Research Article **Novel drug delivery system**

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COMPARATIVE STUDIES ON THE ANTICANCER ACTIVITY OF COLCHICINE BY VARIOUS CONTROLLED DRUG DELIVERY MODES

E.BALASUBRAMANIAN*[1] AND T.GAJENDRAN[2].

*[*1] Department of Biotechnology, Anna University of Technology,Tiruchirappalli-24*
[2] Assistant Professor, Department of Biotechnology, Anna University of Technology,Tiruchirappalli-24.

ABSTRACT

Colchicine is a secondary metabolite, originally extracted from plants of the genius colchicum it consists of the dried ripe seeds of *Colchicum autumnale Linn*., colchicine effectively functions as a mitotic poison or spindle inhibitor but the major disadvantage of the colchicine is toxicity and non target cell (normal cell)effect. That toxicity and targeted drug delivery can be controlled by carriers Vesicular systems are a novel means of drug delivery that can enhance bioavailability of encapsulated drug and provide therapeutic activity in a controlled manner for a prolonged period of time. This review describes all aspects of controlled drug delivery including their different controlled drug delivery (niosomes, proniosomes, liposomes, ethosomes),different compositions, the various methods of preparation, methods of characterization, cytotoxicity analysis(MTT assay) and comparative controlled drug delivery analysis. The review also provides detailed information of the various types of controlled drugs that provide reduced toxicity.

KEY WORDS: colchicine, controlled drug delivery, cytotoxicity analysis,

E.BALASUBRAMANIAN
Department of Biotechnology, Anna University of Technology,Tiruchirappalli-24

Corresponding author

INTRODUCTION

Colchicine is a secondary metabolite, originally extracted from plants of the genius colchicum it consists of the dried ripe seeds of *colchicum luteum* and *colchicum autumnale Linn.,* belong to the family Liliaceae .colchicum corn is also used medicinally colchicum seed contains 0.2 to 1% of amino alkaloids of which colchicine is the main constituent .the seeds contain upto 0.8% of colchicine and in corms ,it is upto 0.6%. colchicum also contain demecolcine. Both the alkaloids contain tropolone or and cycloheptatrien-ol-one ring structure.Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively functions as a mitotic poison or spindle inhibitor. Since one of the defining characteristics of cancer cells is a significantly increased rate of mitosis, this means that cancer cells are significantly more vulnerable to colchicine poisoning than are normal cells. However, the therapeutic value of colchicine against cancer is (as is typical with chemotherapy agents) limited by its toxicity against normal cells. The mitosis inhibiting function of colchicine has been of great use in the study of cellular genetics. To see the chromosomes of a cell under a light microscope,it is important that they be viewed near the point in the cell cycle in which they are most dense.this occurs near the middle of mitosis.so mitosis must be stopped before it completes .Adding colchicine to a culture during mitosis is part of the standard procedure for doing karyotype studies. Colchicine is currently used clinically at low doses for the treatment of acute gout , familial Mediterranean fever (FMF) , and dermatologic and auto-inflammatory diseases . However, the utility of colchicine for cancer therapy is currently limited, as only doses close to the maximal tolerated dose (MTD) can induce reduction in tumor blood perfusion leading to a high risk for toxicity .The major disadvantage of the colchicine is toxicity and non target cell (normal cell)effect. That toxicity and targeted drug delivery can be controlled by carriers. The ideal drug delivery system delivers drug at rate decided by the need of the body throughout the period of treatment and it provides the active entity solely to the site of action. [1]

The concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localized on the targeted site. Hence, surrounding tissues are not affected by the drug. In addition, loss of drug does not happen due to localization of drug, leading to get maximum efficacy of the medication. Different carriers have been used for targeting of drug, such as immunoglobulin, serum proteins, synthetic polymers, microspheres, erythrocytes and controlled drug delivery. [2] Controlled drug delivery (non-ionic surfactant vesicles) are microscopic lamellar structures obtained on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.The non-ionic surfactants form a closed bilayer vesicle in aqueous media based on its amphiphilic nature using some energy for instance heat, physical agitation to form this structure. In the bilayer structure, hydrophobic parts are oriented away from the aqueous solvent, whereas the hydrophilic heads remain in contact with the aqueous solvent. 3 The properties of the vesicles can be changed by varying the composition of the vesicles, size, lamellarity, tapped volume, surface charge and concentration. Various forces act inside the vesicle, e.g., van der Waals forces among surfactant molecules, repulsive forces emerging from the electrostatic interactions among charged groups of surfactant molecules, entropic

repulsive forces of the head groups of surfactants, short-acting repulsive forces, etc. These forces are responsible for maintaining the vesicular structure of niosomes. But, the stability of niosomes are affected by type of surfactant, nature of encapsulated drug, storage temperature, detergents, use of membrane spanning lipids, the interfacial polymerization of surfactant monomers in situ, inclusion of charged molecule.4 Controlled drug delivery may act as a depot, releasing the drug in a controlled manner. The therapeutic performance of the drug molecules can also be improved by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells. It can also be used as vehicle for poorly absorbable drugs to design the novel drug delivery system. It enhances the bioavailability by crossing the anatomical barrier of gastrointestinal tract via transcytosis of M cells of Peyer's patches in the intestinal lymphatic tissues.5 The controlled drug delivery vesicles are taken up by reticulo-endothelial system. Such localized drug accumulation is used in treatment of diseases, such as leishmaniasis, in which parasites invade cells of liver and spleen. Some non-reticulo-endothelial systems like immunoglobulins also recognize lipid surface of this delivery system. Encapsulation of various anti-neoplastic agents in this carrier vesicle has minimized drug-induced toxic side effects while maintaining, or in some instances, increasing the anti-tumour efficacy. Many drugs are administered through niosomes via transdermal route to improve the therapeutic efficacy.6

