# Table of content

A. **Introduction**

B. **Methodology**

C. **Hypothesis**

D. **Experiments**

   D.1 **Section 1: Necessary condition for the colour changes**
      
      D.1.1 Colourimetric analysis on the micro-scale reaction between nano-silver solution and different amino acids under different pH environments
      
      D.1.2 Investigation on the colours of silver plates under different amino acids
      
      D.1.3 **Proposed mechanism**
      
      D.1.3.1 Formation of linkages between cysteine and silver ion

   D.2 **Section 2: The effect of pH on the colour changes**
      
      D.2.1 Investigation on the effect of pH on the colour changes of silver plates in the albumen and cysteine solution
      
      D.2.2 **Proposed mechanism**
      
      D.2.2.1 pH dependence of the complexation mode
      
      D.2.2.2 Diversity of colours in respect to different complexation modes
      
      D.2.2.3 Energy level
      
      D.2.3 Verification of mechanism in macro-scale
      
      D.2.4 Verification of mechanism in micro-scale

   D.3 **Section 3: The effects of ions common in sweat**
      
      D.3.1 Investigation on the effect of phosphate ion, chloride ion and bicarbonate ion on the colour changes of silver plates
      
      D.3.2 Investigation on the effect of phosphate ion in acidic and alkaline environment

E. **Evidence supporting the mechanism**

   E.1 Contribution of carboxyl group and amine group to the colours
   
   E.2 Close agreement between the $pK_a$, $pK_b$ and $pI$ value with colour changes
   
   E.3 Necessity of silver oxide
   
   E.4 Strong interaction between cysteine and silver ion
   
   E.5 High activation energy required

F. **Application - Preliminary test for Chronic Renal Failure**

   F.1 **Screening urine test for proteinuria**
      
      F.1.1 Introduction
      
      F.1.2 Test backgrounds
      
      F.1.3 Test procedure
F.1.4 Results
F.1.5 Discussion

F.2 Diagnostic sweat test
  F.2.1 Introduction
  F.2.2 Suggested procedure
  F.2.3 Result of simulation
  F.2.4 Discussion
  F.2.5 Clinical trials using sweat samples

G. Limitations
H. Conclusion
I. Appendix
  I.1 Colourimetric analysis on the micro-scale reaction between nano-silver solution and different amino acids under different pH environments
  I.2 Investigation on the colours of silver plates under different amino acids
  I.3 Transmittances of cysteine solution of different pH plotted against different wavelengths.
A. Introduction

Referring to a traditional Chinese methodology, illness of patient can be detected by silver, usually in form of coin or strip inside a hard-boiled egg. The silver strip, when rolled with albumen on the area where patient felt sick, shows obvious colour changes from silvery white to yellow, red, blue or purple, depending on the type of illness.

While the correlation between the colour changes and the nature of the illness had remained uncertain, we found linkages between the methodology and silver staining for protein footprinting, which was introduced by Switzer et al. in 1979.\(^1\) First of all, the colour changes matched with what reported by Chao-Ming Tsai and Carl E. Frasch.\(^2\) Secondly, the rate of colour change had increased as the reaction progressed.\(^3\) Furthermore, both colour changes involve the interaction between silver and protein, which is abundant in egg. Hence, we suggested that the classic Chinese methodology might have a mechanism similar to that of colour silver staining. Several experiments were carried out to replicate the methodology, with different external factors being investigated.

In the light of better understanding of nature of science of this ancient technique, we hope to utilize a modern application which will be beneficial to our society.

---

B. Methodology

The procedures of the ancient China methodology are stated as follows: (Fig. 1)

1. A hard-boiled egg was prepared with its egg shell removed.

2. The egg was cut into two halves and egg yolk was removed. A silver coin or a silver plate was inserted into the albumen.

3. The egg was wrapped with a towel, and was rolled on the area where patient felt uncomfortable or pain for 10 minutes.

4. The silver plate was taken out from the crushed egg, and colours of the silver plates rolled on different patient were compared.

(Fig. 1) Procedure for the ancient Chinese Diagnosis
### Result

The colour changes of silver plate after rolling the egg on the affected areas of patients suffering from different diseases.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Silver plates after experiment</th>
<th>Colours of the silver plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rolled on the forehead of a normal person</td>
<td><img src="image1.jpg" alt="Image" /></td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>Rolled on the joint of the arthritic patient</td>
<td><img src="image2.jpg" alt="Image" /></td>
<td>Brown with some purple</td>
</tr>
<tr>
<td>Rolled on the stomach of the patient having stomachache</td>
<td><img src="image3.jpg" alt="Image" /></td>
<td>Blue</td>
</tr>
<tr>
<td>Rolled on the forehead of the patient having headache</td>
<td><img src="image4.jpg" alt="Image" /></td>
<td>Blue</td>
</tr>
</tbody>
</table>

The changed colour of the silver plates which rolled on healthy persons, showed a slight brownish yellow on the silver plates. The plates rolled on patients having headache or stomach showed greenish blue and the arthritic patient revealed a brown and purple colour.

We propose that, due to the different skin environment arise from various sickness, the albumen stain the silver differentially. The hypothesis is described in the next section with further details.
C. Hypothesis

The majorities of the composition of chicken egg are protein and fat. Since colour silver staining was used to stain protein, we suggested that protein is the cause of the colour changes. Since proteins without cysteine were stained negatively (yellow against a yellow background) in silver staining\(^4\), we proposed that cysteine is responsible for the colour change of the silver in the ancient Chinese methodology.

