5.1 Magnetic liposomes as drug targeting carriers

With the growing interest in the field of nanotechnology, it has now become possible to synthesize, characterize and specially tailor the functional properties of nanoparticles for clinical and diagnostic applications. Such an approach would considerably improve the efficiency of drug delivery to the specific target site in a human body, and most importantly minimize the toxic side and after effects of the drug. One of such nanoparticle type is magnetic nanoparticles; the use of magnetic nanoparticles can contribute to a precise delivery of drugs to the exact site (e.g. inflammation, cancer etc.) by application of an external magnetic field (Hofmann *et al.*, 2001).

In the past, chemotherapy targeted by magnetic fields using magnetic particles has shown encouraging results (Widder *et al.,* 1983; Gupta *et al.,* 1993; Lubbe *et al.,* 2001). The concept of magnetoliposomes for various biological and medical applications is in existence since 1980s. As already mentioned earlier (table 7), several groups have investigated the use of magnetoliposomes mainly for hyperthermia, as magnetic resonance contrast enhancing agents, few studies on site-specific drug targeting and for cell sorting.

Although, active targeting of anticancer drugs using magnetically responsive carriers offers very attractive treatment approach for solid tumors, however successful results are limited. In view of the above, the current work was undertaken to develop and optimize a stabilized magnetoliposomal preparation and further evaluate it for targeting drug to solid tumors.

5.1.1 Preparation of Magnetoliposomes (MLs)

To achieve this objective, magnetoliposomes were prepared and characterized as described in materials and methods. The methods reported by Sangregorio *et al.* (1999) and Babincova *et al.* (2002) were used as a base to devise a modified method for preparation of magnetoliposomes. The procedure was optimized and the amount of magnetite that can be entrapped within the core of liposome was determined.

Large unilamellar MLs were prepared by reverse phase evaporation. First, colloidal magnetite was synthesized by alkaline co-precipitation of Fe^{+2} and Fe^{+3} salts. The precipitated magnetite were extensively washed with water, methanol and stored in 100% methanol. Just before the preparation of magnetoliposomes, necessary amounts of magnetite were washed several times in chloroform. The lipid (soyabean phosphatidyl choline), cholesterol and dihexadecyl phosphate were dissolved in chloroform and added to the chloroform-washed magnetite precipitates. Finally, the chloroform was evaporated in a rotary evaporator and held under vacuum for 2 hours to remove the traces of solvent. The film containing magnetic nanoparticles dispersed colloidally in the lipid was immediately reconstituted in isopropanol with rapid vortexing. The solution was then injected in aqueous buffer (preferably 300 mM citric acid, pH 4.0) and extensively sonicated. Organic solvent was evaporated in a rotary evaporator to obtain large unilamellar MLs. Compared to conventional magnetoliposome synthesize procedure where fatty acid coated magnetite particles needs to be dialyzed at 37°C for 2-3 days in

presence of pre-formed liposomes, with the present method the time taken to prepare magnetoliposomes was only 4-5 hours. The MLs thus prepared were then characterized physically and in terms of its integrity and stability.

5.1.2 Physical characterization of the Magnetoliposomes

As seen in figure 45, there is clear difference in color of prepared magnetoliposomes and naked magnetite. So, when magnetite is covered with phospholipid coat the color of the resulting suspension is brown as compared to naked magnetite, which is black. The prepared magnetoliposomes are highly susceptible to the external magnetic field. When the ML suspension was kept in front of a magnet with strength 0.3 Tesla, all of the magnetoliposomes were attracted towards the magnet and forms a pellet in less than 2 min (Fig. 46).

5.1.3 TEM characterization of the Magnetoliposomes

A typical TEM image of a magnetoliposome sample is shown in Fig. 47. The micrograph clearly shows the occurrence of relatively uniform, spherical shaped nanosized particles. The particles are well separated and their average size is of ~25 nm. TEM evidence of the occurrence of the phospholipid coating is obvious by transmission electron micrograph of samples stained with 0.5% uranyl acetate solution. The images show the occurrence of a thin layer surrounding each particle, which is attributed to the liposomes bilayer membrane. The MLs prepared with the present method is comparable to that reported by Sangregoria *et al.* (1999).

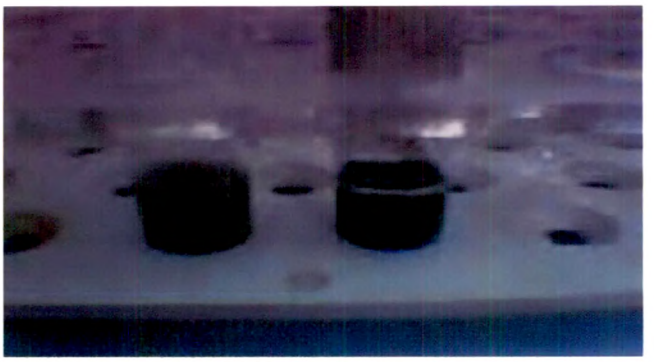

Figure 45: Colour and texture of MLs (left) and magnetite (right)

Figure 46: Magnetic property of MLs

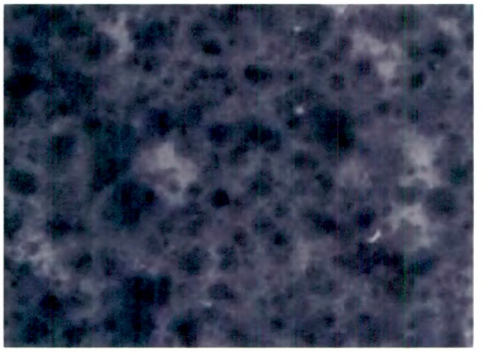

Figure 47. TEM of ML taken at 140,000 X magnification. The average size of the particles is ~25 nm.

