Iraqi Journal of Science
ISSN: 0067-2904
GIF: 0.851

Novel Study of Cyproheptadine Hydrochloride Precipitate Formed by Potassium hexacyanoferrate and Sodium nitroprusside using Atomic Force Microscopy

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Abstract
Atomic Force Microscope is an efficient tool to study the topography of precipitate. A study using Continuous Flow Injection via the use of Ayah 6SX1-T-2D Solar cell CFI Analyser . It was found that Cyproheptadine –HCl form precipitates of different quality using a precipitating agent's potassium hexacyanoferrate (III) and sodium nitroprusside. The formed precipitates are collected as they are formed in the usual sequence of forming the precipitate via the continuous flow .The precipitates are collected and dried under normal atmospheric pressure. The precipitates are subjected to atomic force microscope scanning to study the variation and differences of these precipitates relating them to the kind of response to both precipitates give as. The incident light (i.e. super snow white LED) was scanned and it reveals that is , it compose of three components blue ,green and red color . The obtained spectrum were measured as a percentage area (percentage effect) also different models were study for the incident light irradiation of the measuring cell followed by the study of the effect on the detector area and responses .Various details and theoretical representation were adopted and were taken in to account ,the nodules (grains) on the surface were assumed to be sphere . The probability of radiation of the nodules of the surface of precipitate as the blue color and green color with the red color were 56.73% of green color , 42.12% of blue color and 1.15% of red color effect on the surface of precipitate .Granulation cumulating distribution data for both precipitates were measured also grains (nodules) diameter were taken to concentration .

Keywords: Atomic Force Microscopy, Flow injection analysis

دراسة نبيلة لراسب السايبروهبتادين هايدروكلورايد المتكون بوساطة سداسي سيانيد الحديد(III) البوتاسيوم و نايترو بروسيد الصوديوم باستخدام مجهر القوة الذرية

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الخلاصة
مجهر القوة الذرية هو اداة فعالة لدراسة تضاريس الراسب . اجريت الدراسة باستخدام تقنية الحقن الجرياني المستمر من خلال استخدام محلل الحقن الجرياني Ayah 6SX1-T-2D Solar cell CFI .لوحظ ان السايبروهبتادين هايدروكلورايد يكون رواسب بنوعية مختلفة باسخدام عوامل مرسبة ، سداسي سيانيد الحديد (III) البوتاسيوم وصوديوم نايتروبروسيد .تم جمع الرواسب المتكونة من خلال الحقن الجرياني المستمر، و

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بدورها جففت تحت الضغط الجوي الاعتيادي .تم عرض الرواسب الى مجهر القوة الذرية لاجراء المسح لدراسة التباين والاختلاف للرواسب وربطها بنوع الاستجابة المستحصل عليها لكلا الراسبين . تم اجراء مسح للضوء الساقط (ثنائي وصلة باعث للضوء الابيض الثلجي) ولوحظ انه مكون من ثلاث مكونات الازرق ، الاخضر واللون الاحمر وتم قياس الطيف المستحصل علية كنسبة مئوية للمساحة (النسبة المئوية المؤثرة) .كما درست نماذج مختلفة للضوء الساقط الذي يشعع خلية القياس تليها دراسة تاثيرة على مساحة المتحسس والاستجابات. مختلف التفاصيل والتمثيل النظري اعتمد واخذ بنظر الاعتبار ، العقيدات (الحبيبات) على السطح افترضت انها كرة . الاحتمالية لتشعيع العقيدات على سطح الراسب للضوء الاخضر ، الازرق والاحمر كانت 73 .56% ، 42.12 % و 1.15 % على التوالي تؤثر على سطح الراسب . تم قياس بيانات التوزيع التراكمي لكلا الراسبين وكذلك قطر العقيدات اخذت بالحساب .

Introduction

Atomic force microscopy or AFM is a method to see the shape of a surface in three dimensional (3D) details down to the nanometer scale. AFM can image all materials—hard or soft, synthetic or natural (including biological structures such as cells and biomolecules)—irrespective of opaqueness or conductivity. The sample is usually imaged in air, but can be in liquid environments and in some cases under vacuum[1]. The AFM raster scans a sharp probe over the surface of a sample and measures the changes in force between the probe tip and the sample. A cantilever with a sharp tip is positioned above a surface. Depending on this separation distance, long range or short range forces will dominate the interaction. This force is measured by the bending of the cantilever by an optical lever technique: a laser beam is focused on the back of a cantilever and reflected into a photo detector. Small forces between the tip and sample will cause less deflection than large forces. By raster-scanning the tip across the surface and recording the change in force as a function of position, a map of surface topography and other properties can be generated[2-4].Previous preliminary study was made for two different lead of precipitate [5], also a previous study dealing PAA gel beads was studied [6].

The aim of this study is to establish a basic foundation for the use of the obtained data based on the formation of both precipitates which are mainly CPH-HCl-$[Fe(CN)_6]^{3-}$ and CPH-HCl-$[Fe(CN)_5NO]^{2-}$. A collection of the precipitates was made via the measuring cell outlet, left for 1 week to dry up under normal unattained disturbance, a way from dust and air draught. The explanation will be followed according to the usually given parameter by the AFM-Scan. i.e;

1. Amplitude parameter
2. Hybrid parameter
3. Functional parameter
4. Spatial parameter

a) AFM-Scan exploited by term of nodules (grains) formed after the nuclei of precipitates were formed through the adopted methodology.
b) Terms of the used instrument parameter relating to Ayah 6SX1-T-2D Solar cell CFIA.
c) Logic interface model for AFM-Scan parameter with instrument parameters.

Reagents and Chemicals

All chemicals were of analytical-reagent grade while distilled water was used to prepare the solution .A standard solution of Cyproheptadine hydrochloride ($C_{21}H_{22}ClN.1.5\ H_2O$, M.Wt 350.9 $g.mol^{-1}$, SDI, 0.05 $Mol.L^{-1}$) was prepared by dissolving 4.3863 g in 250 ml methanol . A stock solution (0.1 $Mol.L^{-1}$) of potassium hexacyanoferrate $K_3[Fe(CN)_6]$ (M.Wt 329.26 $g.moL^{-1}$, Fluka) was prepared by dissolving 8.2315 g in 250 ml of distilled water , A stock solution(0.1 $Mol.L^{-1}$) of sodium nitroprusside $Na_2Fe(CN)_5NO.2H_2O$ (M.Wt 298 $g.moL^{-1}$, M&B) was prepared by dissolving 7.4500 g in 250 ml of distilled water.

Apparatus

Peristaltic pump – 2 channels variables speed (Ismatec , Switzerland)and rotary 6-port medium pressure injection valve, (IDEX corporation ,USA) with sample loop(0.7mm i.d.Teflon ,different lengths) The response was measured by a homemade Ayah 6 SX1-T-2D Solar cell-CFI Analyser, which uses a six snow white LEDs for irradiation of the flow cell at 2 mm path length . Two solar cell used as a detector for collecting signals via sample travel for 60 mm length. The readout of the system composed of x-t potentiometric recorder(Kompenso Graph C-1032) Siemens (Germany) (1-500 Volt , 1-500 mV)or digital AVO-meter (auto range) (0-2volt) (China), Atomic Force Microscope (Scanning probe microscope) (SPM –AA3000) Angstrom advanced Inc.,2008 ,U.S.A contact mode ,(0.25 nm Lateral ,0.1 nm vertical) resolution . The flow diagram for this study in which a precipitate is formed is shown in Figure-1.