(Fig. 2) Diagrammatic presentation of our hypothesis

Cysteine consists of an amine group, a carboxylic acid group and a thiol group (-SH) as the side chain. The nucleophilic nature of thiol group (-SH) owing to its diffuse lone pair electrons might induce a reaction with silver and lead to a coloured product. We suspected that the thiol group is attached to the silver plate and form a coloured product. (Fig. 2)

Since different colours could be shown on the silver plate, the complexes formed should vary from one case to another. This suggests that the difference of the resulting colours is due to the change of chelation modes.

D. Experiments

D.1 Section 1: Conditions necessary for the colour changes

D.1.1 Colourimetric analysis on the micro-scale reaction between nano-silver solution and different amino acids under different pH environments

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano-silver solution</td>
<td>12-well plates</td>
</tr>
<tr>
<td>20.0 mM Leucine solution</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td>20.0 mM Methionine solution</td>
<td>droppers</td>
</tr>
<tr>
<td>20.0 mM Cysteine solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Phenylalanine solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Valine solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Histidine solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Alanine solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Aspartic acid solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Glutamic acid solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Glycine solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Proline solution</td>
<td></td>
</tr>
<tr>
<td>20.0 mM Asparagine solution</td>
<td></td>
</tr>
<tr>
<td>12 different buffer solutions of pH 5.0, 6.0, 6.3, 6.7, 7.0, 7.3, 7.7, 8.0, 8.3, 8.7, 9.0, 10.0</td>
<td></td>
</tr>
</tbody>
</table>

Procedure:

1. 5.0 cm³ of buffer solution was added into a sample cell.
2. 5.0 cm³ of nano-silver solution was added.
3. 10 drops of prepared amino acid solution were added and placed in room condition for 10 minutes.
4. The specimen was analyzed by colourimeter.
5. Another container was filled with distilled water had its reading set as zero.
6. The transmittance rates of different wavelengths of the specimen were measured.
7. Steps 1 to 6 were repeated with different pH buffers.
8. Steps 1 to 7 were repeated with different amino acids.
Results:

(Because of the similar behaviour shared all amino acid except cysteine, only the data of leucine and cysteine are shown below. Data of other amino acids are presented in the appendix)

Discussion:
Clear trough had been observed in all amino acids tested at the wavelength of 470 nm, which represented the presence of the original pale yellow colour of the nano-silver solution.

A unique pattern had been observed in the graph of cysteine when compared to the rest of the amino acids was observed. Besides a drop of transmittances rate at the
wavelength of 440 nm – 490 nm, another trough at higher wavelength 550 nm – 590 nm was also reported. This suggested that another coloured complex had been formed in the sample cell by reacting nano-silver with cysteine.

In addition, it was observed that the trends of the transmittances rates varied with pH. This means that under different pH environments, the product will be in different colours.

For the sake of providing a broader picture of the mechanism, UV-visible spectrophotometer, which can measure the transmittance rates continuously, had been used to analyze the colour change of the setup containing cysteine and nano-silver. Since it agreed closely with the result of this experiment, the new data and the experimental procedure is included in the appendix.

**D.1.2 Investigation on the colours of silver plates under different amino acids**

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver plates</td>
<td>Incubators</td>
</tr>
<tr>
<td>Amino acids solutions</td>
<td>Steam bath</td>
</tr>
<tr>
<td>(as Experiment D.1.1)</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>Test tubes</td>
</tr>
</tbody>
</table>

**Procedures:**

1. 10.0 cm$^3$ of 12 different amino acid solutions were added into the corresponding labelled boiling tubes.
2. A clean silver plate was put in each boiling tubes.
3. The tubes were put into the incubator of 70°C for one day.
4. The colours of silver plate were recorded.

**Results:**

(Because of the similar behaviour shared all amino acid except cysteine, only the result of leucine is shown below. Setups of other amino acid is present in the appendix)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Resulting colour of the silver plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Purple and blue</td>
</tr>
</tbody>
</table>

**Discussion:**

Only the silver plate that immersed into the cysteine solution showed a significant colour change from silvery white to bluish purple. This suggested that cysteine is responsible for the colour changes, which agreed with the conclusion in experiment D.1.1.

**D.1.3 Proposed mechanism:**

Base on the result of experiments D.1.1 and D.1.2, it was discovered that cysteine is responsible for the colour changes. The proposed mechanism was shown below.

**D.1.3.1 Formation of linkages between cysteine and silver ion**

First of all, thiol group of cysteine in peptide form or free molecular form would react with the silver oxide on the silver plate or nano-silver molecule to form the Ag-S bond,
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

which is also reported by Sastry et al.\(^5\)

Cys-Ag \(^5\) might possibly be the coloured product.

Nonetheless, more than one colours were observed on the silver plates. As only cysteine gives rise to a colour change but not other amino acids, we can thus conclude that the Cys-Ag complex can express different colours under different conditions.

Cysteine contains carboxyl group and amine group, which can be protonated and deprotonated according to pH. They may cause changes in the complexation mode and result in different colour. Therefore, the effect of pH would be investigated in the next section.

D.2 Section 2: The effect of pH on the colour changes

D.2.1 Investigation on the effect of pH on the colour changes of silver plates in albumen and cysteine solution

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumen</td>
<td>Incubator</td>
</tr>
<tr>
<td>Buffer solutions with</td>
<td>Blender</td>
</tr>
<tr>
<td>pH 5.0, 6.0, 6.3, 6.7, 7.0, 7.3, 7.7, 8.0, 9.0, 10.0</td>
<td></td>
</tr>
</tbody>
</table>

Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

<table>
<thead>
<tr>
<th>Silver plates</th>
<th>Thermometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.20 x 10^{-5} M Cysteine solution</td>
<td>Syringe</td>
</tr>
<tr>
<td></td>
<td>Test tubes</td>
</tr>
</tbody>
</table>

