PROGRESS REPORT FOR AINGRA09132P

<table>
<thead>
<tr>
<th>PROJECT TITLE</th>
<th>Use of radiotracer probes to study the binding of metal ions to wool powders</th>
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<tr>
<td>INVESTIGATOR(S)</td>
<td>Institution and Department</td>
</tr>
<tr>
<td>Chief Investigator</td>
<td>Professor Xungai Wang</td>
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<td>Dr. Suzanne Smith, ANSTO</td>
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<td>Ms. Guiqing Wen, PhD student, Deakin University</td>
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<td>ANSTO Investigators</td>
<td>Suzanne Smith</td>
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<tr>
<td>Specialist Committee</td>
<td>MSD</td>
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</table>

SCIENTIFIC OBJECTIVES

The objective of the project is to examine the potential use of wool as an industrial and/or laboratory adsorbent. This will be carried out by examining the metal sorption and desorption behaviour of a series of wool powders using radiotracer probes. Wool powders will be prepared using various milling techniques, and their metal-binding properties investigated using radiotracers such as $^{57}$Co, $^{64}$Cu and $^{109}$Cd, as well as reactor produced $^{199}$Au, $^{110}$Ag, $^{203}$Hg and $^{51}$Cr. Separation of gold and copper is important for the gold refining industry. Work with mercury will have important environmental implications because of mercury contamination/toxicity in our waterways.

Absorption of metal ions will be monitored over a range of concentrations ($10^{-3}$ to $10^{-6}$M) and pH values (from 3 to 9) at ambient temperature. Particular attention will be paid to the reversibility of the reactions and ability to scale up processes.

PROGRESS REPORT and RESEARCH OUTCOMES

Wool is a reactive material because of its main functional groups, including peptide bonds, side chains of amino acid residues and disulphide crosslinks [1]. Wool is known to bind a wide range of metal ions, such as mercury, copper, aluminium, nickel, zinc, cobalt, chromium, silver and gold [2-8]. There have been many reports where native wool fibres [9], chemically-modified wool fibre [10, 11] and regenerated wool protein [12] have been used to remove metal ions from industrial effluent and/or recover precious metal ions from solution.

Recent studies show that the reactivity of wool can be enhanced greatly by converting the fibre into fine powder [13-16]. For example, the dye uptake of wool powder was found to be rapid, even at room temperature, while no dye was taken by wool fibre under the same condition. The rate and extent of dye uptake by fine wool powder was comparable to that obtained by activated charcoal, even when the surface area of activated charcoal was 100 times greater than that of the wool powder [15]. Naik el al also found that the sorption capacities of fine wool powder for Co$^{2+}$ and Cu$^{2+}$ were up to 10 times higher than those of commercial resins [13].

It is well established that the pH of a metal solution plays an important role in the binding of metal ions to wool [8, 17, 18]. Some researchers have studied metal binding in acidic and alkali conditions, using chemicals such as sulphuric acid [4], acetic acid [19, 20], hydrochloric acid [10], ammonia solution [10, 19-23], and also the universal buffer mixture of Teorell and Stenhagen (1938) [7] to adjust pH. Buffer systems, however, can interact with metal ions in solution. For example, the interactions of acetate and amine buffers with Cu$^{2+}$ form copper diacetate Cu(CH$_3$CO$_2$)$_2$ and tetraammonium copper complexes, respectively [8]. Although the metal ion sorption by wool at various pH values has been investigated extensively, little information about the influence of buffer composition on the metal sorption behaviour of wool is documented.

Techniques used to study the binding of metal ions to wool include atomic absorption spectroscopy [5], X-ray fluorescence spectroscopy [24], Inductive Coupled Plasma –Atomic Emission Spectrometer (ICP-AES) [20], chelate titration [25], polarographic analysis [26], X-ray photoelectron spectroscopy [3], ATR/IR spectroscopy [21] and Electron Spin Resonance (ESR) spectroscopy [27]. Because these techniques either require large test sample
solution, or have comparatively complex testing procedures to determine the amount of metal ions in solution, one alternative approach, using the radioisotope (\(^{57}\)Co, \(^{109}\)Cd and \(^{64}\)Cu) of the stable metal ion to quantitatively track the movement of common metal ions (Co\(^{2+}\), Cd\(^{2+}\) and Cu\(^{2+}\)) onto and off sorbents (wool powders) in solution, was employed recently by Naik et al [28].