5.1.4 Biochemical characterization of the Magnetoliposomes

The magnetoliposomes prepared with the present method was also characterized in terms of phospholipid/Fe_3O_4 ratio. The amount of Fe_3O_4 entrapped within the core of the liposomes was determined from iron estimation (Ramsay, 1957) after digestion of an aliquot with concentrated H_2SO_4. Initially, estimation of iron with α-α dipyridyl method gave problem due to the extreme acidic condition of the assay system. The problem was sorted out by using 1:1 ratio of acetate buffer (pH 4.0) and water to make up the volume after digestion was accomplished. The amount of phospholipid was determined from phosphorus estimation (Fiske and Subbarow, 1925) after digestion of the sample with 70% perchloric acid. The phospholipid to Fe_3O_4 ratio of the present magnetoliposomal preparation was 0.32 ± 0.05 mmol/g.

5.1.5 Drug encapsulation efficiency of magnetoliposomes

Drug loading efficiency of present magnetoliposomal suspension was determined by encapsulation of doxorubicin (DOX). It is one of the anticancer drug possessing good therapeutic efficacy but higher side effects that include cardiotoxicity and nephrotoxicity. However, numerous studies have shown that liposomal encapsulation of doxorubicin reduces its toxic side effects while maintaining or, in some instances increasing antitumor potency. Moreover, the ability to target, control and produce efficient release from the liposomes would be extremely advantageous. In the present study, doxorubicin was

encapsulated within the core of the magnetoliposomes using transmembrane pH gradient method described by authors (Mayer *et al.,* 1990). Briefly, pH of the MLs suspension, initially at 4.0 (300 mM citric acid) was raised to 7.5 with 400 mM sodium carbonate and pre-incubated at 60°C. ML suspension was then mixed with preheated DOX at a drug /lipid ratio of 0.2 (w/w).

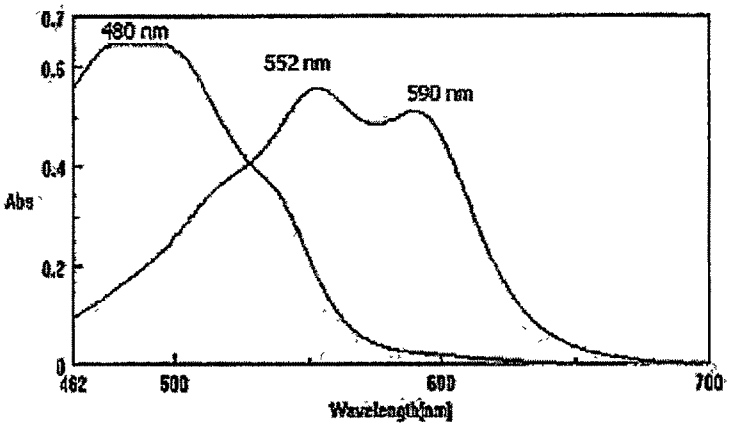

Figure 48. Spectral shift of DOX. At pH 7 5 – DOX shows peak at 480 nm; pH 10 5 – DOX shows peak at 552 and 590 nm

Free and encapsulated doxorubicin in magnetoliposomal systems was determined spectrophotometrically employing a Jasco V-530 spectrophotometer. This method utilizes pronounced change in absorbance (absorbance maxima 480 nm, pH 7.5; 550 and 592 nm, pH 10.5) observed on increasing the pH of doxorubicin solution from 7.5 to 10.5 (Fig.48). The sequence of measurement made is already described in materials and methods. Free/total doxorubicin ratio were calculated as the absorbance at 600 nm upon NaOH addition divided by the absorbance after Triton X-100 treatment. In all

cases, liposomes (without magnetic particles) encapsulated with DOX were simultaneously analyzed.

Table 24. DOX encapsulation efficiency in liposome/ML system

Samples	Liposomes (n=6)	Magnetoliposomes (n=6)
Free DOX (%)	2.21 ± 0.014	2.93 ± 0.016
Encapsulated DOX (%)	97.79 ± 0.013	97.07 ± 0.017

As observed in table 24, the encapsulation efficiency of doxorubicin was found to be greater than 95% with both liposomal and magnetoliposomal suspension. Thus, the magnetoliposomes prepared in the present study compares well with liposomes in terms of its efficiency to entrap DOX.

5.1.6 Storage stability of liposomes/magnetoliposomes-encapsulated doxorubicin

In order to avoid batch-to-batch variation and use the drug loaded liposomal formulation for long-term purposes; a need was felt to investigate the storage stability of liposome/ML-encapsulated doxorubicin. The procedural design for this experiment is already discussed in materials and methods.

Table 25: Stability of Liposome/ML encapsulated doxorubicin stored at 0-4°C

Duration	ENCAPSULATED DOX	
	Liposomes (%) (n=3)	**Magnetoliposomes (%) (n=3)**
0 Day	100	100
7 days	99.01	99.40
14 days	100	98.40
21 days	100	99.50
28 days	99.80	96.52

From the results obtained (table 25), it can be said that more than 95% of DOX remain associated with liposomes and MLs thereby indicating the intactness of the lipid bilayer. Therefore, the liposomal/magnetoliposomal formulation was found to be stable for at least 4 weeks when stored at 4°C. The presence of cholesterol and saturated fatty acids containing phospholipid appears to be the most important factors that contribute to the bilayer rigidity, thereby preventing the leakage of drug from the liposome/ML (Bally *et al.,* 1994; Gabizon *et al.,* 1993). Additionally, other factor such as remote loading of drug against the pH gradient results in high interior concentration of the drug.

5.2 *In vitro* drug release characteristics of liposomes / magnetoliposomes

5.2.1 Dilution induced drug release from liposomes / magnetoliposomes

When the formulation is brought into circulation the liposomes faces the greater dilution due to the larger volume of the circulating fluid. This dilution can either disturb the equilibrium distribution of the drug or it may disrupt the pH gradient across the lipid bilayer. This may cause membrane instability and leakage of drug before reaching the target site. So, there was a need to check whether the preparation is suitable to withstand the effect of dilution and bring about necessary changes in the existing composition if required.