**Procedures:**

1. Albumen was extracted from the eggs and was transferred into a large beaker.
2. The albumen was cooked under boiling water.
3. The cooked albumen was scrambled by a blender.
4. Scrambled albumen was distributed into 10 test tubes.
5. Buffer solutions with different pH and water were added into the test tubes, with the buffer solution just over the surface of the albumen.
6. The test tubes were put into a water bath of temperature 70°C.
7. Continuous stirring was required.
8. After 25 minutes, the silver plates were taken out and their colour changes were observed.
9. Steps 1-8 had been repeated by replacing albumen with cysteine solution.
10. Steps 1-8 had been repeated by rolling the silver plate in albumen, which covered by a towel, on a clean white tile. Buffers solution with different pH values were sprayed on the tile beforehand.
Results:

<table>
<thead>
<tr>
<th>pH values</th>
<th>Silver plates immersed in albumen</th>
<th>Silver plates immersed in cysteine solution</th>
<th>Silver plates rolled in towel on white tile</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5.0</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>pH 6.3</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>pH 6.7</td>
<td>Brown with green</td>
<td>Brown with blue</td>
<td>Brown</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>Brown with blue</td>
<td>Green with blue</td>
<td>Brown with green</td>
</tr>
<tr>
<td>pH 7.3</td>
<td>Green with blue</td>
<td>Blue with purple</td>
<td>Green with blue</td>
</tr>
</tbody>
</table>
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

<table>
<thead>
<tr>
<th>pH</th>
<th>Color Description</th>
<th>Color Description</th>
<th>Color Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7</td>
<td>Deep blue</td>
<td>Blue with purple</td>
<td>Green with blue</td>
</tr>
<tr>
<td>8.0</td>
<td>Deep blue</td>
<td>Blue with purple</td>
<td>Blue with purple</td>
</tr>
<tr>
<td>9.0</td>
<td>Blue with purple</td>
<td>Blue with purple</td>
<td>Blue with purple</td>
</tr>
<tr>
<td>10.0</td>
<td>Blue with purple</td>
<td>Blue with purple</td>
<td>Blue with purple</td>
</tr>
</tbody>
</table>

Discussion:

The silver plate immersed in the albumen turned brown under low pH environment, from 5.0 to 6.7, while blue and green and blue appeared at the pH value 7.3. The blue and green colours were intensified with the increasing pH value. And purple appeared at the pH 9.0. Similar observations were made in the rest of the setups.

The result agreed with our previous finding that cysteine is responsible for the colour change. Moreover, since silver plates in cysteine solution and albumen solution had shown similar colour trends in respect to pH, it suggested that the free cysteine
molecules and the less mobile cysteine in the peptide chain react with silver plate in a very similar way over a wide spectrum of pH.

Higher colour intensity as well as reaction rate were reported in the albumen setup. We reasoned that the amount of cysteine in albumen is higher than that in the cysteine solution. In addition, it may be reasoned by the fact that some cysteine present in albumen in the form of protein. The chelation of one of the cysteine in a protein chain can facilitate the chelation of the others in the same protein chain intramolecularly as Fig. 4.

(Fig. 4) Diagram showing how the complexation of one Cys unit in protein facilitates others'

The setup that was covered by towel showed similar pH dependence about the colour changes. Thus, the external pH outside the towel can affect the internal pH inside the albumen effectively.
D.2.2 Proposed mechanism

D.2.2.1 pH dependence of the complexation mode

The amine group and carboxylic acid group of AgSR would respond differentially according to the pH environment in respect to cysteine’s pKa, pKb and pI, hence the following forms A, B, C of cysteine-silver complex would be formed.

D.2.2.2 Diversity of colours in respect to different complexation modes

The protonation and deprotonation of amine group and carboxylic group would result in different interactions between the functional groups and silver ion. The yellowish and the bluish complexes, with different wavelength absorption, are resulted from different energy levels of the complexation modes.

In experiment D.2.1, silver plate appeared yellow in pH 5.0 – 6.3 and green layer emerges since pH 6.3 and the blue layer becomes distinct in

(Fig. 5) Comparison between pI of cysteine and the trend of colour change
higher pH. The result closely agrees with the pK\textsubscript{a} (pH=1.7), pK\textsubscript{b} (pH=10.8) and pI (pH=6.3) values of cysteine. (Fig. 5)

**D.2.2.3 Energy level**

We have tried to justify our proposed mechanism by immersing the blue silver plate into a pH 4 buffer and putting a yellow silver plate into a pH 10.0 buffer in 70\(^\circ\)C for 8 hours. It was found that the yellow silver plate gradually transformed to blue at pH 10.0, but the blue silver plate showed no observable changes at pH 4.
Consequently, the following energy profile diagram was proposed. (Fig. 7)

We proposed that the blue substance formed in pathway A is a thermodynamic product. Once it was formed, it is energetically unfavourable for it to transform to the yellow product via pathway B.

To verify the energy profile, we have repeated experiment D.2.1 and prolonged the duration to 7 days. It was found that all of the silver plates turned to blue. This supported that the blue complex is a thermodynamic product.
D.2.3 Verification of the proposed mechanism in macro-scale

According to the proposed mechanism, carboxyl group is responsible for the formation of yellow and the amine group is responsible for blue. To prove our hypothesis, we reacted cysteamine and 3-mercaptopropionic acid with silver. These two are the counterparts of cysteine with their carboxyl group or amine group being removed. (Fig. 8)

If our hypothesis is correct, the expected result would be as follow:

<table>
<thead>
<tr>
<th></th>
<th>Cysteine</th>
<th>Cysteamine</th>
<th>3-mercaptopropionic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acidic</strong></td>
<td>Yellow</td>
<td>No observable change</td>
<td>Yellow</td>
</tr>
<tr>
<td><strong>Alkaline</strong></td>
<td>Blue</td>
<td>Blue</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

