#### **Supporting Information**

# Protein Corona Composition of Gold Nanoparticles/Nanorods Affects Amyloid Beta Fibrillation Process

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#### Materials

HAuCl<sub>4</sub>.3H<sub>2</sub>O (99.9%), NaBH<sub>4</sub> (99%), L-Ascorbic acid (99+%), AgNO<sub>3</sub> (99+%), The Aβ (1-42)peptide(MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA) and Thioflavin T were used as purchased (Sigma). Etyltrimethylammonium bromide (CTAB, 99%) was used as purchased (Merck). Ultrapure deionized water (DI; Continental Water Systems) was used for all solution preparations and experiments. Glassware was cleaned by soaking in aqua regia and finally washing with DI water. FBS and HP was purchased from Iranian Blood Transfusion Organization (IBTO).

#### Instrumentation

Absorption spectra were taken on a Shimadzu UVmini-1240 UV-Vis Spectrophotometer (Japan) with the use of quartz cell. Measurements of pH were made with a Denver Instrument Model of 270 pH meter equipped with a Metrohm glass electrode. Size distributions of the particles were obtained using Malvern Zetasizer Nano ZS90 at room temperature. Transmission Electron Microscopy (TEM) images were registered with a BOSCH at an acceleration voltage of 80 KV. Amyloid  $\beta$  fibrillation was recorded using Hybrid Technology<sup>TM</sup> Synergy H4 Performance (BioTek Instruments, Inc., Winooski, Vermont, USA) with 96 well black fluorescence plate, NUNC 96 black Polypropylene MicroWell<sup>TM</sup> Plates (Sigma).

Name	Peak 1 λ (nm)	Absorption	Shift	Thickness[nm]
Without Protein	526.6	0.7568	0	0
0 min	529.6	0.5076±0.013	3	13.22
2 min	531.4	0.5561±0.004	4.8	11.42
4 min	531.9	0.5702±0.009	5.3	11.04
6 min	532.1	0.5802±0.017	5.5	10. 9
8 min	532	0.5831±0.0104	5.4	10.97
10 min	532.4	0.5826±0.014	5.8	10.7
12 min	533.2	0.5816±0.016	6.6	10.2
14 min	532.2	0.5796±0.001	5.6	10.83
16 min	531.9	0.5753±0.002	5.3	11.04
18 min	529.6	0.5708±0.0006	3	13.22
20 min	529.9	0.5661s±0.002	3.3	12.85

#### Table S1. Thickness of protein in NPs-Protein Interaction in situ Sphere ~20 nm FBS10%

#### Table S2. Thickness of protein in NPs-Protein Interaction in situ Sphere ~30 nm FBS

Peak  $\lambda$ Shift Thickness Name Absorption (nm) [nm] [s] (nm) Without 0 0 526 0.601194 protein 0.5  $0 \min$ 526.5  $0.4302{\pm}0.04$ 12.12 2 min 527  $0.4585 \pm 0.03$ 1 10.5 10.5 1 4 min 527  $0.4589 \pm 0.02$ 2 6 min 528 8.9  $0.4609 \pm 0.0167$ 8 min 1.5 527.5  $0.4734 \pm 0.021$ 9.6 10 min 1.5 527.5  $0.4678 \pm 0.0161$ 9.6 12 min  $0.4602 \pm 0.01$ 2.5 528.5 8.4 14 min 0.5 526.5  $0.4626 \pm 0.011$ 10.12 16 min 527  $0.4696 \pm 0.0145$ 1 9.5 1.5 18 min 527.5  $0.4674 \pm 0.0126$ 9.6 20 min  $0.4657 \pm 0.011$ 1.5 527.5 9.6