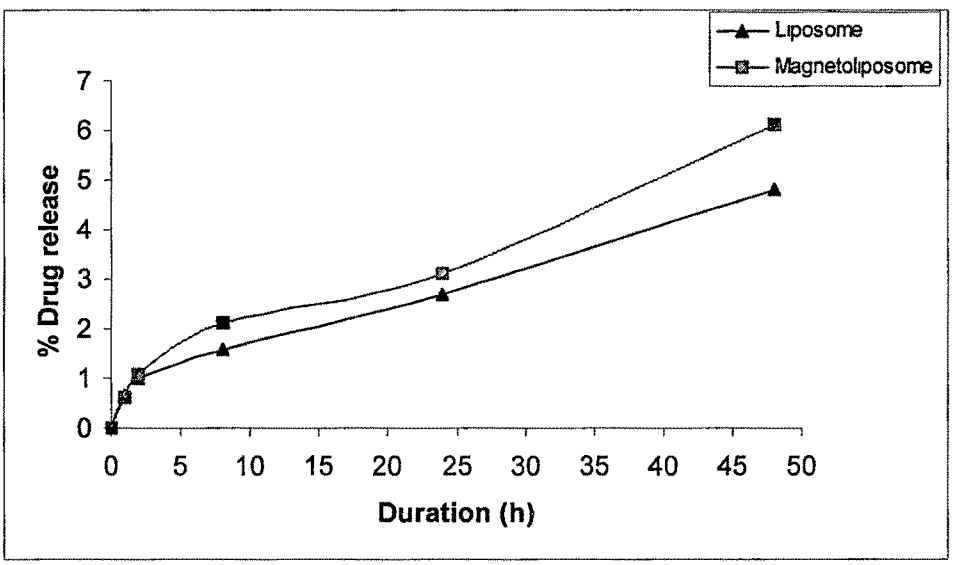

Figure 49: Dilution induced drug leaching from liposomal/magnetoliposomal preparation

In vitro drug release of liposome/ML-encapsulated doxorubicin was monitored by dialyzing samples against large volumes (1 litre) of phosphate buffered saline (pH 7.5) for 48 h at 37°C. At the indicated times, aliquots were removed and entrapped doxorubicin was determined. The result obtained shows greater than 90% of the drug remains associated with liposomes (Fig. 49). This was perhaps due to the fact that no agent was present to disrupt the transmembrane pH gradient. This served as a control for spontaneous drug release, illustrating that the phospholipid-cholesterol bilayer remains tightly packed at 37°C, maintaining the pH gradient and preventing drug transport across the bilayer (Mayer *et al.,* 1986).

5.2.2 Effect of serum component on the stability of liposome / magnetoliposomes-encapsulated doxorubicin

This experiment was performed to examine whether the serum components have any influence on the integrity of liposomes/MLs. The DOX encapsulated liposomal/magnetoliposomal suspension was incubated with 50% fetal calf serum for 24 h at 37°C. As indicative from the Figure 50 and 51, in the presence of serum 15 to 25% of DOX was released, where as with PBS only 2-3% was released. Thus, release of drug appears to be at least partially dependent on protein-lipid interactions (Bonte *et al.,* 1986; Semple *et al.,* 1996). The serum components such as serum proteins, lipoproteins and proteins of the complement system may destabilize the lipid bilayer resulting in drug leakage. The problem could be sorted-out by coating the surface of

liposomes with polyethylene glycol. This will reduce lipid-protein interactions and uptake of liposomes by reticulo-endothelial system, thereby increasing the circulation time in blood. However, due to the financial constraint it was not possible to procure polyethylene glycol tagged phosphatidyl ethanolamine. Additionally, since the present study was undertaken to establish the proof of concept for developing a magnetoliposomal formulation for drug targeting, the liposomes/MLs used were non-PEgylated.