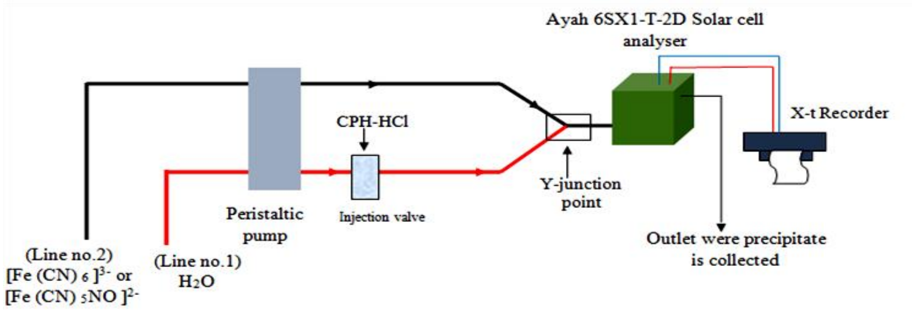


Figure 1- Flow diagram manifold system

Methodology

The manifold system used as shown in Figure-1 which is composed of two lines. The first line at a flow rate of 1.6 ml.min^{-1} (carrier stream distilled water) passing through the injection valve to carry the sample segment (Cyproheptadine –HCl 5 mMol.L^{-1} ,100 µl &104µl for $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_5NO]^{2-}$ respectively) to meet the potassium hexacyanoferrate (III) (10 mMol.L^{-1}) or sodium nitroprusside (5 mMol.L^{-1}) carried by the second line (2.3 ml .min^{-1}) at a Y-junction point to form yellow precipitate of an ion pair $[CPH\text{-}HCl^{+}]_3[Fe(CN)_6]^{3-}$ or white precipitate for an ion pair $[CPH\text{-}HCl^{+}]_2[Fe(CN)_5NO]^{2-}$ before it is introduced to the CFI Analyser. The formed precipitates are collected and dried under normal atmospheric conditions.

Results and Discussions

Terms and Symbols

The study carried out in this section is in four sub division; as it will be described in the next coming paragraphs:

For; 1- AFM parameters
2- Incident hole parameters
3-Incident light parameters
4- Detector and transmitted light fall parameters.

1- AFM – parameters

$A_{Tscan}= A_{Ts}$ = Total scanned area (use 2D-profile)
$A_{TNodules} = A_{TN}$= Total scanned surface area of all sum of nodules
$A_{TLeft} = A_{TL}$= Total scanned surface area that is left
N= Total nodules present in the scanned area

2- Incident hole parameters

s = Single hole area (Φ= 2mm)
S = Area of six hole
R= Number of repeated units
Z_1 = Total available no. of grain /hole
Z_6 = Total available no. of nodules for six holes

3- Incident light parameters

RGB = Red –Green – Blue (main component of white light)
No. % R ; Red light percentage of WLED with λ (660- 697 nm)
No. % G ; Green light percentage of WLED with λ (443-660nm)
No. % B ; Blue light percentage of WLED with λ (421- 480 nm)

4- Detector and transmitted light fall parameters

A_{TDA} = Total available detector area
A_{Hs} = Area of a single detector hole
a_{HTotal} = Used detector area of six hole or total hole area
$A_{TDA} – a_{HT} = \grave{A}_{LDA}$
$\grave{A}_{LDA}$ = Left unused detector area

Calculation Applied to the Used Research Work

Oblong Model

Since the flow cell is cylindrical having the inside diameter 2 mm and outside diameter 4 mm. This flow cell is embedded in a brass metal block that have channel shape of width and depth equivalent to 4mm×4 mm which can occupy by flow cell above this, there is a channel of 14mm×60 mm to hold the detector (30mm ×14mm×1 mm for each solar cell (two)) that is in close contact with flow cell Figure-2. Therefore it is expected and it is more probably logic that the detector will see only an area equivalent to 4mm multiplied 60mm which is equivalent to 4×60= 240 mm^2 =2.4 cm^2.

Total detector area = the surface of the two solar cell having the dimension of 2(14mm×30mm)

A_{TDA} =840 mm^2 = 8.4 cm^2 .

Ratio of used area to the total available detector area equal to

(2.4 /8.4) ×100

= 28.57%

Which leaves %100 – %28.57 = %71.43 theoretically value and hypothetical unused area of detector. The schematic figure as shown in Figure-3

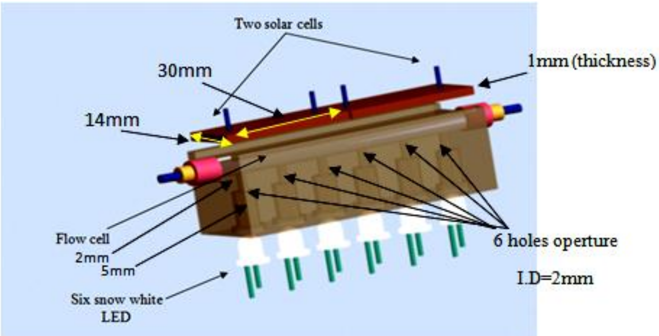


Figure 2-Ayah 6SX1-T-2D solar cell-CFI Analyser

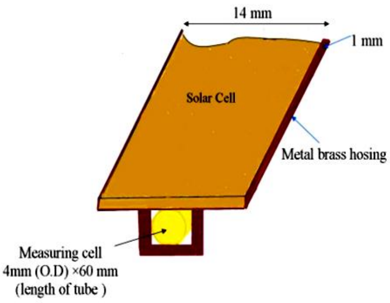


Figure 3- Cross sectional diagram of the measuring unit showing the geometrical arrangement of the flow cell, solar cell and metal housing.

Assuming that regular incidence of white LED as seen by its scanned spectrum Figure- 4A. Composed between seen RGB as unbiased area calculated by triangulation as shown in Figure-4B.

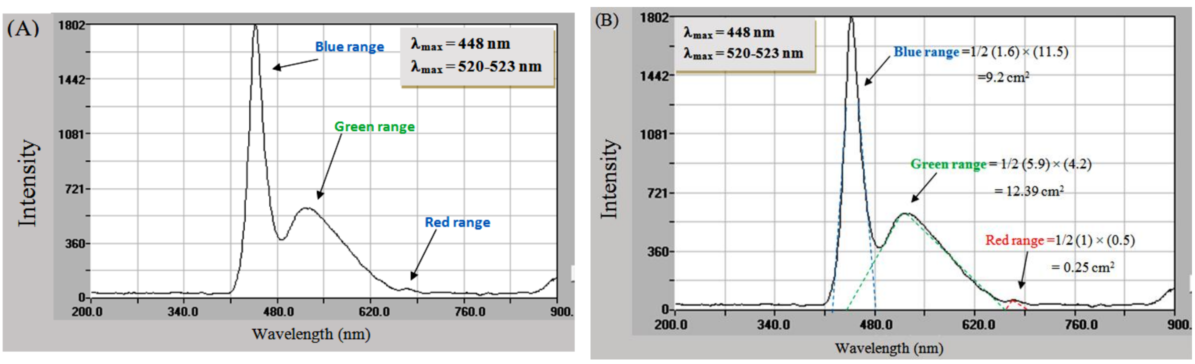


Figure 4- A- Spectrum of snow white LED as recorded by spectrofluorometer (ELICO-INDIA)

B- The contribution of the three main colors RBG on the form of snow white LED.