Approaches to stabilize niosomal drug delivery system without affecting its properties of merits. Proniosomes is dry formulation using suitable carrier coated with non ionic surfactants and can be converted into niosomes immediately before use by hydration. These proniosome-derived niosomes are as good as or even better than conventional niosomes.51

MATERIALS AND METHODS

The materials used were dried ripe seeds of *colchicum autumnale* and methanol ,HPLC, Colchicine, Chloroform,Surfactants : Span 20, 40, 80 , g ,veg .soya lechithin , and phosphate buffer pH 7.4 ,Rotary flash evaporator , bath sonicator, cancer cell line

i. Plant collection

Dried ripe seeds of *colchicum autumnale Linn.*, belong to the family Liliaceae collected from tamilnadu agriculture university Coimbatore

Preparation of plant extract:

Solvents used: Ethyl acetate, Methanol ,Petroleum ether,Ethanol,Chloroform.

ii. Methods of extraction of colchicines

Dried ripe seeds of *colchicum autumnale* collected and sliced into small pieces for freeze drying at -20°c,after 7 days the freeze dried plant material was ground to fine powder and then used for extraction of colchicine,50gm of freeze dried material was extracted using 500ml petroleum ether(1:10) with frequent shaking for 1 hr followed each time by filtration .the solid residues were a dried and then extracted with 10ml of dichloromethane at room temperature for 30 min with frequent shaking. Then 10% solution of ammonia was added to the mixture with vigorous shaking for 10 min ;the mixture was left undisturbed for 30min and then filtered. The residue was washed twice with 10 ml of dichloromethane and then combined with the filtrate. The organic phase was evaporated to dryness and then dissolved in 1ml of 70% ethanol to yield the test sample.

iii. Colchicum autumnale crude extract analysis through HPLC

Identification of colchicine was done by comparing the retention time of the sample with that of the standard obtained from Sigma, USA A water HPLC system equipped with a

binary pump 1525 and porous silica with 5μm diameter C18 4.6×150mm column was for separation .The mobile phase consisted of acteonitrile:3% acetic acid (60:40),at a flow rate of 1ml/min .The pecks eluted were detected at 245 nm and identified with authentic standards,which ensured in sample by comparison with the standard containing 10mg/ml colchicine as control.

iv. Formulation of controlled drugs

This present research described about colchicines with four different controlled drug delivery systems Niosomes, Proniosomes, Liposomes, Ethosomes this are the four different controlled drug delivery systems.

v. Niosomes preparation Thin film hydration method

In this preparation the surfactant and cholesterol mixed with 25 ml of chloroform then add the colchicine at the vortex of rotary flash evaporator at 60°c for 15 min after that thin film will form on the vortex for the re suspension of the layer add 5 ml of phosphate buffer (ph 7.4) on the vortex and shake well and transfer the solution to the centrifuge tube and place tube in centrifuge at 11000rpm speed for 15min after the decant of supernatant take the pellet and resuspend with phosphate buffer then kept the solution at bath sonicator for 10min after the sonication take the solution for centrifuge at 11000rpm for 15 min decant the supernatant take the pellet kept it at dessicator for 24 hours for dryness.

vi. Proniosomes preparation Thin film hydration method

In this preparation the mannitol,surfactant and cholesterol mixed with 25 ml of chloroform then add the colchicine at the vortex of rotary flash evaporator at 60°c for 15 min after that thin film will form on the vortex for the re suspension of the layer add 5 ml of phosphate buffer (ph 7.4) on the vortex and shake well and transfer the solution to the centrifuge tube and place tube in centrifuge at 11000rpm speed for 15min after the decant of supernatant take the pellet and resuspend with phosphate buffer then kept the solution at bath sonicator for 10min after the sonication take the solution for centrifuge at 11000rpm for 15 min decant the supernatant take the pellet kept it at dessicator for 24 hours for dryness .

vii. Liposomes preparation Solvent evaporation method

The lipids and lipophilic compounds are dissolved in an organic solvent which is then removed under vacuum by rotary flash evaporator. the lipid residue form a film on the wall of the container .an aqueous solution generally containing electrolytes and water soluble compound of the product is added to the film ,agitation produce large luv.suv can prepared by sanitation.

viii. Ethosomes preparation Hot methods

In this method phospholipid is dispersed in water by heating in a water bath at 40 c until a colloidal solution is obtained, in a separate vessel ethanol and propylene glycol are mixed and heated to 40°c once both mixture reach 40°c ,the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desired extent using both sonications

ix. Particle size and shape analysis

Particle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. About 200 vesicles were measured individually, average was taken and their size distribution range and mean diameter were calculated. Further microphotographs of optimized vesicles were taken by using 9 megapixel Sony DSC-W110 digital camera. The histogram for particle size distribution and particle size.