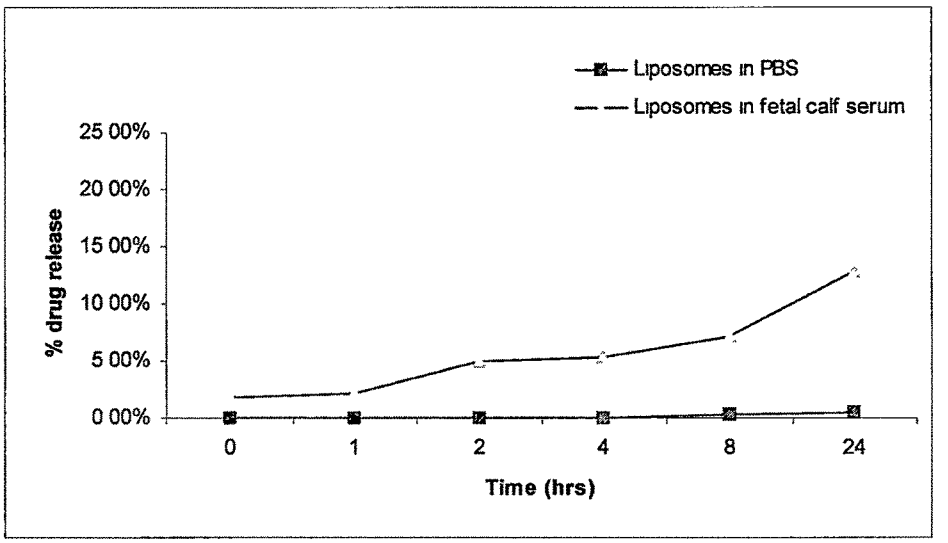

Figure 50. Serum induced DOX release from liposomes

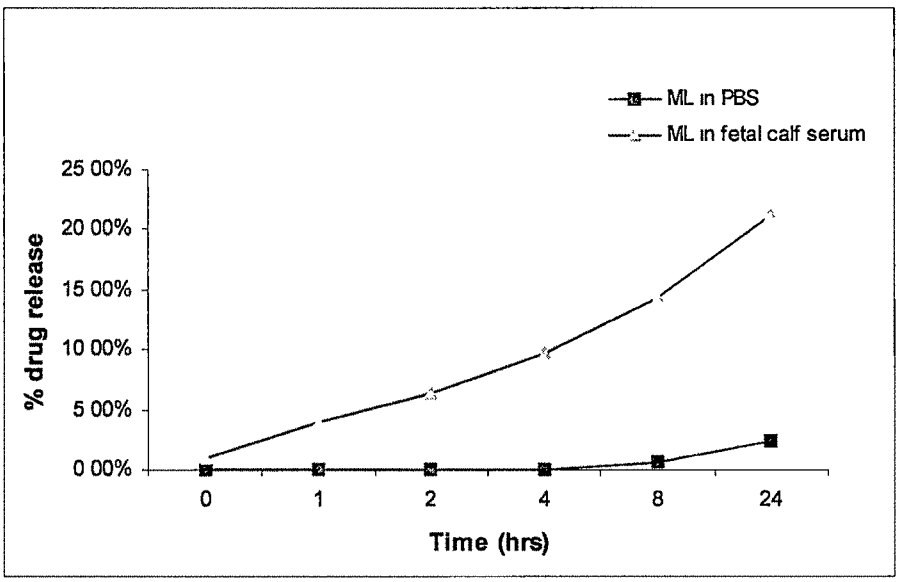

Figure 51. Serum induced DOX release from magnetoliposomes

5.2.3 Stability of liposomes / magnetoliposomes in closed circulatory system (*in vitro* study)

Blood is a complex mixture of cells, proteins, lipoproteins, and ions by which nutrients and wastes are transported. Red blood cells typically comprise approximately 40 -45% of whole blood by volume. Blood is approximately four times more viscous than water. Moreover, blood does not exhibit a constant viscosity at all flow rates and is especially non-Newtonian in the microcirculatory system. The non-Newtonian behavior is most evident at very low shear rates when the red blood cells clump together into larger particles. Blood also exhibits non-Newtonian behavior in small branches and capillaries, where the cells squeeze through and a cell-free skimming layer reduces the effective viscosity through the tube. However, in most arteries, blood behaves in a Newtonian fashion, and the viscosity can be taken as a constant, 4 centipoise (cP). As a reference, the average viscosity of whole blood in a normal subject is considered to be 4.5 ± 0.3 cP (Devereux *et al.,* 1984). Also blood flow and pressure are unsteady, which along with the cyclic nature of the heart pumping creates pulsatile conditions in all arteries. So, when the formulation is introduced in the body several factors come into play in an *in vivo* condition. Therefore, a formulation should be prepared that is able to resist these opposing forces and is able to efficiently transfer the drug to the target with minimal leaching during circulation.

In order to check the flow properties; an *in vitro* circulatory model system was designed whose functioning is described earlier in materials and methods. To study the effect of friction provided by the capillary walls the formulation was passed in saline through the model system. Moreover to study the effect of blood viscosity, the formulation was passed in 5% dextran 500 (4.5 cP) having viscosity equivalent to that of blood.

As inferred from the figures 52 and 53, the frictional drag exerted by the capillary wall on the flow characteristics of the liposomes/MLs was found to be minimal even after passing the formulation in saline through a distance of 20 meters. There was only 3% leakage of the DOX from the liposomes/MLs. However the model was limiting in not being compatible to capillaries of varying diameter.

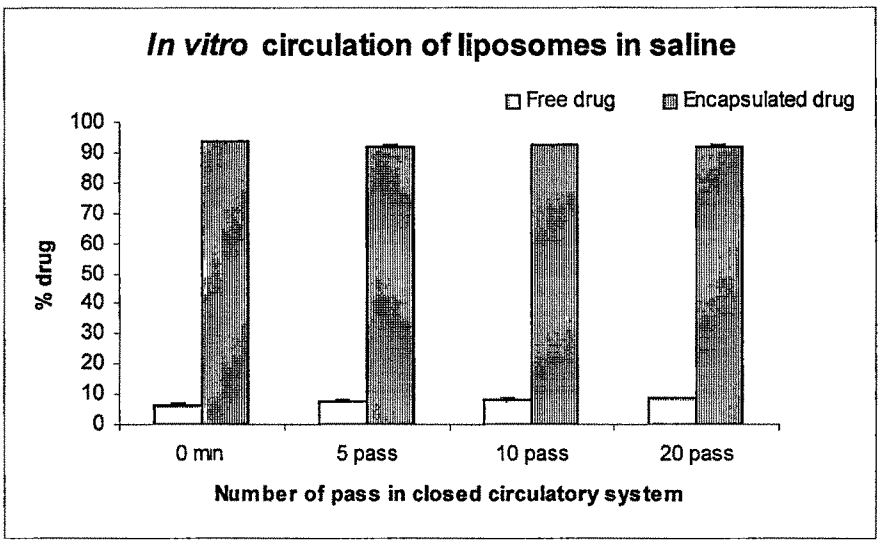

Figure 52: *In vitro* circulation stability of liposome encapsulated DOX in normal saline. Results are expressed as Mean ± SE, (n = 6), Length of capillary used in model system – 0 3 mm, Flow rate – 10 ml/min