**Experiment**

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>Incubator</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>Test tubes</td>
</tr>
<tr>
<td>3-mercaptopropionic acid</td>
<td>Stopper</td>
</tr>
<tr>
<td>pH 4.0 buffer</td>
<td></td>
</tr>
<tr>
<td>pH 10.0 buffer</td>
<td></td>
</tr>
<tr>
<td>Silver plates</td>
<td></td>
</tr>
<tr>
<td>Deionized water</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure:**

1. Test tubes, labelled from I to III, were filled with content 2.0 cm³ pH 4.0 buffer and 2.0 cm³ DI water³. On the other hand, test tubes, labelled from IV to VI, were filled with content 2.0 cm³ pH 10.0 buffer and 2.0 cm³ DI water³.
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

2. 0.009 g of cysteine was added into test tubes I and IV, 0.006 g of cysteamine was added into test tube II and V, and 0.008 g of 3-mercaptopropionic acid was added into test tube III and VI. In other words, the mixtures can be concluded by the following table:

<table>
<thead>
<tr>
<th></th>
<th>Cysteine</th>
<th>Cysteamine</th>
<th>3-mercaptopropionic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH 4.0 buffer</strong></td>
<td>Test tube I</td>
<td>Test tube II</td>
<td>Test tube III</td>
</tr>
<tr>
<td><strong>pH 10.0 buffer</strong></td>
<td>Test tube IV</td>
<td>Test tube V</td>
<td>Test tube VI</td>
</tr>
</tbody>
</table>

3. The mixtures were warmed at 70°C, and a silver plate was put into each test tube and heated for 24 hours.

4. The colours of the silver plates were recorded after 24 hours.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Cysteine</th>
<th>Cysteamine</th>
<th>3-Mercaptopropionic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH 4.0 buffer</strong></td>
<td><img src="image1.png" alt="Yellow" /></td>
<td><img src="image2.png" alt="No observable change" /></td>
<td><img src="image3.png" alt="Yellow" /></td>
</tr>
<tr>
<td><strong>pH 10.0 buffer</strong></td>
<td><img src="image4.png" alt="Blue" /></td>
<td><img src="image5.png" alt="Blue" /></td>
<td><img src="image6.png" alt="Yellow" /></td>
</tr>
</tbody>
</table>
Discussion:

Our proposed mechanism was further verified by reacting the silver plate with cysteamine and 3-mercaptopropionic acid. It was found that cysteamine reacted with Ag₂O on silver plate to form yellow complex in either acidic or alkaline environment. This confirmed that the carboxyl group is responsible for the yellow complex.

Moreover, the blue complex only formed if the non-protonated amino group of 3-mercaptopropionic acid was present at pH > pKₐ. This confirms that the lone pair of amino group is necessary for the formation of blue complex.

Interestingly, once the blue complex formed, the amino group would not be protonated again at pH = 2 for 120 h. The finding suggested that the N,S-bidentate ligand is the thermodynamic product and showed a close agreement with the findings in the previous experiments.
D.2.4 Verification of the proposed mechanism in micro-scale

In section D.2.3, our proposed mechanism is supported by the macro-scale testing with the counterparts of cysteine. Thus, our team would like to find out whether testing in micro-scale can provide further evidence to support our proposed mechanism.

We decided to use nano-silver to replace solid silver strip and analyse the sample with UV-visible spectrometer. According to our proposed mechanism, cysteamine should share a same absorption peak with cysteine when pH > pl, while 3-mercaptopropionic acid should share a same absorption peak as cysteine in any pH.

**Experiment**

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>UV-visible spectrometer with quartz cells</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>Test tubes</td>
</tr>
<tr>
<td>3-mercaptopropionic acid</td>
<td></td>
</tr>
<tr>
<td>pH 4.0 buffer</td>
<td></td>
</tr>
<tr>
<td>pH 10.0 buffer</td>
<td></td>
</tr>
<tr>
<td>Nano-silver</td>
<td></td>
</tr>
<tr>
<td>distilled water</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure:**

1. Excess cysteine is dissolved in pH 4 and pH 10 buffer solution with the aid of sonicator.

2. 1.0 cm³ of the cysteine solution in different pH is syringed in 1.5 cm³ nano-Ag solution in a quartz cell.

3. The content of the quartz cell is then analysed with UV-visible spectrometer
from 800 nm to 200 nm with distilled water as base line and plotted as an absorption spectrum.

4. The test is repeated with 3-Mercaptopropionic acid and cysteamine.

5. After half an hour, the test is repeated and the result is compared to the previous data. Resemblance in absorption spectrum indicates that the reaction had been completed when they are first analysed.

**Results:**

![Absorption Spectrum of nano-Ag in Cysteine at different pH](image1)

![Absorption Spectrum of nano-Ag in 3-Mercaptopropionic acid at different pH](image2)
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

Discussion:
For cysteine, an absorption peak at 400 nm is found, which is identified to be the colour of nano-Ag. An additional peak at 600 nm is observed in acidic environment. This suggests that a new complex formed, which may be due to chelation of the carboxyl group of the cysteine bonds to the silver. While in pH > pI, an upward shifting in base line from 500 to 750 nm indicate a broad absorption peak is found. We reason that to be the bonding between amine group and the silver atom in the [Ag-Cys] complex.

For 3-mercaptopropionic acid in all pH, an obvious shoulder at 600 nm is observed. This value is in close agreement with the absorption peak in [Ag-Cys] complex which corresponds to the bonding between carboxyl group and the silver atom. The extent of reaction is high in acidic condition, which could be due to the proton activation of silver oxide at low pH. On the contrary, no surge in base line observed. This is explained by the absence of amine group.

