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Research article

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Preparation, encapsulation and blood interaction studies of chemically and biologically synthesised magnetic liposomes

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Abstract

Magnetic liposomes are vesicles with encapsulated magnetic nanoparticles which can increase the bioavailability and targeting of a drug and could be beneficial for many therapeutic purposes. The main objective behind this study was to conduct a comparative analysis on encapsulation and blood component interaction efficiency of liposomes with magnetic nanoparticles synthesised by biological and chemical methods. The overall results have shown a gradual increase in the yield of highly pure but toxic magnetic nanoparticles during chemical synthesis. Meanwhile, less toxic level and high encapsulation efficiency was found in the biologically synthesised nanoparticles and their liposomal formulation. The study visualises few parameters that stress the vitality of producing magnetic nanoparticles using biological method and encapsulating those nanoparticles in liposomes for wider usage *in vivo*.

Keywords

Liposomes; Bioavailability; Haemotoxicity; Nanoparticles; Magnetic liposomes

Introduction

Iron oxide nanoparticles have been extensively studied as drug carriers in the pharmaceutical and medical fields.[1,2] Research has expanded considerably over the last 30 years, increasing applications area from drug and gene delivery to diagnostics, cosmetics, long-lasting immune-contraception to food and chemical industry.[3-8] Magnetic nanoparticle has been shown to be a promising therapeutic approach to cancer treatment.[10-12] However, the recent challenges are to develop the magnetic nanoparticles with high heating ability (measured by specific absorption rate (SAR)) and to control the temperature of magnetic nanoparticles *in vivo*.[13-15] Toxicity is another major issue which is seen in these magnetic nanoparticles due to their chemical composition, which includes metals like cobalt, zinc or silver that would highly affect all biological entities.[16]

Recently, the biocompatibility and heating ability are of prime concern for targeted drug delivery experiments as there is a necessity for efficient drug delivery system.[17]

There is a search for a possible approach to control the temperature of the magnetic nanoparticles *in vivo* to avoid the damage to the surrounding normal tissue due to overheating temperature.[18]

One of the crucial solutions for this application is using liposomes. Magnetic liposomes (magnetic nanoparticles encapsulated in phospholipid bilayers) appear to be a versatile delivery system due to biocompatibility, chemical functionality and its potential for combination of drug delivery and treatment.[19]

In order to achieve optimum efficacy, magnetic liposomes with high encapsulation efficiency, stability and long circulating (stealth) ability are desired.[20-22] Thus, the magnetic nanoparticles encapsulated liposomes may act as temperature controlling switch.[23-24] Liposomal encapsulation technology (LET) is used by medical investigators to transmit drugs that act as curative promoters to the assured body organs.[25] This form of delivery system proposal targeted the delivery of vital combinations to the body.[26-29]

LET is a method of generating sub-microscopic foams called liposomes, which encapsulate numerous materials.[30] These liposomes form a barrier around their contents, which is resistant to the enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals.

The contents of the liposomes are therefore protected from oxidation and degradation.[31-33] This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the exact target gland, organ or system where the contents will be utilised.[34-39]

Because of their unique bilayer-structure properties, liposomes are used as carriers for both lipophilic and water-soluble molecules.[40] Hydrophilic substances are encapsulated in the interior aqueous compartments. Lipophilic drugs are mainly entrapped within lipid bilayers. As asserted by different authors, liposomes have attractive biological properties, including the biocompatibility and biodegradability.[41,42] They show promise as active vectors as a result of their capacity to enhance the performance by increasing drug solubility and stability, delivering encapsulated drugs to specific target sites, and providing sustained drug release. Their subcellular size allows relatively higher intracellular uptake than other particulate systems, improving *in vivo* drug bioavailability.[43,44] Other advantages of liposomes include high encapsulation efficiency in spite of drug solubility, low toxicity due to phospholipids content, drug protection against degradation factors like pH and light, and the reduction of tissue irritation.[45] Many studies on the decrease of toxic effect of magnetic nanoparticles with the incorporation of several organic or inorganic polymer have been conducted in the recent past.[46]

Materials and Methods:

Synthesis of magnetic nanoparticles

Chemical process: Ferric chloride and ferrous sulphate were taken in a glass beaker in 1:2 molar concentrations and added with twice the volume of 25% ammonia solution and incubated at 50°C for 2 h in a magnetic stirrer.[47]

Biological process: 0.5 ml of 0.25 M ferric chloride solution and 1.5 ml of green tea extract (prepared by boiling 6 g of green tea leaves with 100 ml distilled water[48]) were added and incubated at room temperature.[49,50] The mixture was then centrifuged at 10,500 rpm for 25 min to remove the plant extract. Then the residue was dissolved in distilled water for further use.

