SIRIM Berhad
Industrial Biotechnology Research Centre, Building 19
Tel: 03-55446953/6960
Fax:03-55446988

STUDY REPORT

EVALUATION OF NANO COLLOIDAL ARGENTUM IN THE *IN VITRO* MEMBRANE BARRIER TEST FOR SKIN CORROSION

Study No. J208/13

Report No. R208/13/B19/11

Sponsor:
Trumer Medicare Sdn Bhd.
No. 16-2, Jalan SS19/1G,
47500 Subang Jaya,
Selangor.

Sponsor Representative:
Mr. KC Yap

Test Facility:
Industrial Biotechnology Research Centre (IBRC),
Bldg 19, SIRIM Berhad.

Study Initiation Date:
21 June 2013

Experimental Start Date:
10 July 2013

Experimental End Date:
10 July 2013

Study Completion Date:
15 July 2013

SIRIM Berhad
(No. Syarikat 367474 - V)
1, Persiaran Dato' Menteri
Seksyen 2, Peti Surat 7035
40700 Shah Alam
MALAYSIA
Tel: 60-3-55446000
Hotline: 60-3-55103535
Faks: 60-3-55108095
Website : www.sirim.my

STUDY REPORT

REPORT NO: R208/13/B19/11	PAGE: 2 of 8

KEY PERSONNEL PARTICIPATING IN THIS STUDY

We, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected throughout the study.

15 JUL 2013

(DR. NEELAM SHAHAB, *AMIC*)
Senior Principal Researcher
Industrial Biotechnology Research Centre

Date

15 JUL 2013

(SUZAINI BADRUDIN)
Researcher
Industrial Biotechnology Research Centre

Date

STUDY REPORT

CONTENTS

APPROVAL SIGNATURES		2
KEY PERSONNEL PARTICIPATING IN THIS STUDY		3
1.0	**SUMMARY**	4
2.0	**BACKGROUND**	4
3.0	**OBJECTIVE**	4
4.0	**TEST SYSTEM**	4
5.0	**APPARATUS AND MATERIALS**	
5.1	**Test Item**	5
5.2	**Corrositex® System**	5
5.3	**Positive Control**	5
5.4	**Negative Control**	5
5.5	**Apparatus**	5
6.0	**METHOD**	
6.1	**Qualification Test**	6
6.2	**Categorization Test**	6
6.3	**Classification Test**	7
7.0	**RESULT**	
7.1	**Qualification Test**	7
7.2	**Categorization Test**	7
7.3	**Classification test**	8
8.0	**DISCUSSION**	8
9.0	**CONCLUSION**	8
10.0	**RETENTION OF RECORDS AND TEST ITEM**	8
11.0	**REFERENCE**	9

SUZAINI BINTI BADRUDIN
Researcher
Bioprocess Programme
Industrial Biotechnology Research Centre
SIRIM Berhad

STUDY REPORT

1.0 SUMMARY

An *in vitro* membrane barrier test method was conducted using Corrositex® to assess the dermal corrosion hazard potential of Nano Colloidal Argentum. Under the condition of this study, the potential of Nano Colloidal Argentum to cause skin corrosion could not be determined. Nevertheless, the Nano Colloidal Argentum Sample is classified non-corrosive according to dermal irritation test report No. R206/13/B19/05.

2.0 BACKGROUND

The United Nations' regulation stipulates that all corrosives in commerce be classified prior to shipment into UN Packing Groups I, II, III or verifies non-corrosivity, according to the US Department of Transportation and International requirement. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) defines skin corrosion as the production of irreversible damage to the skin, manifested as visible necrosis through the epidermis and into the dermis, following the application of a test item.

Packing Groups are assigned according to the degree of danger presented by the corrosive items. Packing Group I, II and III indicate great, medium, and minor danger, respectively. For consistency, these same definitions are used for this test method and are referred to as Group I, Group II and Group III.

3.0 OBJECTIVE

The objective of this study was to predict the potential of Nano Colloidal Argentum to cause skin corrosion. This study was conducted using the *in vitro* membrane barrier test method commercially available as Corrositex® according to OECD Test Guideline No. 435.

