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Short communication

A long-term stability study of Prussian blue: A quality assessment of water content and cesium binding☆

Adil Mohammad, Yongsheng Yang, Mansoor A. Khan, Patrick J. Faustino*

Division of Product Quality Research, Office of Testing and Research, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Silver Spring, MD 20993, United States

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ABSTRACT

Prussian blue (PB) is the active pharmaceutical ingredient (API) of Radiogardase, the first approved medical countermeasure for the treatment of radiocesium poisoning in the event of a major radiological incident such as a "dirty bomb" or nuclear attack. The purpose of this study is to assess the long-term stability of Prussian blue drug products (DPs) and APIs under laboratory storage condition by monitoring the loss in water content and the *in vitro* cesium binding. The water content was measured by thermal gravimetric analysis (TGA). The *in-vitro* cesium binding study was conducted using a surrogate model to mimic gastric residence and intestinal transport. Free cesium was analyzed using a validated flame atomic emission spectroscopy (AES) method. The binding equilibrium was reached at 24 h. The Langmuir isotherm was plotted to calculate the maximum binding capacity (MBC). Comparison of the same PB samples with 2003 data samples, the water content of both APIs and DPs decreased on an average by approximately 12–24%. Consequently, the MBC of cesium was decreased from 358 mg/g in 2003 to 265 mg/g @ pH 7.5, a decrease of approximately 26%. The binding of cesium is also pH dependent with lowest binding at pH 1.0 and maximum binding at pH 7.5. At pH 7.5, the amount of cesium bound decreased by an average value of 7.9% for APIs and 8.9% for DPs (for 600 ppm initial cesium concentration). These findings of water loss, pH dependence and decrease in cesium binding are consistent with our previously published data in 2003. Over last 10 years the stored DPs and APIs of PB have lost about 20% of water which has a negative impact on the PB cesium binding, however PB still meets the FDA specification of >150 mg/g at equilibrium. The study is the first quantitative assessment of the long-term stability of PB and directs that proper long-term and short-term storage of PB is required to ensure that it is safe and efficacious at the time of an emergency situation.

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1. Introduction

PB was first prepared in 1704 and is considered to be the first synthetic coordination compound[1]. Since then, PB, also known as 'insoluble' ferric(III) hexacyanoferrate ($Fe_4^{III}[Fe^{II}(CN)_6]_3 \cdot xH_2O$ (x = bound water molecules coordinated to PB and are optimally 14–16) is employed in a wide variety of applications in industry, pharmaceutics, science and is widely used as an inorganic pigment [2]. In 2003, the FDA approved Radiogardase®, also known as PB, as the first medical countermeasure drug product indicated for internal radioactive contamination in the event a nuclear bomb or a terrorist attack such as a "dirty bomb" [3].

PB was first identified as a metal decorporation agent as early as the 1950s [4–6]. For several decades PB had been used as an investigational agent to enhance the excretion of cesium-137 and thallium from the body [4–7]. Mechanistically, insoluble PB binds to metal ions and enhances the excretion of radioactive isotopes of cesium and thallium ions that are enterically cycled into the gastrointestinal tract. This process prevents reabsorption, thereby reducing the radioactive burden to the body.

A major source of concern for radioactive contamination from a nuclear incident is cesium (Cs). Cs is alkali metal that has 32 radioactive isotopes. There are two isotopes of cesium with considerably long radioactive decay half-lives namely Cs-137 (30.17 years) and Cs-134 (2.06 years). Both of these radio-active isotopes decay by emitting beta particles and gamma radiation, by their decay products Ba-134 and Ba-137. Cs-137 is a major source of

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* Corresponding author. Tel.: +1 301 796 0021; fax: +1 301 796 9816.
E-mail address: patrick.faustinop@fda.hhs.gov (P.J. Faustino).

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concern, because it is a significant byproduct of nuclear fission, it is widely available with a considerable half-life [8].

Cs-137 was first detected in humans in 1956 due to the extensive atmospheric nuclear testing in the 1950s and 1960s [9]. The Chernobyl nuclear accident in 1986 led to widespread environmental contamination with Cs-137 and Cs-134, which ultimately led to an extensive use of PB, to reduce radiocesium levels in humans and animals throughout the regional countries that were affected [10–12]. The Goiânia incident in Brazil in 1987 exposed 250 people to radiocesium. This tragedy provided an opportunity to carry out uncontrolled human trials using PB for the treatment of radiocesium poisoning. These clinical studies have shown that PB treatment significantly reduced the biological half-life of radiocesium from (43–69)% [13]. Recently, PB had been used as an adsorbent for radioactive cesium ions from contaminated water at the Fukushima nuclear plant in Japan in 2011[14].