Preparation of magnetic liposomes

The magnetic liposomes were prepared by thin layer hydration (TLH). Lauric acid (sodium lauryl sulphate) and magnetic nanoparticles (40 wt% of ferrite) were added and kept on a hot plate (100°C) for 10 min with stirring. Then the magnetic fluid was filtered through a Whatman paper and centrifuged at 15000 rpm for 15 min at room temperature. 5 ml of oleic acid was added and heated for 5 h at 55°C. An equal volume of chloroform was then added and incubated. The phase separation was done using separating funnel and finally washed with ethanol. The required amount of cholesterol (in oleic acid) was dissolved in the phase transformed magnetic fluid in a round bottomed flask. Then, the solvent was evaporated under vacuum at room temperature using a rotary evaporator. The magnetic fluid was added to the flask and hydration was done for 30 min at 40°C.[51]

Determination of encapsulation efficiency

For determining the encapsulation efficiency, the excess oleic acid was removed from the solution by treatment with NaOH (20 mM) solution at 50°C for 15 min. The clear brown upper layer of magnetic nanoparticles in chloroform was separated and carefully collected. The chloroform solution was then treated with 0.9% NaCl solution. The precipitated iron nanoparticles were collected, washed twice with water, estimated by UV-Vis Spectrophotometry and finally compared with standard values.[52]

Blood interaction studies

Freshly collected mammalian blood was used to determine the haemotoxicity of the chemically and biologically synthesised magnetic liposomes. Five different test tubes (containing a pinch of EDTA or heparin) with same volume of blood were added with different concentration of magnetic nanoparticles solution such that the overall concentration of the nanoparticles vary over a range [Suitable dilutions of blood were made based on the sampling time and sampling volume with 1X PBS solution (13.7 M NaCl, 2.7 M KCl, 10 M NaH_2PO_4 and 1.8 M K_2HPO_4)]. The testers (blood sample along with control) were incubated over a period of 4 h. Sampling was done every 30 min for cell viability testing. Samples were diluted in distilled water and incubated at room temperature. The absorbance was read at 380, 415 and 450 nm against distilled water blank in a Spectrophotometer. The percentage of haemolysis is determined as[53,54]:

$$\%Haemolysis = 100 \times (free\ Hb\ in\ Control[mg/ml] / (free\ Hb\ in\ Sample[mg/ml])$$

Results and Discussion

Nanoparticle preparation

During synthesis by co-precipitation (chemical synthesis), the ferrous and ferric salts were reduced to unstable ferric hydroxide intermediate which immediately gets reduced to ferric oxide. Thus formed ferric oxide does not get dissolved in the solution and thereby settles down as small, blackish brown particles. On heating at 50°C, the ammonium chloride evaporates, leaving a precipitated solution of ferric oxide which was then filtered and dried.[55]

In the biological reduction process by green tea extract, the polyphenols present in the extract act as reducing agents which replace the chloride or sulphate ions in the ferrous or ferric salts to ferric oxide molecules[56]. Due to the presence of other active oxidising agents, there might be further reactions in the reaction mixture and in order to prevent that, the solution was transferred to cold conditions immediately for terminating the reaction process.

Magnetic liposome preparation

The lauric acid coated and oleic acid based ferrimagnetic (nano) liposomes prepared responded positively to the applied magnetic field while the continuous stirring process enhanced the vesicle formation; hence, encapsulating the magnetic nanoparticles within the nanovesicles.

Encapsulation efficiency

Table 1. The encapsulation efficiency determined by chemical synthesis

Total quantity of magnetic nanoparticles used in the synthesis of liposomes	225.2 mg
Total quantity of unencapsulated nanoparticles (M)	93.41 mg
Total quantity of encapsulated nanoparticles (E_{Np})	131.79 mg
Entrapment efficiency of the process	58.52%
% Loss of magnetic nanoparticles	41.48%

Table 2. The encapsulation efficiency determined by biological synthesis

Total quantity of magnetic nanoparticles used in the synthesis of liposomes	123.7 mg
Total quantity of unencapsulated nanoparticles (M)	40.5 mg
Total quantity of encapsulated nanoparticles (E_{Np})	184.7 mg
Entrapment efficiency of the process	82.01%
% Loss of magnetic nanoparticles	17.99%

The percentage fraction of the entire magnetic nanoparticles that gets entrapped in the liposome is termed as encapsulation efficiency. The encapsulation efficiencies of the liposome formulations are reported in Table 1 and Table 2. The liposomal magnetic nanoparticles prepared by biological synthesis exhibited higher encapsulation efficiency (82.01%) than those prepared by the chemical method. The percentage loss of magnetic nanoparticles in chemical synthesis was seen very high (41.48%), which makes less good synthesis method.