*Cysteine is only slightly soluble in water at pH 7.
For cysteamine, at pH < pI, the baseline showed insignificant change. This matches our observation in the macro-scale test, where there is no colour change on the silver strip, attributable to the protonation of amine group. While for pH > pI, an increase in base line. This broad absorption is explained by the bonding between amine group and silver atom when the lone pair of amine group is available at higher pH. Similar absorption pattern for pH = 7 and pH = 10 indicates that the factors other than amine group may be limiting the extent of reaction, such as active site on nano-Ag solution.

In conclusion, our proposed mechanism is supported in a micro-scale experiment.
D.3 Section 3: The effects of ions common in sweat
D.3.1 Investigation on the effect of phosphate ion, chloride ion and bicarbonate ion on the colour changes of silver plates

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0200 M cysteine solution</td>
<td>Bunsen burner</td>
</tr>
<tr>
<td>2.140 x 10^{-1} M Sodium chloride solution</td>
<td>Wire gauze and tripod</td>
</tr>
<tr>
<td>5.600 x 10^{-2} M Sodium bicarbonate solution</td>
<td>Beaker</td>
</tr>
<tr>
<td>5.000 x 10^{-6} M Trisodium phosphate solution</td>
<td>Timer</td>
</tr>
<tr>
<td>5.000 x 10^{-5} M Trisodium phosphate solution</td>
<td></td>
</tr>
<tr>
<td>5.000 x 10^{-4} M Trisodium phosphate solution</td>
<td></td>
</tr>
<tr>
<td>DI water</td>
<td></td>
</tr>
<tr>
<td>Silver plates</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure**

1. 30.0 cm^3 0.0200 M cysteine solution, 2.00 cm^3 5.00 x 10^{-5} M trisodium phosphate solution, 2.00 cm^3 DI water and a silver plate for 2 minutes under continuous stirring.

2. After the removal of heat, the stirring continued for 5 minutes, and the colour of the silver plate was recorded.

3. To ensure there was no more development of colour, the stirring continued for 10 minutes and the colour of the silver plate was observed.

4. Steps 1-3 were repeated with different solutions in different combinations.

**Result**

<table>
<thead>
<tr>
<th></th>
<th>PO_4^{3-}</th>
<th>HCO_3^-</th>
<th>Cl^-</th>
<th>PO_4^{3-} + Cl^- + HCO_3^-</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO_4^{3-}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCO_3^-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl^-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Concentration for each ion is the same for each setup)
**Discussion**

The concentrations of chloride, bicarbonate and phosphate ions solutions employed were similar to human sweat. It was discovered that phosphate ion turned the silver plate into dark blue, while chloride ion and bicarbonate ion promoted no obvious changes.

Since the concentration of phosphate ion was very low, it was unlikely for the phosphate ion to affect the colour changes by elevating the pH. Measured by pH meter, the pH of the four mixtures ranged from 7.97 to 8.01. Therefore, it is unlikely that phosphate ion affect the reaction by pH.

Nonetheless, based on the result above, it is possible to develop a qualitative test for phosphate ion. Therefore, detection limit was investigated by repeating this experiment with various concentrations of phosphate ion.

**Result**

| Colour of the silver plate |  |
|----------------------------|  |
| **Control (Distilled water was added)** | (Overall conc. of \( \text{PO}_4^{3-} \) : 0 M ) |
| **2.0 cm\(^3\) 5.00 x 10\(^{-6}\) M \( \text{PO}_4^{3-} \) added** | (Overall conc. of \( \text{PO}_4^{3-} \) : 3.125 x 10\(^{-7}\) M ) |
| **2.0 cm\(^3\) 5.00 x 10\(^{-5}\) M \( \text{PO}_4^{3-} \) added** | (Overall conc. of \( \text{PO}_4^{3-} \) : 3.125 x 10\(^{-6}\) M ) |
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

Discussion

It was discovered that the addition of phosphate ion solution with a concentration higher than $5.00 \times 10^{-6}$ M significantly intensified the colour changes on silver plate. Please be noticed that, by altering the ratio between the volume of the cysteine solution and the water sample, it is possible to change the detection limit. This would be further discussed in the application section.

It was discovered that the rate of colour change from silver to yellow and blue was greatly enhanced, hence, there might be the possibility for phosphate being a positive catalyst or promoter of the reaction.

To confirm that phosphate ion was not affecting the reaction by affecting the pH, we repeated our test in cysteine solution with the addition of buffer solution of pH 4 and pH 10, and observed the colour change continuously, in order to observe how the presence of phosphate ion interacts with the effect of pH.
**D.3.2 Investigation on the effect of phosphate ion in acidic and alkaline environment**

**Material**
- Cysteine
- 0.0100 M Trisodium phosphate solution
- Silver plates
- pH 4.0 buffer
- pH 10.0 buffer
- Deionized water

**Apparatus**
- Incubator
- Test tubes
- Stopper
- Timer
- syringe

**Procedure:**

1. Test tubes, labelled from 1 to 4, were filled with content as shown in the table below, with a total volume of 4.0 cm³

2. 0.003 g of cysteine was added into each of the test tubes.