Radioisotope’s decay is accompanied by emission of \(\alpha\), \(\beta\) and \(\gamma\) radiations. The emission of a gamma signal is ideal for tracking the movement of its natural metal ion in solution and material. The gamma emission can be easily detected using a gamma counter equipped with a scintillation crystal. Counts obtained can be readily correlated to the concentration of metal ion present [29]. Due to its high sensitivity, accuracy and simplicity, the isotopic tracer method is widely and safely used in medicine, food preservation, agriculture, mining and archaeology [30, 31].

In the present study, two wool powders with different particle sizes were utilized to sorb and recover Co\(^{2+}\) ions from solution. The effects of buffer type, pH, contact time as well as concentration of Co\(^{2+}\) ions on the sorption behaviour of wool powders were studied. The recovery of bound Co\(^{2+}\) from wool using various chemicals was examined. The efficiency of wool powders repeatedly used to sorb Co\(^{2+}\) was also investigated. Comparisons were made with the original wool fibre. The radioisotope \(^{57}\)Co was used to track the amount of bound Co\(^{2+}\) ions on the wool samples.

### DATA

#### 1 Effect of buffer on the binding of Co\(^{2+}\) on wool powders

It is necessary to use buffers to stabilize the pH of a metal ion solution during metal ion binding to wool. If there is no buffer in the metal ion solution, the pH value will decrease when wool is immersed in the solution, because metal ions (such as Ag\(^{+}\)) can compete with hydrogen ions for the same binding sites on wool [6]. Additionally, buffer is required to equilibrate the internal pH of wool with the pH of the metal ion solution. When there is no electrolyte in the solution, the inner pH of wool is lower than the pH of external solution because of the Donnan effect [32]. Adding salts or buffers will reduce the difference between the internal pH of wool and the pH of the external solution [1].

![Graph 1](image1.png)

Figure 1. Effect of buffer composition and pH value on the Co\(^{2+}\) sorption by wool samples: [Co\(^{2+}\)] of 10\(^{-4}\) M, 23ºC and contact time indicated.
The binding of Co^{2+} by wool powders and the wool fibre at various pH values adjusted with different chemicals is shown in Figure 1. For buffer I, solutions were made up of citric acid and di-sodium hydrogen phosphate in the range of pH 2.61 to pH 7.77, and sodium sulphate/ammonia at pH 10.16. The uptake of Co^{2+} at pH 10.16 was much higher than that at other pH values. In case of buffer II, the amount of Co^{2+} on wool samples significantly increased as the pH was increased from pH 3 to pH 8, and then fell sharply at pH 9.

Tsukada et al [20] also reported the optimum pH for Co^{2+} sorption by native and chemically treated wool fibre is pH 11.4, which was adjusted with ammonia. It is worth noting that even at a similar pH, the sorption capacity of wool for Co^{2+} was markedly different with different buffers. For example, the uptake of Co^{2+} by WP-D in di-sodium hydrogen phosphate buffer (pH 8) (2.36 × 10^{-9} mol/mg) was much higher than that in citric acid/di-sodium hydrogen phosphate buffer (pH 7.77) (0.22 × 10^{-9} mol/mg). These results illustrated that the sorption behaviour of wool for Co^{2+} depends on both the chemical composition of the buffers and the pH values.

The process of metal ion binding to wool can be partly explained in terms of the charged functional groups of wool [33]. In the case of pH values below the isoelectric point of wool (IEP) (pH 4-5), the surface of wool is positive due to the positively-charged ammonium groups, which inhibit the sorption of metal ions. When the pH is higher than the IEP, the surface of the wool carries negative charges because of the negative carboxyl groups, which enhance the attraction of the metal ions. The carboxyl groups of aspartic and glutamic acid are believed to be the main active binding sites for metal ions [2]. Other factors, such as the formation of metal-buffer complexes, the hydrolysis of metal ions in solution [33], and the possible oxidation of Co^{2+} to Co^{3+} under alkaline conditions [20], also affect the sorption behaviour of wool for Co^{2+}.

Fourier-transform infrared (FTIR) spectroscopy was employed to observe the changes in chemical structure of wool samples under various binding conditions. The pH 8 (phosphate buffer) and pH 10 (sodium sulphate/ammonia buffer) were selected because of the significant sorption of Co^{2+} by wool at both of these pH values.