Interactions with blood

Interactions with blood proteins: The concentration of proteins in the blood varies from 45-65 g/l. Under *in vitro* incubation, the blood proteins and peptides in the solution further split into monomeric units (amino acids).[57] The reactive nature of the magnetic liposomes with the blood proteins has been studied and the degradation profile of the proteins was measured using the spectrophotometric absorbance at 480 nm. From the results, the magnetic liposomes synthesised using chemical method were found to fasten the rate of disintegration of proteins to amino acids (Table 3 and Graph 1) than the biologically synthesised magnetic liposomes (Table 4 and Graph 2) and the control. Chemically prepared magnetic liposomes were found to be more interactive in protein disintegration.[56]

Interactions with bilirubin: The chemical and biological magnetic liposomes have almost the same effect on the bilirubin concentration in the blood. The effect of the magnetic liposomes degraded the bilirubin in the blood; thereby, there is a steady fall in its concentration (Table 5 and 6; Graph 3 and 4).

Interactions with lipids: The lipid concentration in blood almost remained the same throughout the period of experimentation. There was a very slight increase in the concentration of lipids in the blood that might be due to the disintegration of magnetic liposomes and successive release of their lipid components in the reaction mixture containing chemically synthesised magnetic liposomes (Table 7 and Graph 5). However, such lipid disintegration in biologically synthesised nanosomes (Table 8 and Graph 6) is lesser comparatively.

Interactions with RBCs: During the four hours incubation of RBCs with magnetic liposomes, around 70-80% of red blood cells disintegration was observed in the concentration of 10 mg/ml of chemically synthesised magnetic liposomes (Table 9 and Graph 7). In contrast, biologically synthesised magnetic liposomes induced 50% of cell lysis (Table 10 and Graph 8).

The reduction in the hemotoxicity of the biologically synthesised nanosome is positively reduced by the presence of phyto-capping agents present in the green tea extract used during the synthesis process.[59]

Table 3. Impact of chemical magnetic liposomes on proteins/ lipoproteins of blood

S. No.	Period of incubation (h)	Proteins (g/l) in samples containing liposomes (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	63.4	58.12	54.95	50.62	47.25	43.79	40.36
2.	0.5	61.01	54.85	51.90	48.23	44.8	41.37	38.2
3.	1	58.2	50.5	49.04	4593.	42.4	38.38	33.5
4.	1.5	56.43	48.46	45.28	40.84	37.8	33.7	31.8
5.	2	49.0	44.8	41.40	36.29	32.82	31.24	26.3
6.	2.5	45.7	40.36	39.93	30.73	29.8	26.8	23.5
7.	3	37.09	37.06	28.26	25.04	25.93	23.5	17.7
8.	3.5	32.28	29.35	23.96	21.49	21.2	17.2	13.46
9.	4	24.35	22.70	19.83	17.84	16.59	13.9	10.2
10.	4.5	18.87	16.3	15.36	13.79	11.29	10.3	9.9

Table 4. Impact of biological magnetic liposomes on proteins/ lipoproteins of blood

S. No.	Period of incubation (h)	Proteins (g/l) in samples containing liposomes (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	63.4	61	57.83	53.37	49.87	46.48	42.99
2.	0.5	61.01	57.73	54.78	50.98	47.42	44.06	40.83
3.	1	58.2	53.38	51.92	45.75	45.02	41.07	36.13
4.	1.5	56.43	51.34	48.16	43.59	40.42	36.39	34.43
5.	2	49.0	47.68	44.28	39.04	35.44	33.93	28.93
6.	2.5	45.7	43.24	42.81	33.48	32.42	29.49	26.13
7.	3	37.09	39.94	31.14	27.79	28.55	26.19	20.33
8.	3.5	32.28	32.23	26.84	24.24	23.82	19.89	16.09
9.	4	24.35	25.58	22.71	20.59	19.21	16.59	12.83
10.	4.5	18.87	19.18	18.24	16.54	13.91	12.99	12.53

Table 5. Impact of chemical magnetic liposomes on bilirubin/ albumin complexes of blood

S. No.	Period of incubation (h)	Bilirubin (g/l) in samples containing liposomes (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	54.1	50.72	48.6	45.52	42.7	39.36	35.4
2.	0.5	50.95	47.23	46.01	43.4	40.52	34.3	31.21
3.	1	49.65	46.5	45.31	41.17	39.32	36.4	33.24
4.	1.5	46.23	44.7	42.37	38.66	36.82	32.89	29.47
5.	2	44.0	40.26	37.87	36.58	33.36	28.71	26.5
6.	2.5	41.20	37.65	34.25	31.67	29.8	25.2	22.22
7.	3	39.36	34.86	29.37	27.66	24.5	21.8	17.65
8.	3.5	37.91	27.46	25.7	23.3	19.84	16.4	13.2
9.	4	32.42	22.8	20.4	18.8	15.8	12.9	10.24
10.	4.5	29.21	19.83	16.7	14.52	11.25	9.6	4.3

Table 6. Impact of biological magnetic liposomes on bilirubin/ albumin complexes of blood