3. The mixtures were warmed at 70°C, and a silver plate was put into each test tube and heated for 18 hours.

4. The colours of the silver plates were recorded every 2 hours.

<table>
<thead>
<tr>
<th>Volume (cm³)</th>
<th>Setup 1</th>
<th>Setup 2</th>
<th>Setup 3</th>
<th>Setup 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.0 buffer</td>
<td>2.0 cm³</td>
<td>2.0 cm³</td>
<td>2.0 cm³</td>
<td>2.0 cm³</td>
</tr>
<tr>
<td>pH 10.0 buffer</td>
<td>2.0 cm³</td>
<td>2.0 cm³</td>
<td>2.0 cm³</td>
<td>2.0 cm³</td>
</tr>
<tr>
<td>pH (by pH meter)</td>
<td>4.02</td>
<td>4.30</td>
<td>10.03</td>
<td>10.34</td>
</tr>
</tbody>
</table>
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

Results:

<table>
<thead>
<tr>
<th>Heating duration</th>
<th>Setup 1</th>
<th>Setup 2</th>
<th>Setup 3</th>
<th>Setup 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9 hours</td>
<td>No observable changes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 hours</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>12 hours</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>14 hours</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>16 hours</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
</tr>
<tr>
<td>18 hours</td>
<td><img src="image17.png" alt="Image" /></td>
<td><img src="image18.png" alt="Image" /></td>
<td><img src="image19.png" alt="Image" /></td>
<td><img src="image20.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Discussion

In the presence of phosphate ion, the silver plate gradually changed from silvery, brownish red, to blue. Concerning about the formation of blue complex, the reaction rate was higher in higher pH and the presence of phosphate ion, and the later factor was found to be more significant. This confirms phosphate ion's ability to promote the reaction in either alkaline condition or acidic condition. Yet, the mechanism of the promotion effect remains unclear despite efforts devoted on extensive literature reviews.
E. Evidence supporting the proposed mechanism

Here is some evidence supporting the proposed mechanism.

E.1 Contribution of carboxyl group and amine group to the colours

As shown in section D.2.4, only with the presence of amine group a purplish blue will be observed. The same relationship is true for carboxyl group and yellow. It is a strong evidence for our mechanism, in which the group that silver bonds with determines the overall colour of the complex.

E.2 Close agreement between the pK_a, pK_b and pI value with colour changes

The close agreement between the pK_a, pK_b and pI value with the pH dependence of the colour changes reveals the high possibility for the protonation of amine group and carboxyl group affect the resultant colour of the complexation by altering the complexation mode.

E.3 Necessity of silver oxide

It was observed that heating the silver plate with Bunsen flame before the experiment gave rises to a more uniform colours development. The lost of lustre of silver plate after the heating indicated the formation of silver oxide. On the other hand, if the silver plate is not put under Bunsen flame, it was reported that the liquid junction of the silver plate had its colour developed most quickly. Base on our proposed mechanism, it can be explained by the fact that oxygen diffusion is more effective near the atmospheric oxygen, which favours the formation of silver oxide and initiation of the reaction.
In addition, while performing the experiments, it was discovered that blocking the oxygen supply slows down the reaction dramatically. (Fig. 9) We may conclude that the formation of silver oxide, which requires oxygen, is essential for the development of the colour.

(E.4 Strong interaction between cysteine and silver ion)
As shown in experiments D.1.1 and D.1.2, and agreed by the rest of the experiments, cysteine is responsible for colour changes on silver plate. This is reasoned by the outstanding strong interaction between cysteine and silver ion, which had previously been reported by Gruen LC (1975). This makes the complexation between silver oxide and cysteine a very plausible reason for the colour changes. Please be noticed this mechanism diverges from silver staining technique, which shows no colour change without the addition of reducing agents. We explain this by the differences of reaction condition.

(E.5 High activation energy required)
No observable changes were recorded if the experiments, including the conversion between the yellow and the blue described in our proposed mechanism, were performed in room temperature. This shows that the activation energy is high. This agrees with the fact that silver oxide has a moderately high bond enthalpy of 220.1 ± 20.9 KJ/mol, which must be overcome for the formation of the coloured complexes.

---

F. Application - Test for Chronic Renal Failure

F.1 Screening urine test for proteinuria

F.1.1 Introduction

CRF patients generally exhibit serious proteinuria (urine protein concentration > 0.43 g/L). Since protein is the substrate of the reaction investigated, it is possible to test for the excessive amount of protein in them by the reaction. High level of protein would readily react with nano-Ag solution in the presence of phosphate to bring about an observable color change.

To confirm the applicability of the proposed CRF Preliminary test, 50 patients and 50 healthy controls have been contacted through hospital referral. They have agreed to be the test subject of our proposed test under the supervision of licensed doctors of the hospital.

F.1.2 Test procedure

First morning urine samples were collected from the patients, and was stored in sealed vial immediately. The samples were then refrigerated at 4°C for less than 4 hours before test. The test subjects were selected through randomized process. Primary disease was not specified, while patients with co-morbidities are not selected as test subject.

The ratio between urine and testing solution is 1-to-1. The mixture was allowed to heat under boiling water bath for 30 min. Since protein acts as the substrate of the
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

reaction, a decolourization of the yellow solution indicates a positive result. If no observable change was observed, it is said to be a negative test.