It was noticed from Figure-4B that
RGB (21.84): 0.25: 12.39: 9.2
(R/RGB)×100 = (0.25/21.84)×100
= 1.15%
(G/RGB) ×100 = (12.39/21.84)×100
= 56.73%
(B/RGB)×100 = (9.2 /21.84) ×100
= 42.12 %
The range of the three main color represented in Figure-5 in which as seen
Blue = 421- 480 nm
Green = 443- 660 nm
Red = 660 – 697 nm
Therefore: White LED is composed of 42.12% B, 56.73 % G, 1.15% R

Ratios	R	:	G	:	B
	1.15	:	56.73	:	42.12
	1	:	49.33	:	36.63

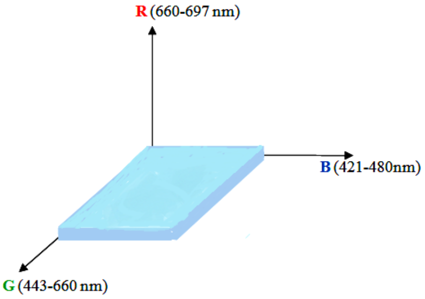


Figure 5-Color axis representation of the white LED used in whole project research studies scanned by spectrofluorometer (ELICO-INDIA)

The ratio of color index axis and the ratios indicate that in addition to the main contribution of these main colors RGB there is the mixed color region form the remaining visible spectrum. Since the green region forms about 56.73% of the white LED i.e; 56.73% of the incident light will suffer more reflection than the blue or even the 1.15% red region of the white LED this will definitely contribute to the positive response(reverse response) obtained from the instrument (spectrofluorometer).
The area of 2.4 cm^2 will represent the whole area that the detector can see (there is no need to multiply by six), therefore;
The blue portion will affect an area of the detector = 2.4 ×0.42
= 1.008 cm^2
The green portion will affect an area of the detector =2.4×0.57
= 1.368 cm^2
The red portion will affect an area of the detector = 2.4×0.012
= 0.029 cm^2

Circular Model

If the assumption were made that the detector will receive circular transmitted light having maximum diameter of 10 mm of each single beam of light .The area of a circular (A_C) diameter of 10 mm will be equivalent to
$A_C = \pi r^2$
$= 3.14(5mm)^2$
$= 78.5\ mm^2 = 0.785\ cm^2$
This is equivalent to single LED irradiation
So, 0.785×6= 4.71cm^2 = total area of expected theoretical and hypothetical of the transmitted light that might affect on the detector response.

Since the detector can seen only 4 mm as the flow cell is embedded as shown in Figure-2 therefore; it can be postulated and assumed that a channel of 4mm width (diameter of expected area affected on area of the detector) which is equal to 4mm ×10 mm as shown inFigure-6

So: Expected area = 4 mm ×10mm

= 40 mm^2=0.4 cm^2

This can be multiplied by six which is equal to the total affected area of the detector

6×0.4 = 2.4 cm^2

Total detector area = the surface of two solar cell having the dimension of 2(14mm×30mm)

A_{TDA}=840 mm^2 = 8.4 cm^2 .

Ratio of used area to the total available detector area equal to

(2.4 /8.4) ×100 = 28.57%

Which leaves %100 – %28.57 = %71.43 theoretically, hypothetical unused area of detector .The schematic figure shown in Figure-6

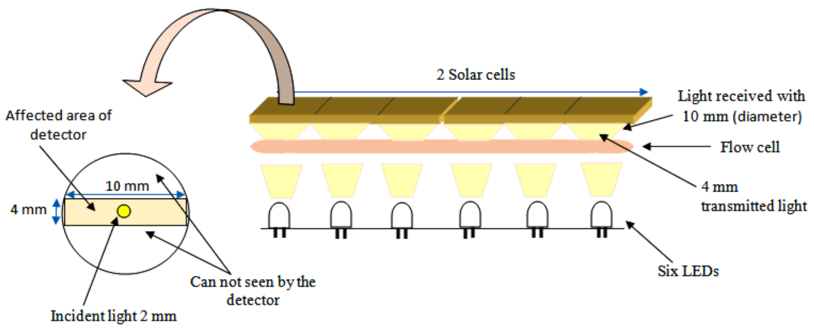


Figure 6- Diagram of the measuring unit showing the diameter of receive circular transmitted light (10 mm) and 4 mm diameter as seen by detector for six source and 2 solar cell

The result of the circle affected area cannot be seen by the detector due to non transparency of the brass metal block that is used as housing.

Therefore; the blue portion will affect an area of the detector for one hole

= 0.4×0.42

=0.168 cm^2

The green portion will affect an area of the detector for one hole

= 0.4 ×0.57

= 0.228 cm^2

The red portion will affect an area of the detector for one hole

= 0.4×0.012

=0.0048 cm^2

For six (6×0.168) + (6×0.228) + (6×0.0048)

1.008 + 1.368 + 0.0288

= 2.4 cm^2

2.4 cm^2 that has been counted by previous section calculation assuming oblong area is the affected part which is equivalent to 2.4 cm^2 counted circular area with assumed postulation

Study of Precipitate from CPH-HCl-$[Fe(CN)_6]^{3-}$ System Using Atomic Force Microscopy

The study was carried out through the preparation of calibration graph for twenty successive injection .A collection of turbid solution for CPH-HCl (5mmol.L^{-1})-$[Fe(CN)_6]^{3-}$(10mmol.L^{-1}) reaction system . The product was left for one week; after a complete eye seen dry precipitate product was used for AFM study. The obtained data Table-1 from this study is represented in Figure-7. While the topographic 2D and 3D of CPH-HCl –$[Fe(CN)_6]^{3-}$ system is shown in Figure-8 by atomic force microscopy.

Table 1- Granularity cumulation distribution data for CPH-HCl-$[Fe(CN)_6]^{3-}$

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
200.00	0.52	0.52	380.00	6.19	31.96	560.00	5.67	77.32
220.00	0.52	1.03	400.00	4.64	36.60	580.00	4.12	81.44
240.00	2.06	3.09	420.00	6.19	42.78	600.00	1.55	82.99
260.00	1.55	4.64	440.00	5.15	47.94	620.00	5.15	88.14
280.00	2.06	6.70	460.00	4.64	52.58	640.00	2.06	90.21
300.00	3.61	10.31	480.00	3.09	55.67	660.00	4.12	94.33
320.00	6.70	17.01	500.00	8.25	63.92	680.00	1.55	95.88
340.00	5.15	22.16	520.00	3.09	67.01	700.00	3.09	98.97
360.00	3.61	25.77	540.00	4.64	71.65	720.00	1.03	100.00

Grain No.:194 , **Avg. Diameter:** 455.71 nm

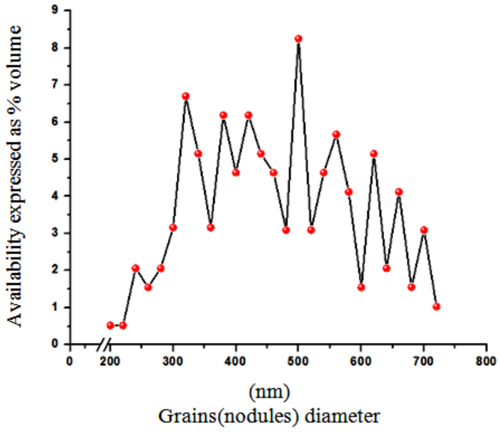


Figure 7-Relationship between diameter of nodules (nm) and occupied area expressed as a volume (%).

