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Original Article

Formulation and Evaluation of Magnetic Microspheres of Cytarabine

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Abstract

Cytarabine is the drug of choice for treatment of leukemia. However, many formulations have been prepared, due to certain limitations they could not prove to be effective ones, therefore magnetic microspheres are formulated as they minimise the Reticuloendothelial clearance and target site specificity can be increased. The current study aimed to utilize nanotechnology to develop magnetic microspheres. Magnetic microspheres of Cytarabine were prepared using two polymers are chitosan and sodium alginate by the continuous solvent evaporation method. Optimization was done and nine different formulations were prepared. Particle sizes, encapsulation efficiency, magnetic responsiveness, *in vitro* release of all the formulations were determined. The studies demonstrated that F7 was the best formulation and drug can thus be reached the targeted site as magnetic responsiveness was also good. Novel magnetic microspheres were developed with less Reticuloendothelial clearance and target site specificity; however, further clinical investigations are necessary to evaluate its therapeutic effectiveness.

Keywords: Magnetic, microspheres, Cytarabine, Reticuloendothelial, target, specificity

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1. Introduction

Administering the drug or pharmaceutical product, in order to achieve the desired therapeutic effect comes under drug delivery [1]. Cancer is the most common cause of death now a day's worldwide. In order to design drug delivery system for cancer, various challenges are encountered. To increase drug bioavailability and to minimize drug loss as well as degradation, various drug delivery, and drug targeting systems are currently under development. The major limiting factor is the hindrance to deliver these chemotherapeutics in required optimal concentrations at affected tissues, without exhibiting severe side effects on healthy tissues [2]. Combination of carefully monitored chemotherapeutic regimens, surgical excisions as well as radiation therapy in certain cases is involved in the current chemotherapeutic regimen. Nanotechnology helps in developing an appropriate delivery system. Several nano-

vehicle such as polymeric micelles, nanoparticles, solid lipid nanoparticles, liposomes, dendrimers, have been developed to deliver these highly hydrophobic chemotherapeutic drugs to the target tumor sites. Thus, by these strategies, the mean residence time of exposure of these drugs at the site of action, coupled with a favorable distribution profile, can be affected [3, 4].

Magnetic microspheres are the supramolecular particles that are small enough to circulate through the capillaries but are sufficiently susceptible to be encaptured in microvessels by applying magnetic fields of 0.4 T-0.8 T. Targeted drug deliveries can be done via magnetic drug delivery and non magnetic drug delivery. Magnetic drug delivery is an excellent method in which a drug is directly delivered to the diseased area. Nonmagnetic drug delivery systems are successfully utilized for drug targeting but do not show satisfactory site specificity and are rapidly cleared off by Reticuloendothelial system. Magnetic microspheres are composed of magnetic cores to ensure a strong magnetic response and polymeric shells to protect from particle aggregation. These microspheres exhibit features such as small and proper size, different shapes, and various functional groups on the surface. They have therefore received much attention in recent years for wide potential applications and various drug delivery processes. Thus magnetism plays an important role in living beings metabolism [5].

1.1. Basic Problems Of Chemotherapeutic Agents

Conventional chemotherapeutic agents (conventional) are small molecules that act on tumor cells by multiple mechanisms. Mechanisms involved in inhibiting the normal functioning of tumour cells involve apoptosis. Drugs such as Paclitaxel, demonstrate their effects by stabilization of microtubules and preventing further mitotic processing from metaphase to anaphase [6].

Chemotherapeutic agents belonging to the class of anthracyclines, such as Doxorubicin and Daunorubicin, exert their efficacious effects on the tumor cells by acting upon the topoisomerase II-DNA complex, thus preventing the replication at the cellular level. Toxicity problems are caused as the chemotherapeutic agents lack the ability to differentiate between healthy tissue and a tumorigenic tissue. These agents thus target any both types of masses, whether it is malignant or benign [7, 8].

This is the root cause of major toxicity symptoms that are manifested during a regimen of chemotherapeutic treatment. Doxorubicin, which is included in most of the first-line therapies and is generally considered as a very efficacious chemotherapeutic agent suffers from many dose-limiting limitations such as nausea, drowsiness, vomiting, fatigue, and cardiotoxicity. In wake of such instances, an effective balance must be sought between the toxicity exerted by the drug and its overall efficacy to attack and kill tumor cells, thus prolonging a patient's survival. Nanotherapeutics can be used effectively to ameliorate these toxic effects by minimizing or preventing the distribution of chemotherapeutic agent to the healthy tissues

1. Orally administrable pharmaceutical compositions, however, have been found ineffective as a result of absorption of

pharmacologically active agent in the digestive tract before the target site is reached.