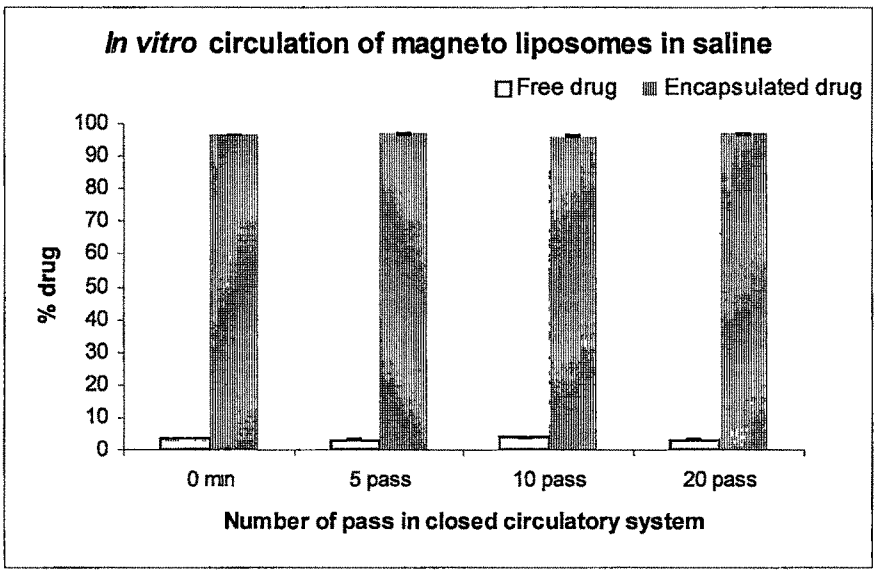

Figure 53: *In vitro* circulation stability of magnetoliposomes encapsulated DOX in normal saline Results are expressed as Mean ± SE, (n = 6), Length of capillary used in model system – 0.3 mm, Flow rate – 10 ml/min

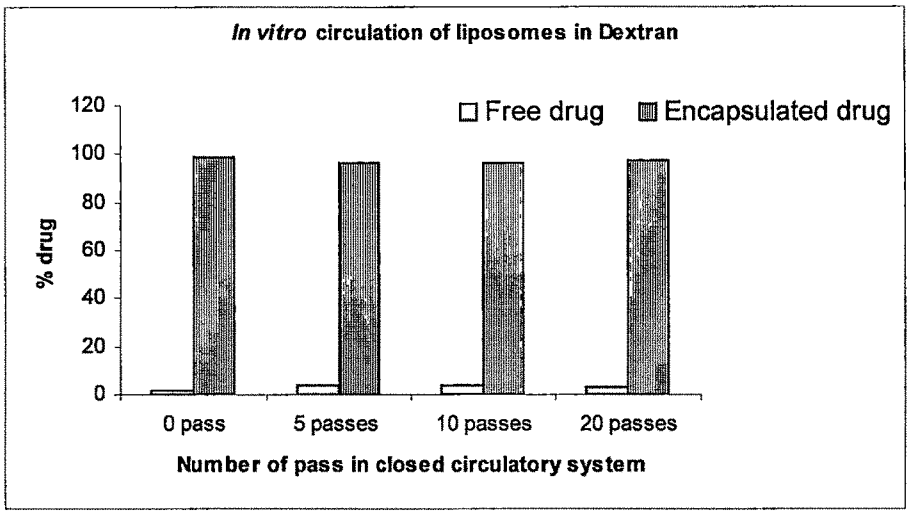

Figure 54: *In vitro* circulation stability of liposomes encapsulated DOX in 5% dextran Results are expressed as Mean ± SE, (n = 6); Length of capillary used in model system – 0 3 mm, Flow rate – 10 ml/min

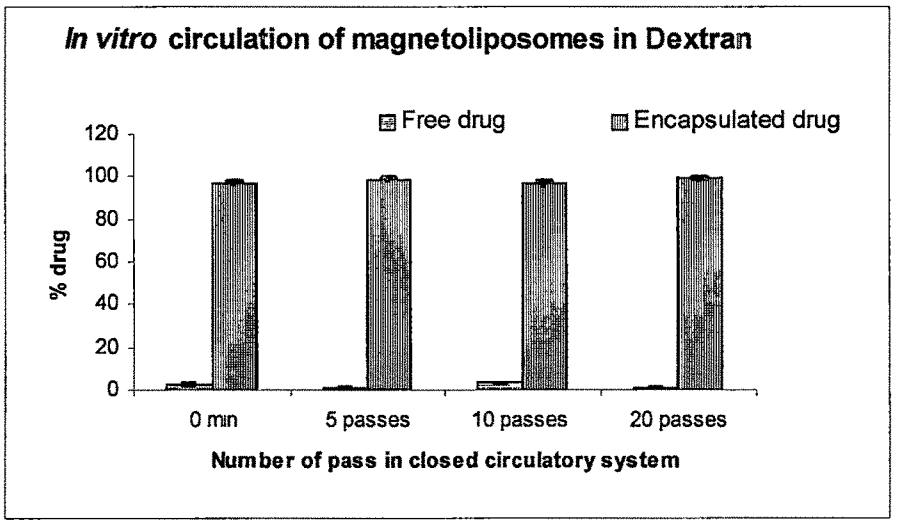

Figure 55: *In vitro* circulation stability of magnetoliposomes encapsulated DOX in 5% dextran Results are expressed as Mean ± SE, (n = 6), Length of capillary used in model system – 0 3 mm, Flow rate – 10 ml/min

As already mentioned, in addition to the frictional force the viscosity may also provide hindrance in the free flow of the liposomes in circulation. To study the effect of viscosity the formulation was suspended in 5% dextran T500 solution (viscosity 4.5cP) and then circulated through the model system. After passing through a length of 20 meters at 10 ml/min no substantial leakage of the drug from the formulation was observed as judged by free drug levels (Figure 54 and 55). Thus, it indicates the ability of the drug-encapsulated liposomes/MLs to withstand the viscosity of blood during flow within the capillaries.

5.3 *In vivo* biodistribution of DOX

After the optimization of magnetoliposomal formulation in terms of characteristics, *in vitro* stability during storage and circulation, the next goal was check the *in-vivo* distribution and efficacy of this formulation.

