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Degree of Labeling of Folate-BSA with Rhodamine Dye

Prathibha Yazala
Governors State University

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DEGREE OF LABELING OF FOLATE-BSA WITH RHODAMINE DYE

A Project Submitted

To

Governors State University

Under the supervision

Of

Dr Walter Henne

In partial Fulfillment of the requirements for the Degree

Of

Masters in Science

Governors State University

By

Prathibha Yazala

Dedicated

To

My parents

ACKNOWLEDGEMENTS

It's a pleasure to thank those who made this thesis possible.

I owe my deepest gratitude to my advisor Dr. Walter Henne who has made his support available in a number of ways, from the starting to the completion of this project and thesis. This thesis would not have been possible without the support and encouragement of Dr Henne.

I want to express my gratitude to my committee members Dr. Patty Fu and Dr Darcy for their guidance. All the chemistry department grad professors were very motivating and I thank them all for their support.

I thank Governors state University for giving such an opportunity to help present my work.

I take privilege in thanking my parents for their unconditional love and support in every aspect of my life.

I thank my group members Hari kirshna kosuri and Sravanth Laxmareddygari and all my friends for their co-operation and support.

And last but not least, I thank the Almighty for reasons too numerous to mention and for the countless blessings He has bestowed on me all my Life.

Prathibha Yazala.

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ABSTRACT:

Folic acid is a very important vitamin. It is needed by cells for various physiological functions. It plays an important role in growth and multiplication of cells and therefore is needed very much by cancerous cells. One of the ways folate reaches the cells is the folate receptor mediated endocytosis. Therefore folate binding proteins are abundant on cancerous cells. Clinical trials proved that the folate receptor targeted drug delivery is very promising to increase the drug exposure to malignant cells and to reduce the toxicity to healthy cells. It was recently discovered that folate receptor is also over expressed on activated macrophages which could contribute to diseases such as crohn's disease, rheumatoid arthritis, lupus, inflammatory osteoarthritis, vasculitis, diabetes, ischemia, reperfusion injury, sarcoidosis, glomerulonephritis, psoriasis and sjogren's disease.^{2-5,8} Therefore folate receptor targeted drug delivery would also be beneficial for above diseases. Folic acid needs a carrier to take it to folate receptor. Serum Albumin is found to be suitable carrier for folate. In this project we make several conjugates of Folate-BSA and determine the simple way of making Folate-BSA for commercial use. The project also focuses on determining the degree of labeling of Folate on BSA. Finally Folate-BSA is labeled with dyes fluorescein and Rhodamine which can be used in future studies as fluorescent imaging agents.²⁵⁻²⁶

INTRODUCTION:

In this project, we prepare different fluorescein and Rhodamine folate-BSA conjugates. We discovered a method to prepare folate-BSA for commercial purposes. The conjugates are used as imaging agents, as a surface for adsorption of gold nanoparticles, to discover effective folate receptor targeted drug delivery many malignancies (in chemotherapy particularly).

Folic acid:

Folic acid (Fig 1) is a water soluble vitamin B9. Folic acid is the man made form of Folate which occurs naturally in the body. It is used for various physiological functions. It is very important for cell growth and maintenance. It also acts as a coenzyme for the synthesis of DNA and RNA. It also plays important role in the rapid growth and multiplication of cells in the fetus. Deficiency of folate in pregnant woman will lead to child birth defects. Amino acids histidine, serine, glycine and methionine require folate for metabolism. Unavailability of folate for methionine metabolism may lead to excess amounts of homocysteine which may lead to heart problems. Folate is also required for the proper maintenance of nervous system. In case of folate deficiency, of all, nucleic acids synthesis is the most affected process which may lead to cancer.¹⁻

3, 11

Folate Receptor:

There are two mechanisms for the transport of folate into cells:

1. Reduced folate carrier (RFC) (Fig-2): This system has high affinity for reduced folate. The RFC produces an electrochemical potential difference for folates across the cell membranes. RFC is a transmembrane protein. It comprises of 591 amino acid residues and it uses an anion exchange mechanism to transport the folates into cells.³⁻⁶

2. Endocytosis of GPI linked membrane Folate receptor alpha: The Folate receptor internalizes the bound Folate or Folate conjugated compounds via receptor mediated endocytosis. The folates are then retained in the cytoplasm by polyglutamation. This mechanism has high affinity for oxidized folate, folic acid. Figure 3 explains the transport of folate conjugates. On the plasma membrane there are small non-clathrin coated invaginations called caveolae which serve to concentrate folates. The caveolae seal and acidification of the caveolae results in the dissociation of the folates from MFR α . Folates are then translocated into the cytoplasm by putative carrier protein.⁸⁻¹²

BSA:

Serum albumin is a protein in plasma. The concentration is around 5g/100ml. It contributes a total of 80% to the colloid osmotic blood pressure. It is mainly responsible for maintaining the blood pH. The precursor of the albumin is the preproalbumin synthesized by liver. From preproalbumin the signal peptide is removed to form proalbumin. The six-residues of propeptide are removed from the new N-terminus of the proalbumin and the final product is the albumin. The half-life of albumin is 19 days. In general albumin has tryptophan and methionine. It has high amounts of cystine, charged amino acids, aspartic and glutamic acids, lysine and arginine.²³⁻²⁶

Folate conjugates:

Folate can be conjugated with variety of physiologically active compounds. Some of them are immunotherapeutic agents, protein toxins, chemotherapeutic agents, drug nanoparticles, liposomes, drug carriers etc. In this report we describe the attachment of folate to BSA. We make several different concentrations of folate-BSA. Folate BSA can be used as an imaging agent.

Imaging agents are chemicals made to detect if a tissue in the body is either benign or malignant.⁷

In United States, the second leading cause of death is cancer. Research was done and is being done to find a way to treat cancer. As a result of this there are many treatments (often not complete) available today. The most efficient and common one is the chemotherapy. In chemotherapy the number of cells killed is directly proportional to the amount of drug the cells are exposed to. But then high doses of the drug exposure are hindered because of the poor selectivity and the severe toxicity it causes to the normal cells. Therefore research is being progressed towards the tumor specific treatment.

The ideal solution to achieving tumor specific treatment is to deliver biologically effective doses of anticancer agents to the tumor tissues with very high specificity. Efforts were made to develop selective drug by conjugating anti-cancer drugs to hormones, antibodies and vitamin derivatives. Folic acid which is of low-molecular weight promises as a tumor-homing agent. Folate also happens to be a high affinity ligand which increases differential specificity of conjugated anti-cancer drugs by targeting Folate receptor-positive cancer cells.⁴⁻⁶

Research has shown that FR level and density accumulates in the tumor cells (Fig-4) and therefore it can be hypothesized that Folate conjugated anti-cancer drugs will improve drug selectivity and decrease the negative side effects on the normal cells. By considering the above it can be said that undestructive delivery of macromolecules to the living cells is difficult but is absolutely necessary in cancer treatment. Since the natural endocytosis pathway for vitamin Folate is known, it can be exploited to deliver the macromolecules if the macromolecules are first covalently linked to Folate. For example, Folate conjugated ribonuclease, horse radish

peroxidase, IgG or ferritin. Treatment of cells with above allowed delivery of more than 10^6 copies of the macromolecules within a 2 hour period.¹⁰ Drug coupling to inert carriers may be designed with the only purpose of prolonging the permeance in blood to get a passive distribution into some tissues and also conjugating provides a targeting system that selectively recognizes specific biological structures.