S. No.	Period of incubation (h)	Bilirubin (g/l) in samples containing liposomes (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	54.1	53.6	51.35	48.14	45.39	41.99	38.17
2.	0.5	50.95	50.11	48.76	46.02	43.21	36.93	33.98
3.	1	49.65	49.38	48.06	43.79	42.01	39.03	36.01
4.	1.5	46.23	47.58	45.12	41.28	39.51	35.52	32.24
5.	2	44.0	43.14	40.62	39.2	36.05	31.34	29.27
6.	2.5	41.20	40.53	37	34.29	32.49	27.83	24.99
7.	3	39.36	37.74	32.12	30.28	27.19	24.43	20.42
8.	3.5	37.91	30.34	28.45	25.92	22.53	19.03	15.97
9.	4	32.42	25.68	23.15	21.42	18.49	15.53	13.01
10.	4.5	29.21	22.71	19.45	17.14	13.94	12.23	7.07

Table 7. Impact of chemical magnetic liposomes on lipids/fatty acids complexes of blood

S. No.	Period of incubation (h)	Lipids (g/l) in samples containing liposomes (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	2.47	2.46	2.45	2.47	2.44	2.42	2.46
2.	0.5	2.45	2.46	2.46	2.47	2.45	2.46	2.48
3.	1	2.40	2.47	2.47	2.48	2.45	2.48	2.49
4.	1.5	2.36	2.47	2.47	2.48	2.46	2.49	2.52
5.	2	2.31	2.47	2.47	2.49	2.47	2.50	2.54
6.	2.5	2.25	2.47	2.48	2.49	2.49	2.51	2.55
7.	3	2.20	2.48	2.49	2.49	2.51	2.53	2.56
8.	3.5	2.13	2.48	2.49	2.50	2.52	2.55	2.58
9.	4	2.09	2.49	2.5	2.52	2.54	2.56	2.60
10.	4.5	2.05	2.51	2.54	2.56	2.57	2.58	2.64

Table 8. Impact of biological magnetic liposomes on lipids/ fatty acids of blood

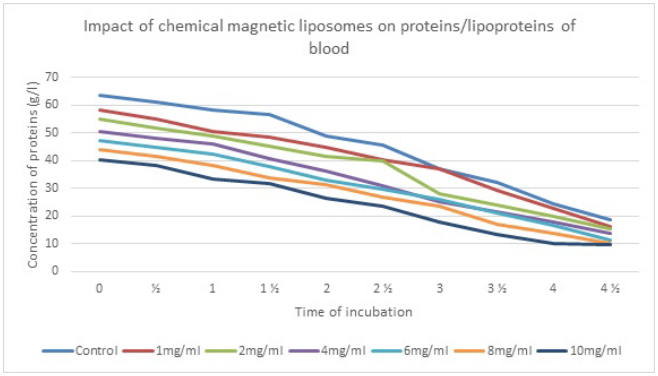
S. No.	Period of incubation (h)	Lipids (g/l) in samples containing liposomes (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	2.47	2.45	2.45	2.47	2.48	2.48	2.49
2.	0.5	2.45	2.45	2.45	2.47	2.48	2.48	2.49
3.	1	2.40	2.45	2.45	2.47	2.48	2.49	2.49
4.	1.5	2.36	2.45	2.45	2.47	2.49	2.49	2.50
5.	2	2.31	2.45	2.45	2.48	2.49	2.49	2.50
6.	2.5	2.25	2.46	2.46	2.48	2.49	2.49	2.51
7.	3	2.20	2.46	2.46	2.48	2.49	2.50	2.53
8.	3.5	2.13	2.46	2.46	2.48	2.49	2.50	2.53
9.	4	2.09	2.46	2.46	2.49	2.49	2.50	2.54
10.	4.5	2.05	2.46	2.47	2.49	2.49	2.51	2.55

Table 9. Effect ofchemically synthesised magnetic liposomes on RBC lysis

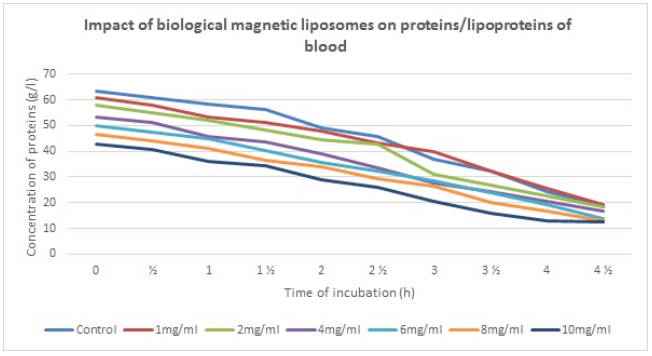
S. No.	Period of incubation (h)	% Haemolysis of the blood cells containing nanoparticles (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	0.24	1.52	1.98	2.84	3.68	4.96	8.74
2.	0.5	0.89	1.97	2.85	3.39	6.18	6.72	12.46
3.	1	1.12	2.83	3.59	7.7	9.48	13.16	16.96
4.	1.5	2.47	3.48	6.72	9.58	11.97	17.12	19.78
5.	2	4.10	6.28	9.35	10.47	14.45	20.9	25.2
6.	2.5	6.2	7.5	9.75	11.42	15.78	24.64	30.17
7.	3	7.36	9.14	11.72	14.7	29.39	39.94	43.8
8.	3.5	8.9	11.29	14.02	20.36	34.58	42.99	58.72
9.	4	10.03	13.09	27.15	31.73	40.94	57.18	64.25
10.	4.5	11.11	24.79	32.75	41.69	56.92	63.06	71.28