2. Rectal delivery by use of either suppositories or enemas is less convenient and less acceptable to the patient than even oral administration. So this route is also ineffective.

3. Colon-specific drug delivery technologies such as enzyme controlled system, pH controlled system, and time controlled system and combination approaches that employ two of these approaches. These technologies also do not deliver the right amount of drug to the colon due to Reticuloendothelial clearance.

Hence to overcome the inherent drawbacks associated with other drug delivery forms of Cytarabine, an attempt has been made to develop magnetic microspheres of Cytarabine.

To get desired results with minimum experiments, optimization method was adopted in the present study. A 3^2 full factorial design was chosen to optimize the variables. Encapsulation efficiency, magnetic responsiveness and *in vitro* release are taken as dependent variables and polymer concentration, and stirring speed is taken as independent variables [9].

2. Material and Methods

2.1. Materials

Cytarabine was obtained as a sample from Avanscure life sciences Private Ltd. Magnetite was obtained as a gift sample from Manmohan International Private Ltd. Chitosan and Sodium alginate were obtained from Sigma Aldrich Private Ltd. and Central Drug House Ltd (CDH), New Delhi respectively. Liquid paraffin was obtained from Zeenish pharma, Ahmedabad and n-hexane from Alpha Chemika, Andheri, Mumbai, India.

2.2. Methods

FTIR (Bruker) studies were carried out for the identification of the drug. Cytarabine was standardized by using UV/Vis spectrophotometer (Shimadzu 1700). FTIR spectral analysis of the physical mixture of drug and excipients was performed to assess drug excipients compatibility.

2.3. Formulation of Magnetic Microspheres

Formulation of magnetic microspheres is shown in figure 1.

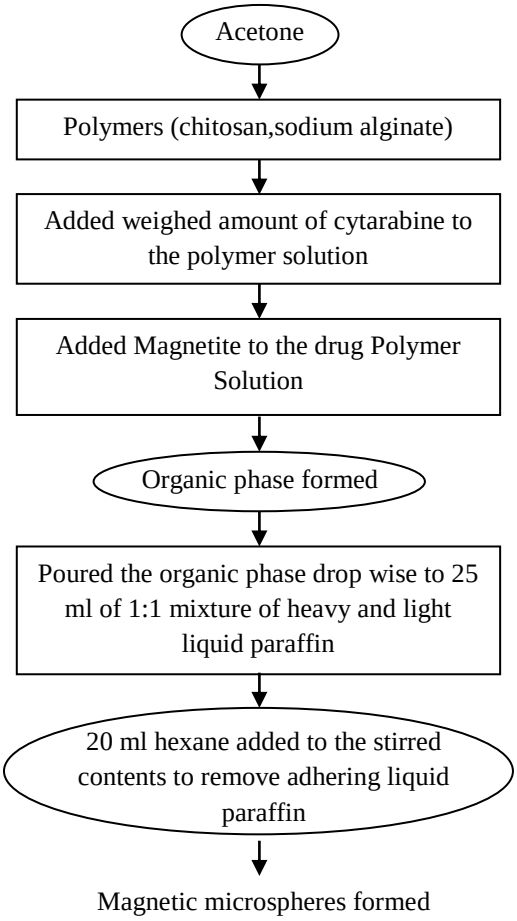

Figure 1. Method of formulation of magnetic microspheres by continuous solvent evaporation method.

Batches of magnetic microspheres were prepared by the continuous solvent evaporation method. Samples tested for changes in encapsulation efficiency, Magnetic responsiveness, and dissolution release profile.

In total nine formulations were prepared. Formulations were prepared using chitosan as polymers having different concentrations of drug and polymer, and different speeds, similarly by using sodium alginate as polymer.-1 is coded for formulations having less concentration of polymer, less speed, while 0 is for medium concentration of polymer, medium speed, and +1 is coded for formulations having high concentration of polymer and high speed. Table 1 shows the Factorial design for formulations.

