# The role of Glutathione, Cysteine and D-Penicillamine in exchanging Palladium and Vanadium metals from albumin metal complex

**Muhammad Mukhtiar[1], Syed Umer Jan[2]*, Ihsan Ullah[3], Abid Hussain[1], Izhar Ullah[1], Rahman Gul[2], Essa Ali[4], Abdul Jabbar[5], Zulfiqar Hussan Kuthu[5], Muhammad Wasim[6] and Muhammad Farid Khan[7]**

[1]Department of Pharmacy, The University of Poonch, Rawalakot, AJK, Pakistan
[2]Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Pakistan
[3]Department of Pharmacology, Poonch Medical College, Rawalakot. AJK, Pakistan
[4]Department of Agronomy, Faculty of Agriculture, The University of Poonch, Rawalakot. AJK, Pakistan
[5]Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot. AJK, Pakistan
[6]Department of Pharmacy, Abasyn University, Islamabad Campus, Pakistan
[7]Faculty of Pharmacy, Gomal University, DI Khan, Pakistan

**Abstract**: Thiol groups are extensively present across biological systems being found in range of small molecules (e.g. Glutathione, Homo-cysteine) and proteins (e.g. albumin, haemo-globin). Albumin is considered to be a major thiol containing protein present in circulating Plasma. Albumin contains a single thiolate group located at cysteine-34(cys-34) at its active site. Albumin also binds a wide variety of metals and metals complexes at various sites around the protein. Usually heavy metals are preferentially attached with the thiol group of albumin. The binding of heavy metals at cys-34 provides a mechanism by which the residence time of potentially toxic species in the body can be increased. In this research we have assessed the oxidative modification of and metal binding capacity of cys-34 with heavy metals Palladium and Vanadium to investigate the ease with which it is possible to effect disulfide-thiol exchange at this sites/or remove a metal bound at this position. Both the metals were treated with albumin and then the albumin metals (Pd and V) complexes were treated with small thoil molecules like Glutathione, Cysteine and D-Penicillamine. Our finding showed that the albumin thiol group retained the metals with itself by forming some strong bonding with the Thiols group, it is concluded from this finding that if by chance both the metals enter the living system; strongly disturb the chemistry and physiological function of this bio-molecule.

**Keywords**: Albumin, Glutathione, Cysteine, D-Penicillamine, Palladium (Pd), Vanadium (V).

## INTRODUCTION

Human serum albumin is a significant, multifunctional and non-glycosylated plasma protein (Ankita *et al.,* 2010). It is found in the concentration of about 42mg/ml in normal individual (Kratz & Keppler, 1993). Due to such significant concentration in the human plasma, it is responsible for something like 80% of the colloid osmotic of the plasma fluid (Ulrich *et al.,* 2006). It also has the quality of enzymatic activities and serves as a transporter of various compounds. HSA comprises of a single non-glycosylated alpha (α) chain from containing 585 amino acids arranged in three comparative domains (I, II and III), each of which is further divided into two sub-domains (IA, IB). Structurally it has two important drug binding domain sites i.e. II-A and III-A, corresponding to domain A proposed by Sudlow. Sudlow site I is responsible for attaching heterocyclic anion whereas aromatic carboxylates usually attached with Sudlow site-II with an extended confirmation (Paolo & Mauro, 2010). Remarkably, warfarin and ibuprofen need aid the prototypical ligands of Sudlow's site-I and site-II (Petitpas *et al.,* 2001, Sudlow *et al*., 1976). It also contains a single free thiol (-SH) group on a cysteine-residue (Cys-34), account for 80% of its total thiols concentration in plasma. The thiol moiety of Cys-34 is highly reactive and capable for most of its detoxification and antioxidant potential i.e. removing most of reactive oxygen species and heavy metals (Droge, 2002). Palladium like other PGMs is in small concentration in the environment (Renner *et al*., 1992 & Renner and Schmuckler, 1991). Palladium occurs normally in traces in drinking water (Johnson *et al*., 1976). Palladium may occur in food, like fishes, aquatic invertebrates, meet, bread and certain plants, in very small quantities (Yang, 1989; Whyte and Boutillier, 1991). The chances of human exposure to Palladium are generally through jewelry, dental alloys, food and emission from automobile converters (Wataha *et al*., 1991a). Exposure of gums to Palladium in dental alloys is the most important one. Similarly skin may expose to Palladium containing jewelry (Wirz *et al*., 1993). Workers in Palladium refineries may also exposed to Palladium directly and there are reports about manifestation of 0.006µg/l Palladium in their urine (Johnson *et al*., 1976). Palladium, usually, accumulates in leaves and roots of marine plants (Farago and Parsons, 1994). The general population is exposed to background