Table 10. Effect of biologically synthesised magnetic liposomes on RBC lysis

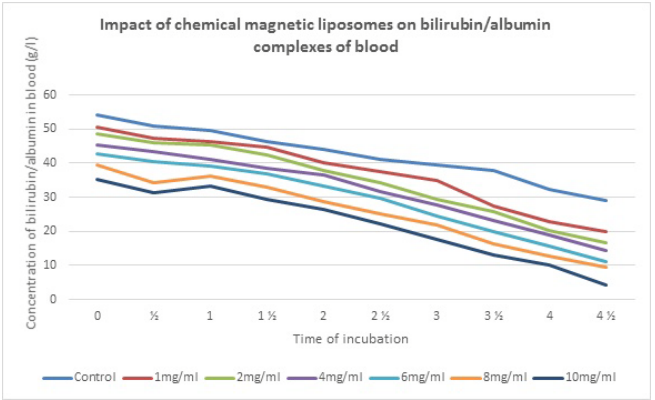
S. No.	Period of incubation (h)	% Haemolysis of the blood cells containing nanoparticles (mg/ml)						
		Control	1.0	2.0	4.0	6.0	8.0	10.0
1.	0	0.24	1.03	1.43	2.38	3.10	4.40	8.13
2.	0.5	0.89	1.48	2.3	2.93	5. 6	6.16	11.85
3.	1	1.12	2.34	3.04	7.24	8.9	12. 6	16.35
4.	1.5	2.47	2.99	6.17	9.12	11.39	16.56	19.17
5.	2	4.10	5.79	8.8	10.01	13.87	20.34	24.59
6.	2.5	6.2	7.01	9.2	10.96	15.2	24.08	29.56
7.	3	7.36	8.65	11.17	14.24	18.81	29.38	33.19
8.	3.5	8.9	10.8	13.47	19.9	24.0	32.43	38.11
9.	4	10.03	12.6	16.6	21.27	30.36	36.62	43.64
10.	4.5	11.11	14.3	22.20	31.23	36.34	42.5	50.67



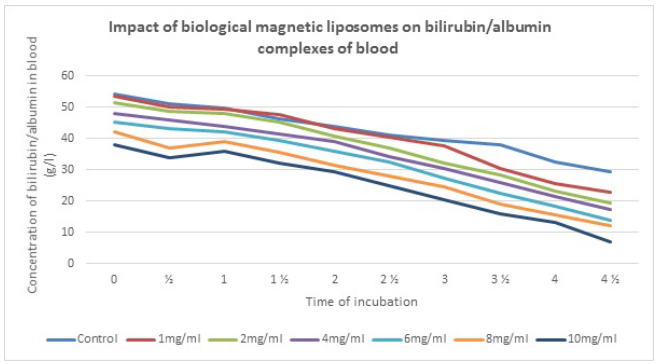
Graph 1. Impact of chemical magnetic liposomes on proteins/ lipoproteins of blood



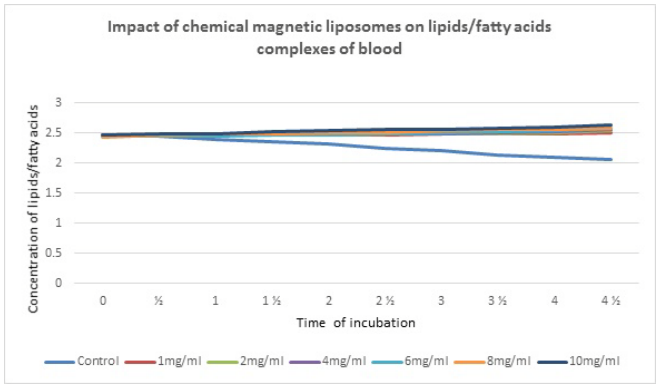
Graph 2. Impact of biological magnetic liposomes on proteins/ lipoproteins of blood



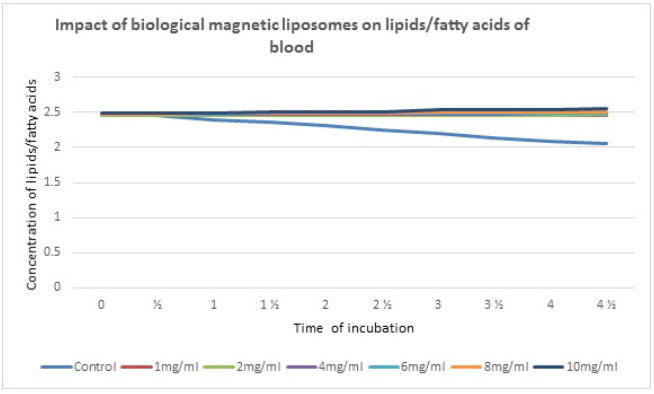
Graph 3. Impact of chemical magnetic liposomes on bilirubin/albumin complexes of blood