3. Result and Discussion

3.1. Optimization of Magnetic Microspheres

Optimization process was done and the optimized formulation was prepared. Desirability was calculated by using the software Design Expert software (Version 10.1). Table 2 shows the composition of the optimized batch.X_1 is the concentration of the polymer, X_2 is the speed; these two are independent variables.Y_1, Y_2, Y_3 represents percentage encapsulation efficiency, magnetic responsiveness and *in vitro* release respectively.

Table 1. Factorial design for formulations.

Code	**Independent variables**	**Level Low(-1)**	**Middle(0)**	**High(+1)**	**Codes**	**Dependent variables**	**Low**	**High**
X_1	Concentration of polymer	5	10	15	Y_1(%)	% encapsulation efficiency	65.10	93.08
X_2	Speed	5	10	15	Y_2(%)	Magnetic responsiveness or microsphere content retained	58.66	91.96
					Y_3(%)	In vitro release	76.78	95.67

Experimental trials

Experimental trails	S1	S2	S3	S4	S5	S6	S7	S8	S9
X_1	-1	-1	-1	0	0	0	+1	+1	+1
X_2	-1	0	+1	-1	0	+1	-1	0	+1
Y_1 (%)	70.23	68.16	65.10	78.21	82.09	80.13	93.08	85.20	84.30
Y_2 (%)	62.70	58.66	60.45	69.87	74.55	73.46	91.96	77.56	76.78
Y_3(%)	80.97	78.34	76.78	85.76	89.98	87.55	95.67	91.32	91.02

Table 2. Composition of optimized batch**.**

Solution					
X_1	**X_2**	**Y_1**	**Y_2**	**Y_3**	**Desirability**
1.00	-1.00	90.1583	88.0968	93.7231	0.898

3.2. Preformulation Studies

The interaction between drug and excipient is studied by FTIR. The studies showed that there is no interaction between cytarabine and chitosan, also there is not any interaction between cytarabine and sodium alginate. The graphs depicting the same are shown in figure 2a and 2b.

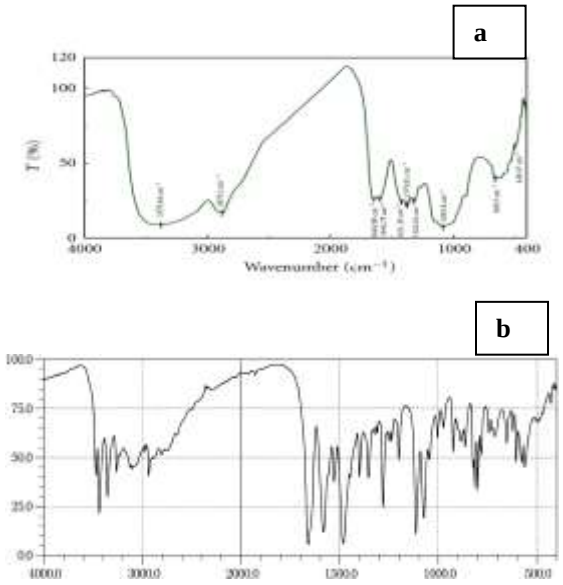

Figure 2. FTIR Spectrum of a)chitosan, b)cytarabine.

3.3. Preparation of Magnetic Microspheres

Magnetic microspheres were prepared by the continuous solvent evaporation method. Nine (F1 to F9) formulations of microspheres were prepared with two polymers sodium alginate and chitosan. Method for preparation of magnetic microspheres is given in figure 1.

3.4. Evaluation

Percentage yield, flow properties, particle size, drug content, entrapment efficiency, dissolution release were the parameters calculated. In the cytarabine: sodium alginate and cytarabine: chitosan physical mixture study, IR bands were identified and no significant shift in peak was observed with respect to drug and polymer, which means very less to no chemical interaction between polymers used and the drug.

Determination of magnetite content in prepared magnetic microspheres was done by employing a conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis. It was observed that the entrapment of magnetite increased with increase in the concentration of polymer added in consecutive formulations. Drug content and entrapment efficiency were calculated. Figure 3 shows Scanning Electron Microscopy of optimized batch.