F.1.3 Results

<table>
<thead>
<tr>
<th>#</th>
<th>Protein (g/L)</th>
<th>Our diagnosis</th>
<th>Category</th>
<th>#</th>
<th>Protein (g/L)</th>
<th>Our diagnosis</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.72</td>
<td>1</td>
<td>TP</td>
<td>38</td>
<td>0.13</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>2</td>
<td>1.43</td>
<td>1</td>
<td>TP</td>
<td>39</td>
<td>0.33</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>3</td>
<td>0.14</td>
<td>0</td>
<td>TN</td>
<td>40</td>
<td>2.15</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>4</td>
<td>11.87</td>
<td>1</td>
<td>TP</td>
<td>41</td>
<td>0.15</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>5</td>
<td>3.00</td>
<td>1</td>
<td>TP</td>
<td>42</td>
<td>2.44</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>6</td>
<td>0.19</td>
<td>0</td>
<td>TN</td>
<td>43</td>
<td>0.58</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>7</td>
<td>0.21</td>
<td>0</td>
<td>TN</td>
<td>44</td>
<td>1.41</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>8</td>
<td>1.57</td>
<td>1</td>
<td>TP</td>
<td>45</td>
<td>0.11</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>9</td>
<td>0.28</td>
<td>0</td>
<td>TN</td>
<td>46</td>
<td>0.16</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>10</td>
<td>0.09</td>
<td>0</td>
<td>TN</td>
<td>47</td>
<td>0.1</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>11</td>
<td>0.31</td>
<td>1</td>
<td>TP</td>
<td>48</td>
<td>3.53</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>12</td>
<td>2.33</td>
<td>1</td>
<td>TP</td>
<td>49</td>
<td>0.13</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>13</td>
<td>0.09</td>
<td>0</td>
<td>TN</td>
<td>50</td>
<td>0.15</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>14</td>
<td>1.52</td>
<td>1</td>
<td>TP</td>
<td>51</td>
<td>1.97</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>15</td>
<td>0.13</td>
<td>0</td>
<td>TN</td>
<td>52</td>
<td>2.03</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>16</td>
<td>0.08</td>
<td>0</td>
<td>TN</td>
<td>53</td>
<td>0.13</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>17</td>
<td>2.27</td>
<td>1</td>
<td>TP</td>
<td>54</td>
<td>0.11</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>18</td>
<td>0.80</td>
<td>1</td>
<td>TP</td>
<td>55</td>
<td>0.09</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>19</td>
<td>0.09</td>
<td>0</td>
<td>TN</td>
<td>56</td>
<td>0.87</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>20</td>
<td>2.58</td>
<td>1</td>
<td>TP</td>
<td>57</td>
<td>0.19</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>21</td>
<td>0.3</td>
<td>0</td>
<td>TN</td>
<td>58</td>
<td>0.29</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>22</td>
<td>0.12</td>
<td>0</td>
<td>TN</td>
<td>59</td>
<td>3.96</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>23</td>
<td>0.48</td>
<td>0</td>
<td>FN</td>
<td>60</td>
<td>0.57</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>24</td>
<td>2.26</td>
<td>1</td>
<td>TP</td>
<td>61</td>
<td>0.25</td>
<td>0</td>
<td>TN</td>
</tr>
<tr>
<td>25</td>
<td>0.22</td>
<td>0</td>
<td>TN</td>
<td>62</td>
<td>0.50</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>26</td>
<td>0.08</td>
<td>0</td>
<td>TN</td>
<td>63</td>
<td>0.73</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>27</td>
<td>0.09</td>
<td>0</td>
<td>TN</td>
<td>64</td>
<td>1.43</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>28</td>
<td>1.77</td>
<td>1</td>
<td>TP</td>
<td>65</td>
<td>1.54</td>
<td>1</td>
<td>TP</td>
</tr>
<tr>
<td>29</td>
<td>3.23</td>
<td>1</td>
<td>TP</td>
<td>66</td>
<td>3.81</td>
<td>1</td>
<td>TP</td>
</tr>
</tbody>
</table>
### Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.09</td>
<td>0</td>
<td>TN</td>
<td>67</td>
<td>0.20</td>
</tr>
<tr>
<td>31</td>
<td>1.18</td>
<td>1</td>
<td>TP</td>
<td>68</td>
<td>0.31</td>
</tr>
<tr>
<td>32</td>
<td>0.1</td>
<td>0</td>
<td>TN</td>
<td>69</td>
<td>0.98</td>
</tr>
<tr>
<td>33</td>
<td>0.37</td>
<td>0</td>
<td>TN</td>
<td>70</td>
<td>0.26</td>
</tr>
<tr>
<td>34</td>
<td>5.92</td>
<td>1</td>
<td>TP</td>
<td>71</td>
<td>0.26</td>
</tr>
<tr>
<td>35</td>
<td>2.18</td>
<td>1</td>
<td>TP</td>
<td>72</td>
<td>3.77</td>
</tr>
<tr>
<td>36</td>
<td>1.16</td>
<td>1</td>
<td>TP</td>
<td>73</td>
<td>0.24</td>
</tr>
<tr>
<td>37</td>
<td>0.45</td>
<td>0</td>
<td>FN</td>
<td>74</td>
<td>2.69</td>
</tr>
</tbody>
</table>

Sensitivity = 94.6% (CI95% = 88.5% to 100%)

Specificity = 97.3% (CI95% = 92.9% to 100%)

**Key:**

*Red grid = patient with proteinuria (protein conc. in urine > 0.43 g/L)*

*Green grid = health subject with no proteinuria (protein conc. in urine < 0.43 g/L)*

*Gray = Test positive*

*Yellow = Test negative*

### F.1.4 Discussion

It is disappointing to see that the preliminary test we suggested showed comparatively lower specificity than the test employed currently in hospital. It was reasoned by the fact that urine protein content may fluctuate due to diet and other physiological conditions. For instance, orthostatic proteinuria might cause false-positive result.

Yet, taking into account various advantages of the test, the potential of this test should not be undermined. First, the test is non-invasive and low-risk, when compared to the current test which requires blood sampling. Second, the test can be done by high-risk people themselves regularly without specialized training. In addition to regular hospital checkup, they may also conduct this test as an extra secure method.
F.2 Diagnostic sweat test

F.2.1 Introduction

Chronic renal failure can be indicated by the significantly elevated phosphate ion concentration in sweat. A normal level of phosphate ion concentration in sweat is about $4.68 \times 10^{-4} \text{ M} \ (4.5 \text{ mg/dL})$. Since phosphate ion speeds up the reaction, the high sweat phosphate level in CRF patients may trigger an intense colour during the reaction. This can be potentially be developed into an alternative test method for CRF.