*Corresponding author: e-mail: suj55@yahoo.com

levels of Vanadium primarily through ingestion of food (Sepe *et al*., 2003). Fuel oils may contain Vanadium in concentrations ranging from 1 to 1,400 ppm, depending on their origin (Byerrum *et al*., 1974). Natural sources of atmospheric Vanadium include continental dust, marine aerosol, and volcanic emissions (Byerrum *et al*., 1974). Combustion of heavy fuels, especially in oil-fired power plants, refineries, and industrial boilers, and coal are the major source of anthropogenic emissions of Vanadium into the atmosphere (Mamane and Pirrone 1998; Sepe *et al*., 2003). The importance of metal compounds in medicine is undisputed, as can be judged by the use of compounds of antimony (anti-protozoal), bismuth (anti-ulcer), gold (anti-arthritic), iron (anti-malarial), silver (anti-microbial) Vanadium (anti cancer anti-diabetic), Palladium (anticancer) and Platinum (anti-cancer) in the treatment of various diseases. In terms of anti-tumor activity, a wide range of compounds of both transition metals and main group elements have been investigated for efficacy (Desoiz, 2004). The earliest reports on the therapeutic use of metals or metal-containing compounds in cancer and leukemia date from the sixteenth century (Desoiz, 2004). More, transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicities associated with these metals may be due at least in part to oxidative tissue damage. Recent studies have shown that metals such as Palladium, Vanadium and other elements exhibit the ability to produce reactive oxygen species, resulting in lipid per oxidation. (Khan H *et al.,* 2010, 2011a, 2011b and 2012; Muktiar *et al., 2012,* 2013 and (2017 accepted); Shah *et al.,* 2013 and 2013a; Khan J *et al.,* 2012; Naseem *et al*., 2015; Hashmat *et al*., 2015, 2015a, 2016 and 2016a). Bio-Thiols which are characterized by having a very reactive (SH) functional group are biologically the most important antioxidant constituent, responsible for protecting the cell from various oxidative damages. Glutathione is one among them extensively studied, comprises of three amino acids: L-glutamyl, L-cysteinyl and l-glycine, this antioxidant have extreme potential to protect cells against oxidative stress. It is synthesized in two steps: synthesis of γ-glutamyl cysteine by glutathione synthetase which intern react with glycine catalyzed by GSH synthetase to form GSH. It is the Glutathione that play a major role in the reduction processes necessary for protein and DNA synthesis. GSH also play very important role in the transport and storage of cysteine, act as a co-enzyme in various reactions of exogenous species (Demirkol *et al.,* 2004). GSH is found mainly in cytosol content of cells in the considerable concentration ranges from 1-10mM; it's also present in the plasma where its concentration is approximately in the range of about 1-3μM (Rees *et al.,* 2008). GSH is also found in the most of the plant cells, microorganisms and all mammalian tissues (Hami *et al*., 2013). N-Acetylcysteine (NAC) has been used as an antioxidant precursor to glutathione (GSH) in the treatment of acetaminophen overdoes for several years (Kundala *et al.,* 2007). It has numerous pharmacological, pharmaceutical and clinical applications i.e. it is widely used as a mucolytic agent, in the treatment of HIV. It has also been found to be effective in chronic obstructive pulmonary diseases and contrast-induced nephropathy (Sadowska *et al*., 2012). It has also been observed to possessing considerable efficacy against some brain disorders specifically in Alzheimer disease patients (Unnithan *et al.,* 2012). The chemical name of D-Pinicillamine is ß-ß- dimethylcysteine or 3-mercapto-D-Valine.It is an amino acid containing degradation product of penicillin and sulfhydryl group. Its D-isomer is useful while the L-Isomer can cause optic neuritis. D-Pinicillamine chelates heavy metals and decreases their toxicity (Roussaeux and MacNabb, 1992). D-Penicillamine can be used in treatment of the toxicity of different metals like Gold, Lead, Copper and Arsenic (Master, 2008). The main purpose of the study was to evaluate the function of Glutathione, N-Acetylcysteine, D-penicillamine, whether they can detached Pd and V from the Albumin-Pd and Albumin-V complex respectively, and make it available for further function.

