was constantly stirred on magnetic stirrer during this process for 12 h. The reaction mixture was centrifuged and supernatant was decanted. The resulting blue solid was placed in a 2 L Erlenmeyer flask and rinsed twice with 1.5 L of H\textsubscript{2}O and 300 mL of MeOH. Product was collected and dried on filter paper at 80 °C for 2 h. The sample was then grounded with a mortar and pestle and stored in a glass ampoule. The yield of PB from this method was approximately 17.72 g.

2.2.2 Indirect Method (PB-2)

The indirect method for the synthesis of PB-2 involves a two-step reaction. A solution of 0.3 M ferrous sulfate and 0.1 M potassium ferrocyanide in 250 ml was prepared separately. After this, the solution of potassium ferrocyanide was poured into the solution of ferrous sulfate in equal volumes (1:1) with continuous mixing. It resulted in the formation of Berlin White and was kept for 2 hours at 60°C. In this condition, the greenish-blue material slowly settled down. After 2 hours, the extra water/supernatant was decanted using a pipette without disturbing or shaking the settled greenish-blue material. After aging, Berlin white was oxidised to PB (Ferric(III) hexacyanoferrate(II)) by adding drop by drop 70 % hydrogen peroxide and allowed to rest for 15 minutes. The formed product was washed with Milli-Q water 5–6 times after centrifugation. After washing, the solid or semi-solid paste was evenly poured into a glass or stainless steel tray and dried for 2 hours at 80 °C. The yield of PB from this method was approximately 11 g.

3. CHARACTERISATION OF PB

3.1 Particle Size Analysis

Synthesised PB (PB-1 and PB-2), different APIs (API-1, API-2, and API-3), and Radiogardase\textsuperscript{®}-Cs were analysed for particle size distribution using laser diffraction particle size analysers (Mastersizer 3000E, Malvern Panalytical Ltd., UK). The particle size of PB samples was determined in terms of D\textsubscript{90}.

3.2 Moisture Content

The moisture content and thermal decomposition were determined by Thermogravimetric analysis (TGA) using TGA 4000 system Thermal Gravimetric Analyzer (PerkinElmer, USA) as the method given by Aparicio.\textsuperscript{11} Moisture-induced weight loss was measured using a temperature increase of 20 °C/min.

3.3 Iron Content by Atomic Absorption Spectrophotometry

3.3.1 Analytical Method and Validation

A LABINDIA AA8000 atomic absorption/emission spectrophotometer equipped with an air-acetylene burner
was used to analyse. All standards and samples for iron and Tl content AAS standard solution (1000 mg/L) were used as the standard stock solution. The sample solutions aspirated into air-acetylene flame for 5s. In triplicate, samples and standards were prepared using 5% HNO₃. An absorption peak of 248 nm and 276.8 nm was detected for iron and Tl. Following ICH Q2(R1) guidelines 12, the AAS analytical method met precision, accuracy, linearity, range, and specificity criteria. As per the standard deviation (σ) of the blank response and the slope (m) of the calibration curve, the limit of detection (LOD) and limit of quantification (LOQ) were calculated using the formula, LOD =3σ/m with LOQ =10σ/m.¹³

3.3.2 Estimation of Iron Content in PB

PB Samples were digested completely using acidic and basic treatment before analysis. 0.01g of PB samples were weighed and mixed with 10ml of sodium hydroxide solution (10N). Samples were incubated for 10 min at room temperature. A reddish brown precipitate was formed. Sodium hydroxide was decanted and 10 ml of concentrated hydrochloric acid was added to the residue and kept in the water bath until a clear solution was obtained. Acidic and basic solutions were analysed for iron content using AAS.

3.4 Maximum Binding Capacity for Tl

Thallium chloride 1.173 grams was dissolved in 1000 ml Millipore water to prepare the Standard stock solution (1000 mg/L). A total of six calibration standards were prepared with concentrations ranging from 2, 4, 8, 12, 16, and 20 mg/L. The calibration curve was plotted for the determination of Tl content in unknown samples.