Fluorescein Isothiocyanate and Rhodamine Dye:

Fluorescein and Rhodamine are fluorescent dyes used to trace the flow of proteins, drugs or imaging agents in the living cells. Rhodamine is red in color and Fluorescein orange. They impart pink and yellow color respectively to Folate-BSA. The absorption maxima of fluorescein is 494nm and emission maxima is 521nm. For Rhodamine, the absorption maximum is 570nm and emission maxima at 590nm.

Folate conjugates labeled with FITC or Rhodamine can be used in imaging the cancerous growth. Targeted delivery of imaging agents can be achieved by use of folate conjugates and the stage of the disease can be identified. Folate conjugates can be attached to dyes and drugs. Folate-BSA can also be used as adsorption surface in synthesis of Gold nanoparticles. This adsorption is explained in figure 4. Gold has high light scattering and absorbing ability. The detection of cancer would be much easier when gold nanoparticles are made using the folate-BSA as the surface for adsorption. Folate conjugates can also to be attached to drugs to provide targeted drug delivery in case of cancer.³⁻⁷

MATERIALS AND METHODS:

Materials and Reagents:

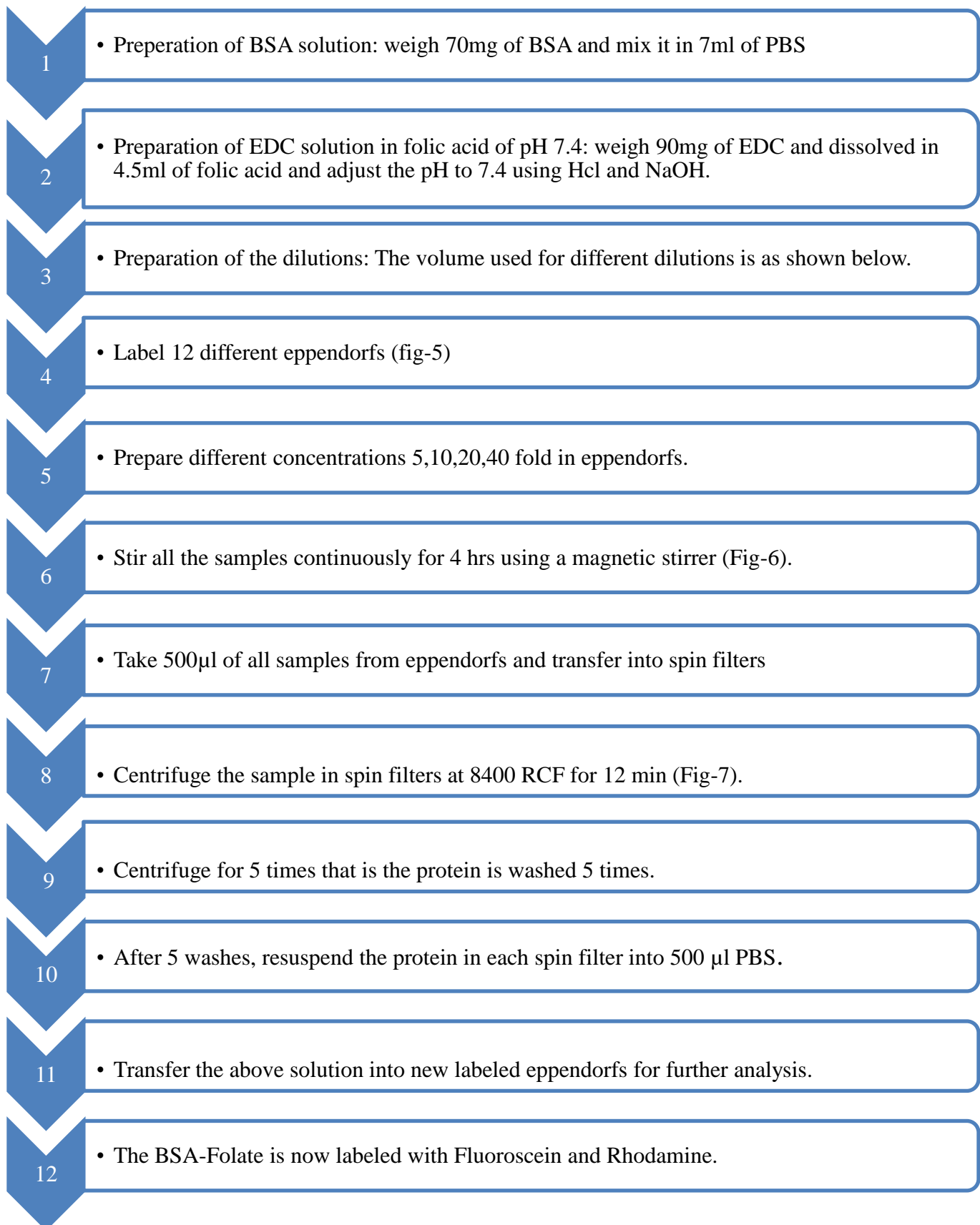
Albumin from Bovine serum A4503-10G Lot # 029k1394 p code: 1000528230; $\geq 96\%$ (agarose gel electrophoresis) powder lyophilized 5.0-5.6; 1 % w/v in H₂O, Phosphate Buffered saline without calcium and magnesium Cat No: 21-040-CV Lot No: 21040174 exp 07/11 volume 500ml, Biocinchonic acid solution (10ml) from Sigma Life science B9643-1L, Copper sulfate (Cu₂SO₄) 4% w/v prepared from copper (II) sulfate pentahydrate, EDC, N-((3-Dimethylaminopropyl)-N-ethyl carbodamide hydrochloride CAS No: 25952-53-8 Mfg by Sigma Aldrich, Eppendorfs fisher Brand premium 1.5 ml MCT Graduated natural Cat No: 05-408-129 Lot No: 10110134, Beckman VWR Centrifuge filter Modified PES 3k 500 μ l Low protein Binding, Epoch plate reader, mini-PROTEAN^R TGXTM catalog # 456-1096 4-20% 15 well comb 15 μ l, Precision Plus proteinTM catalog # 161-0373 500 μ l, Laemmli sample buffer 30ml catalog # 161-0737 by Bio-Rad laboratories.

Methodology:

Preparation of Protein samples:

The concentrations prepared in step 5 in the flow chart are shown below:

- a. 5 fold: 500 μ l of BSA+PBS, 37 μ l of FA+EDC and 463 μ l of PBS
- b. 10 fold: 500 μ l of BSA+PBS, 75 μ l of FA+EDC and 425 μ l of PBS
- c. 20 fold: 500 μ l of BSA+PBS, 150 μ l of FA+EDC and 350 μ l of PBS
- d. 40 fold: 500 μ l of BSA+PBS, 300 μ l of FA+EDC and 200 μ l of PBS



Different concentrations of Folate, BSA and EDC are made. EDC is used as a coupling agent (Fig-8).