## MATERIAL AND METHOD

### *Materials*

All materials (reagent) were purchased commercially. Bovine serum Albumin (>98%), Ellman's Reagent, agarose gel electrophoresis lyophilized) and Sephadex (G25 coarse) were purchased from Sigma Aldrich. U.V-visible spectra were recorded on a Unicam U.V.300 spectrophotometer.

### *Preparation of standard curve for BSA solution*

A 50uM solution of Bovine Serum albumin (BSA) was prepared by dissolving 16.75mg of BSA in 5ml of (0.1 M $KH_2PO_4$, pH 7.4), then 5 different dilution i.e. 10,20,30,40,50 from this stock solution were prepared by the addition of further (0.1 M $KH_2PO_4$, pH 7.4) to stock solution. UV spectrum (200-600nm) of the solution was recorded after each addition. Plotting the absorbance of the solution at λ280 nm as a function of BSA concentration gives a straight line (R2=0.993) with an intercept (0.0911) derived from the natural background of fluorescence of the albumin solution. The unknown concentration of BSA in the BSA–Pd and BSA-V mixtures were then calculated on the bases of molecular weight, extent co-efficient of BSA and using beer lambert law as shown in fig 1.

### *The calculation of free thiolate content of BSA*

Ellman's reagent (13.2mg, 3ml in BPS was titrated into solution of Albumin (1.98mg, 50µl) (the UV spectrum (200-600nm) of the solution were recorded after each addition. Plotting the absorbance of the solution at λ412 nm as a function of Ellman's reagent concentration gives a straight line (R2=.993) with an intercept (0.0911)

derived from the natural background of fluorescence of the albumin solution. The thiolate content of albumin is obtained from the concentration of the Ellman's anion released for BSA based on a molecular weight of 66,000.

***Preparation of ellman's modified BSA (BSA-SSE)***

Solutions of BSA (200mg, 1mL) and Ellman'sreagent (1mg, 1ml) in PBS (0.1M $KH_2PO_4$, pH 7.4) were mixed and allowed to react overnight. The solution was carefully applied to a column (10cm x 2cm) packed with swollen Sephadex (G25 coarse). The mixture was eluted with PBS. The appearance of the protein in the eluent was identified by testing the liquors with tri-chloroacetic acid (which precipitates denatured protein) whereupon collection commenced. Periodic sampling identified when the eluent was protein free. The residual Ellman's reagent and anion (identified as a yellow band) eluted second and were washed from the column with further aliquots of PBS. The concentration of protein in solution was calculated using Beers Law at ($\lambda_{max}$ max = 280 nm; $\varepsilon$ = 43,824 $cm^{-1}M^{-1}$) (Peters, 1975.)