Name	Peak 1λ (nm)	Absorption	Shift [nm]	Thickness [nm]	Peak 2λ (nm)	Absorption	Shift [nm]	Thickness [nm]
Without Protein	510.5	0.664681	0	0	674	1.434005	0	0
0 min	510.4	0.4310±0.002	0.1	10.74	687.7	0.7789±0.0011	13.7	10.82
2 min	511.9	0.4305±0.009	1.4	6.74	689.2	0.7635±0.0007	15.2	10.25
4 min	514.9	0.4222±0.005	4.4	4.6	689.8	0.7537±0.005	15.8	10.04
6 min	515.2	0.4231±0.007	4.7	4.5	692.2	0.7708±0.006	18.2	9.3
8 min	515.4	0.4228±0.003	4.9	4.44	692.5	0.7646±0.0015	18.5	9.2
10 min	515.2	0.4203±0.006	4.7	4.5	691.9	0.7649±0.007	17.9	9.4
12 min	513.4	0.4217±0.006	2.9	5.3	691	0.7666±0.002	17	9.65
14 min	513	0.4161±0.008	2.5	5.53	692.8	0.7590±0.004	18.2	9.3
16 min	515.5	0.4139±0.002	5	4.41	693.4	0.7482±0.002	19.4	8.94
18 min	512.8	0.4025±0.001	2.3	5.7	692.8	0.7267±0.005	18.8	9.1
20 min	514.9	0.3947±0.012	4.4	4.6	693.1	0.7248±0.0077	19.1	9.02

Table S3. Thickness of protein in NPs-Protein Interaction in situ Short rods FBS 10%

## Table S4. Thickness of protein in NPs-Protein Interaction in situ Long rods FBS 10%

Name	Peak 1 λ (nm)	Absorption	Shift [nm]	Thickness[nm]
Without Protein	528.7	0.9275	0	0
0 min	514.9	0.7788±0.004	13.4	4.8
2 min	522.7	0.7562±0.008	6	7
4 min	520	0.7070±0	8.7	6
6 min	518.2	0.7065±0.0001	10.5	5.5
8 min	522.7	0.6982±0.0007	6	7
10 min	523.3	0.6862±0.0018	5.4	7.25
12 min	521.5	0.6752±0.0003	7.2	6.5
14 min	518.5	0.6658±0.0008	10.5	5.5
16 min	523.6	0.6629±0.0005	5.1	7.4
18 min	520.9	0.6616±0.0003	6.8	6.6
20 min	521.8	0.6651±0.0003	6.9	6.6

## Table S5. Thickness of protein in NPs-Protein Interaction in situ Sphere $\sim 20$ nm FBS

Name	Peak 1 λ (nm)	Absorption	Shift [nm]	Thickness [nm]
Without Protein	526	1.8035	0	0
0 min	526.5	0.9297±0.005	0.5	12.12
2 min	526.5	0.9766±0.04	0.5	12.12
4 min	525.5	0.8706±0.032	1.5	9.58
6 min	526.5	0.9154±0.01	0.5	12.12
8 min	525.5	0.9427±0.043	0.5	12.12
10 min	526.5	0.9633±0.065	0.5	12.12
12 min	526.5	0.9784±0.082	0.5	12.12
14 min	527	0.9886±0.0036	1	10.5
16 min	527	0.9936±0.027	1	10.5
18 min	525.5	0.9960±0.006	0.5	12.12
20 min	527	1.0035±0.027	1	10.5

## 100%

### Table S6. Thickness of protein in NPs-Protein Interaction in situ Sphere ~30 nm FBS

Name	Peak 1 λ (nm)	Absorption	Shift	Thickness [nm]
Without Protein	526.6	1.0175	0	0
0 min	527.1	0.7596±0.02	0.5	20.08
2 min	527.9	0.6977±0.003	1.3	16.42
4 min	528	0.7339±0.02	1.4	16.14
6 min	528.8	0.7519±0.026	2.2	14.41
8 min	528.9	0.7641±0.015	2.3	14.24
10 min	529	0.7708±0.002	2.6	13.7
12 min	529.2	0.7762±0.002	2.8	13.5
14 min	529.9	0.7802±0.005	3.3	12.85
16 min	528.2	0.7816±0.0092	2.1	14.6
18 min	529.9	0.7858±0.0096	3.3	12.85
20 min	530.6	0.7878±0.01	4	12.12