Preparation of BSA standards:

BSA standards are prepared according to the thermo scientific instructions as follows:

Vial	Diluent Volume (μL)	BSA Source and Volume (μL)	Concentration (μg/mL)
A	0	500 of stock	2,000
B	125	375 of stock	1,500
C	200	200 of stock	1,000
D	200	200 of vial B	750
E	200	200 of vial C	500
F	200	200 of vial E	250
G	200	200 of vial F	125

The standard BSA curve is shown in figure 9.

Folate-BSA labeling with Fluorescein and Rhodamine:

Samples 10 fold and 20 fold are taken and are centrifuged to remove the PBS. To the washed protein 0.5ml of sodium carbonate is added. The protein in sodium carbonate is taken in a different tube. To this add 10μl of fluorescein in DMSO (This is prepared by adding 3.25μl of the dye in 250μl of DMSO). The protein is then washed with DMSO and is resuspended in PBS. Fluorescein and Rhodamine are weighed and added to the sample and stirred for four hours using a magnetic stirrer. After adding the dye, it is allowed to mix overnight. The dye labeled protein samples are again spin filtered and washed with PBS 5 times. The BCA assay is done on

the samples. Degree of labeling is calculated. Fluorescein imparts yellow color and Rhodamine violet color to the protein BSA samples. (Fig-10)

INSTRUMENTATION:

UV-VIS Plate reading:

- In a UV-VIS plate reader, take 100µl of PBS as blank in A1 and A2 wells.
- In B1 and B2 wells, take 100µl of the washed pure protein
- Read at 280nm and 363nm wavelength at corrected path length.

BCA Analysis:

BSA standards are prepared as per the instructions given in the thermo scientific.

1. BCA standard solution is prepared by mixing 200µl of Cu_2SO_4 in 10ml of BCA reagent.
2. 10µl of standards in triplicates and 10µl of samples in duplicates are added in the wells of the BCA plate. (Fig 11)
3. To all the above wells 200µl of BCA reagent is added.
4. The plate is covered with par film and is incubated for 30 minutes.
5. BCA analysis is run and plate is read at 562nm and the data is recorded.

For those protein samples labeled with Fluorescein, both uv-vis and BCA readings are taken but those samples with Rhodamine, only uv-vis is done because the emission wavelength of Rhodamine is around 562nm which make it overlap with BCA readings taken at 582nm.

Gel electrophoresis (fig-12) was done on the protein samples based on Bio Rad. From the gel we could conclude that there were no impurities (fig-13) since there were no large aggregates formed. A standard curve of different molecular weights is calculated (fig-14)

RESULTS AND DISCUSSION:

Preparation of Folate-BSA:

Different approaches were tried in preparation of Folate-BSA. EDC was used as a binding agent. 10 μ l of EDC is added to 5mg of BSA in an eppendorf and is stirred overnight for 24 hours with a magnetic stirrer and then the centrifugation is done 5 times. But this showed protein precipitation in the 40 fold and the 60 fold. Even when the stirring time was reduced from overnight or 24 hrs to 6hrs time, the protein in 40 fold and 60 fold concentrations precipitated. And when 2hrs time for stirring was used the 60 fold protein concentration still precipitated. Therefore finally a 4hr stirring time was determined as optimum. The 60 fold still showed precipitation but was much less relatively. To make the preparation method easy, the compounds were mixed in different order. First BSA and EDC were weighed in equal quantities and then folate and PBS were added. This resulted in the formation of crystals in the 40 and 60 fold and the dissolving process was also not that good. After several trials, it was found out that EDC dissolved well in Folate and BSA dissolved in PBS and both mixed well. Finally to make the preparation much easier, EDC in folate and BSA in PBS were prepared in bulk and mixed in quantities as per the calculations and were made up with PBS.

Degree of labeling of Folate-BSA:

The binding of Folate-BSA was calculated using the following spreadsheet:

Protein	BCA Reading	Molarity	Molarity x DF	Abs at 363nm	Molarity	Degree Of Labeling
5 Fold 1	0.131	1.97E-06	1.97E-05	0.73	6.94E-05	3.519253278
5 Fold 2	0.082	1.23E-06	1.23E-05	0.64	5.21E-05	4.216666275
5 Fold 3	0.337	5.08E-06	5.08E-05	0.94	0.00010993	2.166029033
10 Fold 1	0.454	6.84E-06	6.84E-05	1.68	0.00025265	3.695172877
10 Fold 2	0.359	5.41E-06	5.41E-05	1.63	0.00024301	4.494645203
10 Fold 3	0.404	6.08E-06	6.08E-05	1.66	0.00024879	4.089099363
20 Fold 1	0.388	5.84E-06	5.84E-05	3.61	0.00062488	10.69381344
20 Fold 2	0.332	0.000005	0.00005	3.11	0.00052845	10.56894889
20 Fold 3	0.34	5.12E-06	5.12E-05	2.51	0.00041273	8.060355097
40 Fold 1	0.123	1.85E-06	1.85E-05	3.02	0.00051109	27.59053241
40 Fold 2	0.245	3.69E-06	3.69E-05	4.41	0.00077917	21.1171157

Moles of folate per mole of BSA= No. of moles of Folate/No. of moles of BSA

In the above calculations, Volume used is 1ml, Dilution factor is 10, Molecular weight of BSA is 66400 and molar extinction coefficient of Folate is 5185. It was calculated as shown in Fig-11

Labeling of Folate-BSA with fluorescein and Rhodamine:

With the molar extinction coefficient of $70,000 \text{ M}^{-1}\text{cm}^{-1}$, it was calculated that there were ~ two fluorescein molecules attached to each BSA. Rhodamine molar extinction coefficient is $85,000\text{M}^{-1}\text{cm}^{-1}$. There was ~ 1 Rhodamine attached to each BSA.

CONCLUSION:

A simple way of preparation of different protein concentrations is determined. It is found out that the 10 and 20 fold concentration is the optimum concentration for Folate BSA. Concentrations above 40 fold showed precipitation. The degree of Folate labeling on BSA is determined to be about roughly double for each concentration increased. Fluorescein and Rhodamine dyes are attached to Folate BSA and it is found that on an average, two fluorescein molecules and one Rhodamine molecule are attached to each BSA molecule. In future, research can be focused on preparing Folate BSA for commercial purposes which can in turn be tested for use with gold nanoparticles, as imaging agents, targeted drug deliver etc.