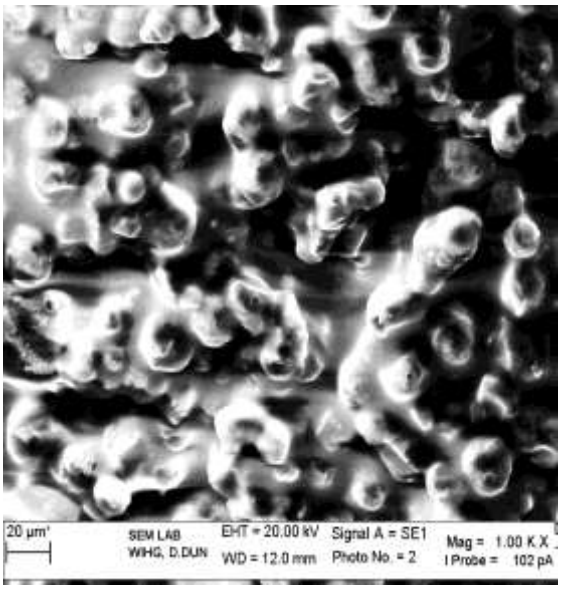

Figure 3. SEM of optimized formulation.

3.4.1. In vitro release

The dissolution release studies of the prepared magnetic microspheres were carried out. Samples (5 ml) were collected periodically at different time intervals (1, 2, 4,

6, 8, 10, 15, 20, 25, 30, 60, 90,120, 240, 360, 420 minutes) and replaced with fresh dissolution medium. The absorbance was determined spectrophotometrically at 280 nm. Dissolution release profiles were constructed as shown in figure 4. Concentrations were calculated using calibration curves developed in respective media.

3.5. Experimental Design

A two factor, three level (3^2) full factorial design was used in the optimization of magnetic microspheres. The levels of the factors studied were chosen on the basis of results of preliminary studies, so that their relative difference was adequate to have a measurable effect on the response, along with the information that the selected levels are within practical use. Effect of independent variables on dependent variables was studied [10-12].

The factorial design is a simplified representation in the analytical form of a given reality. In this mathematical approach, each experimental response Y_1, Y_2, Y_3 can be represented by a quadratic equation of the response surface:

The Regression equation for (% encapsulation efficiency)

$Y_1= +80.875+9.833X_1-2.000\ X_2-1.000X_1\ X_2-3.025X_1^2-0.525\ X_2^2$

The Regression equation for (magnetic responsiveness)

$Y_2= +72.618+10.748X_1-2.306X_2-3.232X_1\ X_2-2.182X_1^2+1.372X_2^2$

The Regression equation for (% released)

$Y_3= +88.692+6.986X_1-1.175X_2-0.115X_1\ X_2-2.535X_1^2-0.71071X_2^2$

The equations enabled the study of the effects of each factor and their interaction over the considered responses.

3.6. Optimization

Optimization was done employing a 3^2 FFD. The dependent and independent variables were related using quadratic equations obtained with the Design Expert software (Version 10.1). For each dependent variable, a summarizing equation was generated. ANOVA was performed to identify insignificant factors. Model selection was based on lower p values than the assigned significance level, high F value, an absence of

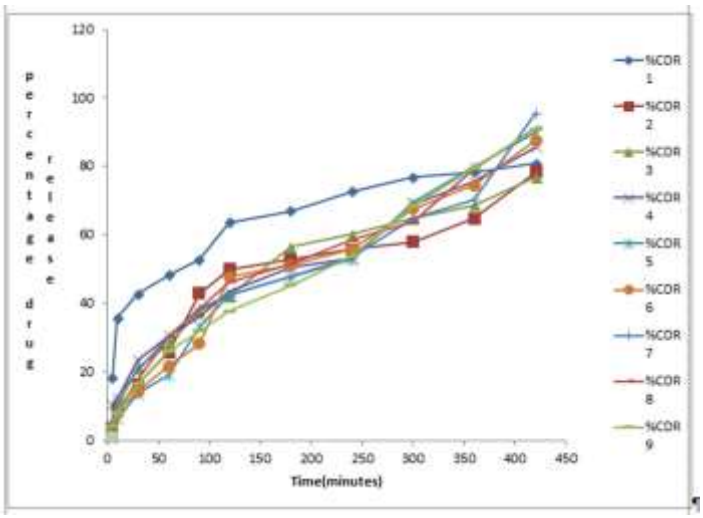

Figure 4. *In vitro* release of different formulations.

lack of fit, highest level of adjusted R^2 and predicted R^2, low standard deviation.

3.6.1. Response Surface Analysis

Response surface plots were generated for each response to study the behavior of the system. Response surface plot for percentage encapsulation efficiency in figure 5 shows that with the increase in the concentration of polymer and decrease in speed, the encapsulation efficiency increases. In the case of magnetic responsiveness surface plot (Figure 6) shows that with the increase in the concentration of polymer, magnetic responsiveness increases. The response surface plot (Figure 7) of percent drug release shows a linear increase with the increase in the concentration of polymer.