x. FTIR analysis

FTIR spectrum of the drug Colchicine, physical mixture of cholesterol and soya lecithin ,span 20,mannitol with colchicine.

xi. Cell line and culture

MCF-7 cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 go /ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

xii. In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on MCF-7 were determined by the MTT assay (*Mosmann et al*.,1983). Cells (1 × 10^5/well) was plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester,NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of MCF-7 were expressed as the % cell viability, using the formula:

% cell viability = A570 of treated cells / A570 of control cells × 100

RESULTS

1. Extraction of colchicum seeds:
Extracted the colchicum seeds and obtain the yield of the test sample 1.59gm
2. *Colchicum autumnale* crude extract analysis through HPLC.
Identification of a compound this is done by comparing the retention time of the sample peck 3.353 with standard(colchicine) peck 3.233
3. Biochemical test:

i. ***Colchicine give yellow colour with dil.sulphuric acid***
ii. ***Alcoholic solution of colchicine taken and treated with ferric chloride gives red colour.***

This article can be downloaded from www.ijpbs.net

Figure 1
Biochemical test

Particle size and shape analysis

Table 1
Particle size

Formulation	Size(μm)
Niosomes	9.14μm
Proniosomes	8.8μm
Liposomes	24.6μm
Ethosomes	6.7μm

Figure 2 NIOSOMES (size- 9.14μm)

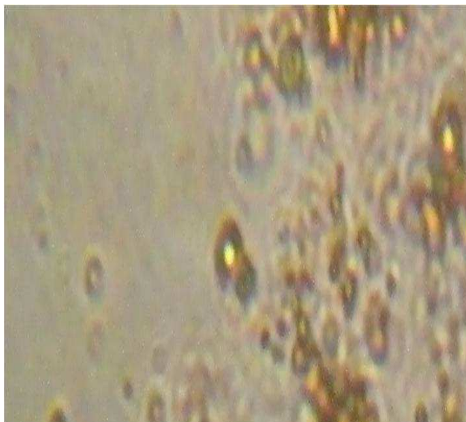


Figure 3 PRONIOSOMES (size-8.8μm)

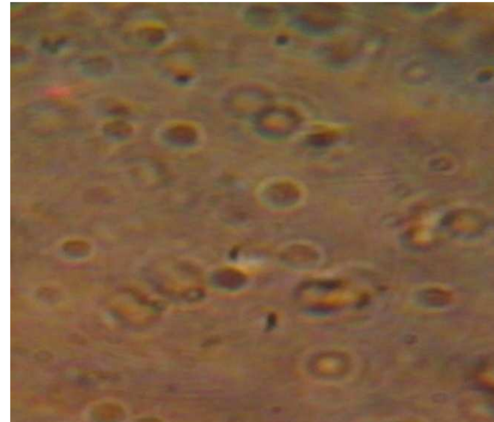


Figure 4 LIPOSOME (size-24.6μm)

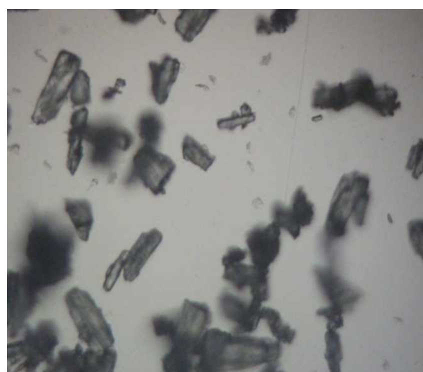
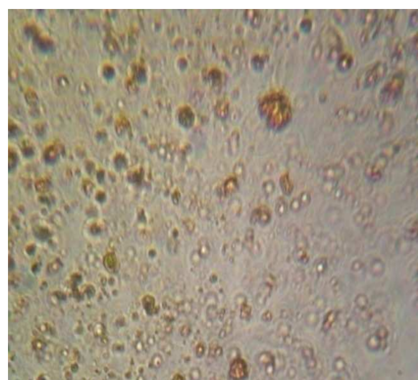


Figure 5 ETHOSOME (size-6.7μm)



Controlled drug delivery formulation preparation:

Table 2
Formulation preparation

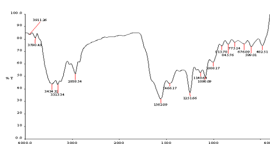
Formulation	Colchicines(mg)	Chloroform (ml)	ethanol(ml)	Phosphate buffer(ml)	Span20(mg)	Soya lechithin(mg)	Cholesterl(mg)	Mannitol(mg)
Niosomes	99	25	_	5	155	677	193	_
Proniosomes	99	25	_	5	155	677	193	500
Liposomes	99	25	_	5	155	677	193	_
Ethosomes	99	_	25	_	155	677	193	155

FTIR analysis results

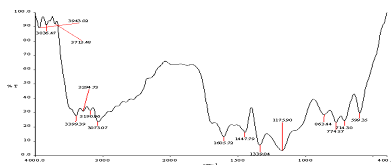
There were some mild interactions in the wave number 3600–3200 cm^{-1} and 1500–800 cm^{-1}. The region 3600–3200 cm^{-1} is a stretching region of the functional group N-H, C-H of aromatic ring (3100–3000 cm^{-1}), O-H (3200 cm^{-1}) and C-H of alkenes (3100–3000 cm–1) and C-H of alkane (~3000 cm–1). The region 1500–800 cm^{-1} is the stretching region of the functional group C-OH of alcohol (1400–1075 cm^{-1}). In the region 1500–800 cm^{-1}. The drug interaction was noticed at 3911.26, 3313.34 and 1466.17, 775.14 cm^{-1}.