REFERENCES:

1. Henne WA, Doorneweerd DD, Hilgenbrink AR, Kularatne SA, Low PS. Synthesis and activity of a folate peptide camptothecin prodrug. *Bioorg Med Chem Lett*. Oct 15 2006;16(20):5350-5355.
2. Leamon CP. Folate-targeted drug strategies for the treatment of cancer. *Curr Opin Investig Drugs*. Dec 2008;9(12):1277-1286.
3. Low PS, Henne WA, Doorneweerd DD. Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. *Acc Chem Res*. Jan 2008;41(1):120-129.
4. Leamon CP, DePrince RB, Hendren RW. Folate-mediated drug delivery: effect of alternative conjugation chemistry. *J Drug Target*. 1999;7(3):157-169.
5. Leamon CP, Reddy JA, Vetzal M, et al. Folate targeting enables durable and specific antitumor responses from a therapeutically null tubulysin B analogue. *Cancer Res*. Dec 1 2008;68(23):9839-9844.
6. Esmaili F, Ghahremani MH, Ostad SN, et al. Folate-receptor-targeted delivery of docetaxel nanoparticles prepared by PLGA-PEG-folate conjugate. *J Drug Target*. Jun 2008;16(5):415-423.
7. Low PS, Kularatne SA. Folate-targeted therapeutic and imaging agents for cancer. *Curr Opin Chem Biol*. Jun 2009; 13(3):256-262.

8. Viola-Villegas N, Rabideau AE, Cesnavicious J, Zubieta J, Doyle RP. Targeting the folate receptor (FR): imaging and cytotoxicity of ReI conjugates in FR-overexpressing cancer cells. *ChemMedChem*. Sep 2008;3(9):1387-1394.
9. Zhao J, Cao SL, Zheng XL, Zhao B. [Folate receptor-mediated antitumor drugs]. *Yao Xue Xue Bao*. Feb 2009;44(2):109-114. 16
10. Nutt JE, Razak AR, O'Toole K, et al. The role of folate receptor alpha (FRalpha) in the response of malignant pleural mesothelioma to pemetrexed-containing chemotherapy. *Br J Cancer*. Feb 2 2010;102(3):553-560.
11. Corona G, Giannini F, Fabris M, Toffoli G, Boiocchi M. Role of folate receptor and reduced folate carrier in the transport of 5-methyltetrahydrofolic acid in human ovarian carcinoma cells. *Int J Cancer*. Jan 5 1998;75(1):125-133.
12. Sanchez-del-Campo L, Montenegro MF, Cabezas-Herrera J, Rodriguez-Lopez JN. The critical role of alpha-folate receptor in the resistance of melanoma to methotrexate. *Pigment Cell Melanoma Res*. Oct 2009;22(5):588-600.
13. Zhao R, Min SH, Wang Y, Campanella E, Low PS, Goldman ID. A role for the proton-coupled folate transporter (PCFT-SLC46A1) in folate receptor-mediated endocytosis. *J Biol Chem*. Feb 13 2009; 284(7):4267-4274.
14. Tong, L., Y. Lu, et al. (2007). "Imaging receptor-mediated endocytosis with a polymeric nanoparticle-based coherent anti-stokes Raman scattering probe." *J Phys Chem B* **111**(33): 9980-9985.
15. Kelemen LE. The role of folate receptor alpha in cancer development, progression and treatment: cause, consequence or innocent bystander? *Int J Cancer*. Jul 15 2006;119(2):243-250.
16. Theti DS, Jackman AL. The role of alpha-folate receptor-mediated transport in the antitumor activity of antifolate drugs. *Clin Cancer Res*. Feb 1 2004;10(3):1080-1089.
17. Reddy JA, Westrick E, Vlahov I, Howard SJ, Santhapuram HK, Leamon CP. Folate receptor specific anti-tumor activity of folate-mitomycin conjugates. *Cancer Chemother Pharmacol*. Aug 2006;58(2):229-236.

18. Hahn F, Schepers U. Versatile procedure for asymmetric and orthogonal protection of symmetric polyamines and its advantages for solid phase synthesis. *J Comb Chem*. Mar-Apr 2008;10(2):267-273.
19. Sabharanjak, S. and S. Mayor (2004). "Folate receptor endocytosis and trafficking." *Adv Drug Deliv Rev* **56**(8): 1099-1109.
20. Yang, J., H. Chen, et al. (2007). "Characterization of the pH of folate receptor-containing endosomes and the rate of hydrolysis of internalized acid-labile folate-drug conjugates." *J Pharmacol Exp Ther* **321**(2): 462-468.
21. Rijnboutt, S., G. Jansen, et al. (1996). "Endocytosis of GPI-linked membrane folate receptor-alpha." *J Cell Biol* **132**(1-2): 35-47.
22. Zhao, R., L. H. Matherly, et al. (2009). "Membrane transporters and folate homeostasis: intestinal absorption and transport into systemic compartments and tissues." *Expert Rev Mol Med* **11**: e4.
23. Ifergan, I., A. Shafran, et al. (2004). "Folate deprivation results in the loss of breast cancer resistance protein (BCRP/ABCG2) expression. A role for BCRP in cellular folate homeostasis." *J Biol Chem* **279**(24): 25527-25534.
24. Henne, W. A., D. D. Doorneweerd, et al. (2006). "Detection of folate binding protein with enhanced sensitivity using a functionalized quartz crystal microbalance sensor." *Anal Chem* **78**(14): 4880-4884.
25. Qu, X., C. Yang, et al. (2010). "In vitro evaluation of a Folate-bovine serum albumin-doxorubicin conjugate." *J Drug Target* **18**(5): 351-361.
26. Shinoda, T., A. Takagi, et al. (1998). "In vivo fate of folate-BSA in non-tumor- and tumor-bearing mice." *J Pharm Sci* **87**(12): 1521-1526

LIST OF FIGURES:

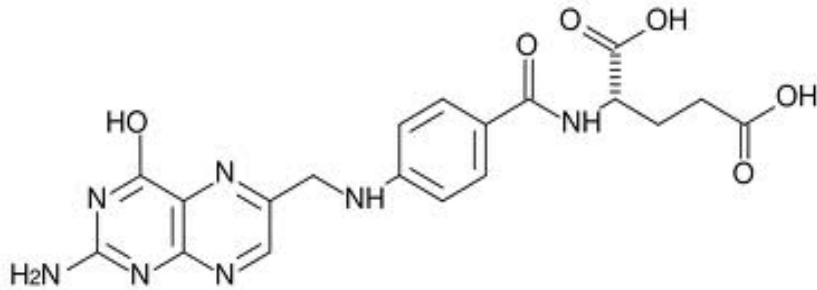


Figure-1 Structure of Folic acid

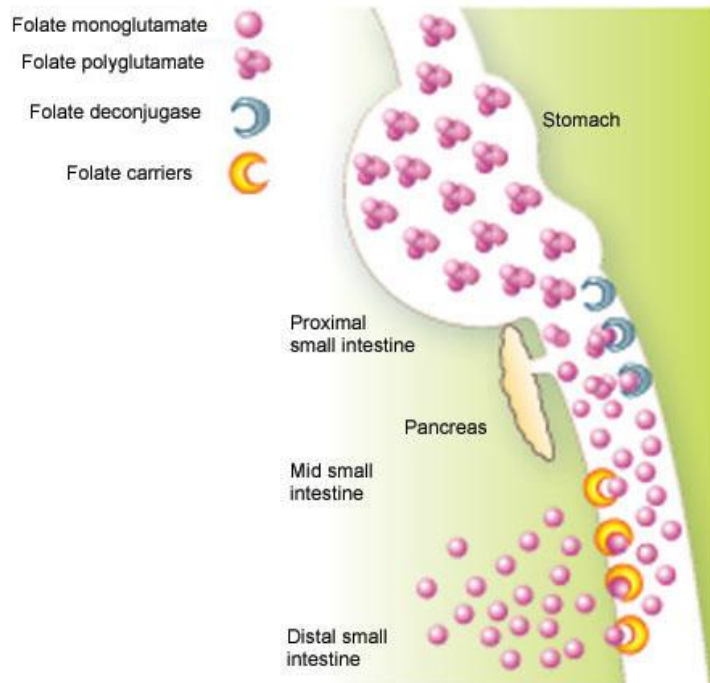


Figure -2: RFC mediated transport of folate into cells. (Medscape)