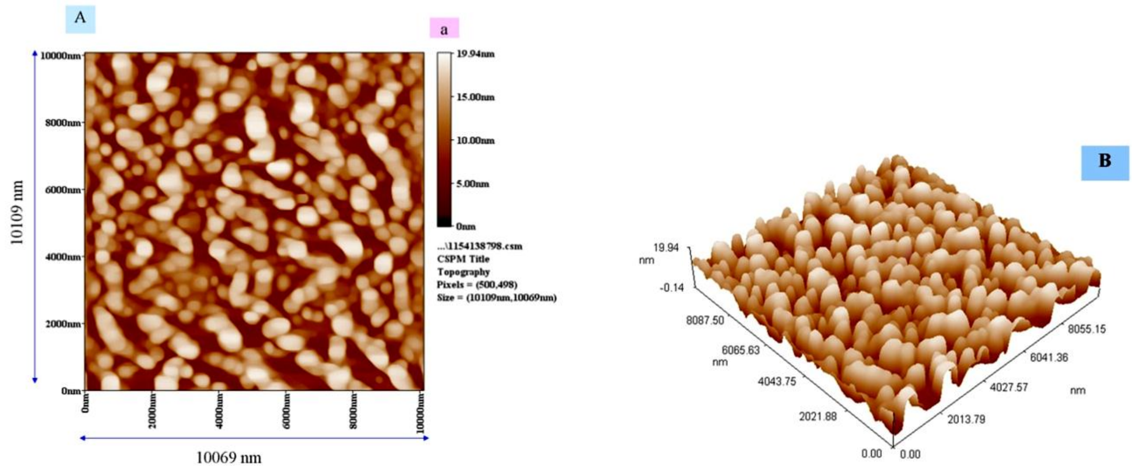


Figure 8- (A): 2D, (B): 3D profile for CPH-HCl-$[Fe(CN)_6]^{3-}$ system a: Depth profile

From the outlet of this study the following calculation is conducted:
Average diameter = Φ nm
Average radius =Φ/ 2 nm
Assume that the nodules are sphere
Surface area of sphere = $4\pi r^2$
A one nodule surface area $=4\pi\ (\Phi/2)^2$
Since the average nodules diameter =455.71 nm
Radius = 455.71/2 = 227.855 nm
Surface area of a single nodule = $4\times3.14\times(227.855)^2$
$= 652088.8369\ nm^2$

Assumption is made on the basis of compactness of grains or nodules as shown in Figure-9a or random scattered nodules as represented in Figure-9b.

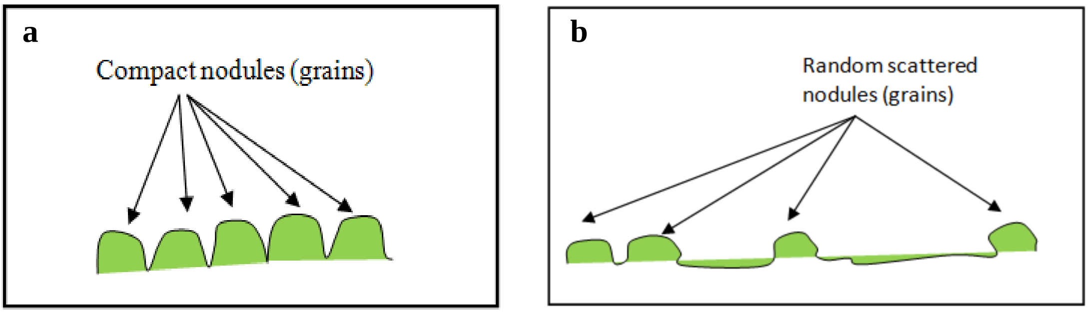


Figure 9- Variation of the distribution of nodules (grains) on precipitate surface

And since there are 194 nodules
Surface area occupied by 194 nodules
A_{TN} = 194× 652088.8369 = 126505234.4 nm ^{2}
= 1.26505×10^{-6} cm^2 Total counted area as predicted from nodules average diameter
Scanned area from 2D –depth profile equal to image size Figure-8A
A_{Ts} =10109 nm ×10069 nm
=1.01787×10^{-6} cm^2 total calculated area
1.01787×10^{-6} - 1.26505×10^{-6} = - 0.24718×10^{-6} cm^2
Average diameter of nodules calculated manually = 464.482 nm
Reference is made to Table-2

Table 2-Tabulation of the average diameter, standard deviation of precipitated particle for CPH-HCl-$[Fe(CN)_6]^{3-}$ system

Average diameter manually ($\bar{\Phi}$) (nm)	Standard deviation (σ_{n-1})	Confidence interval at (95%) $\bar{\Phi} \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
464.482	158.7451	464.482± 62.93

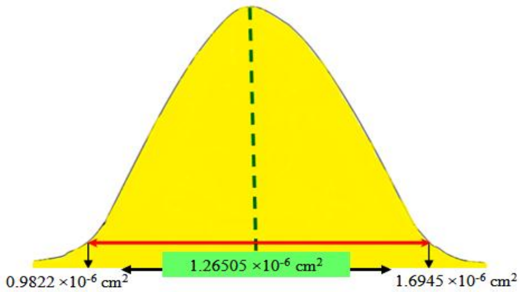
n=27 , $t_{0.05/2,n-1}$= 2.056

Average diameter calculated manually equal to 464.482 ±62.93 nm at 95% confidence interval
464.482 - 62.93 = 401.552 nm
464.482+ 62.93 = 527.412 nm
Confidence interval
Average diameter calculated by atomic force software equal to 455.71 nm

($\bar{\Phi}$) = 455.71 nm
401.552 nm 527.412 nm

Confidence interval of calculated surface area of nodules , the right part of confidence interval representation i.e; for $[CPH\text{-}HCl^{+}]_3$ $[Fe(CN)_6]^{3-}$ precipitate equal to 62.93 which mean that a fluctuation of average diameter($\bar{\Phi}$) is a fluctuating within ± 62.93nm. Since the average diameter calculated manually equal to 464.482 nm whiles the average diameter given by software program of AFM equal to 455.71 nm, therefore;
Average diameter $[CPH\text{-}HCl^{+}]_3[Fe(CN)_6]^{3-}$ precipitate =464.482 nm
So: 464.482 – 62.93 = 401.552 nm
Radius = 401.552/2
= 200.776 nm
Surface area of single nodule = $4\pi r^2$
= 4×3.14× (200.776)^2
= 506306.1873 nm^2
Since there are 194 nodules
Surface area occupied by 194 nodules = 506306.1873 ×194
= 98223400.34 nm^2

And: $= 0.9822\times10^{-6}$ cm^2
464.482 + 62.93 = 527.412 nm
Radius = 527.412 /2 = 263.706 nm
Surface area of single nodule = $4\times3.14 \times (263.706)^2$
= 873433.1317 nm^2
Surface area occupied by 194 nodules= 873433.1317×194
= 169446027.6 nm ^{2}
= 1.69446×10^{-6} cm^2



The compactness of small nuclei (fine crystal nuclei) forms mirror and this mean forms a compact surface due to the formation of a reflecting surface and increase intensity of incident light In other words, there is a light coming from the reflection of the surfaces of the crystals or planar surfaces which will works as a reflective mirrors in all directions ,but the detector will receive a vertically submission in diameter equal to the area of six holes each with an area equivalent to πr^2.