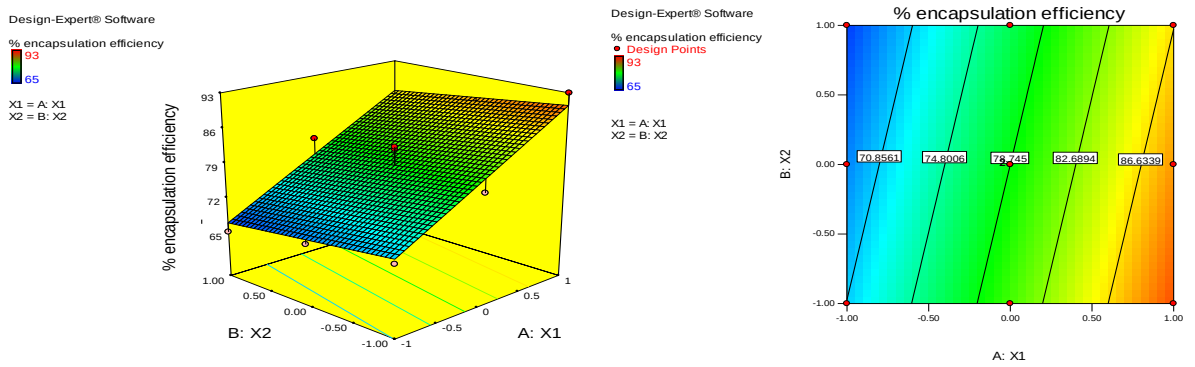

Figure 5. Response surface graph for encapsulation efficiency

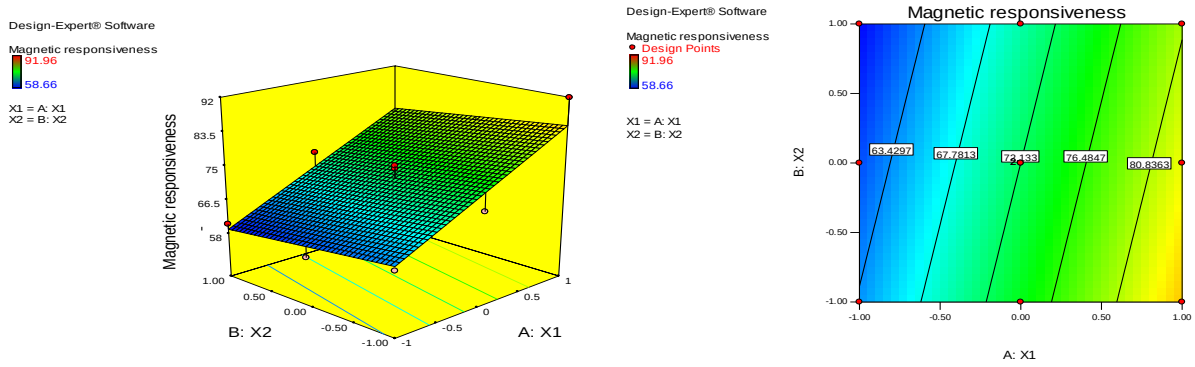

Figure 6. Response surface graph for magnetic responsiveness.