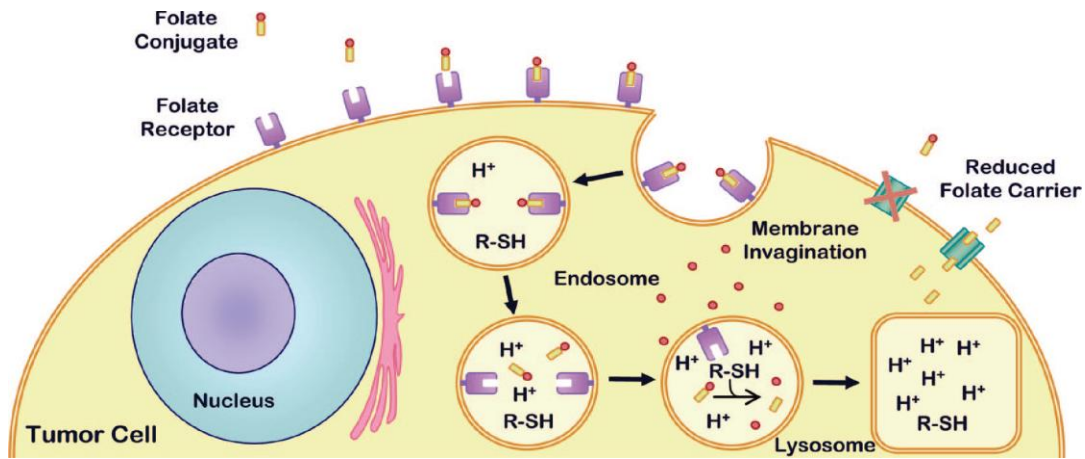
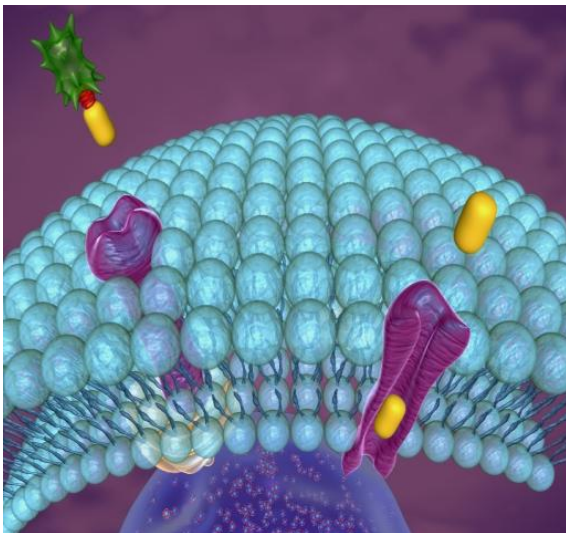
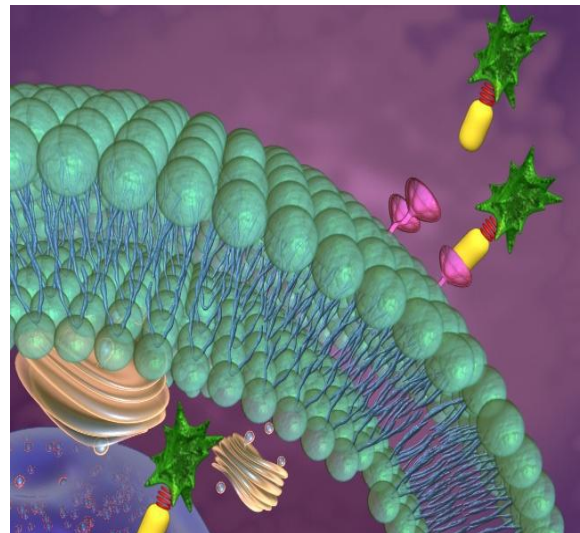


Figure 3: Folate receptor mediated endocytosis of folic acid conjugate. Different folate conjugates bind to the folate receptor with high affinity and are then internalized into endosomes. (Low, Henne, Accounts of Chemical Research).



Reduced folate carrier in normal cells.



alpha-FR in malignant cells.

Figure-4: Comparison of alpha-FR on normal and malignant cells. (Endocyte Inc.)