-Incident hole parameter:

Total area that seen from the detector
Since the area of operture = 2mm (Φ)
$s=\pi r^2 \rightarrow 3.14(1)^2$
= 3.14 mm^2 = 0.0314 cm^2
Since there are six holes
$S = 6\times3.14 (1)^2$
= 18.84 mm^2 = 0.1884 cm^2
No. of repeated area as seen through passage in front of 2mm aperture
$R= 0.0314 / 1.01787\times10^{-6}$
$= 0.30849\times10^5$
Since each area have 194 nodules
$Z_1 = R\times N$
$=0.30849\times10^5\times194$
$= 5.98465\times10^6$ total available no. of nodules (grains) / hole
$Z_6 = Z_1 \times6$
$= 5.98465\times10^6 \times6$
$= 35.9079\times10^6$ Nodules (grains)/ six hole.

- For detector and light fall:

Since the green region forms about 56.73% of the white LED i.e; 56.73% of the incident light will suffer more reflection than the blue or even the 1.15% red region of the white LED this will definitely contribute to the positive response(reverse response) obtained from the instrument (spectrofluorometer).

The diameter of operture for the emerged light from the measuring cell falling on the detector surface Φ= 5mm.

The blue portion will affect an area of the detector for one single hole
$= (\pi r^2) \times0.42$
$= 3.14\times (2.5)^2\times0.42$
=8.2425 mm^2 = 0.082425 cm^2
The green portion will affect an area of the detector for one single hole
$= (\pi r^2) \times 0.57$
$= 3.14 \times (2.5)^2\times0.57$
= 11.18625 mm^2 = 0.11186cm^2

The red portion will affect an area of the detector for one single hole

$= (\pi r^2) \times 0.012$

$= 3.14\times (2.5)^2\times 0.012$

$= 0.2355\ mm^2 = 0.002355\ cm^2$

For six holes:

$(8.2425\times 6) + (11.18625\times 6) + (0.2355\times 6)$

$= 1.179855\ cm^2$ total area irradiated by RGB

Solar cell used 30mm×14mm

Two cell were used

Total available area for detection

$A_{TDA} = 2\times 30 \times 14 = 840\ mm^2 = 8.4\ cm^2$

$A_{TDA} - a_{HT} = A_{LDA}$

$8.4 - 1.179855 = 7.22015\ cm^2$ left unused detector area

Portion of irradiated of the white LED relative to the total available surface by the solar cell (2 cell) equal to

$(1.179855 / 8.4)\times 100 = 14.05\ \%$

Only 14.05 of the detector surface area were used.

Total no. of grain / single hole $Z_1 = 5.9846\times 10^{6}$

Irradiated unshared (overlapped scanned area-shared area between bands c.f. Figure-4) absolute no. of grains or no. of nodules seen by source equal

For B: $0.42\times 5.9846\times 10^6 = 25.135\times 10^5$

For G: $0.57\times 5.9846\times 10^6 = 34.113\times 10^5$

For R: $0.012 \times 5.9846\times 10^6 = 0.7182\times 10^5$

If it is assumed that the no. of nodules without the participation of any source or band of the spectrum interferes i.e; taken absolute value alone for each color of the spectrum of irradiation, which represents 57% green, 42% blue, 1.15 % red so the total no. of nodules for each spectrum of irradiation will be as calculated above (with an approximation).

3×10^6 nodules irradiated with blue color of the snow white light which used as a source (3,000,000 nodules).

3.4×10^6 nodules irradiated with green color of the snow white light which used as a source (3,400,000nodules).

0.72×10^5 nodules irradiated with red color of the snow white light which used as source (72,000 nodules).

For six holes:

Blue color

$(25.135\times 10^5)\times 6 = 15.0813\times 10^6$= total grains no. irradiation by blue portion of the spectrum of the white LED for six holes.

Green color

$(34.113\times 10^5)\times 6 = 20.4675\times 10^6$ = total grains no. irradiation by green portion of the spectrum of the white LED for six holes.

Red color

$(0.7182\times 10^5)\times 6 = 0.4309\times 10^6$ = total grains no. irradiation by red portion of the spectrum of the white LED for six hole.

Study of Precipitate of CPH-HCl – $[Fe(CN)_5NO]^{2-}$System Using Atomic Force Microscopy

The study was carried out as the same of previous section. A collection of turbid solution for CPH-HCl (5 $mmol.L^{-1}$) –$[Fe(CN)_5 NO]^{2-}$ (5 $mmol.L^{-1}$) reaction system .

The obtained data tabulated in Table-3 and represented in Figure-10, while the topographic of 2D and 3D shown in Figure-11.

Table 3-Granularity cumulation distribution data for CPH-HCl-$[Fe(CN)_5NO]^{2-}$

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
160.00	8.46	8.46	260.00	8.46	89.05
180.00	18.41	26.87	280.00	4.48	93.53
200.00	18.41	45.27	300.00	4.48	98.01
220.00	22.89	68.16	340.00	1.49	99.50
240.00	12.44	80.60	360.00	0.50	100.00

Grain No.:201 , Avg. Diameter:208.48 nm

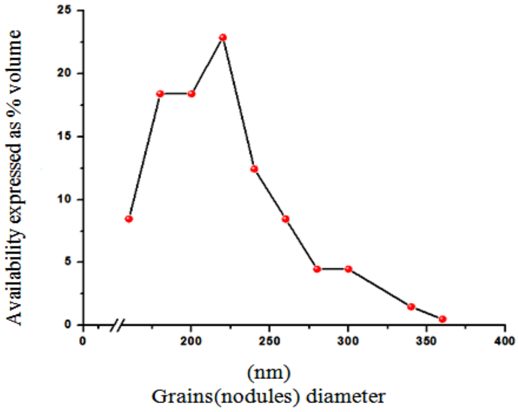


Figure 10-Relationship between diameter of nodules (nm) and occupied area expressed as a volume (%)