The Further stock solution of Tl (1000 mg/L) was used to assess the adsorption capacity of PB samples. Accurately weighed (0.1 g) quantity PB samples were mixed separately in a flask containing 50 ml Tl solution ranging from 200-1000 mg/L. For 24 h, a shaking water bath was used to incubate the flask, which was tightly closed. After 24 h of incubation, Accrodisc® syringe filters (Pall Corporation, USA) were used to filter samples, and then 0.5 ml of the filtrate was taken out and diluted up to 10 ml with 5 % Nitric acid solution. These diluted samples were analysed for Tl content using AAS. The Ce and Qe values were calculated as described in the literature.¹¹,¹²

3.5 Adsorption Isotherm Models

The data for PB were analysed using the Langmuir adsorption isotherm model to determine its maximum Tl adsorption capacity (mg/g). In the Langmuir adsorption isotherm, equation (1) provides the linear equation for the adsorption isotherm.

\[
\frac{C_e}{q_e} = \frac{L}{q_mK_L} + \frac{C_e}{q_m}
\]

A constant \( K_L \) (L/mg) relate to the energy of adsorption, and \( C_e \) (mg/L) provides the concentration of Tl at equilibrium, \( q_e \) (mg/g) the amount of Tl adsorbed at equilibrium, \( q_m \) (mg/g) the MBC, and \( q_m \) (mg/g) the maximum amount of Tl adsorbed. A plot of \( C_e/q_e \) vs. \( C_e \) was used to calculate the constants \( q_m \) and \( K_L \).¹³,¹⁴

4. RESULTS AND DISCUSSION

The PB was synthesised by both direct and indirect methods. The direct method involved a single step process whereas indirect method involved two step processes. The maximum yield value was obtained by direct synthesis method as 17.72 g whereas by indirect method it was 11 g. Further, chemical as well as physical characteristics of PB was studied.

The quantitative estimation of four parameters namely particle size, moisture content, iron content and thermal decomposition which may affect the MBC of PB was carried out. A direct correlation between the MBC and moisture content was observed. Particle size and iron content were also estimated and correlated with MBC.

As per USFDA the minimum MBC of PB to qualify as an antidote to Cs and Tl contamination is 150 mg/g.¹⁵ Three samples PB-1, PB-2 and API-2 had MBC of 192.31, 200.00 and 166.67 mg/g respectively. However, the particle size, moisture content and iron content of these PB samples showed significant variation as compared to Radiogardase®-Cs. The MBC of Radiogardase®-Cs was maximum, i.e 238.10 mg/g for Tl.

The \( D_90 \) of PB-1, PB-2, API-1, API-2 and API-3 was 462, 465, 315, 165 and 230 \( \mu m \) (Table 1). Radiogardase®-Cs had the highest \( D_90 \) value of 785 \( \mu m \). API-2 with the lowest \( D_90 \) value of 165 \( \mu m \) was found to have 166.67 mg/g MBC (Figure 1). Fustino, et al. 2008 recommended \( D_90 \) to be more than 160\( \mu m \) for achieving higher MBC values. The present study showed that all samples have \( D_90 \) more than 160 mg/g but API 1 and API-3 have MBC less than the acceptable limits. These two APIs has the lowest moisture content (Table 1) which may lead to lower MBC.

![Figure 1. Particle size as D90 (µm) and MBC (mg/g of Tl) of PB samples.](image-url)
The moisture content was determined as the loss in weight between temperatures 62-272 °C by subjecting the samples to thermogravimetric analysis. The weight loss between temperatures 62-272 °C corresponds to the loss of water available in the PB molecule. This is an important factor which affects the MBC of PB molecules. The present results also showed a direct correlation between the MBC and moisture content. The API-1 and API-2 samples also followed this correlation as shown in figure 2.