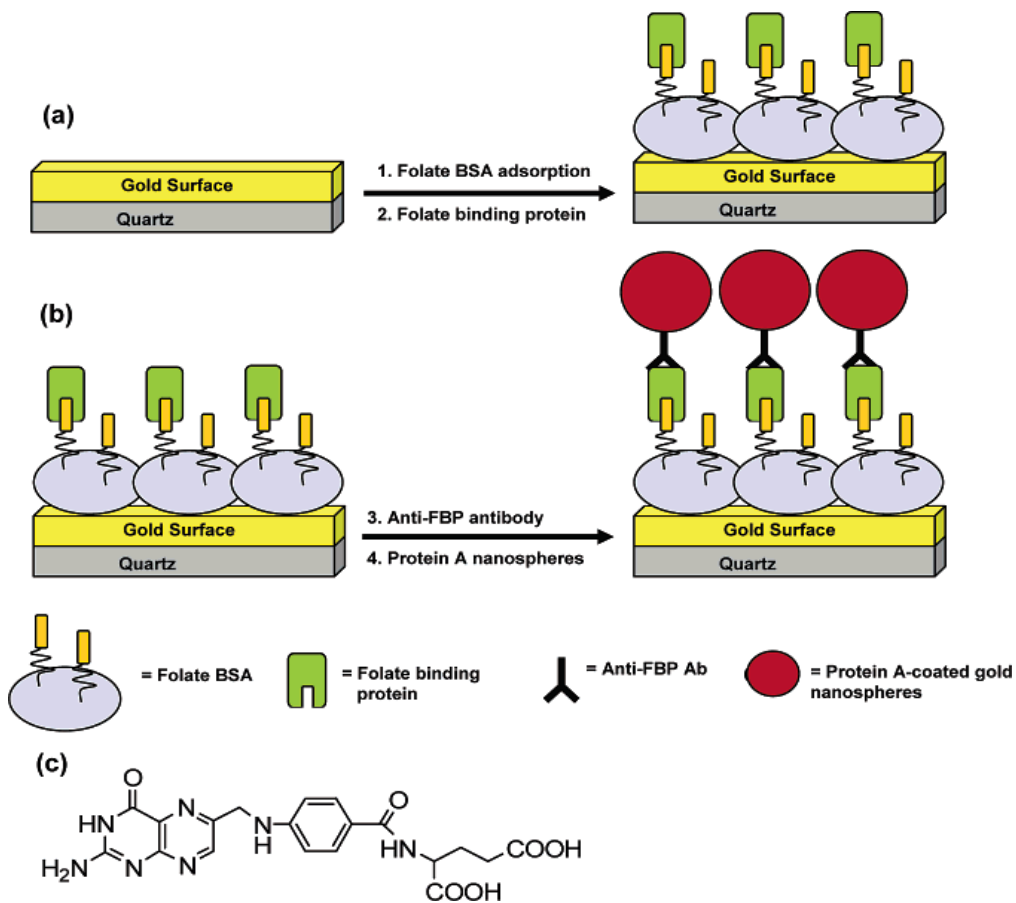


Figure 4: Diagrammatic representation of adsorption of Folate BSA on gold surface. (Walter A. Henne,[†] Derek D. Doorneweerd, Folate binding detection by enhance quartz balance.)

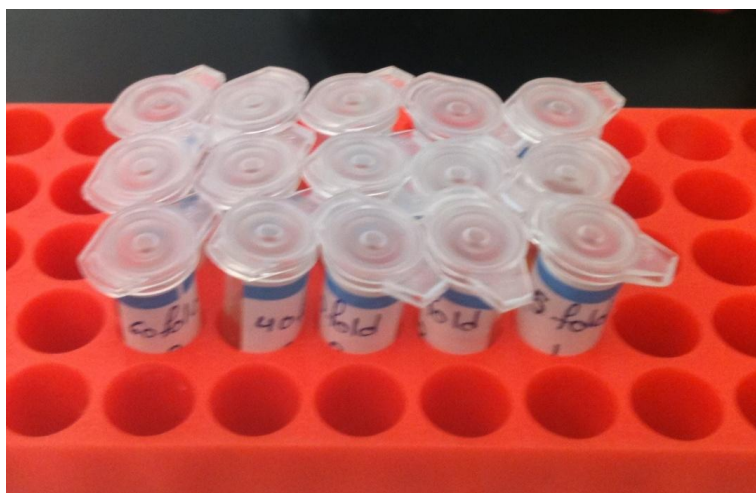


Figure-5: Labeling of eppendorfs for 5 fold, 10 fold, 20 fold, 40 fold, 60 fold.



Figure-6 Mixing of all the samples using a magnetic stirrer.



Figure-7: Beckman Centrifuge used to wash the protein in spin filters.

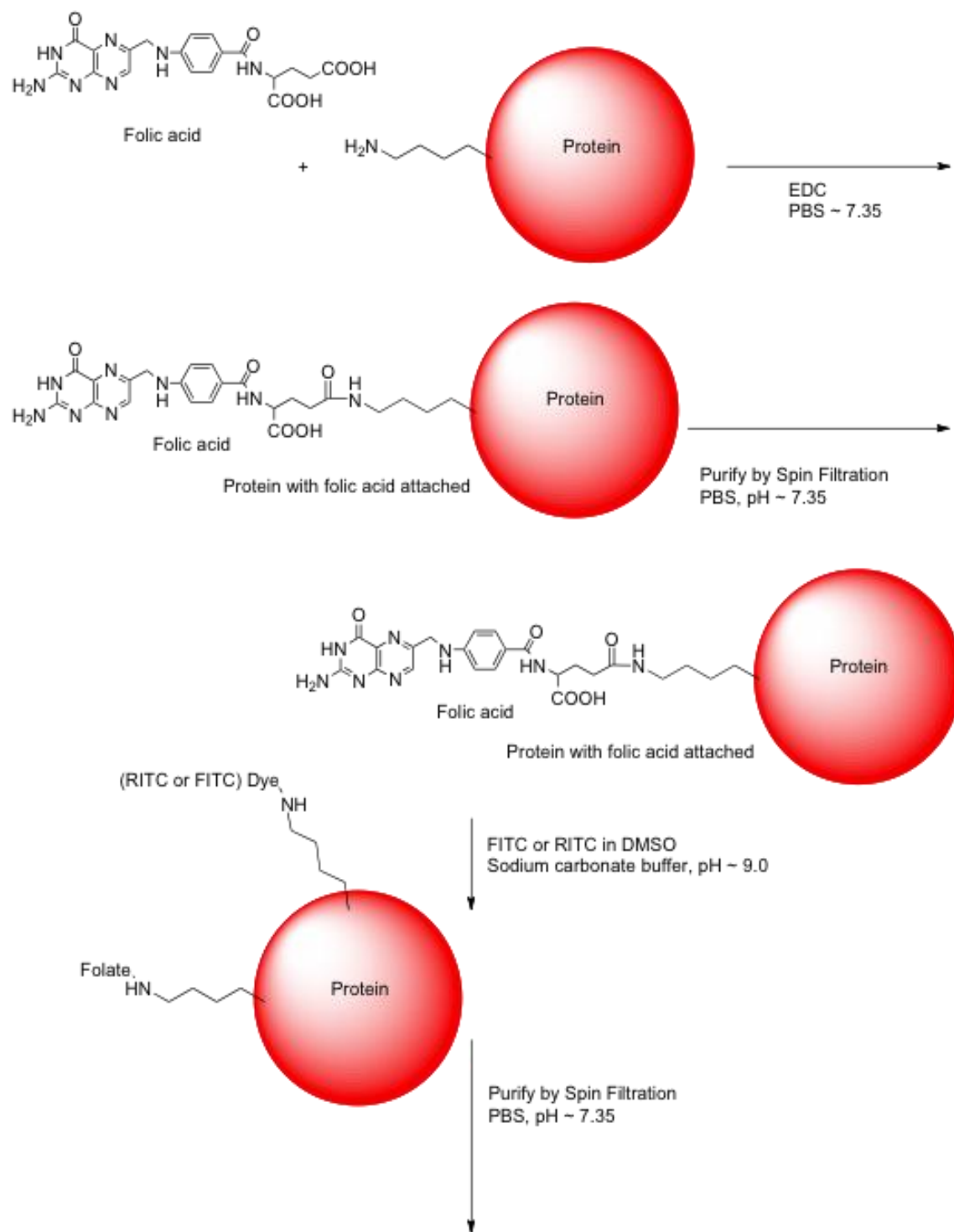


Figure-8: schematic representation of EDC coupling.