4.0 TEST SYSTEM

Corrositex® is a commercially available validated *in vitro* membrane barrier test method that can be used to identify corrosive substances. The test method utilizes an artificial membrane designed to respond to corrosive substances in a manner similar to animal skin *in situ*, and classifies the level of corrosivity in chemicals, formulations and waste. Based on its acknowledged validity, this validated reference test method has been recommended for use as part of a tiered testing strategy for assessing the dermal corrosion hazard potential of chemicals.

The *in vitro* assay system is composed of two components, a synthetic macromolecular biobarrier and a Chemical Detection System (CDS). Test items are applied to the upper surface of the macromolecular biobarrier. Corrosive items are able to disrupt the integrity of the biobarrier, leading to the penetration of the test items through the biobarrier into the CDS located beneath. The presence of the test items in the CDS results in a colour change that is detected visually. The time it takes a test item to penetrate (or break through) the biobarrier into the CDS is inversely proportional to its corrosivity; the more corrosive a test item, the shorter the time required for a colour change. Non-corrosive test items do not disrupt the biobarrier, or disrupt the biobarrier too slowly to be identified as corrosive.

SUZAINI BINTI BADRUDIN
Researcher
Bioprocess Programme
Industrial Biotechnology Research Centre
SIRIM Berhad

5.0 APPARATUS AND MATERIALS

5.1 Test Item

5.1.1 Test Item: Nano Colloidal Argentum

5.1.2 Physical appearance: Liquid

5.1.3 Colour: Clear

5.1.4 Physical chemical properties data: Not provided

5.1.5 Quantity: Approximately 100 mL

5.1.6 Storage condition: Refrigerated

5.1.7 Stability: Not provided

5.1.8 Expiration date: Not provided

5.2 Corrositex® System

5.2.1 Lot No: CT 052112 (Exp 05-14)

5.2.2 Qualify Test Tubes

5.2.3 Categorize Tubes (Tube A, Tube B and Confirm Reagent)

5.2.4 A vial containing Biobarrier Matrix

5.2.5 Biobarrier Diluent

5.2.6 A rack of seven vials filled with Chemical Detection System (CDS)

5.2.7 Membrane Discs

5.3 Positive Control

5.3.1 Sulphuric acid 95%

5.4 Negative Control

5.4.1 Citric acid 10% (w/v)

5.5 Apparatus

5.5.1 Stirring hot plate

5.5.2 Thermometer

5.5.3 Digital timer

SUZAINI BINTI BADRUDIN
Researcher
Bioprocess Programme
Industrial Biotechnology Research Centre
SIRIM Berhad

5.5.4 Eppendorf repeat pipettor

5.5.5 Eppendorf combitips 2.5 ml

5.5.6 Positive displacement pipettor and tips

5.5.7 Spatula

5.5.8 Forcep

5.5.9 Water bath

6.0 METHOD

6.1 Qualification Test

The first step was to evaluate compatibility between the test item and the test system. Test item (150 µL) was placed in the Qualifying Test Tube containing a small amount of CDS fluid. A detectable change in colour or consistency will indicate that the test item is qualified and can be tested for corrosivity using this test system. Otherwise, the test item is considered to be incompatible with this test system and corrosivity will need to be assessed using other methods.

6.2 Categorization Test

The next step was to categorize the test item in order to determine interpretation of the cut-off times. Categorization is based on the test item's ability to induce a pH change in the defined buffers.

The test item (150 µL) was added into Tube A (acidic buffer) and Tube B (base buffer). The colour changes were compared to the corresponding colour charts on the Corrositex® Testing Protocol Poster, which will match to the group of colours either in Category 1 or Category 2. If a colour change is not observed in either tube, two drops of Confirm Reagent are added to Tube B and the resulting colour is then matched again to the corresponding colour charts for categorization.

If a colour change is still not observed, the pH of a 10% (v/v or w/v) aqueous test item solution is measured. If the pH is < 7.0, Tube A is utilized for categorization. If the pH is > 7.0, Tube B is utilized for categorization. Test item (100 mg) was then added into the selected tube, thoroughly mixed and the pH measured. Tube A with a pH ≤ 5.0 or Tube B with a pH ≥ 9.0 indicates the test item is Category 1. Tube A with a pH > 5.0 or Tube B with a pH < 9.0 indicates the test item is Category 2.