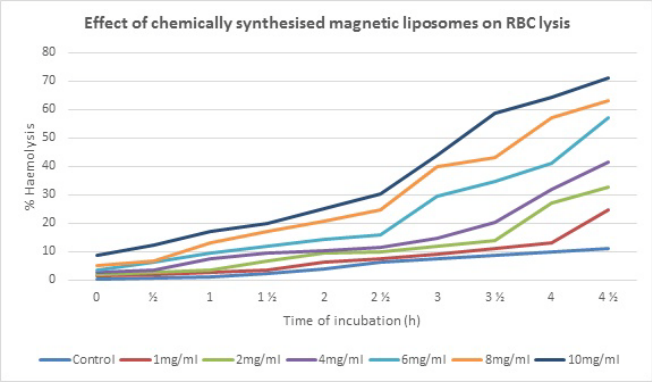
Graph 4. Impact of biological magnetic liposomes on bilirubin/albumin complexes of blood



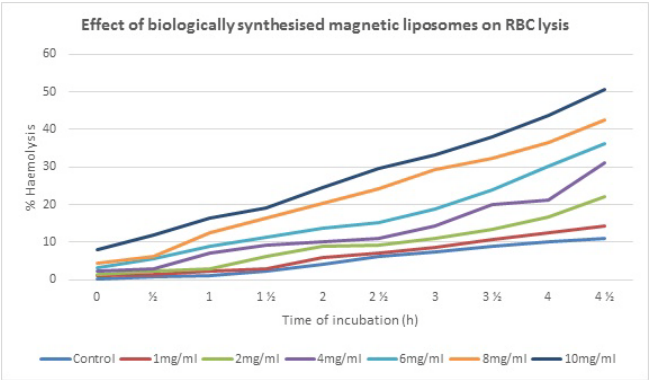
Graph 5. Impact of chemical magnetic liposomes on lipids/ fatty acids complexes of blood



Graph 6. Impact of biological magnetic liposomes on lipids/ fatty acids of blood



Graph 7. Effect of chemically synthesised magnetic liposomes on RBC lysis



Graph 8. Effect of biologically synthesised magnetic liposomes on RBC lysis

On the whole, the haemotoxicity of the chemically synthesised magnetic liposomes is very much lower when compared to the chemically synthesised magnetic nanoparticles. About 20-25% of lower toxicity of these magnetic liposomes compared with that of their precursors is mainly due to the liposomes that act as shielding or capping agents to the blood cells from the toxic nature of the magnetic nanoparticles.

Conclusion:

The present research study compared the method of preparations of magnetic liposomes and their efficiency in encapsulation of magnetic nanoparticles and their blood toxicity. From the results obtained, it can be concluded that green synthesis of magnetic lipsomes was better than chemical synthesis in terms of its toxicity in blood. The green tea polyphenols were identified as the efficient phytocomponents for producing magnetic nanoparticles with lesser toxicity and better encapsulation into liposomes. Thus, green synthesised ferrite-based magnetic liposomes made up of cholesterol can be used for the treatment of human disorders. This formulation can be further explored for targeted thermo-chemotherapy of cancers.

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References:

1. Abduz Z & Abdul R. Evaluation of different extracts and synthesized silver nanoparticles from leaves of *Euphorbia prostrata* against *Haemaphysalis bispinosa* and Hippobosca maculate. Veterinary Parasitology. 2012. (187): 511–20.
2. Abe M, Schechter D, Schechter RS, Wade WH, Weerasooriya U & Seang Yiv. Microemulsion formation with branched tail polyoxyethylene sulfonate surfactants. Journal of Colloid and Interface Science. 1986. (114): 342–356.
3. Alexiou C, Arnold W, Klein RJ, Parak FG, Hulin P, Bergemann C et al. Locoregional cancer treatment with magnetic drug targeting. Cancer Research. 2001. 60 (23): 6641–8.
4. Aniansson EAG & Wall SN. Kinetics of step-wise micelle association. Journal of Physical Chemistry. 1974. 78: 1024–30.
5. Ansari SG, Ansari ZA, Wahab R, Kim YS, Khang G & Shin HS. Glucose sensor based on nano-baskets of tin oxide templated in porous alumina by plasma enhanced CVD. Biosensors and Bioelectronics. 2008. (23).
6. Aqil M, Ahad A, Sultana Y & Ali A. Status of terpenes as skin penetration enhancers. Drug Discovery Today. 2007. (12): 1061–7.
7. Azaroff LV, Kaplow R, Kato N, Weiss RJ, Wilson AJC & Young RA. X-ray diffraction. New York: McGraw-Hill; 1974.
8. Bharali DJ, Khalil M, Gurbuz M, Simone TM & Mousa SA. Nanoparticles and cancer therapy: a concise review with emphasis on dendrimers. International Journal of Nanomedicine. 2009. (4): 1–7.
9. Brewer E, Coleman J & Lowman A. Emerging technologies of polymeric nanoparticles in cancer drug delivery. Journal of Nanomaterials. 2011.
10. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT & Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens; Colloids and Surfaces B: Biointerfaces. 2010. (76): 50–56.
11. Cheong I, Huang X, Thornton K, Diaz LA Jr & Zhou S. Targeting cancer with bugs and liposomes: ready, aim, fire. Cancer Research. 2007. 67(20): 9605–8.
12. Chowdary KP & Rao YS. Mucoadhesive microspheres for controlled drug delivery. Biological and Pharmaceutical Bulletin. 2004. 27(11): 1717–24.
13. Davis ME, Chen ZG & Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nature Reviews Drug Discovery. 2008. 7: 771–782.
14. DeGennes PG & Taupin C. Microemulsions and the flexibility of oil/water interfaces. The Journal of Physical Chemistry. 1982. 86: 2294–304.
15. Deitel M, Friedman KL, Cunnane S, Lea PJ, Chaiet A, Chong J et al. Emulsion stability in a total nutrient admixture for total parenteral nutrition. Journal of the American College of Nutrition. 1992. 11(1): 5–10.
16. Markides H, Rotherham M & El Haj AJ. Biocompatibility and toxicity of magnetic nanoparticles in regenerative medicine. Journal of Nanoscience and Nanotechnology. 2012.
17. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nature Reviews Cancer. 2005. 5: 161–71.
18. Gao ZG, Fain HD & Rapoport N. Controlled and targeted tumor chemotherapy by micellar-encapsulated drug and ultrasound. Journal of Controlled Release. 2005. 102: 203–22.