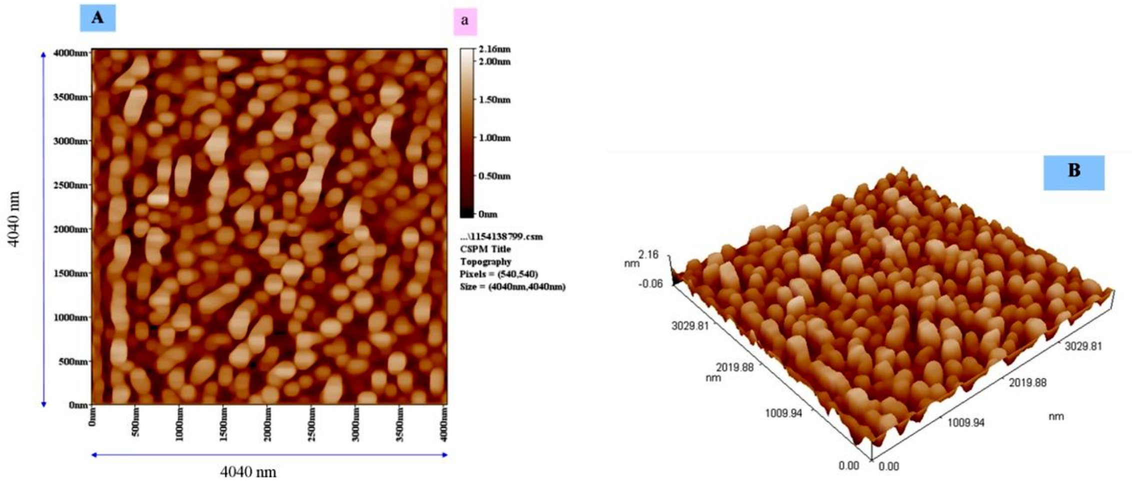


Figure 11- (A): 2D, (B): 3D profile for CPH-HCl-$[Fe(CN)_5NO]^{2-}$ system, a: Depth profile

From this study, the following calculation is conducted:
Since the average nodules diameter =208.48 nm
Radius = 208.48/2 = 104.24 nm
Surface area of a single nodule = $4\times3.14 \times (104.24)^2$
$= 136476.6787\ nm^2$
And since there are 201 nodules
Surface area occupied by 201 nodules
$A_{TN} = 201\times136476.6787 = 27431812.42\ nm^2$
$= 2.74318\times10^{-7}\ cm^2$ Total counted area as predicted from nodules average diameter
Scanned area from 2D –depth profile equal to image size
$A_{Ts} = 4040\ nm \times 4040\ nm$
$= 1.63216\times10^{-7}\ cm^2$ total calculated area
$1.63216\times10^{-7} - 2.74318\times10^{-7} = -1.11102\times10^{-7}\ cm^2$

Average diameter of nodules calculated manually = 218.554 nm
Reference is made in Table-4

Table 4-Tabulation of the average diameter, standard deviation of CPH-HCl-$[Fe(CN)_5NO]^{2-}$

Average diameter manually ($\overline{\Phi}$) (nm)	Standard deviation (σ_{n-1})	Confidence interval at (95%) $\overline{\Phi} \pm t_{0.05/2,n-1}\, \sigma_{n-1}/\sqrt{n}$
218.554	66.70	218.554 ± 47.67

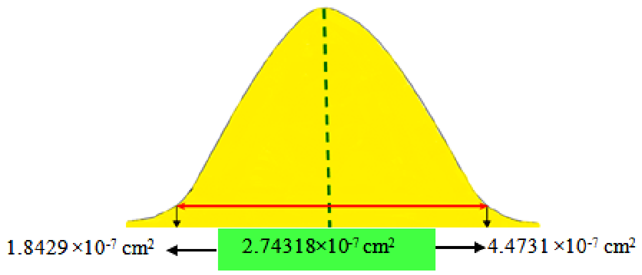
n= 10 , $t_{0.05/2,n-1}$= 2.26

Average nodules diameter calculated manually equal to 218.554 nm
218.55 ± 47.67 nm
Confidence interval (95%)
218.55 – 47.67 = 170.88 nm
218.55+ 47.67 = 266.22 nm

170.88 nm ← ($\overline{\Phi}$)=208.48 nm → 266.22 nm

Confidence interval of calculated surface area of nodules , the right part of confidence interval representation i.e: for $[CPH\text{-}HCl^{+}]_2[Fe(CN)_5NO]^{2-}$ equal to 47.67 which mean be a fluctuation of average diameter is a fluctuating between ± 47.67 nm . Since the average diameter calculated manually equal to 218.55 nm whiles the average diameter given by software program of AFM equal to 208.48 nm, therefore;

Average diameter of CPH-HCl -$[Fe(CN)_5NO]^{2-}$ system =218.55 nm
*218.55 – 47.67 = 170.88 nm
Radius =170.88 /2
= 85.44 nm
Surface area of single nodule = $4\pi r^2$
= 4×3.14× $(85.44)^2$
= 91687.9196 nm^2
Since there are 201 nodules
Surface area occupied by 201 nodules = 91687.9196 ×201
= 18429271.84 nm^2
= 1.842927 $\times 10^{-7}$ cm^2
*218.55 + 47.67 = 266.22 nm
Radius = 266.22 /2 = 133.11 nm
Surface area of single nodule = 4×3.14× $(133.11)^2$
= 222541.4976 nm^2
Surface area occupied by 201 nodules = 222541.4976 ×201
= 44730841.01 nm^2
= 4.47308 $\times 10^{-7}$ cm^2



Incident hole parameters:
S = 6× πr^2
= $6\times 3.14(1)^2$
= 18.84 mm^2 =0.1884 cm^2

Since total area of scanned region = 1.63216×10^{-7} cm^2
Area of operture (2mm Φ) s= $3.14mm^2 = 0.0314cm^2$
Since there are six them S = 6×0.0314
= $0.1884\ cm^2$
No. of repeated area as seen through passage in front of 2mm aperture
R= $0.0314/ 1.63216\times10^{-7}$
= 0.192383×10^6
Since each area have 201 nodules
$Z_1 = R\times N$
= $0.192383\times10^6\times201$
= 3.86689×10^7 total available no. of nodules (grains) / hole
$Z_6 = Z_1\times6$
= $3.86689\times10^7\times6$
= 23.20134×10^7 Nodules (grains)/ six hole

Since the green region forms about 57 % of the white LED i.e; 57% of the incident light will suffer more reflection than the blue or even the 1.15% red region of the white LED this will definitely contribute to the positive response (reverse response obtained from the instrument (spectrofluorometer).

The diameter of operture for the emerged light from the measuring cell falling on the detector surface Φ = 5mm
The blue portion will affect an area of the detector = $(\pi r^2)\times0.42$
= $3.14\times(2.5)^2\times0.42$
= $8.2425\ mm^2 = 0.08245\ cm^2$
The green portion will affect an area of the detector area = $(\pi r^2)\times0.57$
= $3.14\times(2.5)^2\times0.57$
= $11.18625\ mm^2 = 0.11186\ cm^2$
The red portion will affected an area of the detector = $(\pi r^2)\times0.012$
= 3.14 × (2.5)2×0.012
= $0.2355\ mm^2 = 0.002355cm^2$

For six holes:
$(0.082425\times6) + (0.11186\times6) + (0.002355\times6) = 1.179855\ cm^2$ total area affected by RGB
Solar cell used 30mm×14mm
Two cell were used
Total available area for detection (2 solar cells)
$A_{TDA} = 2\times30\times14 = 8.4\ cm^2$
$A_{TDA} - a_{HT} = A_{LDA}$
$8.4 - 1.179855 = 7.220\ cm^2$ left unused detector area
Portion of irradiated of the white LED relative to the total available surface by the solar cell (2 cell) equal to (1.179855/8.4)×100 = 14.06 %
Only 14.06% of the detector surface area was used.
Total no. of grain / single hole $Z_1=3.86690\times10^7$
Irradiated unshared absolute no. of grains or no. of nodules seen by source equal
For B: $0.42\times3.86690\times10^7 = 1.6241\times10^7$
For G: $0.57\times3.86690\times10^7 = 2.2041\times10^7$
For R: $0.012\times3.86690\times10^7 = 0.4640\times10^6$

If it is assume that the no. of nodules and without participation with any source or part of the spectrum interferes i.e; taken absolute value alone for each color of the spectrum of irradiation, that represents 57% green, 42% blue, 1.15% red so the total no. of nodules for each spectrum of irradiation will be as calculated above.
2×10^7 nodules irradiated with blue color of the snow white light which used as a source (20,000,000).
2.2×10^7 nodules irradiated with green color of the snow white light which used as a source (20,000,000).
0.5×10^6 nodules irradiated with red color of the snow white light which used as source (500,000).