***Preparation of ellman's modified BSA with Palladium and Vanadium***

Solutions of BSA (200mg/1mL) and Palladium Nitrate (1mg /1ml), and 1mg /1ml) Ammonium Vanadate in PBS (0.1M $KH_2PO_4$, pH 7.4) was mixed and allowed to react overnight respectively. The solutions were carefully applied to a column (10cm x 2cm) packed with swollen Sephadex (G25 coarse). The mixture was eluted with PBS. The appearance of the protein in the eluent was identified by testing the liquors with tri-chloroacetic acid (which precipitates denatured protein) whereupon collection commenced. Periodic sampling identified when the eluent was protein free. The residual free Palladium Nitrate, Ammonium Vanadate eluted second due to their smaller molecular sizes and was washed from the column with further aliquots of PBS. The concentration of protein in solution was calculated using Beers Law at ($\lambda_{max}$ max = 280 nm; $\varepsilon$ = 43,824 $cm^{-1}M^{-1}$) (Peters, 1975).

***Treatment of BSA-Pd and BSA-V with thiolates***

The modified Palladium, Vanadium BSA protein were freeze dried in order to reduce the volume of water content up to 1.5ml and the mixed with thiolate (reduced Glutathione, N-acetyl cysteine and D-Penicillamine. Again pass through the column in order to remove the free thiols if any. The modified protein (BSA-Pd, BSA-V) solutions collected above were diluted by taking 1ml of elute and adding 4ml of ml (0.1M $KH_2PO_4$, pH 7.4) in order to generate a solution of known concentration (typically 60µM) which produced a solution with an absorbance at 280 nm of approximately 2.5, the spectrum (200-600nm) was recorded. These solutions were then titrated with Ellman's reagent the spectra were being recoded after each addition. The release of Ellman's anion is assess at $\lambda_{max}$ max = 412 nm ($\varepsilon$ = 14,150 $cm^{-1}M^{-1}$).

## RESULTS

***Construction of standard curve for BSA and BSA-ES-***

The standard curve for both BSA-ES-, simple Bovine serum Albumin (BSA) were constructed in order to calculate the amount of protein in the eluent sample as shown in (fig. 1) and to insure the quantity of thiols (SH) on BSA used in the current sample and was found to be 30%, as shown in (fig. 2, equation 1).

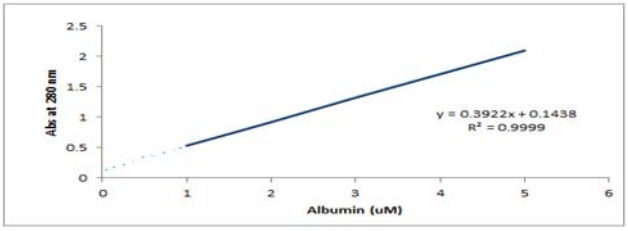


**Fig. 1**: Calibration curve of BSA at 280 nm

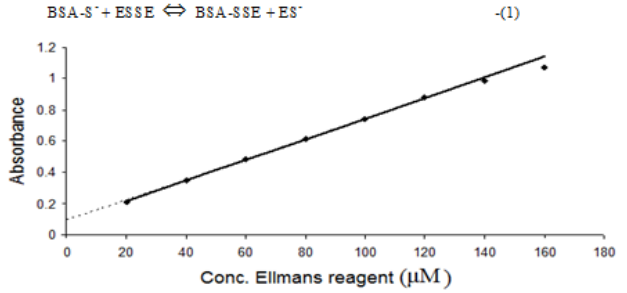


**Fig. 2**: The titration of BSA (13.2mg/3 ml) with Ellman's reagent (1.98mg/ml 5ul).The expected deviation from linear behaviour at high Ellman's reagent concentrations (150, 1.98mg/ 5µl) is evident. An intercept is found which is consistent with residual absorbance by BSA at 412 nm.