FTIR analysis

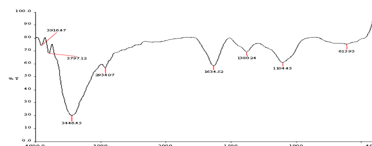
Graph 1
Colchicine (control)-FTIR analysis



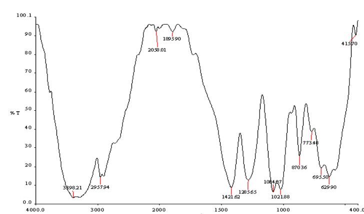
Graph 2 Niosomes-FTIR



Graph 4 Liposomes-FTIR



Graph 3:Proniosomes-FTIR



Graph 5:Ethosomes-FTIR

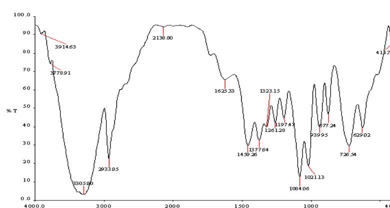


Table 3
Anticancer effect of Niosomes on MCF-7 cell line

S.No	Concentration (mg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	10	Neat	0.08	16.67
2	5	1:1	0.10	20.83
3	2.5	1:2	0.17	35.41
4	1.25	1:4	0.23	47.91
5	0.625	1:8	0.26	54.16
6	0.312	1:16	0.30	62.50
7	0.156	1:32	0.35	72.91
8	Cell control	-	0.48	100

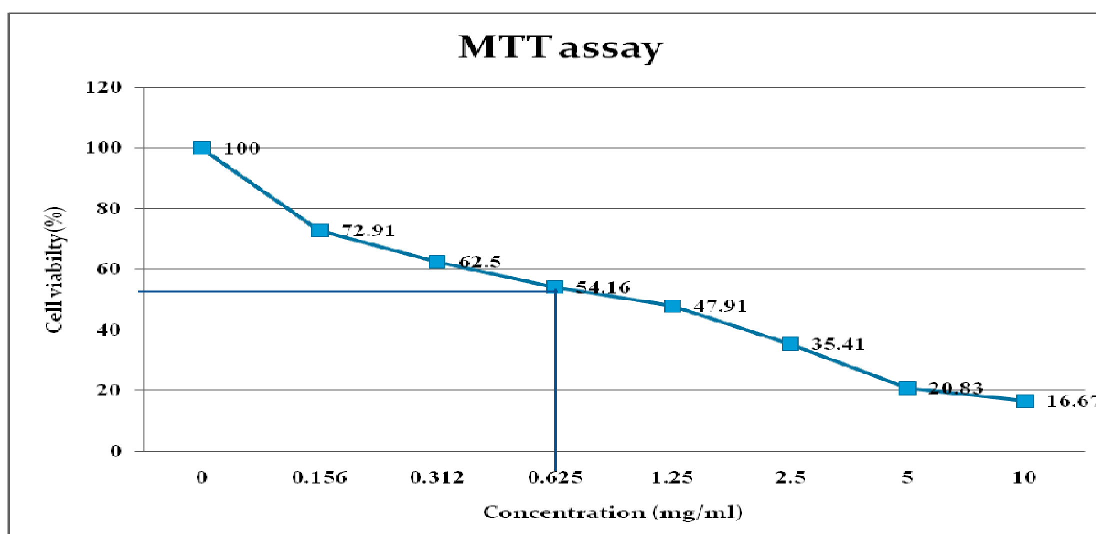


Table 4
Anticancer effect of Proniosomes on MCF-7 cell line

S.No	Concentration (mg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	10	Neat	0.12	25.00
2	5	1:1	0.20	41.67
3	2.5	1:2	0.23	48.11
4	1.25	1:4	0.28	58.33
5	0.625	1:8	0.31	64.58
6	0.312	1:16	0.35	72.91
7	0.156	1:32	0.37	77.08
8	Cell control	-	0.48	100