For six holes:

Blue color

$(1.6241\times10^{7})\times6 = 9.7446\times10^{7}$= total grain no. irradiation by blue portion of the spectrum of the white LED for six holes.

Green color

$(2.2041\times10^{7})\times6 = 13.2248\times10^{7}$= total grain no. irradiation by green portion of the spectrum of the white LED for six holes.

Red color

$(0.4640\times10^{6})\times6 = 2.7842\times10^{6}$= total grain no. irradiation by blue portion of the spectrum of the white LED for six hole.

There was convent difference between the average diameters given by AFM soft ware which give 208.48 nm while manually calculated average diameter which gave a value of 218.554 nm. Therefore; calculation based on 218.554 nm as an average diameter shows that the confidence interval of 218.554± 47 .6688 causes variation of total nodules surface area lays between1.8429×10^{-7} – 4.4731×10^{-7} while the calculated value based of the average diameter of 208.48 nm is equal to 2.74318 $\times10^{-7}$ cm^2. This indicate that the above shown calculation indicate an error that can be expected within the range shown above.

A final conclusion can be drown that scanned surface area of the nodules can be regarded as an equivalent to the total scanned area(A_{TS}) by AFM at confidence level of 95% (α=0.05) that was calculated manually or calculated by average diameter which was given by AFM .

Comparison Between CPH-HCl-$[Fe(CN)_6]^{3-}$ System & CPH-HCl $[Fe(CN)_5NO]^{2-}$System or Study Parameters

Granularity Cumulative Distribution Chart

Shows that there is a clear difference between the precipitate formed CPH-HCl with $[Fe(CN)_6]^{3-}$ &$[Fe(CN)_5NO]^{2-}$ .It was noticed that the probe where incursion of greater depth in case with $[Fe(CN)_6]^{3-}$ rather than the precipitate with $[Fe(CN)_5NO]^{2-}$. Where the nodules with small diameter deposited and appeared as one nodule. The precipitate of CPH-HCl-$[Fe(CN)_6]^{3-}$ has larger nodules diameter i.g.;Φ= 700 nm with the limit 3.09 (volume %) & 720 nm with limit (1.03) , while greater number nodules for CPH-HCl- $[Fe(CN)_5NO]^{2-}$ with diameter 360 nm in the limit 0.5 (volume%) .

Amplitude parameters

Roughness average:

Gives the deviation in height. Different profiles can give the same roughness average [7] .It was noticed that the value of average roughness for CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to 3.56nm while the value equal to 0.366nm when using $[Fe(CN)_5NO]^{2-}$ as precipitating agent with CPH-HCl as shown in Table 5 , this indicate that the roughness average for CPH-HCl-$[Fe(CN)_6]^{3-}$ > the roughness average for CPH-HCl- $[Fe(CN)_5NO]^{2-}$ because the second precipitate form a compact and small nodule crystals .This compactness causes less roughness and give a finesse to a degree of acting as extended surface.

Root mean square:

Represent the standard deviation of surface heights [7]. It was noticed in the case of use $[Fe(CN)_6]^{3-}$ the value of root mean square equal to 4.09 nm Compared with value when use of $[Fe(CN)_5NO]^{2-}$ because the nodules diameters were large and the distance between them was large, so; the standard deviation value was high while when use $[Fe(CN)_5NO]^{2-}$ the value equal to 0.435nm as shown in Table-5 , it indicates that the value is less than 4.09 because the diameters of nodules was small and compact so; it leads to lesser standard deviation.

Surface skeweness:

Is used to measure the symmetry of probability distribution of a real – valued random variable about its mean. When the height distribution is a symmetrical R_{sk} is zero. If the height distribution is symmetrical ,and the surface has more peaks than valleys the skewness moment is positive and if the surface is more planer and valleys are predominant the skewness is negative[7] . It was noticed in the case of use $[Fe(CN)_6]^{3-}$ the value of R_{sk} equal to -0.0939 compared with value when use of $[Fe(CN)_5NO]^{2-}$ equal to -0.354 as shown in Table 5 , this indicate negatively skewed distribution of grains indicate that the crystal growth is toward increased diameter of grain but not enough time was given for them to grow due to constant and continuous flow mode of working and that the surface is more planer and valleys are predominant.

Kurtosis R_{ku}:

Measure surface sharpness. When (R_{ku}) is three indicates a Gaussian amplitude distribution, and the surface is called mesokurtic , but if kurtosis is smaller than 3 the surface is flat and called platykurtic . If the kurtosis is higher than 3, the surface has more peak than valleys [7] .

The value of kurtosis for CPH-HCl- $[Fe(CN)_6]^{3-}$ equal to 1.76 & equal to 2.13 for CPH-HCl-$[Fe(CN)_5NO]^{2-}$ as shown in Table 5 , both value less than 3 this indicates that the surface was flat , and it was noticed the value of kurtosis for CPH-HCl- $[Fe(CN)_6]^{3-}$ < CPH-HCl-$[Fe(CN)_5NO]^{2-}$ that mean the precipitate with $[Fe(CN)_6]^{3-}$ have more valleys because the nodules with average diameters 455.71 nm and a total number of 194 compared with the diameters of nodules for CPH-HCl-$[Fe(CN)_5NO]^{2-}$ were 208.48 nm.Also it indicate a wider spread of nodules i:e.; platykurtic distribution since it is <3 for both precipitate with a difference (2.13-1.76 = 0.37) for a wider spread of nodules in case of CPH-HCl –$[Fe(CN)_6]^{3-}$.

Peak to peak:

The value of CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to 15.2 nm while the value equal to 1.93 nm for precipitate of CPH-HCl-$[Fe(CN)_5NO]^{2-}$ as shown in Table-5, in the case of first precipitate the value was large this shows that , the distance between peak and another was larger compared with second precipitate , the average diameters of nodules were small (208.48nm) and it was compact so; the distance between peak s were small .

Ten point height:

The difference in height between the average of the average of the five highest peaks and five lowest valleys along the assessment length of the profile [7]. It was noticed the value of ten point height for CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to 7.5 nm while for CPH-HCl-$[Fe(CN)_5NO]^{2-}$ equal to 0.978 nm, from these values, it was found that the value of the first deposit 10 times greater than the second precipitate because the standard deviation of CPH-HCl-$[Fe(CN)_6]^{3-}$> standard deviation of CPH-HCl-$[Fe(CN)_5NO]^{2-}$.