Table 26: Tissue distribution of doxorubicin after intravenous administration

Groups	Liver (μg/gm)	Spleen (μg/gm)	Kidney (μg/gm)	Heart (μg/gm)	Lung (μg/gm)
Dox	0.971 ± 0.109	12.05 ± 1.20	6.38 ± 0.86	3.32 ± 0.20	7.17 ± 2.93
Lip-DOX	16.18 ± 3.15	13.76 ± 2.26	2.28 ± 0.12	2.003 ± 0.64	3.799 ± 0.9
ML-DOX	17.75 ± 6.37	20.23 ± 6.47	3.43 ± 0.71	1.48 ± 0.25	9.85 ± 2.92

Results are mean ± SE; n=6

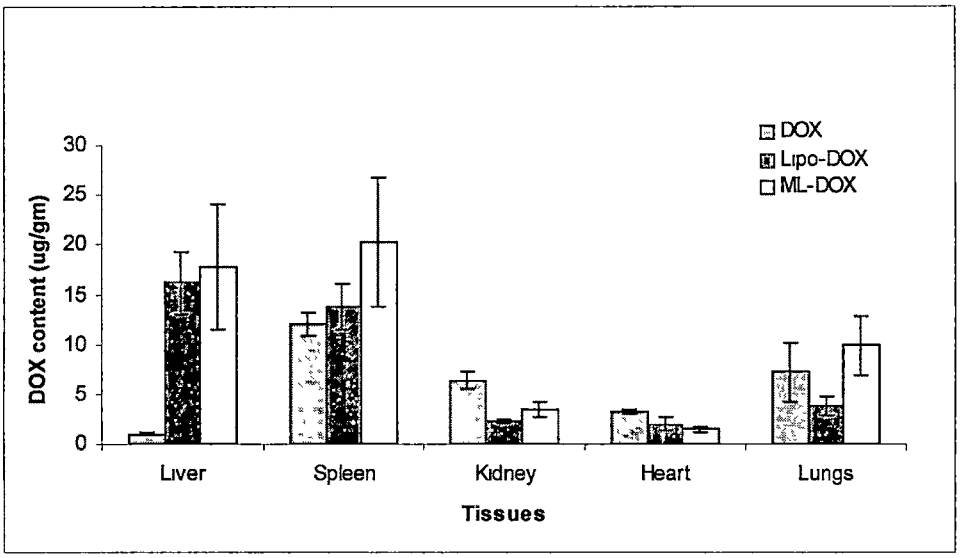

Figure 56: Biodistribution of DOX after intravenous administration in rat

To determine the *in vivo* tissue distribution of DOX; animals were injected intravenously with 5 mg/kg body weight of free DOX, liposome-DOX and ML-DOX. 24 hours following treatment, the animals were sacrificed. This time point was selected because earlier reported studies have demonstrated that maximum hepatic liposomal doxorubicin uptake occurs 24 hours after administration (Gabizon *et al.,* 1989). The fluorescent property of doxorubicin was used for quantification of the amount of tissue doxorubicin. Tissues (liver, kidney, heart, spleen and lung) were harvested, weighed, and homogenized in acid alcohol (0.3-N hydrochloric acid, 50% alcohol), and extraction was continued for 24 hours at 5°C. The supernatant separated from the tissue homogenate after 24 hours was used to estimate doxorubicin concentration. Spectrofluorometric estimation of DOX was performed with an excitation wavelength of 470 nm, and emission at 590 nm. Doxorubicin extraction from

blood was also attempted with the reported method (Bally *et al.,* 1994); however the amount of drug extracted was too low to fall in the linear range of the detection limit.

Some of the inference drawn from results presented in table 26 and figure 56 are given below:

- A significant uptake of free DOX by different tissues is observed. As compared to kidney, heart and lungs more amount of free drug is present in spleen. The amount of free drug in liver is significantly low possibly indicating its metabolism by liver.
- Higher amount of liposome/ML associated drug in liver and spleen compared to other tissues probably hints at RES uptake of this liposomes. However, as previously mentioned PEgylation of liposomes may reduce this problem. But it is important to mention here that the idea of present magneting targeting approach is to allow much of the drug loaded magnetoliposomes to be trapped at the site of the tumor, if a sufficiently strong magnet is used. Nonetheless, PEgylation may help to increase the circulation time of magnetoliposomes that are less susceptible to the applied magnetic field.
- Although not very significant, a higher amount of free drug is present in heart as compared to liposome-DOX and ML-DOX. This is thought to be due to inability of liposomes to cross the endothelial cell barrier in the heart and the low availability of the free drug due to its encapsulation in

liposomes. This is an advantage provided by target oriented drug delivery systems over conventional drug administration in use of drugs having toxic side effects.

In contrast to the distribution of free DOX; the liposomal and magnetoliposomal DOX formulation showed somewhat similar pattern of drug distribution. However, it was not possible to account the amount of DOX injected, because of the problem associated with estimation of DOX in blood. Hence, a detailed *in vivo* pharmacokinetics and toxicity study could not be undertaken.

5.3.1 *In vivo* efficacy of DOX loaded liposomes / magnetoliposomes in tumor regression

As mentioned above, the actual tissue distribution of DOX was difficult to determine. So, the next set of experiments was done to directly investigate the therapeutic efficacy of DOX entrapped in liposomes/MLs against fibrosarcoma tumor in BALBc mice.

Fibrosarcoma cell line (WEHI 164) was used to introduce tumor in BALBc mice. 5 x 10^5 cells were injected intradermally and tumor development was monitored. Usually after 6-7 days the tumor becomes palpable, diameter in three perpendicular planes were measured on alternate days with vernier calipers. After two weeks the mouse was killed and the tumor was excised and cut into small pieces to be retransplanted into nude mice so as to establish an experimental model (Fig. 57).

Treatment was performed at a dose of 9 mg/kg DOX entrapped in liposomes/MLs, given IV via a lateral tail on every alternate day for a week. Tumor growth was assessed by vernier caliper measurement and tumor volume was calculated. In the ML-DOX group animals, a small button shape magnet was implanted subcutaneously near the site of the tumor on the zero day of the experiment (Fig. 58).