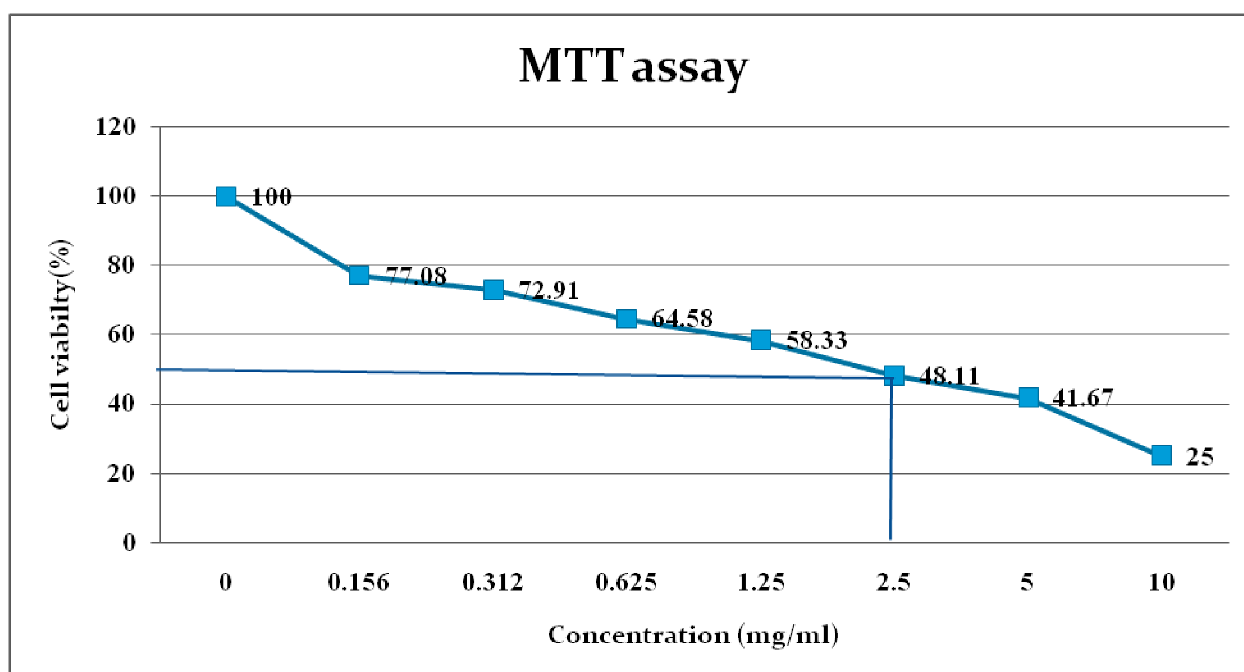


Table.5
Anticancer effect of Liposomes on MCF-7 cell line

S.No	Concentration (mg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	10	Neat	0.10	20.83
2	5	1:1	0.15	31.25
3	2.5	1:2	0.20	41.67
4	1.25	1:4	0.25	52.08
5	0.625	1:8	0.30	62.08
6	0.312	1:16	0.35	72.91
7	0.156	1:32	0.38	79.16
8	Cell control	-	0.48	100

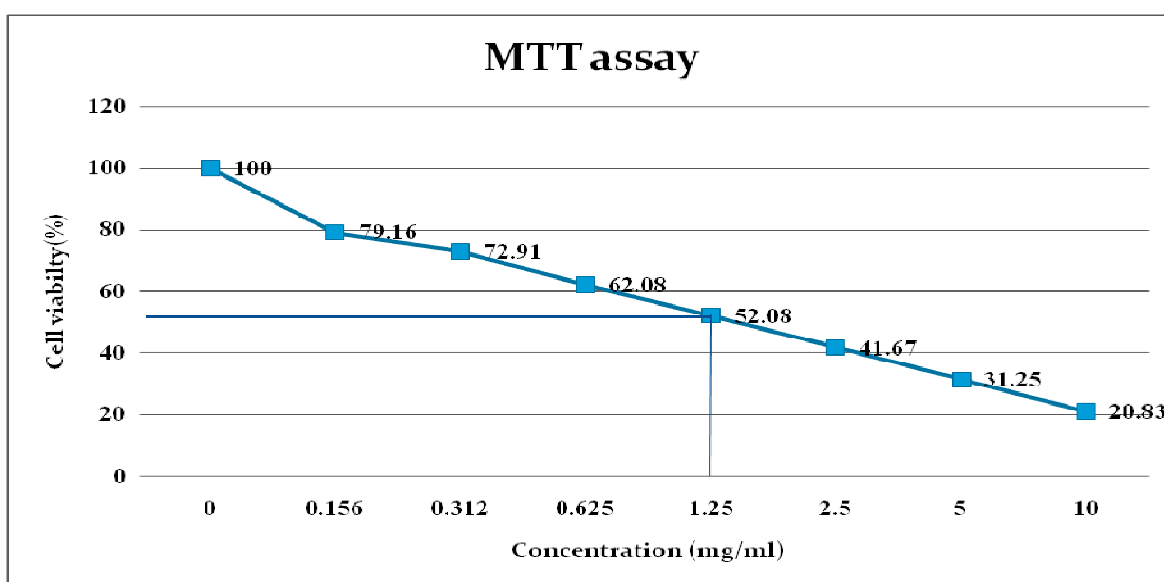


Table.6
Anticancer effect of Ethosomes on MCF-7 cell line

S.No	Concentration (mg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	10	Neat	0.07	14.58
2	5	1:1	0.14	29.16
3	2.5	1:2	0.16	33.33
4	1.25	1:4	0.23	47.91
5	0.625	1:8	0.28	58.33
6	0.312	1:16	0.33	62.50
7	0.156	1:32	0.39	81.25
8	Cell control	-	0.48	100