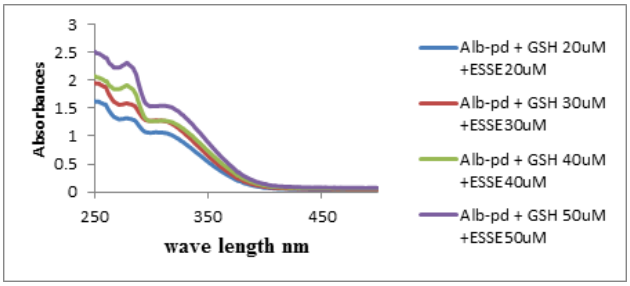


**Fig. 3**: The titration of BSA-Pd 50uM) with Glutathione solution (50uM) the formation of No Ellman's anion (equation 3) is evident from the appearance of a band at 412 nm.

***The exchange reactions of either palladium /or vanadium with either glutathione, or N-acetylcysteine, or D-penicillamine***

The exchange reactions of Palladium and Vanadium with Glutathione, N-Acetylcysteine, D-Penicillamine are shown in (fig. 3, to 8) respectively. From the figs. it was evident that Albumin gave absorbance at 280nm, Ellman'sreagent gave absorbance at 325nm while there

was almost no absorbance at 412nm at which TNB anions give absorbance usually produced during the interaction of free SH group of Albumin with Ellman's reagent (ESSE). The result suggested that both Palladium and Vanadium form stable complexes with the thiol group of the albumin as shown from (fig 3 to 8).

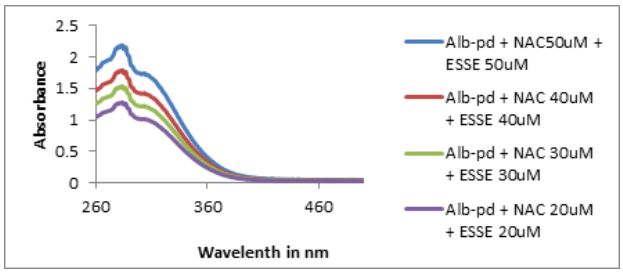


**Fig. 4**: The titration of BSA-Pd 50uM) with N-Acetylcysteine solution (50uM) the formation of No Ellman's anion (equation 4) is evident from the appearance of a band at 412 nm.

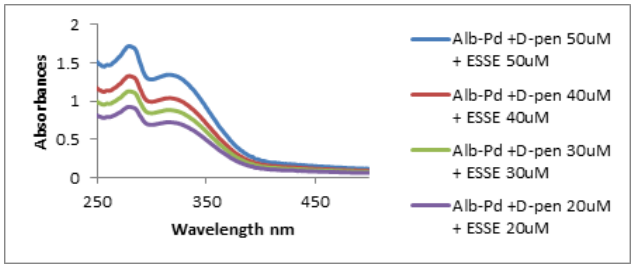


**Fig. 5**: The titration of BSA-Pd 50uM) with D-penicillamine (50uM) the formation of No Ellman's anion (equation 5) is evident from the appearance of a band at 412 nm.

***Albumin palladium exchange reaction***

$$(BSA\text{-}SH) + Pd^{+2} \longleftrightarrow (BSA\text{-}S)_2\text{-}Pd + 2H^{+} \quad -(2)$$

$$(BSA\text{-}S)_2\text{-}Pd + GS^{-} \longleftrightarrow \text{No exchange} \quad -(3)-(6)$$

$$(BSA\text{-}S)_2\text{-}Pd + NACS^{-} \longleftrightarrow \text{No exchange} \quad -(4)$$

$$(BSA\text{-}S)\ 2\text{-}Pd + D\text{-}pen^{-} \longleftrightarrow \text{No exchange} \quad -(5)$$

***Albumin Vanadium exchange reaction***

$$2(BSA\text{-}SH) + VO_2^{+} \longleftrightarrow 2(BSA\text{-}S)\text{-}V + H_2O \quad -(6)-(9)$$

$$(BSA\text{-}S)\text{-}V + GSH \longleftrightarrow \text{No exchange} \quad -(7)-(10)$$

$$(BSA\text{-}S)\text{-}V + NAC \longleftrightarrow \text{No exchange} \quad -(8)$$

$$(BSA\text{-}S)\text{-}V + D\text{-}pen^{-} \longleftrightarrow \text{No exchange} \quad -(9)$$