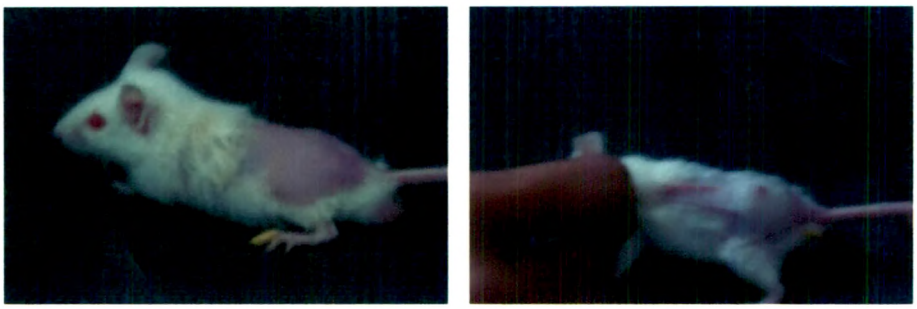

Figure 57: Mice tumor model. Control mice (left image) and tumor bearing BALBc mice (right image)

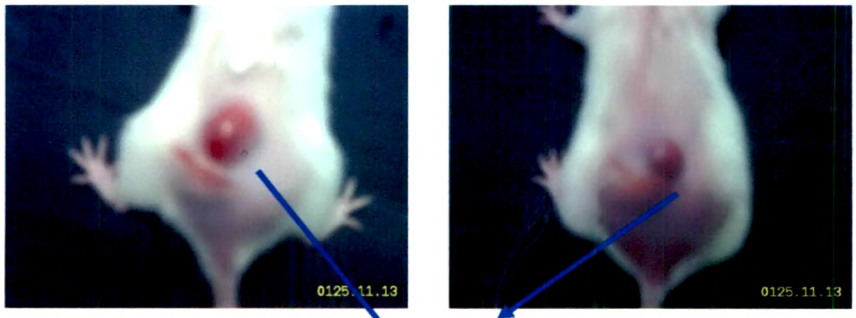

Figure 58: ML-DOX group animal with subcutaneously implanted magnet (left image); ML-DOX group animal with regressed tumor (right image).

Table 27 Tumor regression study in fibrosarcoma bearing BALBc mice

Tumor volume in mm^3 per days	Control group	ML-DOX group	Lipo-DOX group
0	51.75 ± 0.70 (100%)	51.41 ± 3.77 (100%)	48.75 ± 1.86 (100%)
2	61.94 ± 0.27 (120%)	45.33 ± 2.54 (88%)	-
4	64.28 ± 13.48 (124%)	48.07 ± 1.78 (93.5%)	53.35 ± 2.81 (109%)
6	71.88 ± 12.38 (138.9%)	41.71 ± 4.66 (81%)	-
8	87.76 ± 14.32 (170%)	37.10 ± 1.61[a] (72%)	43.83 ± 1.59[b] (89.9%)

Results are Mean ± SE, (n=3), Values with superscript a and b are significantly different (P<0 05), Values in parenthesis are % tumor volume

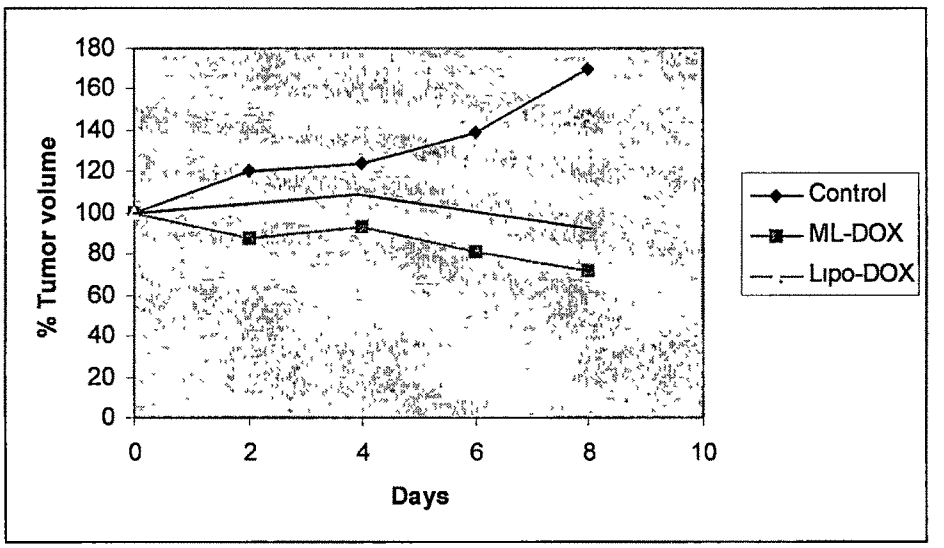

Figure 59 % Reduction in the size of the tumor, after injection with saline, ML-DOX and Lipo-DOX

As seen in the table 27 and figure 59, magnetoliposomal-DOX was more efficacious compared to liposomal-DOX in the treatment of mice injected with rapidly growing WEHI 164 fibrosarcoma cells. The tumor size reduction was seen in 24 hours in case of ML-DOX group as compared to 8 days for Lipo-DOX. Even after 8 days the tumor regression with Lipo-DOX group was lesser than ML-DOX group. The ML-DOX injected animals showed 28% reduction as against 10% reduction of tumour size observed in lipo-DOX animals. This is possibly attributed to the rapid accumulation of ML in tumors due to the influence of external magnetic field. Whereas in the control group (treated with saline) the tumor volume increased to about 170% during the treatment period (8 days).

Thus, the preliminary experiments indicate the potential of ML-DOX as a better treatment option than Lipo-DOX. However, conclusions on this line could be drawn only after performing a systematic preclinical efficacy and toxicity study in tumor bearing animal models. In the present work, due to the limitation of facilities for maintenance of tumor bearing animals further work in this direction could not be pursued. However, it is a part of the future prospect to carry out preclinical studies and if the results are found to be satisfactory then a clinical studies of this formulation would be performed.