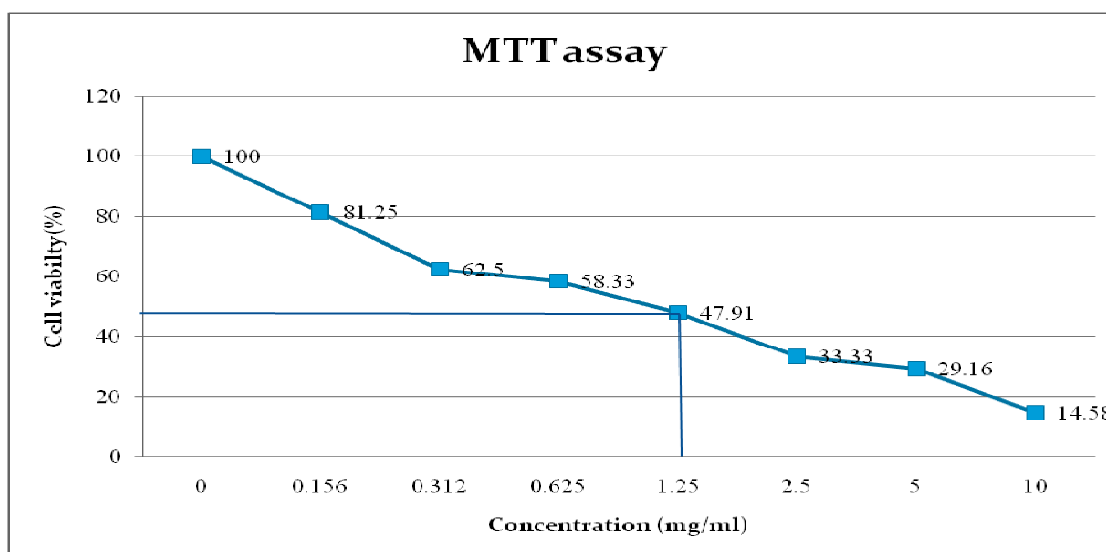


Table.7
Anticancer Effect of Control Sample on MCF-7 Cell line

S.No	Concentration (mg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	10	Neat	0.08	19.0
2	5	1:1	0.10	23.8
3	2.5	1:2	0.12	28.5
4	1.25	1:4	0.15	35.7
5	0.625	1:8	0.16	38.0
6	0.312	1:16	0.21	50.0
7	0.156	1:32	0.26	61.9
8	Cell control	-	0.42	100