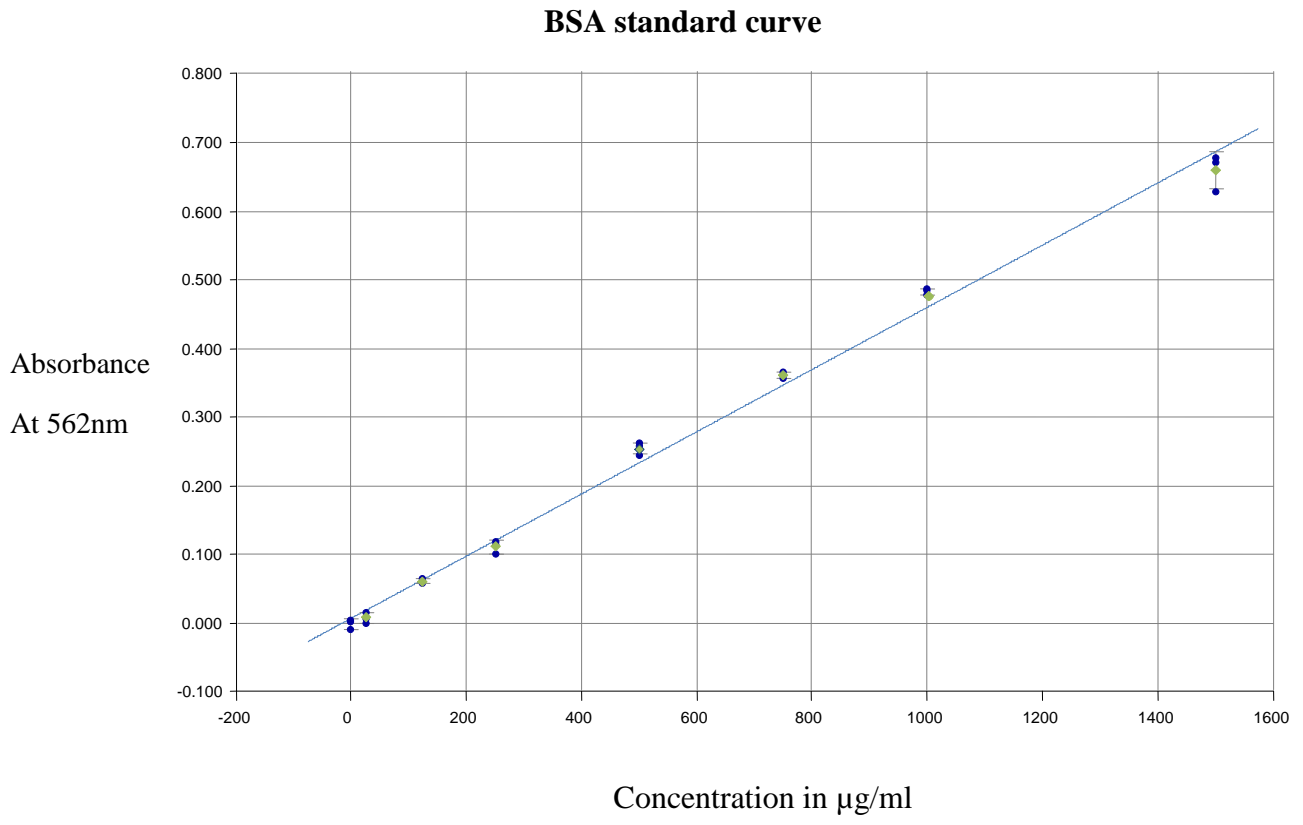


Figure -9: BSA standard Curve

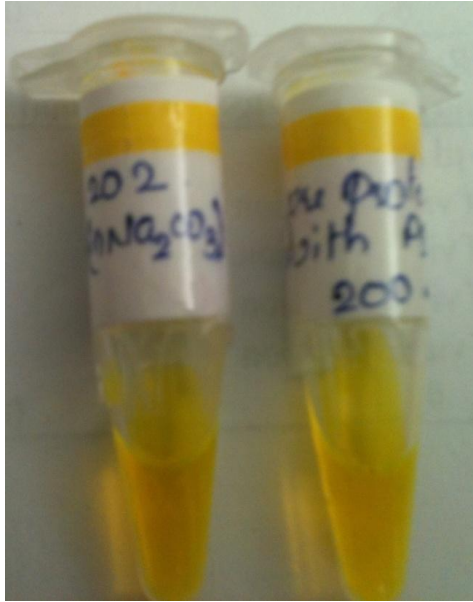


Figure -10: 10 fold and 20 fold Folate-BSA after adding Fluorescein and Rhodamine.

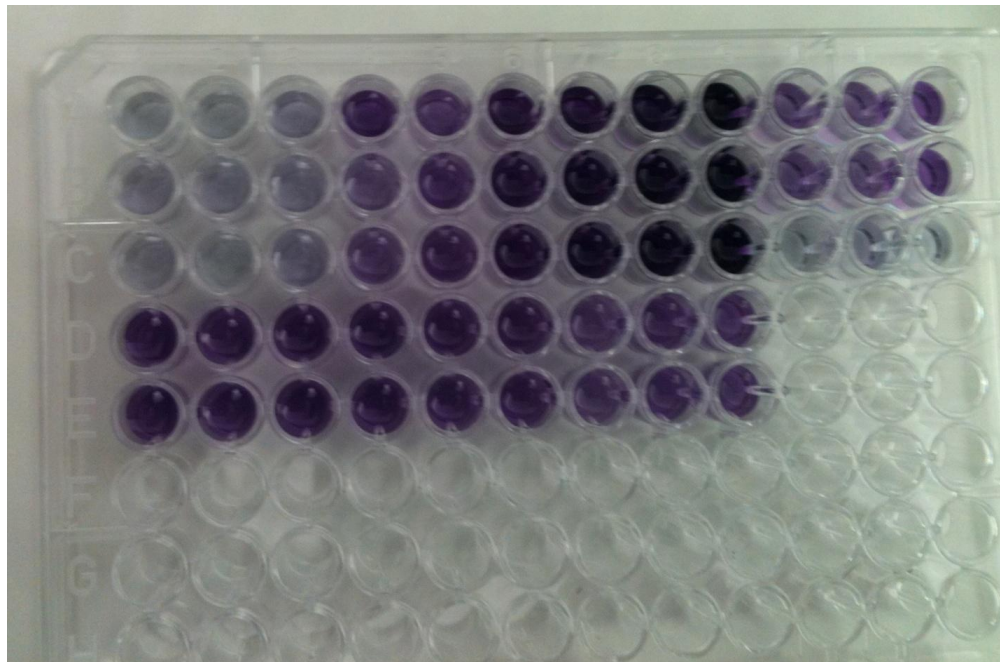


Figure-11: BCA analysis for blank, BCA standards and protein standards.