The FTIR spectra of untreated and treated wool samples are shown in Figure 2. The absorption bands in the range of 1700 – 1600 cm^{-1}, at ~1540 cm^{-1}, and from 1220 to 1300 cm^{-1} correspond to Amide modes I, II and III, respectively [34]. Amide I band is primarily related to the C=O stretching vibration, Amide II is mainly connected to the in-plane N-H bending, C-H stretching and C-C stretching vibrations, while Amide III derives mainly from in-phase combination of N-H in-plane bending and C-N stretching with contributions from C-C stretching and C-O bending [35].

The Amide I mode of proteins at 1650 -1657 cm^{-1}, ~1630 and 1685 cm^{-1}, and ~1655 cm^{-1}, indicates that the conformation of proteins is α-helix, β-sheet and disordered, respectively [36]. In this study, the Amide I mode for wool powders appears at ~1654 cm^{-1}. This indicates that the surface of wool powders has a high proportion of amides in the α-helix conformation. This suggests that the cortical cells were exposed on the surface of wool powders, because the proteins in outer cuticle cells of wool are richer in β-sheet and disordered conformation.
while that in inner cortical cells have much higher $\alpha$-helix content [36, 37]. This result is consistent with the morphology of wool powders observed from their SEM images[16].

Compared to wool fibre and untreated wool powders, the peaks at $\sim$1100 cm$^{-1}$ for both WP-C and WP-D at pH 8 and pH 10, with and without binding of Co$^{2+}$, are probably related to S-O stretching [37]. It is observed that the intensity of band frequency at $\sim$1400 cm$^{-1}$ was dramatically increased, and an unassigned band at $\sim$618 cm$^{-1}$ appeared for wool powders in pH 10 buffer, before and after binding of Co$^{2+}$. The differences in the chemical structure of buffered WP-C and WP-D may contribute to their high sorption capacity for Co$^{2+}$ at pH 8 and pH 10 buffers to some extent.

2 Effect of contact time

![Graph showing binding of Co$^{2+}$ by wool samples as a function of contact time. Initial Co$^{2+}$ concentration of 10$^{-4}$ M, pH 8 (phosphate buffer) and 23ºC.](image)

Figure 3. Binding of Co$^{2+}$ by wool samples as a function of contact time: initial Co$^{2+}$ concentration of 10$^{-4}$ M, pH 8 (phosphate buffer) and 23ºC.

The sorption of Co$^{2+}$ by wool powders and fibre at various contact times ranging from 15 minutes to 22 hours was performed at pH 8 (phosphate buffer) with an initial Co$^{2+}$ concentration of 10$^{-4}$ M at 23ºC. The amount of Co$^{2+}$ on wool versus contact time is shown in Figure 3. It can be seen that the rate and extent of uptake of Co$^{2+}$ by wool powders were considerably higher than those for wool fibre. Prolonging the contact time did not significantly increase the sorption capacity of WP-C and WP-D for Co$^{2+}$, but dramatically increased that of the wool fibre. For example, the amount of Co$^{2+}$ on WP-D was increased from 2.24 ($\times$10$^{-9}$ moles/mg) at a contact time of 15 minutes to 2.52 ($\times$10$^{-9}$ moles/mg) after binding for 22 hours, while that on wool fibre was increased from 0.12 ($\times$10$^{-9}$ moles/mg) to 1.03 ($\times$10$^{-9}$ moles/mg) when the binding time was increased from 15 minutes to 22 hours.

A similar trend for dye sorption by wool powders and fibre was observed previously [16]. The result could be related to the disruption of outer cuticle cells and the exposure of inner cortical cells as a result of the powdering process [16]. The cuticle cells of wool are known to act as barriers for the diffusion of chemicals into wool. The disruption of cuticle cells, because of mechanical milling during powder production, allows Co$^{2+}$ to readily penetrate into the interior of wool, which contributes to the enhanced sorption capacity of wool powders for Co$^{2+}$. The exposure of cortical cells provided more active binding sites on the surface of wool powders for Co$^{2+}$, which led to the rapid sorption rate of wool powders. Compared to WP-C, the higher sorption capacity of WP-D for Co$^{2+}$ may be due to the negative cysteic acid groups, introduced from the chlorination treatment during powder production [16], which favour the Co$^{2+}$ binding with wool.

The sorption time was set at 2 hours in subsequent experiments, because there was no significant increase in the uptake of Co$^{2+}$ by wool powders beyond this time.