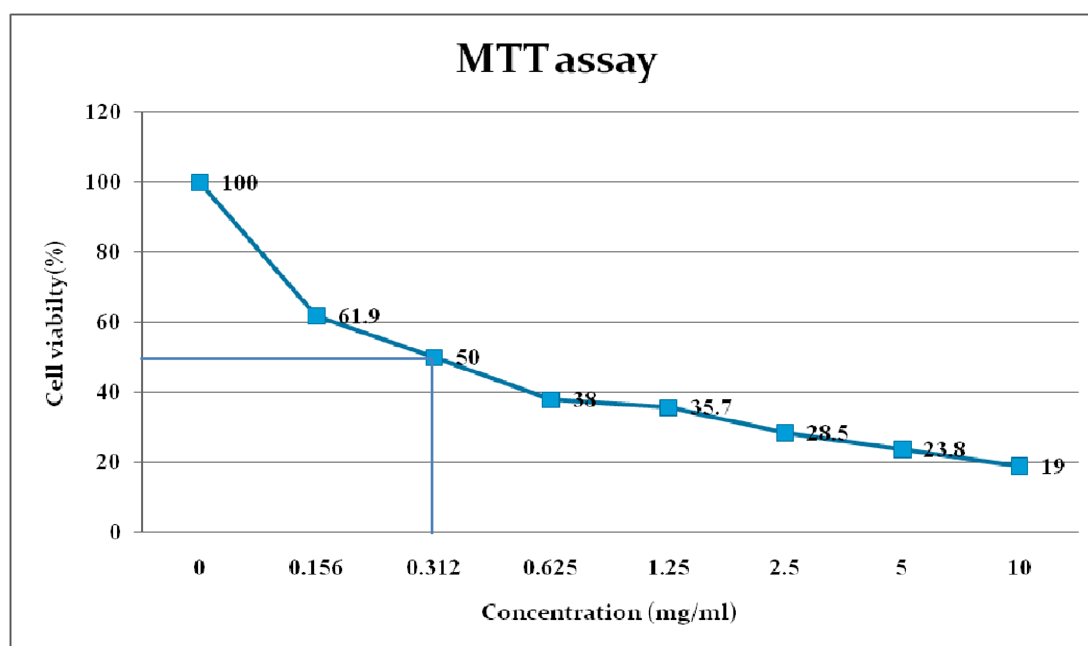


Figure 6
Anticancer effect of Niosomes on MCF-7 cell line

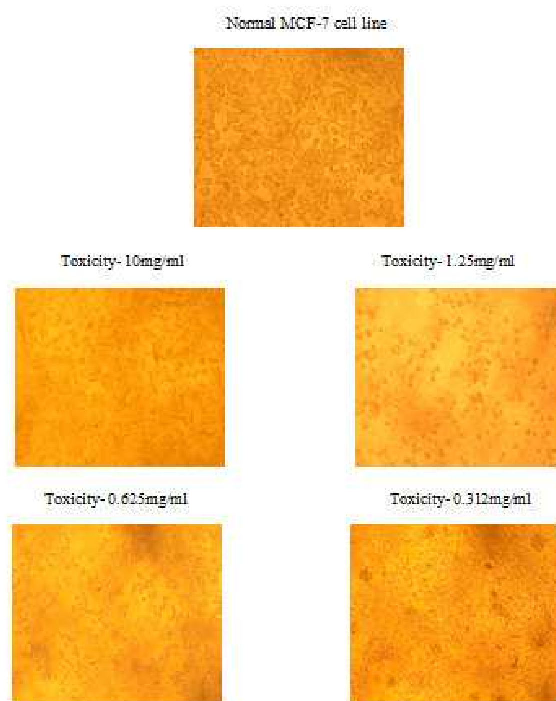


Figure 7
Anticancer effect of Proniosomes on MCF-7 cell line

Figure 8
Anticancer effect of Liposomes on MCF-7 cell line

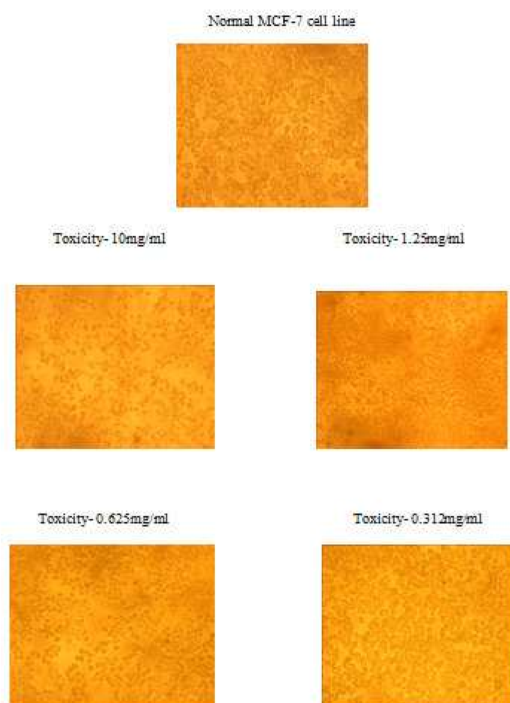


Figure 9
Anticancer effect of Ethosomes on MCF-7 cell line

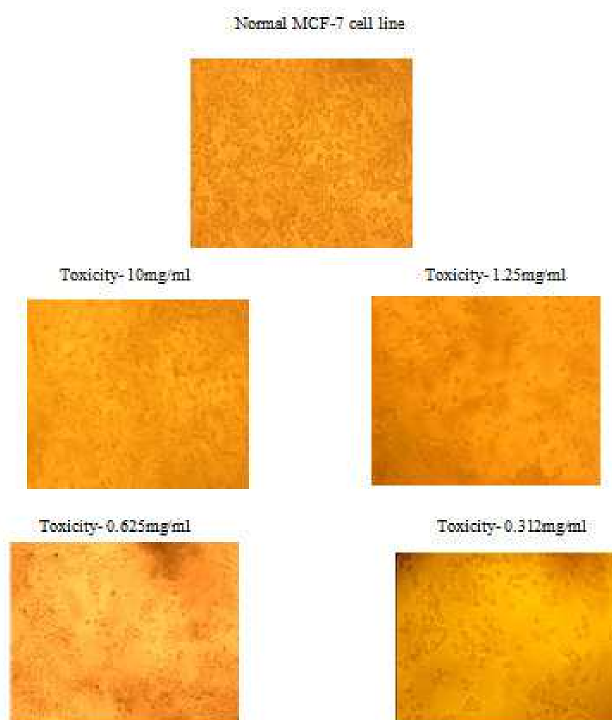
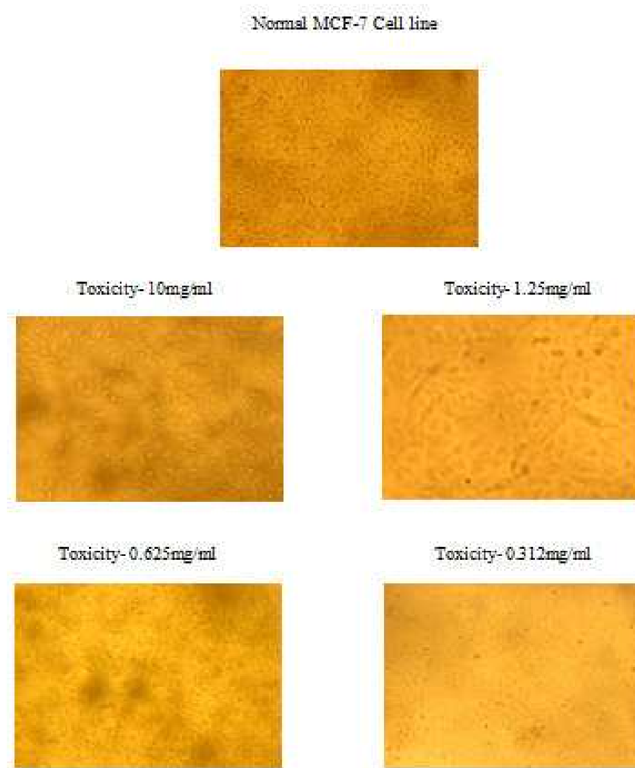