Name	Peak 1 λ (nm)	Absorption	Shift [nm]	Thickness [nm]	Peak 2 λ (nm)	Absorption	Shift [nm]	Thickness [nm]
Without Protein	510.5	0.6646±0.001	0	0	674	1.4340	0	0
0 min	517	0.6627±0.001	6.5	3.75	683.3	0.6552±0.05	9.3	12.88
2 min	514.6	0.3702±0.002	4.1	4.45	684.1	0.7283±0.14	10.1	12.5
4 min	514	0.5184±0.04	3.5	4.7	683.5	0.7827±0.14	9.5	12.77
6 min	513.1	0.5433±0.02	3.1	4.88	682.6	0.8197±0.12	8.6	13.3
8 min	511	0.5569±0.01	0.5	7.66	683.5	0.8490±0.1	9.5	12.77
10 min	512.5	0.5445±0.03	2	5.5	682.6	0.8819±0.09	8.6	13.3
12 min	510.4	0.5503±0.0006	0.1	10.11	681.7	0.8999±0.07	7.7	13.9
14 min	510.4	0.5537±0.014	0.1	10.11	682	0.9203±0.05	8	6.85
16 min	510.4	0.5422±0.007	0.1	10.11	682.3	0.9371±0.03	8.3	13.5
18 min	510.1	0.5440±0.013	0.4	8.00	683.1	0.9482±0.05	9.1	13.00
20 min	511	0.5595±0.002	0.5	7.66	683.2	0.9586±0.01	9.2	12.94

 Table S7. Thickness of protein in NPs-Protein Interaction in situ Short Rods FBS 100%

## Table S8. Thickness of protein in NPs-Protein Interaction in situ Long Rods FBS 100%

Name	Peak 1 λ (nm)	Absorption	Shift [nm]	Thickness [nm]
Without Protein	527.5	0.6563±0	0	0
0 min	522.4	0.5875±0.007	5.1	7.4
2 min	519.4	0.6059±0	8.1	6.15
4 min	518.8	0.5829±0	8.7	6
6 min	520.3	0.5524±0.0003	7.2	6.5
8 min	513.7	0.5205±0.009	13.8	4.7
10 min	507.4	0.5754±0.0001	20.1	3.7
12 min	512.2	0.5195±0.0007	15.3	4.44
14 min	509.8	0.4691±0.0005	17.7	4
16 min	509.5	0.4167±0.0003	18	4
18 min	508.6	0.3856±0.0001	18.9	3.9
20 min	505.9	0.3505±0	21.6	3.5

#### Table S9. Thickness of protein in NPs-Protein Interaction in situ Sphere ~20 nm HP

#### Shift [nm] Thickness [nm] Name Peak 1 Absorption Without Protein 526 1.8719 0 0 0 min 527 0.7821±0.02 1 10.51 1.5 2 min 527.5 0.84460.05 9.58 528 2 8.91 4 min 0.8920±0.04 6 min 529 3 0.9095±0.02 8 8 min 527.5 0.9234±0.02 1.5 9.58 1.5 9.58 10 min 527.5 0.9351±0.02 12 min 530 0.9434±0.014 3 8 14 min 530.5 0.9518±0.018 3.5 7.6 16 min 530 0.9559±0.012 3 8 3 18 min 530 $0.9589 \pm 0.014$ 8

#### 10%

#### Table S10. Thickness of protein in NPs-Protein Interaction in situ Sphere ~30 nm HP

Name	Peak 1	Absorption	Shift [nm]	Thickness [nm]
Without Protein	543	0.3758	0	0
0 min	545	0.1353±0.0008	2	14.9
2 min	545.5	0.1678±0.0008	2.5	14.04
4 min	545	0.1928±0.0008	2	14.9
6 min	545.5	0.2076±0.0008	2.5	14.04
8 min	545.5	0.2156±0.0008	2.5	14.04
10 min	546	0.2225±0.0008	3	13.34
12 min	544.5	0.2282±0.0008	1.5	15.9
14 min	545	0.2313±0.0008	2	14.9
16 min	545.5	0.2385±0.0008	2.5	14.04
18 min	545	0.2382±0.0008	2	14.9
20 min	545.5	0.2421±0.0008	2.5	14.04