3 Sorption capacity of wool powders for Co$^{2+}$
When metal ions bind to wool, the metal ions can not only penetrate the interior of wool [28], but also bind on the wool surface [17]. Langmuir adsorption isotherm was employed to examine whether the Co$^{2+}$ ions on the wool surface were in the form of a monolayer or multiple layers. The widely used Langmuir equation is given below:

$$\frac{C_e}{q_e} = \frac{1}{q_{\text{max}}K_L} + \frac{C_e}{q_{\text{max}}}$$

(4)

where $q_e$ is the amount of Co$^{2+}$ adsorbed on the adsorbent at equilibrium (mg/g)

$C_e$ is the equilibrium concentration of Co$^{2+}$ in solution (mg/L)

$q_{\text{max}}$ is the maximum monolayer capacity of the adsorbent (mg/g)

$K_L$ is the Langmuir adsorption constant (L/mg).

The plot of $C_e/q_e$ versus $C_e$, with the slope of $1/q_{\text{max}}$ and the intercept of $1/q_{\text{max}}K_L$, is shown in Figure 5. It illustrates that the sorption of Co$^{2+}$ by wool substrates fits Langmuir isotherm very well. This result indicates that the Co$^{2+}$ ions bound on the surface of wool substrates are in the form of a monolayer. The maximum amounts of Co$^{2+}$ sorbed on the surface of wool at equilibrium were calculated to be 0.913 mg/g, 0.858 mg/g and 0.18 mg/g for WP-C, WP-D and wool fibre, respectively.
4 Reversibility and re-sorption ability of wool powders

Three reagents, EDTA (0.01 M), HCl (0.1 M) and pH 3 buffer (glycine/sodium chloride), were chosen to elute Co²⁺ from wool substrates in this study. EDTA is widely used as a chelating agent in various industries to sequestrate di- and tricationic metal ions from solution [38, 39]. It is found to be an effective agent to recover metal ions, such as mercury and copper ions, from wool [7, 22, 40]. HCl was selected in this study because hydrogen ions in solution compete with metal ions for the same binding sites on wool [6]. When the metal ion bound wool is immersed in an acidic solution, some metal ions are released from wool. Acids such as acetic acid [19, 20], hydrochloric acid [11, 41] and citrate acid [7], have been used to release metal ions from wool. Additionally, the desorption of metal ions from wool at a particular pH value, which gives the lowest metal uptake by wool, is thought to be a possible method to reverse metal ions from wool [7]. From Figure 3, the uptake of Co²⁺ at pH 3 (glycine/sodium chloride buffer) was at a minimum, so the efficiency of pH 3 buffer to release Co²⁺ was also examined.

After releasing bound Co²⁺ from wool, the ability of the substrates to resorb Co²⁺ ions was investigated, and the results are given in Table III.

### Table III. Releasing and re-sorption capacity of wool samples for Co²⁺

<table>
<thead>
<tr>
<th>Wool sample</th>
<th>Pre-sorption</th>
<th>Releasing</th>
<th>Re-sorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount of Co²⁺ on wool (×10⁻⁹ moles/mg)</td>
<td>Releasing per cent (%)</td>
<td>Amount of re-sorbed Co²⁺ on wool (×10⁻⁹ moles/mg)</td>
</tr>
<tr>
<td></td>
<td>eluting solution</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>WP-C</td>
<td>2.13 EDTA (0.01 M)</td>
<td>69.26</td>
<td>71.32</td>
</tr>
<tr>
<td></td>
<td>2.21 HCl (0.1 M)</td>
<td>74.38</td>
<td>77.37</td>
</tr>
<tr>
<td></td>
<td>2.17 pH 3 buffer</td>
<td>69.56</td>
<td>76.51</td>
</tr>
<tr>
<td>WP-D</td>
<td>2.51 EDTA (0.01 M)</td>
<td>87.11</td>
<td>87.10</td>
</tr>
<tr>
<td></td>
<td>2.56 HCl (0.1 M)</td>
<td>93.86</td>
<td>94.27</td>
</tr>
<tr>
<td></td>
<td>2.51 pH 3 buffer</td>
<td>88.63</td>
<td>91.45</td>
</tr>
<tr>
<td>Wool fibre</td>
<td>0.56 EDTA (0.01 M)</td>
<td>59.93</td>
<td>54.50</td>
</tr>
<tr>
<td></td>
<td>0.58 HCl (0.1 M)</td>
<td>61.33</td>
<td>65.82</td>
</tr>
<tr>
<td></td>
<td>0.59 pH 3 buffer</td>
<td>63.14</td>
<td>68.11</td>
</tr>
</tbody>
</table>