The moisture content of 23.245 and 24.243 % of Radiogardase®-Cs and API-2 respectively corresponds to 14.4 and 15.4 water molecules in the unit-cell of PB molecule.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PB-1</th>
<th>PB-2</th>
<th>API-1</th>
<th>API-2</th>
<th>API-3</th>
<th>Radiogardase®-Cs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size, D90 (µm)</td>
<td>462</td>
<td>465</td>
<td>315</td>
<td>165</td>
<td>230</td>
<td>785</td>
</tr>
<tr>
<td>Iron content (%)</td>
<td>31.70</td>
<td>35.08</td>
<td>33</td>
<td>25</td>
<td>24.50</td>
<td>31</td>
</tr>
<tr>
<td>Langmuir Isotherm model qm (mg/g)</td>
<td>192.31</td>
<td>200.00</td>
<td>90.91</td>
<td>166.67</td>
<td>111.11</td>
<td>238.10</td>
</tr>
<tr>
<td>$K_L$ (L/mg)</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.95</td>
<td>0.98</td>
<td>0.95</td>
<td>0.89</td>
<td>1.00</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Where, $D_{90}-90$ % of the particles have particle size below $D_{90}$ value, amount of adsorption capacity indicated by $q_m$, Langmuir constant (adsorption energy) indicated by $K_L$.

The moisture content was determined as the loss in weight between temperatures 62-272 °C by subjecting the samples to thermogravimetric analysis. The weight loss between temperatures 62-272 °C corresponds to the loss of water available in the PB molecule. This is an important factor which affects the MBC of PB molecules. The present results also showed a direct correlation between the MBC and moisture content. The API-1 and API-2 samples also followed this correlation as shown in figure 2.

The moisture content of 23.245 and 24.243 % of Radiogardase®-Cs and API-2 respectively corresponds to 14.4 and 15.4 water molecules in the unit-cell of PB molecule.

Results shown in table 2 indicate that the iron content of each sample of PB was between 25 to 35 %. However, the recommended iron content for the PB should be more than 31 %. This assures the quality of the PB samples and also the presence of impurities. Less than 31 % iron content was observed in two samples API-2 and API-3 however, API-2 has an MBC of more than 150 mg/g, and API-1 with higher content did not meet the minimum requirement of MBC. It is important to mention here that API-1 had the lowest moisture content. Thus it can be concluded that although iron content is an important indicator of the purity level of PB but, the most influential factor is moisture content which significantly affect MBC.

Table 1. Particle size, moisture content, iron content and adsorption isotherm model (Langmuir) for adsorption of Tl on synthesised PB (PB1-2), commercially available APIs (API1-3) and Radiogardase®-Cs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PB-1</th>
<th>PB-2</th>
<th>API-1</th>
<th>API-2</th>
<th>API-3</th>
<th>Radiogardase®-Cs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron content (%)</td>
<td>31.70</td>
<td>35.08</td>
<td>33</td>
<td>25</td>
<td>24.50</td>
<td>31</td>
</tr>
<tr>
<td>Langmuir Isotherm model qm (mg/g)</td>
<td>192.31</td>
<td>200.00</td>
<td>90.91</td>
<td>166.67</td>
<td>111.11</td>
<td>238.10</td>
</tr>
</tbody>
</table>

Table 2. Percentage purity of PB sample based on its iron content

<table>
<thead>
<tr>
<th>PB Samples</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>API-1</td>
<td>106.4516</td>
</tr>
<tr>
<td>API-3</td>
<td>79.03226</td>
</tr>
<tr>
<td>API-2</td>
<td>80.64516</td>
</tr>
<tr>
<td>PB-1</td>
<td>102.2581</td>
</tr>
<tr>
<td>PB-2</td>
<td>113.1613</td>
</tr>
<tr>
<td>Radiogardase®-Cs</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 2. Moisture content (% weight loss) vs MBC (mg/g) of PB samples.

5. CONCLUSIONS

According to the results of this study, the compositional and structural properties of PB are important to qualify it as an antidote to Tl/Cs. The particle size, impurities and moisture content play a crucial role in its therapeutic efficacy. Therefore, quality control during the manufacturing and storage of PB must be monitored. Significant variations in the MBC of PB samples were observed with the highest being determined for Radiogardase®-Cs. The Radiogardase®-Cs met all the optimisation parameters with respect to iron content, moisture content and MBC. However, the higher particle size of Radiogardase®-Cs as compared to other PB samples could have an inverse affect on the MBC. Thus reducing the particle size of Radiogardase®-Cs may further enhance its MBC. Based on the work done, the antidote PB should have a moisture content of at least 24 % w/w, iron content not less than 31 % and MBC not less than 150 mg/g for Tl/Cs.

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REFERENCES


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She contributed in the conception and design of the work, experimentation, data analysis, interpretation, and proof reading.