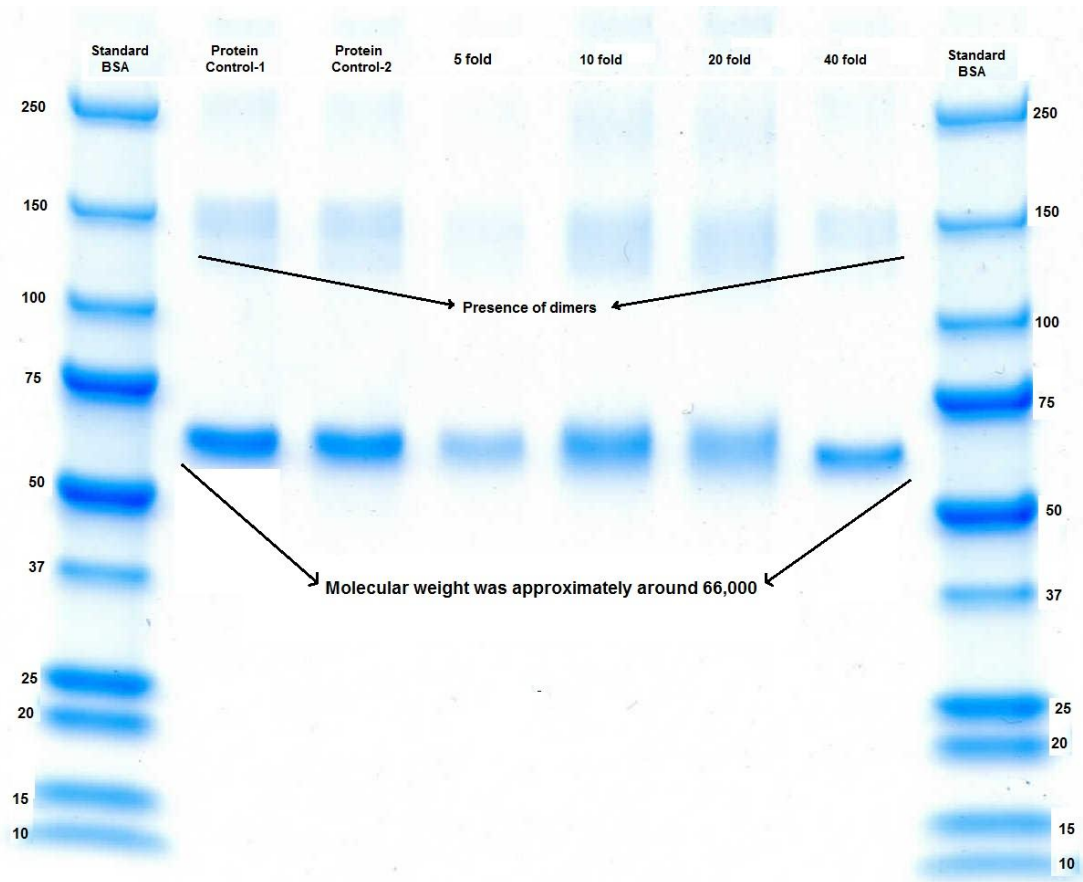


Figure-12: Gel electrophoresis of the Folate-BSA protein sample.

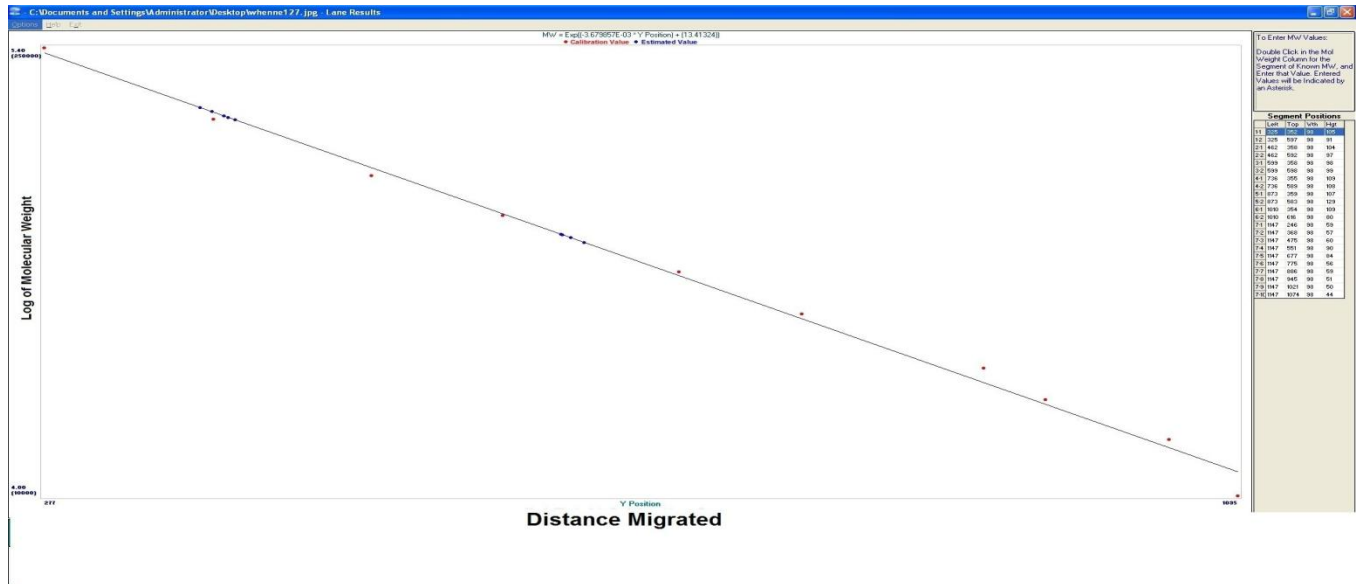


Figure 13: Distance migrated by the proteins on a gel

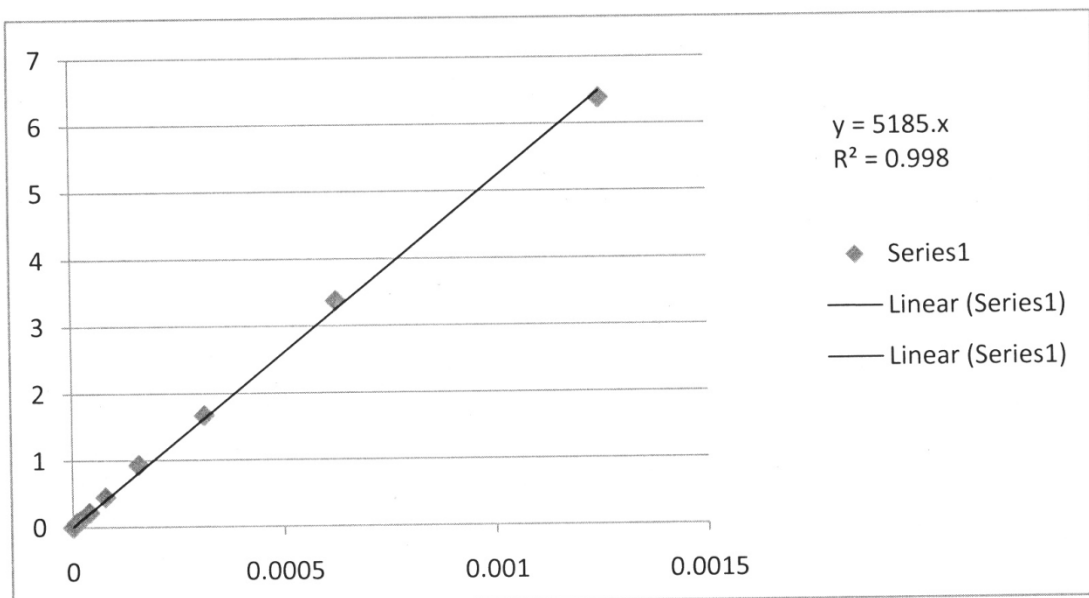


Figure -14: Calculation of molar extinction coefficient.