It can be seen from Table III the releasing efficiencies of HCl and pH 3 buffer are similar, and better than that of EDTA. This result indicates that the competition between hydrogen ions and Co²⁺ played a more important role than chelating of Co²⁺ with EDTA, in releasing of Co²⁺ from wool. Desorption of Co²⁺ from wool in dilute acid suggests that the carboxyl groups were the major binding sites. The unreleased Co²⁺ was likely to bind on wool in the form of cobalt mercaptide [2]. Among these three wool samples, the releasing percentage for WP-D is the highest, and more than 90% of Co²⁺ was removed from WP-D using HCl (0.1M) and pH 3 buffer, while that for wool fibre was the lowest (60% - 70%). The amount of released Co²⁺ was increased with prolonged eluting time from 1 hour to 2 hours.

The amounts of re-sorbed Co²⁺ by wool samples, after releasing by HCl and pH 3 buffer, were similar, and higher than that by EDTA. This is expected because the former have the higher releasing percentages, and can provide more active sites to re-sorb Co²⁺. The sorption capacity of reused wool powders (WP-C and WP-D), after releasing by HCl and pH 3 buffer, was more than 80% of that for the fresh wool powders. This fact indicates that wool powder can be repeatedly used as an effective sorbent to remove Co²⁺ from solution.

REFERENCES

CONCLUSIONS

Binding of Co\(^{2+}\) to wool powders is heavily dependent on the pH value and the chemical nature of buffer. Wool has a remarkable sorption ability for Co\(^{2+}\) at pH 8 (phosphate buffer) and pH 10 (ammonium sulphate/ammonia buffer). Many factors, such as the surface charge of wool in acidic or alkali media, the changes in the chemical structure of buffered wool, the formation of Co-buffer complex, the hydrolysis and the oxidation of metal ion, can contribute to this complicated binding process.

When wool was milled into ~5 µm powder form, its sorption capacity for Co\(^{2+}\) was increased about 4 times. There was no significant increase in the amount of sorbed Co\(^{2+}\) by wool powders when the binding time was increased from 0.25 hour to 22 hour. The disruption of the cuticle cells and the exposure of the cortical cells in wool powders contribute to their rapid sorption rate and enhanced sorption ability for Co\(^{2+}\).

More than 70% of Co\(^{2+}\) was recovered from wool powders using HCl (0.1M) and pH 3 buffer (glycine/sodium chloride). The sorption ability of re-used wool powders, after releasing Co\(^{2+}\), was 80% of that of fresh wool powders.

ACKNOWLEDGMENTS

We are grateful to Australian Institute of Nuclear Science and Engineering (AINSE), Australian Wool Innovation (AWI), and the Australian Research Council for supporting this research.
Compared to wool fibre, fine wool powder was more efficient for removing and recovering some metal ions (such as Co$^{2+}$) from solution.

Table I. Composition of buffer I

<table>
<thead>
<tr>
<th>pH value</th>
<th>Composition</th>
<th>di-sodium hydrogen phosphate (0.2 M) (mL)</th>
</tr>
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<tbody>
<tr>
<td>2.61</td>
<td>citric acid (0.1 M) (mL)</td>
<td>39</td>
</tr>
<tr>
<td>3.60</td>
<td>461</td>
<td></td>
</tr>
<tr>
<td>4.61</td>
<td>351</td>
<td>149</td>
</tr>
<tr>
<td>5.67</td>
<td>275</td>
<td>225</td>
</tr>
<tr>
<td>7.77</td>
<td>218</td>
<td>282</td>
</tr>
<tr>
<td>10.16</td>
<td>42</td>
<td>458</td>
</tr>
</tbody>
</table>

66 g ammonium sulphate and 25 mL ammonia (30%) solution in 500 mL Milli-Q water

Table II. Composition of buffer II

<table>
<thead>
<tr>
<th>pH value</th>
<th>Composition</th>
<th>Total volume (mL)</th>
<th>Chemical used to adjust pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.75 g glycine 2.92 g sodium chloride</td>
<td></td>
<td>HCl / NaOH</td>
</tr>
<tr>
<td>4</td>
<td>13.5 g sodium succinate</td>
<td>add Milli-Q water to 500mL</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9.76 g morpholino ethane sulfonic acid (MES)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.10 g disodium hydrogen phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.75 g glycine 2.92 g sodium chloride</td>
<td></td>
<td></td>
</tr>
</tbody>
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