F.2.2 Suggested procedure

<table>
<thead>
<tr>
<th>Material</th>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0200 M cysteine solution</td>
<td>Container for heating</td>
</tr>
<tr>
<td>Silver plate (reusable for over 30 times)</td>
<td>Heating setup</td>
</tr>
<tr>
<td>DI water</td>
<td>Clothing</td>
</tr>
<tr>
<td>4.5 mg/dL Trisodium phosphate solution</td>
<td></td>
</tr>
<tr>
<td>5.0 mg/dL Trisodium phosphate solution</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure**

1. $0.80 \text{ cm}^3$ of saturated cysteine solution in 0.30 M acetic acid was mixed with $0.8 \text{ cm}^3$ sweat sample from the patient.
2. A piece of clean silver strip (2.0 x 1.0 cm$^3$, 0.5 mm) was put into the reaction mixture, such that the solution just cover the silver strip.
3. The solution was warmed at $70^\circ\text{C}$ for 45 min.
4. The colour of silver strip was examined afterwards. An intense blue colour indicates a positive test. A light brown colour indicates a negative test.

---

F.2.3 Result of simulation

(To analyze the colour of silver plate in different concentrations of phosphate ion, sodium triphosphate solutions of different concentration were used to simulate sweat.)

<table>
<thead>
<tr>
<th>[PO₄³⁻]</th>
<th>DI water</th>
<th>4.5 mg/dL</th>
<th>6.0 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colours</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>(after 10 minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F.2.4 Discussion

Chronic renal failure can be indicated by the excessive amount of phosphate ion in sweat. As mentioned previously, the normal level value range of phosphate is about 4.5 mg/dL, and the majority of CRF patients have phosphate level higher than that. Although, as determined by experiment D.3.1, the detection limit is 4.5 mg/L, it was found 4.5 mg/L was unable to trigger the intensification of colour if the egg is covered by the towel. This is explained by the fact that the diffusion is not completely effective. Nevertheless, obvious colour intensification was discovered when the phosphate concentration is abnormal, which indicates high possibility of CRF.

There are several advantages about this test. Firstly, since the test is handy and affordable, it is possible for the test to be carried by individual regularly. Secondly, the test is non-invasive.

The detection, however, is definitely less quantitative and precise when compared to modern methods for detection of CRF, such as blood test for estimated glomerular filtration rate.
filtration rate (eGFR). Nevertheless, the conveniences of the test shall not be overlooked.

**F.2.5 Clinical trials using sweat samples**

**Introduction**

Because only 10 samples were provided by the hospital, it is unable to calculate the sensitivity and specificity with confidence intervals narrow enough to be meaningful. Still, the trial was carried out as a proof of idea.

**Testing procedure**

Using nickel electrodes and 0.5% pilocarpine solution, sweating is stimulated on the arm of human subjects artificially. Then, the site is properly cleaned and sweat is collected using a filter paper. The filter paper is then sealed in a capsule and used for testing within 1 hour.

**Result**

All 5 patients suffering from CRF was diagnosed correctly but 1 false-positive result was observed in healthy control. According to the central limit theorem, a sample mean obtained from a sample size n below 30 does not accurately represent the population mean. Thus the sensitivity and specificity cannot be calculated.
G. Limitations

Sample size
Due to the limitation of resources, we are not able to test our application on a large number of CRF patients. Thus, we are unable to determine the sensitivity and specificity of our diagnostic sweat test. Still, 9 correct diagnoses out of 10 suggest that the test has its potential to be further developed.

Egg
Eggs from different sources often have slightly different content. To obtain a comparable result, all eggs purchased are from the same source.

Isolating single product
Despite repeated attempts to isolate and crystallize the product, we have not successfully obtained a pure sample of the product for structure analysis using X-ray crystallography and NMR.
H. Conclusion

Inspired by silver staining technique, we investigate the mechanism of an ancient Chinese medical procedure, in which a silver plate embedded inside a hard-boiled egg was used to detect disease of patient.

It was discovered that cysteine is responsible for the colour changes, and the colour generally changes from silvery to yellow and eventually blue. The reaction rate differs according to pH. Also, the presence of phosphate ion can catalyze the reaction. A mechanism for the reaction was proposed. Because the reactions allow low concentration of phosphate ion to be tested qualitatively, the possibilities of establishing two kinds of phosphate detector were discussed.

In spite of several limitations, the sensitivity and the selectivity of the applications had been confirmed. We hope that the applications can protect the ecosystem and the health of the citizens.
I. Appendix

1.1 Colourimetric analysis on the micro-scale reaction between nano-silver solution and different amino acids under different pH environments

Aspartic acid

Valine
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

Asparagine

![Chart showing the relationship between wavelength and transmittance for different pH levels of asparagine]

Glycine

![Chart showing the relationship between wavelength and transmittance for different pH levels of glycine]

Leucine

![Chart showing the relationship between wavelength and transmittance for different pH levels of leucine]
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

Phenylalanine

![Phenylalanine chart]

Proline

![Proline chart]

Histidine

![Histidine chart]
Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

I.2 Investigation on the colours of silver plates under different amino acids

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Resulting colour of the silver plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Methionine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Purple and blue</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Valine</td>
<td>Silvery white</td>
</tr>
</tbody>
</table>
### Mechanism of Chinese silver staining diagnosis and its application as rapid test for Chronic Renal Failure

<table>
<thead>
<tr>
<th>Protein</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Alanine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Glycine</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Proline</td>
<td>Silvery white</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Silvery white</td>
</tr>
</tbody>
</table>
1.3 Transmittances of cysteine solution of different pHs plotted against different wavelengths.

The graph showed that there is a significant increase of transmittance at 390 – 420 nm (violet) with a considerable drop of transmittance at 500 – 600 nm (greenish yellow) in pH 9 – 10.