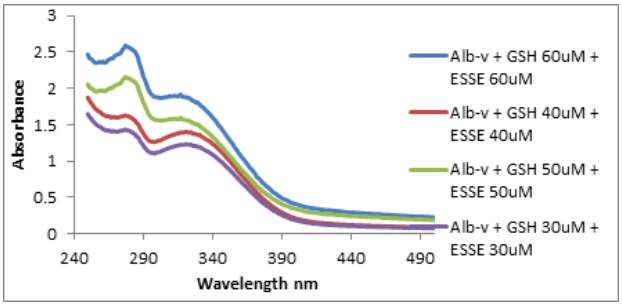


**Fig. 6**: The titration of BSA-V 60uM) with Glutathione solution (60uM) the formation of No Ellman's anion (equation 7) is evident from the appearance of a band at 412 nm.

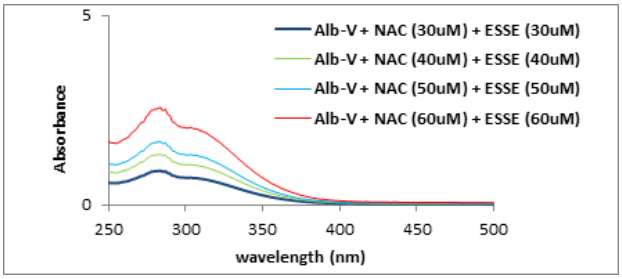


**Fig. 7**: The titration of BSA-V60uM) with N-Acetylcysteine solution (60uM) the formation of No Ellman'sanion (equation 8) is evident from the appearance of a band at 412 nm.

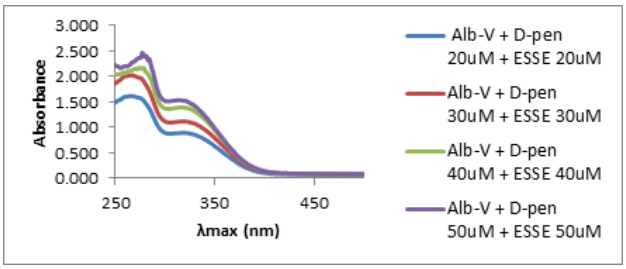


**Fig. 8**: The titration of BSA-V 50uM) with D-penicillamine solution (50uM) the formation of No Ellman's anion (equation 9) is evident from the appearance of a band at 412nm.

## DISCUSSION

The result suggested that both Palladium and Vanadium form stable complexes with the thiol group of the albumin which could not be broken by smaller thiols like GSH, NAC, and D-Penicillamine as shown from (fig 3 to 8). The data in this study also suggested that Palladium and Vanadium showed more toxicity on protein level by forming somewhat strong coordination which could not be exchanged by free thiolate group like Glutathione, N-acetyl-cysteine and D-Penicillamine usually present in the biological fluids. On the other hand it can be anticipated from this model of study that besides the propound toxicity of either Palladium or Vanadium towards GSH, Vanadium or Palladium can also render the albumin malfunctioning. Albumin being present in rich

concentration in plasma may not play its critical physiological roles in the living system in high Palladium and Vanadium environment.

## CONCLUSION

As the exposure of experimental animals and human to inorganic or organic forms of Vanadate and Palladium accompanied by the induction of oxidative stress as the level of both deplete the level of Glutathione. The disturbances in the oxidative status may be a result of an independent effect of Vanadium and/or Palladium. Since it is bound with albumin strongly and cannot be displaced by antioxidant like GSH, Cysteine and D-Penicillamine. So exposure of human to these metals may disturb their normal physiology.

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