Nama	Peak 1	Abcorntion	Shift	Thickness	Peak 2	Absorption	Shift	Thickness
Name	λ (nm)	Absorption	[nm]	[nm]	λ (nm)	Absorption	[nm]	[nm]
Without Protein	510.5	0.6646	0	0	674	1.4340	0	0
0 min	508	0.3158±0.005	2.5	5.21	685	0.4155±0.005	13	11.1
2 min	514	0.5455±0.031	3.4	4.74	685	0.5988±0.0006	13	11.1
4 min	506	0.5994±0.04	3.5	4.7	679.5	0.6342±0.002	5.5	15.5
6 min	509	0.5173±0.025	1.5	6	679.5	0.6512±0.0004	5.5	15.5
8 min	510.6	0.5247±0.026	0.1	10.11	681.5	0.6597±0.0007	7.5	14.01
10 min	510.6	0.5284±0.03	0.1	10.11	681.5	0.6654±0.002	7.5	14.01
12 min	511	0.5292±0.03	0.5	7.7	682	0.6688±0.0008	8	13.73
14 min	509	0.5294±0.03	0.5	7.7	681.5	0.6702±0.000	7.5	14.01
16 min	511	0.5274±0.03	1.5	6	681.5	0.6699±0.0005	7.5	14.01
18 min	509	0.5266±0.03	1.5	6	682	0.6704±0.0002	8	13.73
20 min	514	0.5238±0.03	3.5	4.7	682.5	0.6697±0.0005	8.5	13.4

 Table S11. Thickness of protein in NPs-Protein Interaction in situ Short rods HP 10%

Table S12. Thickness of protein in NPs-Protei	in Interaction in situ Long rods HP	10%

Time (min)	Peak 1	Absorption	Shift [nm]	Thickness [nm]
Without Protein	528.7	0.9275	0	0
0	527.5	0.6684±0.02	1.2	11.31
2	520	0.6501±0.02	8.7	6
4	522.7	0.6016±0.02	6	7
6	515.2	0.6011±0.02	13.5	4.8
8	518.5	0.5942±0.02	10.2	5.53
10	515.2	0.5842±0.02	13.5	4.8
12	519.7	0.5704±0.02	9	5.9
14	517.6	0.5609±0.02	11.1	5.3
16	516.4	0.5586±0.04	12.3	5
18	521.2	0.5568±0.04	7.5	6.4
20	521.2	0.5603±0.02	7.5	6.4

#### Table S13. Thickness of protein in NPs-Protein Interaction in situ Sphere ~20 nm HP

#### Time (min) Peak 1 Absorption Shift Thickness[nm] Without Protein 526 0.6239 0 0 11.34 526.7 0.5421±0.001 0.7 0 2 527.9 0.5589±0.0007 1.9 9.03 4 8.14 528.8 0.5583±0.0.0007 2.8 6 530.2 0.5589±0.0008 4.2 7.2 8 529.6 0.5606±0.0008 3.6 7.55 10 530.2 0.5583±0.0008 4.2 7.2 12 0.5577±0.0008 2.4 8.5 528.4 0.5571±0.0008 1.3 9.91 14 527.3 16 527.2 0.5526±0.0008 1.2 10.1 18 529.7 0.5472±0.0008 3.7 7.5 20 0.5773±0.0008 3.4 529.4 7.67

#### 100%

#### Table S14. Thickness of protein in NPs-Protein Interaction in situ Sphere ~30 nm HP

Name	Peak 1	Absorption	Shift [nm]	Thickness [nm]
Without Protein	543	0.3758	0	0
0	540.3	0.3071±0.0007	2.7	13.75
2	540.2	0.3064±0.0007	2.8	13.6
4	539.9	0.3048±0.0007	3.1	13.21
6	539.6	0.3048±0.0008	3.4	12.86
8	539.9	0.3047±0.0008	3.1	13.21
10	539.6	0.3035±0.0011	3.4	12.86
12	540.9	0.3147±0.0008	4.1	12.14
14	538	0.3045±0.0008	5	11.4
16	538	0.3051±0.0008	5	11.4
18	540.6	0.3059±0.0008	4.4	11.9
20	540.3	0.2960±0.0008	4.7	11.62