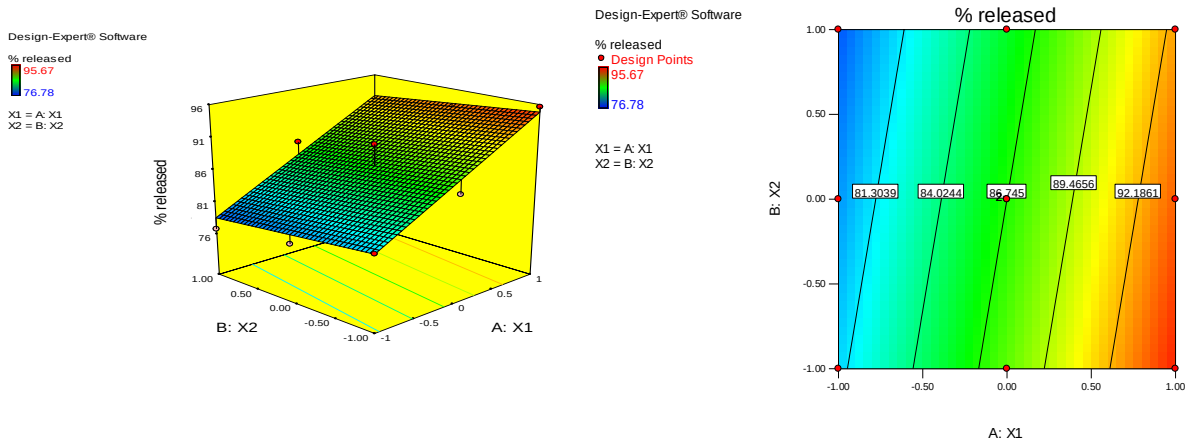

Figure 7. Response surface graph for *in vitro* release.

4. Conclusion

In an attempt to develop magnetic microspheres of cytarabine; chitosan and sodium alginate were used as polymers as they do not show interaction with the drug. Formulation F7 showed high encapsulation. Efficiency with less particle size and drug release over a prolonged time interval. It was concluded that implementation of a suitable experimental design results in achievement of an appropriate formulation. The magnetic microspheres of cytarabine prepared with sodium alginate and chitosan were found to be a better alternative than non-magnetic forms.

It has been observed that magnetic microspheres are among the best novel drug delivery systems, as it has the advantage of target specificity and better patient compliance. Applications are also enormous as they are not only used for delivering drugs but also for imaging tumors. So in future by combining various other strategies, magnetic microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective *in vivo* delivery and supplements as miniature versions of diseased organ and tissues in the body.

It might be possible in near future that magnetic particles would be used as detection probes for a variety of assays, replacing labeling techniques such as fluorescence, chemiluminescence and radioactivity.

Future prospects of magnetic microspheres look bright particularly in the area of medicinal field because of its wide spectrum of application in molecular biology, e.g. microsphere based genotyping platform is used to detect six single nucleotide polymorphism, yittrium-90 microspheres are used to prevent tumor.

References

[1] Agrawal P. Significance of Polymers in Drug Delivery System. *J Pharmacovigil*. (2015) 3:e127.

[2] Nikalje AP. Nanotechnology and its Applications in Medicine. *Med. chem*. (2015) 5: 081-089.

[3]Vinod KR, Reddy R, Banji D, Reddy V, Sandhya S. Critical review on mucoadhesive drug delivery systems. *Hygeia J. D. Med.* (2012) 6 (1): 7-12.

[4] Hee Jae yoon, Woo Dong Jang. Polymeric supramolecular systems for drug delivery. *JMC*. (2010) 2: 211-222.

[5] A-J, Ohlendorf D., Huhnt, W., Huhn, D. Clinical experiences with magnetic drug targeting: A Phase I study with 4'-Epidoxorubicin in 14 patients with advanced solid tumors. *Cancer* Res. (1996) 56 (20): 4686–4693.

[6] Khar RK, Diwan M. *Advances in Novel and Controlled Drug Delivery*, New Delhi, CBS Publishers And Distributors (2001): 452-462.

[7]Alexiou C, Arnold W, Hulin P,Klein RJ, Renz H, Parak FG. Magnetic mitoxantrone nanoparticles detection by histology, X Ray and MRI after magnetic tumour targeting. *J Magn. Magn. Mater* (2001)225(1-2):187-193.

[8]Jain NK. *Controlled and Novel Drug Delivery*, New Delhi, CBS Publishers and Distibutors (2002)1:14.

[9]Kakar Satinder, Singh Ramandeep, Batra Deepa. Preparation and evaluation of magnetic microspheres of 5-aminosalicyclic acid. *J. Acute Dis.* (2013) 2 (3): 226-231.

[10]Winter JN, Variakojis, D, Gaynor ER. Low-dose cytosine arabinoside (Ara-C) therapy in myelodysplastic syndromes and acute leukemia. *J. Cancer*. (1985) 56: 443- 449.

[11]Working PK, Newman MS, Huang SK, Mayhew E, Vaage J, Lasic DD. Pharmacokinetics, biodistribution, and therapeutic efficacy of doxorubicin encapsulated in Stealth® liposomes (Doxil®). *J. Liposome. Res*. (1994) 4: 667-687.

[12]Yuan F, Lwunig, M, Huang SK, Berk DA, Papahadjopoulos D, Jain RK. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. *Cancer Res*. (1994) 54: 3352-3356.

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