19. Hamaguchi T, Kato K, Yasui H, Morizane C & Ikeda M. A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation. British Journal of Cancer. 2007. 97: 170–76.
20. Heyes ME, Bourrel MU, El-Emary MM, Schechter RS & Wade WH. Interfacial tension and behavior of nonionic surfactants. SPE Journal. 1979. 19: 349–356.
21. Hoar TP & Schulman JH. Transparent water-in-oil dispersions: the oleopathic hydro-micelle. Nature. 1943. 152: 102–103.
22. Devi JS & Bhimba BV. Silver nanoparticles: antibacterial activity against wound isolates and *in vitro* cytotoxic activity on human caucasian colon adenocarcinoma. Asian Pacific Journal of Tropical Disease. 2012. 87–90.
23. Jain RK & Stylianopoulos T. Delivering nanomedicine to solid tumors. Nature Reviews Clinical Oncology. 2010. 7: 653–664.
24. Kumara KM, Sinhaa M, Mandala BK, Ghoshb AR, Kumar KS & Reddy PS. Green synthesis of silver nanoparticles using *Terminalia chebula* extract at room temperature and their antimicrobial studies. Spectrochimica Acta Part A. 2012. 91: 228–33.
25. Kikumori T, Kobayashi T, Sawaki M & Imai T. Anti-cancer effect of hyperthermia on breast cancer by magnetite nanoparticle-loaded anti-HER2 immunoliposomes. Breast Cancer Research and Treatment. 2009. 113: 435–441.
26. Kim DW, Kim SY, Kim HK, Kim SW, Shin SW, Kin JS et al. Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel with cisplatin in patients with advanced non-small-cell lung cancer. Annals of Oncology. 2007. 18: 2009–14.
27. Kiwada H, Sato J, Yamada S & Kato Y. Feasibility of magnetic liposomes as a targeting device for drugs. Chemical and Pharmaceutical Bulletin. 1986 34 (10): 4253–8.
28. Kornek GV, Ulrich-Pur H, Penz M, Haider K, Kwasny W, Depisch D et. al. Treatment of advanced breast cancer with vinorelbine and docetaxel with or without human granulocyte colony-stimulating factor. Journal of Clinical Oncology. 2001. 19 (3): 621–7.
29. Kumarasamyraja D, Jeganathan NS & Manavalan R. Phytochemical investigation and antimicrobial activity of *Acalypha indica*. International Journal of Pharmaceutical Science. 2012. 1(6): 313–16.
30. Kwon GS & Forrest ML. Amphiphilic block copolymer micelles for nanoscale drug delivery. Drug Development Research. 2006. 67: 15–22.
31. Licciardi M, Giammona G, Du J, Armes SP, Tang Y & Lewis AL. New folate functionalized biocompatible block copolymer micelles as potential anti-cancer drug delivery systems. Polymer. 2006. 47: 2946–55.
32. McBain SC, Yiu HH & Dobson J. Magnetic nanoparticles for gene and drug delivery. International Journal of Nanomedicine. 2008. 3(2): 169–80.
33. Mehta RC, Head LF, Hazrati AM, Parr M, Rapp RP & DeLuca PP. Fat emulsion particle-size distribution in total nutrient admixtures. American Journal of Hospital Pharmacy. 1992. 49: 2749–55.
34. Mikhail AS & Allen C. Block copolymer micelles for delivery of cancer therapy: transport at the whole body, tissue and cellular levels. Journal of Control Release. 2009. 138: 214–23.
35. Morse MA. Supportive care in the management of colon cancer. Support Cancer Therapy. 2006 3 (3): 158–70.
36. Mukunthan KS, Elumalai EK, Patel TN & Murty VR. *Catharanthus roseus*: a natural source for the synthesis of silver nanoparticles. Asian Pacific Journal of Tropical Biomedicine. 2011. 270–74.
37. Muller RN, Roch A, Colet JM, Ouakssim A & Gillis P. The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging. New York: Wiley J & Sons; 2001. pp. 417–435.
38. Narang AS & Varia S. Role of tumor vascular architecture in drug delivery. Advanced Drug Delivery Reviews. 2011. 63: 640–658.
39. Nie S. Understanding and overcoming major barriers in cancer nanomedicine. Nanomedicine. 2010. 5: 523–528.
40. Nishyama N & Kataoka K. Polymeric micelle drug carrier systems: PEGPAsp (Dox) and second generation of micellar drugs. Polymer Drugs in the Clinical Stage. 2003. 519: 155–177.
41. Petros RA & DeSimone JM. Strategies in the design of nanoparticles for therapeutic applications. Nature Reviews Drug Discovery. 2010. 9: 615–627.
42. Pounder RJ, Willcock H, Ieong NS, O'Reilly RK & Dove AP. Stereocomplexation in novel degradable amphiphilic block copolymer micelles of poly (ethylene oxide) and poly(benzyl a-malate). RSC Soft Matter. 2011. 7: 10987–93.
43. Pouton CW, Wagstaff KM, Roth DM, Moseley GW & Jans DA. Targeted delivery to the nucleus. Advanced Drug Delivery Reviews. 2007. 59: 698–717.
44. Recht A, Come SE, Gelman RS, Goldstein M, Tishler S, Gore SM et al. Integration of conservative surgery, radiotherapy, and chemotherapy for the treatment of early-stage, node-positive breast cancer: sequencing, timing, and outcome. Oncology. 1991. 9(9): 1662–7.
45. Rey JB, Faure C & Brion F. Stability of all-in-one standard formulae for paediatric parenteral nutrition. PDA Journal of Pharmaceutical Science and Technology. 2005. 59: 206–20.
46. Banu S, Thamarai RS, Farzana ZS & Baskar V. Polymer coated magnetic nanoparticles mediated delivery of anti-Alzheimer drug and their characterisation. Journal of Biological and Information Sciences. 2013. 2(3). 21–27.
47. Wu W, He Q & Jiang C. Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies. Nano Research Letters. 2008. 3(11): 397–415.
48. Gramza A, Pawlak-Lemañska K, Korczak J, Wasowicz E & Rudzinska M. Tea extracts as free radical scavengers. Polish Journal of Environmental Studies. 2005. 14(6): 861–867.
49. Mahdavi, Namvar, Ahmad & Mohamad. Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using seaweed (*Sargassum muticum*) aqueous extract. Molecules. 2013. 18: 5954–5964.
50. Han V, Serrano K & Devine DV. A comparative study of common techniques used to measure haemolysis in stored red cell concentrates. International Society of Blood Transfusion. 2009.
51. Malagoli D. A full-length protocol to test haemolytic activity of palytoxin on human erythrocytes. Modena: University of Modena and Reggio Emilia; 2007. pp. 92–94.
52. Downing DT, Abraham W, Wegner BK, Willman KW & Marshall JL. Partition of sodium dodecyl sulfate into stratum corneum lipid liposomes. Archives of Dermatological Research. 1993. 285: 151–157.
53. Kakar S & Singh R. Preparation of magnetic microspheres of mesalamine by phase separation emulsion polymerisation technique. African Journal of Pharmacy and Pharmacology. 2014. 8(9): 246–258.
54. Kandpal ND, Sah N, Loshali R, Joshi R & Prasad J. Co-precipitation method of synthesis and characterisation of iron oxide nanoparticles. Journal of Scientific and Industrial Research. 2014. 73: 87–90.
55. Herlekar M, Barve S & Rakeshkumar. Plant-mediated green synthesis of iron nanoparticles. Journal of Nanoparticles. 2014: 1–9.
56. Elsaesser A & Howard VC. Toxicology of nanoparticles. Advanced drug delivery reviews. 2012. 64: 129–137.
57. Mahdavi M, Namvar F, Ahmad M & Mohamad R. Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using seaweed (*Sargassum muticum*) aqueous extract. Molecules. 2013. 18: 5964–5964.