The test item is defined into four groups:

a) Tube A Category 1 are items that produce a large change in pH when they are added to the acidic buffer. This change in pH is indicated by a strong colour change.

b) Tube A Category 2 are items that produce little or no pH changes when added to the acid buffer and, therefore, little or no colour change in the buffer solution is observed.

SUZAINI BINTI BADRUDIN
Researcher
Bioprocess Programme
Industrial Biotechnology Research Centre
SIRIM Berhad

c) Tube B Category 1 are items that produce a large change in pH when they are added to the base buffer. This change in pH is indicated by a strong colour change.

d) Tube B Category 2 are items that produce little or no pH changes when added to the base buffer and, therefore, little or no colour change in the buffer solution is observed.

6.3 Classification Test

This final step was to determine the appropriate Packing Group for the test item. A macromolecular biobarrier was prepared by adding the entire contents of the Biobarrier Diluent to the vial containing the Biobarrier Matrix under controlled conditions. The biobarrier solution was then carefully transferred into Membrane Discs, avoiding air bubbles formation and ensuring the entire membrane is covered.

The biobarrier discs were individually placed on the top of each vials filled with Chemical Detection System (CDS). The test item (150 µL) was immediately added onto the top of the barrier disc and the timer was started. Vials 1-4 were utilized for test item replicate testing. Sulphuric acid (500 µL) and citric acid (500 µL) were added to vials labelled (+) as positive control and (-) as negative control, respectively. The vial labelled C served as a CDS colour control.

As soon as a colour change was observed beneath the centre of each biobarrier disc, the detection time was recorded. Corrositex® Time and the avearge of the four test item replicates were calculated (Corrositex® Time = Detection Time – Start Time). The appropriate Packing Group was assigned based on the test item category and Corrositex® Time according to Table 1.

Table 1. The UN Packing Group Assignment

Category	**Time Required for CDS change (minutes)**			
Category 1	0 to 3	>3 to 60	>60 to 240	>240
Category 2	0 to 3	>3 to 30	>30 to 60	>60
	Packing Group 1	Packing Group 2	Packing Group 3	Non-Corrosive

7.0 RESULT

7.1 Qualification Test

The Qualifying Test Tube did not show a colour change.

7.2 Categorization Test

The Categorization Test was not carried out.

7.3 Classification Test

The Classification Test was not carried out.

SUZAINI BINTI BADRUDIN
Researcher
Bioprocess Programme
Industrial Biotechnology Research Centre
SIRIM Berhad

8.0 DISCUSSION

Nano Colloidal Argentum did not show a colour change in the Qualification Test. Therefore it is considered to be incompatible with this test system.

A limitation of this validated in vitro membrane barrier test method is that many non-corrosive chemicals and chemicals mixtures and some corrosive chemicals and chemical mixtures do not qualify for testing, as seen in Nano Colloidal Argentum. Test chemicals and chemical mixtures are considered non-qualifying if they do not cause a colour change in the CDS. Aqueous substances with a pH in the range of 4.5 to 8.5 often do not qualify for testing. However, 85% of chemicals tested in this pH range were non-corrosive in animal tests.

9.0 CONCLUSION

The Nano Colloidal Argentum is classified as non-corrosive according to dermal irritation test report No. R206/13/B19/05.

10.0 RETENTION OF RECORDS AND TEST ITEM

Two original, signed final reports were prepared. One report will be forwarded to the Sponsor. The other report, together with all generated raw data, is maintained at the Industrial Biotechnology Research Centre Archives. IBRC will maintain these records for a period of ten years. After this time, the Sponsor will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by IBRC.

11.0 REFERENCE

11.1 OECD (2006). Test Guideline 435. OECD Guideline for Testing of Chemicals. *In Vitro* Membrane Barrier Test Method for Skin Corrosion.

11.2 Corrositex® Testing Procedures (DOT-E 10904). In Vitro International.

11.3 Corrositex® Testing Protocol Poster

SUZAINI BINTI BADRUDIN
Researcher
Bioprocess Programme
Industrial Biotechnology Research Centre
SIRIM Berhad