Name	Peak 1 λ (nm)	Absorption	Shift [nm]	Thickness [nm]	Peak 2 λ (nm)	Absorption	Shift [nm]	Thickness [nm]
Without Protein	520.3	0.6607	0	0	674	1.8737	0	0
0 min	528.7	0.6183±0.001	8.4	3.4	680	0.9089±0.0007	6	15.3
2 min	520.6	0.6163±0.0001	0.3	8.5	680	0.9164±0.0007	6	15.3
4 min	521.8	0.6089±0.002	1.5	6.01	683	0.9215±0.0008	9	13.09
6 min	521.5	0.6157±0.005	1.2	6.35	682.6	0.9114±0.0008	8.5	13.4
8 min	520.6	0.6101±0.01	0.3	8.5	682.5	0.9042±0.0008	8.2	13.6
10 min	519.7	0.6029±0.005	0.7	7.17	680.3	0.8786±0.0008	6.3	15.02
12 min	521.5	0.5961±0.002	1.2	6.35	687	0.8673±0.0008	13	11.1
14 min	518.8	0.5824±0.04	1.5	6.01	687	0.8321±0.0008	13	11.1
16 min	521.2	0.5698±0.007	0.9	6.8	686.3	0.8307±0.0008	12.3	11.4
18 min	517.6	0.5269±0.007	2.7	5.12	684.2	0.7606±0.0008	10.2	12.4
20 min	521.2	0.5222±0.007	0.9	6.8	683	0.7538±0.0008	9	13.09

 Table S15. Thickness of protein in NPs-Protein Interaction in situ Short Rods HP 100%

## Table S16. Thickness of protein in NPs-Protein Interaction in situ Long Rods HP 100%

Time (min)	Peak 1	Absorption	Shift [nm]	Thickness[nm]	
Without Protein	528.7	0.9275	0	0	
0	518.5	0.6452±0.001	10.2	5.53	
2	519.5	0.6583±0.001	9.2	5.81	
4	520.5	0.6574±0.0001	8.2	6.12	
6	520	0.6557±0.0017	8.7	6	
8	520.5	0.6516±0.0005	8.2	6.12	
10	517	0.6504±0.0014	11.7	5.16	
12	518.5	0.6425±0.0016	10.2	5.53	
14	522.5	0.6384±0.0026	6.2	6.88	
16	515	0.6596±0.0009	13.7	4.75	
18	521.5	0.6524±0.0008	7.2	6.5	
20	521	0.6587	7.8	6.3	



Figure S1. SPR spectra of the protein–nanoparticles bioconjugates, obtained from incubation of Small Sphere (20 nm) GNPs with various protein sources ((a) FBS 10%, (b) FBS 100%), (c) HP 10%, (d) HP 100%)) at different incubation times and (e) their calculated protein

corona thicknesses.



Figure S2. SPR spectra of the protein–nanoparticles bioconjugates, obtained from incubation of Big Sphere (30 nm) GNPs with various protein sources ((a) FBS 10%, (b) FBS 100%), (c) HP 10%, (d) HP 100%)) at different incubation times and (e) their calculated protein corona thicknesses.



Figure S3. SPR spectra of the protein–nanoparticles bioconjugates, obtained from incubation of Short Rods (AR~4) GNPs with various protein sources ((a) FBS 10%, (b) FBS 100%), (c) HP 10%, (d) HP 100%)) at different incubation times and (e) their calculated protein corona thicknesses.



**Figure S4.** SPR spectra of the protein–nanoparticles bioconjugates, obtained from incubation of Long Rods (AR~20) GNPs with various protein sources ((a) FBS 10%, (b) FBS 100%), (c) HP 10%, (d) HP 100%)) at different incubation times and (e) their calculated protein corona

thicknesses.



**Figure S5.** Kinetics of A $\beta$  fibrillation in presence of NMs A) Fibrillation kinetics of A $\beta$  (1 $\mu$ M), B) Fibrillation kinetics of A $\beta$  (10 $\mu$ M).



Figure S6. Kinetics of Aβ fibrillation in presence of NMs a) Fibrillation kinetics of Aβ (1μM) and GNPs with one centrifuge (1C), b) Fibrillation kinetics of Aβ (1μM) thrice centrifuge (3C) and c) Fibrillation kinetics of Aβ (10μM) thrice centrifuge (3C).





**Figure S7.** Kinetic of Aβ fibrillation (1μM) on bare GNPs (once and thrice centrifuge). A, B, C) Small Spheres, D, E, F) Big Spheres, G, H, I) Short Rods and J, K, L) Long Rods.









Figure S8. Kinetic of A $\beta$  fibrillation (1 $\mu$ M) on bare GNPs (thrice centrifuge). a, b, c, d, e, f)

Small Spheres, g, h, I, j, k, l) Big Spheres, m, n, o, p, q, r) Short Rods and s, t, u, v, w, y)

Long Rods.