Functional parameters:

Valley fluid retention:

Bearing and fluid retention properties of surface [7].The value of valley fluid retention for CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to 0.0656 while value of it for CPH-HCl-$[Fe(CN)_5NO]^{2-}$ equal to 0.11 as shown in Table 5, this value shows that CPH-HCl- $[Fe(CN)_5NO]^{2-}$ have a large value ;this mean it was amorphous & the precipitate with -$[Fe(CN)_6]^{3-}$ have a small value this indicate that it was a crystal (liquid loses quickly). Thus the large value indicates large fluid retention .A value of 0.15 or larger indicates a good fluid retention in the vally zone which was not available at the measurements made.

Information obtained from section analysis

Vertical distance (nm):

The value of vertical distance for CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to 12.73 nm this value was 9 time greater than the value for CPH-HCl- $[Fe(CN)_5NO]^{2-}$ (1.42 nm) as shown in Figure-12 and Table-5, from this ratio shows that the probe in case with CPH-HCl-$[Fe(CN)_6]^{3-}$ attained to depth more than comparison with CPH-HCl- $[Fe(CN)_5NO]^{2-}$ because the last one have a small and compact nodules that made reflection surface due to high response and detection limit of 1.825 µg/sample but the first precipitate have a large and dispersed nodules with detection limit 0.28µg/sample, so; there was a need for a coil in the manifold for crystalline growth.

Roughness radius (nm):

The value of roughness radius for CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to 1.67 nm this value is 8 time greater than the value for CPH-HCl- $[Fe(CN)_5NO]^{2-}$ (0.203 nm) as shown in Figure-12 and Table-5.

Height [Greeb] (nm):

It was found that the value of height for CPH-HCl-$[Fe(CN)_6]^{3-}$ (5.09 nm) three time greater than the value for CPH-HCl- $[Fe(CN)_5NO]^{2-}$ (1.48nm) as shown in Figure-12 and Table-5 .

Image height (nm):

It was clear difference between image height for the precipitate of CPH-HCl with $[Fe(CN)_6]^{3-}$ &$[Fe(CN)_5NO]^{2-}$, it was noticed that the value equal to 20.09 nm when useing hexacyanoferrate (III) while equal to 2.21 with sodium nitroprusside as shown in Figure-12 and Table-5, this indicates that in case with hexacyanoferrate(III) the probe falling to distance 9 times greater than the case with Sodium nitroprusside because the last one formed from compact and small nodules .

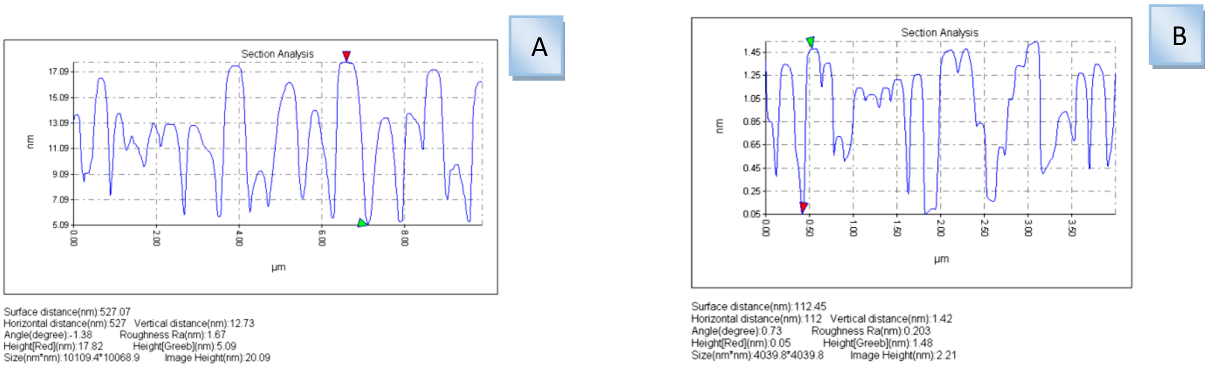


Figure 12- Section analysis for (A):CPH-HCl-$[Fe(CN)_6]^{3-}$system ,(B) : CPH-HCl-$[Fe(CN)_5NO]^{2-}$system

Scanned area representation as 2D-depth profile

Area from 2D –depth profile for CPH-HCl-$[Fe(CN)_6]^{3-}$ equal to image size (10109nm×10069 nm) (Length × Width) while incase with CPH-HCl- $[Fe(CN)_5NO]^{2-}$ (4040 nm×4040 nm) as shown in Figure-8A &-11A).

Scanned area taken for the first one 2.5 times greater than the area taken for the second one , depending on the average diameters of nodules ,in case with $[Fe(CN)_6]^{3-}$ the average diameter of nodule 455.71 nm compared with the CPH-HCl- $[Fe(CN)_5NO]^{2-}$ average diameters of nodule 208.48 nm , in these value indicate that the average diameters for first precipitate twice the average diameters for the second precipitate . Therefore it was needed to larger area for scanned.

Description of 2D- profile image and 3D –depth profile methodology

2D- depth profile Figure-8a shows that the depth of scanning for CPH-HCl with hexacyanoferrate (III) was 19.94 nm while with sodium nitroprusside Figure-11a equal to 2.16 nm this shows clearly that the volume of crystals which formed with $[Fe(CN)_6]^{3-}$ larger, clearer, more systematical and higher compared with the crystals which formed with $[Fe(CN)_5NO]^{2-}$.

It was noticed there was no clear difference between the no. of nodules present in scanned area except for the nodules diameter were very small incase with sodium nitroprusside as shown in granularity cumulation distribution chart Figure-10. While from 3D-depth profile It was noticed from the Figure-8B that the probe of AFM Falling to distance 19.94 nm for CPH-HCl-$[Fe(CN)_6]^{3-}$ while with CPH-HCl-$[Fe(CN)_5NO]^{2-}$ the value was 2.16 nm Figure-11B this indicate that the ppt. with hexacyanoferrate(III) the nodules were prominent, clearer and highest comparison with use of $[Fe(CN)_5NO]^{2-}$.

Table-5 tabulated the summary of result for AFM of CPH-HCl-$[Fe(CN)_6]^{3-}$system and CPH-HCl-$[Fe(CN)_5NO]^{2-}$ system

Table 5- Result of different parameters for CPH-HCl-$[Fe(CN)_6]^{3-}$ & CPH-$[Fe(CN)_5NO]^{2-}$ obtained by AFM

Parameters		**CPH-HCl-$[Fe(CN)_6]^{3-}$**	**CPH-HCl-$[Fe(CN)_5NO]^{2-}$**	**Ratio of CPH-HCl-$[Fe(CN)_6]^{3-}$ to CPH-HCl-$[Fe(CN)_5NO]^{2-}$**
Amplitude parameters	Roughness average	3.56	0.366	9.7267
	Root mean square	4.09	0.435	9.4022
	Surface skewness	-0.0939	-0.345	0.2722
	Kurtosis	1.76	2.13	0.8263
	Peak-peak	15.2	1.93	7.8756
	Ten point height	7.5	0.978	7.6687
Functional parameter	Valley fluid retention	0.0656	0.11	0.5964
Section analysis	Vertical distance	12.73	1.42	8.9647
	Roughness radius	1.67	0.203	8.2266
	Height [Greeb]	5.09	1.48	3.4391
	Image height	20.09	2.21	9.0904

Conclusion

This novel study indicates that combination of precipitation and Atomic Force Microscopy can add details to reveals the kind of obtained responses in turbidimetry or other precipitation methods.

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