Since the FDA's approval of Radiogardase® in 2003, there have been significant quantities of PB drug products placed in storage in the Strategic National Stockpiles (SNS) and also in hospital pharmacies and clinics. PB is considered a thermodynamically stable molecule and historically in the scientific community a very stable molecule, based on a few reports that state that PB is not affected by storage conditions [6,15,16]. However, there is no long-term scientific data available to support this claim. Additionally there is no scientific data on the long-term physiochemical stability of PB. Previous scientific studies of PB, by our group in 2003 led to its approval for marketing and also provided the FDA with a historically and scientifically unique set of pharmaceutically characterized samples to accurately study the long-term stability of PB. These previous FDA studies also found that water loss negatively impacted the product performance of PB by reducing its cesium binding capacity [17]. Bound water in the PB crystal was significantly reduced at higher temperatures (35–105 °C), leading to a dramatic decrease in cesium binding. This change in a physiochemical property of PB suggested that the product quality of PB may also be affected over time as a result of long-term storage.

The goal of this current study was to access the long-term stability of PB stored over a period of 10 years, under laboratory storage conditions (that mimics ideal warehouse storage conditions) by monitoring the loss in bound water and the *in vitro* cesium binding capacity. These parameters were analyzed and compared to the data from 2003. This is the first long-term stability study and scientific evaluation of physiochemical quality attributes of PB. FDA's primary regulatory concern is to ensure that this essential medical countermeasure medicine is safe and efficacious to use in the event of a medical emergency even after long-term storage.

2. Materials and methods

2.1. Chemicals and reagents

Cesium single element standard (1000 ppm, 1 mg/mL Cs) certified was purchased from High-Purity Standards (Charleston, SC). Cesium chloride was purchased from Aldrich (Milwaukee, WI, USA). Certified buffer solutions (pH 1 and 5), hydrochloric acid and sodium hydroxide (10 N) and desiccant (Drierite) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Dibasic and monobasic potassium phosphate and potassium hydroxide were purchased from J. T. Baker Inc. (Phillipsburg, NJ, USA). PB active pharmaceutical ingredients (API) were provided by HEYL Corporation (Berlin, Germany). Drug products (500 mg/capsule) were provided by HEYL Corporation (Berlin, Germany). Filtered 18 MΩ water was supplied in house by a Millipore Milli-Q System (Bedford, MA, USA). All other chemicals were of reagent grade.

2.2. Preparation of media solutions and standards

2.2.1. Preparation of pH solutions

The solutions of pH 1.0 and 5.0 were prepared by diluting corresponding certified buffer solutions 5-fold 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 and was also diluted 5-fold with deionized water for use.

2.2.2. Preparation of calibration standards

Cesium standard (1000 ppm Cs) was used as standard stock solution. Six calibration standard solutions were prepared by transferring 1.0, 1.5, 2.5, 5.0, 7.5, 10.0 and 15.0 mL of stock solution to 50-mL volumetric flasks and then adding the corresponding pH solution to 50 mL resulting in the final concentrations of 20, 30, 50, 100, 150, 200 and 300 ppm Cs, respectively. Two standard curves, which included standard blanks, were prepared daily.

2.2.3. Preparation of quality control standards

The quality control (QC) cesium stock solution (1 mg/mL Cs, 1000 ppm Cs) was prepared by dissolving 1.2667 g of cesium chloride in 1000 mL of deionized water. The lower limit of quantification (LLOQ) of 20 ppm, low QC of 30 ppm, intermediate QC of 150 ppm and high QC of 300 ppm were prepared by transferring 1.0, 1.5, 7.5 and 15 mL of stock solution to 50-mL volumetric flasks and then adding the corresponding pH solution to 50 mL. Five standards at each QC level were prepared daily.

2.3. Analytical method and validation

All standards and samples were analyzed by atomic emission spectroscopy (AES) using a PerkinElmer (Shelton, CT, USA) AAnalyst 800 atomic absorption/emission spectrometer equipped with an air-acetylene burner. Cesium was detected at an emission line of 455.5 nm. The sample solution was aspirated into an air-acetylene flame for a time period of 3 s with read delay of 10 s each. Each sample was analyzed in triplicate by AES. Sample blanks were run between every five samples or standards to assess carry over.

2.4. Water loss from Prussian blue

Water loss from Prussian blue APIs and DPs was determined by TGA using a model 2950 TA Instruments (New Castle, DE, USA). Approximately 10 mg of sample was placed in an open platinum TGA pan. The ramp method was used to heat the samples at the rate of 20 °C/minute. The final temperature was 375 °C. All samples were run in triplicate with a nitrogen flow of 40 mL/min.

2.5. pH-profile (600 ppm)

A 0.1 g amount of each PB APIs (2, 3 and 4) or drug products (1, 2 and 4) sample batch was added individually to a 100-mL glass flask, 49 mL of corresponding pH solution and 1.0 mL of cesium stock solution (30 mg/mL Cs) was added resulting in a final concentration of 600 ppm. The flask was tightly stopped and incubated in a shaking water bath at 37 °C with 75 shakes/min for 24 h. Samples were tested at pH 1, 5 and 7.5. All sample batches were prepared in triplicate.

2.6. Time dependent profile (600 ppm)

Four different API's batches (API-1, API-2, API-3, and API-4) and three different drug product batches (DP-1, DP-2, DP-3) were selected for analysis. A 0.1-g amount of each PB API or drug product sample batch was added individually to a 100-mL glass flask,