**Figure S9.** Kinetic of A $\beta$  fibrillation (10 $\mu$ M) on bare GNPs (thrice centrifuge). a, b, c, d, e, f) Small Spheres, g, h, i, j, k, l) Big Spheres, m, n, o, p, q, r) Short Rods and s, t, u, v, w, y) Long Rods.







Figure S11. Size distribution function of 20 nm FBS10%–NP bioconjugates showing monodispersed population



Figure S12. Size distribution function of 30 nm FBS10%–NP bioconjugates showing monodispersed population



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Fig. S13. Comparison between FBS 10% and FBS 100% for all of NPs concentration; (a, b,

c) Long Rods, (d, e, f) Short Rods, (g, h, i) Small Spheres~20 nm and (j, k, l) Big Spheres~30

nm. A $\beta$  concentration is (1 $\mu$ M)





Fig S14. Comparison between HP 10% and HP 100% for all of NPs concentration; (a, b, c) Long Rods, (d, e, f) Short Rods, (g, h, i) Small Spheres~20 nm and (j, k, l) Big Spheres~30 nm. Aβ concentration is (1μM)





Fig. S15. Comparison between FBS 10% and FBS 100% for all of NPs concentration; (a, b, c) Long Rods, (d, e, f) Short Rods, (g, h, i) Small Spheres~20 nm and (j, k, l) Big Spheres~30 nm. Aβ concentration is (10µM)







Fig. S16. Comparison between HP 10% and HP 100% for all of NPs concentration; (a, b, c) Long Rods, (d, e, f) Short Rods, (g, h, i) Small Spheres~20 nm and (j, k, l) Big Spheres~30 nm. Aβ concentration is (10µM).





Fig. S17. Kinetic of Aβ fibrillation at difference concentrations of GNPs and GNRs (a) Small spheres, (b) Big spheres, (c) Short rods and (d) Long rods. [(after once centrifuged); Aβ

concentration is  $(1\mu M)$ ]





Fig. S18. Kinetic of Aβ fibrillation at difference concentrations of GNPs and GNRs (a) Small spheres, (b) Big spheres, (c) Short rods and (d) Long rods. [(after thrice centrifuged); Aβ

concentration is (1µM)]



Fig. S19. Fitting charts for calculation of lag times, (a) Amyloid  $\beta$  (1  $\mu$ M) and (b) Amyloid  $\beta$ 

(10 µM).



Fig. S20. Fitting Chart for small spheres ~20 nm (a) 80µg/mL (without centrifuged), (b) 80µg/mL (once centrifuged), (c) 80µg/mL (thrice centrifuged), (a1) 25µg/mL (without centrifuged), (b1) 25µg/mL (once centrifuged), (c1) 25µg/mL (thrice centrifuged), (a2) 80µg/mL (without centrifuged), (b2) 5µg/mL (once centrifuged) and (c2) 5µg/mL (thrice centrifuged).



Fig. S21. Fitting Chart for big spheres ~30 nm (a) 80µg/mL (without centrifuged), (b) 80µg/mL (once centrifuged), (c) 80µg/mL (thrice centrifuged), (a1) 25µg/mL (without centrifuged), (b1) 25µg/mL (once centrifuged), (c1) 25µg/mL (thrice centrifuged), (a2) 80µg/mL (without centrifuged), (b2) 5µg/mL (once centrifuged) and (c2) 5µg/mL (thrice centrifuged).



Fig. S22. Fitting Chart for short rods (a) 80µg/mL (without centrifuged), (b) 80µg/mL (once centrifuged), (c) 80µg/mL (thrice centrifuged), (a1) 25µg/mL (without centrifuged), (b1) 25µg/mL (once centrifuged), (c1) 25µg/mL (thrice centrifuged), (a2) 80µg/mL (without centrifuged), (b2) 5µg/mL (once centrifuged) and (c2) 5µg/mL (thrice centrifuged).



Fig. S23. Fitting Chart for long rods (a) 80µg/mL (without centrifuged), (b) 80µg/mL (once centrifuged), (c) 80µg/mL (thrice centrifuged), (a1) 25µg/mL (without centrifuged), (b1) 25µg/mL (once centrifuged), (c1) 25µg/mL (thrice centrifuged), (a2) 80µg/mL (without centrifuged), (b2) 5µg/mL (once centrifuged) and (c2) 5µg/mL (thrice centrifuged).