Thus, our results suggest that this treatment approach, which involves a combination of magnet implantation at the diseased site and intravenous

administration of magnetoliposomes, could provide improved therapeutic option for treatment of cancer tissues.

5.4 SUMMARY

As mentioned earlier, drug targeting using ferrofluids or magnetic particles reversibly bound to drugs has been attempted. The concept of using magnetoliposomes is a more refined rather than the use of particles per se. The entrapping of the particles within the core of the liposomes increases their stability and also prevents their aggregation and possible blockage of the capillaries. Although active targeting of drugs using magnetoliposomes offers very attractive treatment approach, however todate successful results are limited. This gives a scope to further modify the formulation and investigate its efficacy as a drug delivery carrier.

In the present study, preparation procedure for magnetoliposomes was standardized. The ML preparation methods reported earlier were used as a base to devise a modified and an improved method (Sangregorio *et al.*, 1999; Babincova *et al.*, 2002). In the present developed method direct encapsulation of the magnetic particles with in the liposome core was performed by rehydration of the lipid film harboring colloidally dispersed magnetite particles with isopropanol. The lipid suspension thus obtained was injected in an aqueous buffer under constant sonication to yield large unilamellar magnetoliposomes. In contrast, previously reported methods involve dialysis of fatty acid coated magnetite particles at 37°C for 2-3 days in the presence of

sonicated phospholipid vesicles (De Cuyper and Noppe, 1996; De Cuyper and Joniau, 1988). Thus, the present standardized method resulted in reduction of the procedural time to only 4-5 hours as against 2-3 days with conventional procedure. Additionally, the magnetoliposomes prepared with the present method were found consistent in terms of their stability and were comparable in characteristics to that reported earlier. The following table discusses the characteristic of the prepared magnetoliposomes:

Table 28. Characteristics of prepared magnetoliposomes

Parameters	Results
Colour of ML suspension	Brown
Magnetic susceptibility	Attracted by a magnet having strength of 0.3 Tesla
Texture	Velvety and viscous
Shape	Spherical
Mean size of entrapped particles	25 nm
Magnetoliposome size	200-500 nm
Phospholipid/Fe_3O_4 ratio	0.32 ± 0.05 mmol/g
Fe_3O_4 concentration	0.4 mg/ml

The drug encapsulation efficiency of the prepared magnetoliposomes was determined by entrapping doxorubicin, which was loaded in the liposomes against the pH gradient. The encapsulation efficiency was found to be greater than 95%, which is comparable to reported for liposomal doxorubicin (Mayer *et al.*, 1990). Although, Babincova *et al.* in 2002, had attempted entrapping DOX within the core of magnetoliposomes, but neither the % drug

encapsulation efficiency nor the characteristic of the prepared magnetoliposomes were reported. Thus, from the present study it can be said that the presence of magnetic particles within the core of the liposomes doesn't influence the drug loading efficiency.

This drug loaded MLs were also characterized in terms of its storage as well as circulation stability. The results indicated that the formulation was stable against drug leakage for at least 28 days when stored at 4°C. The prepared magnetoliposomes were also found to sustain the frictional forces in an *in vitro* circulatory model system in a fluid having viscosity similar to that of whole blood, thereby indicating the intactness of the phospholipid cholesterol bilayer. Additionally, the magnetoliposomes were able to maintain the integrity when diluted in PBS (pH 7.4); more than 90% of the drug remained associated with liposomes. These results indicated that the formulation is stable enough to withstand the physical and mechanical shearing forces within the circulation. However, in the presence of serum 15-25% of drug release was seen which could be attributed to the protein-lipid interactions. Once the preparation and characterization was done, the next step was to check the *in vivo* efficacy, pharmacokinetics and toxicity of the prepared formulation.

An *in vivo* study of the present formulation was performed to check the tissue distribution of drug loaded liposomes/magnetoliposomes in rats. However, due to lack of facilities and problem associated with extraction of doxorubicin from blood, it was rather difficult to account the total amount of doxorubicin

injected and recovered. Keeping in view the limitation of the method, the results obtained hints the higher uptake of liposomes by reticulo-endothelial system. This problem could be easily overcome by incorporation of PEgylated phospholipids instead of simple phospholipid used in the current study. As mentioned earlier, it is important to mention that the idea of present magneting targeting approach is to concentrate magnetoliposomes using a strong magnet at the site of the tumor. Nonetheless, PEgylation may help to increase the circulation time of magnetoliposomes that are less susceptible to the applied magnetic field. Also, interestingly with this method, decreased drug concentration of liposome/ML DOX in the heart tissue is observed compared to free DOX. This agrees with previous reports where accumulation of free drug in heart tissue is minimized when entrapped within the liposomes, thus reducing the cardiotoxicity of doxorubicin.

A preliminary in vivo efficacy study of this formulation was done in fibrosarcoma tumour bearing BALBc mice. On day zero of the treatment, a small magnet with strength of 0.2 Tesla was subcutaneously implanted near the site of the tumour. Actually a strong magnet with a magnetic field strength equal or greater than 0.45 T could have been a better option, however small button shaped magnet with such high field strength was not available. Therefore, in the present experiment magnetoliposome-DOX injected animals were compared with liposome-DOX group. The results indicated a faster tumour regression in magnetoliposomal groups compared to liposome group, which might be attributed to the rapid accumulation of ML entrapped drug in

tumours due to the influence of external magnetic field. However, confirmation on these lines was difficult to obtain due to the lack of proper imaging techniques that can provide the actual concentration of particles at the site of tumour. Nonetheless, the preliminary findings suggest this targeted approach with magnetoliposomes may be better than normal liposomes or the use of magnetoliposomes as hyperthermia causing agent in which case targeting the liposomes is achieved by the external magnetic field and the tumour cells are killed using hysteresis property of the magnetite particles. However the method does not seem to work satisfactorily at the clinical stage. As a part of future prospect one plan to carry out required modification in the present magnetoliposomal composition and use a combination of drug targeting as well as hyperthermia to eliminate cancerous cells.