Figure 10
Anticancer Effect of Control Sample on MCF-7 Cell line



Comparative controlled drug delivery analysis of control drugs:
Niosomes shows 54.16% cell viability at the concentration 0.625mg/ml
Proniosomes shows 48.11% cell viability at the concentration 2.5 mg/ml
Liposomes shows 52.08% cell viability at the concentration 1.25mg/ml
Ethosomes shows 47.91% cell viability at the concentration 1.25mg/ml
Control drug shows 50.0% cell viability at the concentration 0.312mg/ml

i. Comparative controlled drug studies of colchicine for anticancer activity was identify by IC50 value
ii. Compare to control(colchicine) drug all controlled drug delivery shows very low toxicity
iii. Compare to other controlled drug delivery proniosomes shows very low toxicity
iv. Low toxicity level and anticancer activity : Proniosomes>liposomes>Ethosomes> Niosomes> Control (colchicine)

DISCUSSION

Colchicine is a secondary metabolite, originally extracted from plants of the genius colchicum it consists of the dried ripe seeds of *colchicum luteum* and *colchicum autumnale Linn*., belong to the family Liliaceae Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively functions as a mitotic poison or spindle inhibitor. This means that

cancer cells are significantly more vulnerable to colchicine poisoning than are normal cells. However, the therapeutic value of colchicine against cancer is (as is typical with chemotherapy agents) limited by its toxicity against normal cells. Colchicine was extracted through alcohol and the crude extract was obtained, purified and quantified through A water HPLC system equipped with a binary pump 1525 and porous silica used .The mobile phase consisted of acteonitrile:3% acetic acid (60:40),at a flow rate of 1ml/min .The pecks eluted were detected at 245 nm and the retenion time was observed and that was compared with standard biochemical test conformed through two test and its compared with pharmacognosy by c.k kokate the colchicine present and formulation drug interaction with excipients was observed through FTIR analysis observed through FTIR spectrum of the drug Colchicine, physical mixture of cholesterol and soya lecithin ,span 20,mannitol with colchicine. The drug interaction was noticed at 3911.26, 3313.34 and 1466.17, 775.14 cm^{-1}. The major disadvantage of the colchicine is toxicity and non target cell (normal cell) effect. That toxicity and targeted drug delivery can be controlled by carriers .The ideal drug delivery system delivers drug at rate decided by the need of the body throughout the period of treatment and it provides the active entity solely to the site of action. The concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localized on the targeted site. Hence, surrounding tissues are not affected by the drug. In addition, loss of drug does not happen due to localization of drug, leading to get maximum efficacy of the medication. Different carriers have been used for targeting of drug, such as immunoglobulin, serum proteins, synthetic polymers, liposome, microspheres, erythrocytes and controlled drug delivery. The four different drug delivery system shows various potent action against cancer cells and normal cells(cyto toxicity assay) and observe the results through mcf-7 cell line and compared with other controlled drugs .The following controlled drugs shows very less toxicity when compared with colchicine test and standard Comparative controlled drug studies of colchicine for anticancer activity was identify by IC50 value Compare to control(colchicine) drug all controlled drug delivery shows very low toxicity .Compare to other controlled drug delivery proniosomes shows low toxicity second liposomes shows low toxicity third ethosomes shows low toxicity finally niosomes shows the low toxicity

CONCLUSION

Colchicine effectively functions as a mitotic poison or spindle inhibitor but the major disadvantage of the colchicine is toxicity and non target cell (normal cell) effect. That toxicity and targeted drug delivery can be controlled by carriers Vesicular systems such a iosomes,proniosomes,liposomes,ethosomes are prepared and size evaluation ,FTIR analysis, biochemical and the cytotoxicity analysis (MTT assay) carried out by normal cell line and mcf-7 cell line This review describes all aspects of controlled drug delivery Compare to other controlled drug delivery proniosomes shows low toxicity second liposomes shows low toxicity third ethosomes shows low toxicity finally niosomes shows the low toxicity The review also provides detailed information of the various types of controlled drugs that provide reduced toxicity .

REFERENCE

1. Lange U, Schumann C, Schmidt KL. Current aspects of colchicine therapy. Classical indications and new therapeutic uses. Eur J Med Res 2001;6:150–60.
2. Molad Y. Update on colchicine and its mechanism of action. Curr Rheumatol Rep 2002;3:252–6.
3. Biju, SS; Talegaonkar, S; Mishra, PR and Khar, RK (2006), “Vesicular Systems: An Overview”, Indian Journal of Pharmaceutical Sciences, 68(2), 141-153
4. Vyas, SP and Khar, RK (2004), “Targeted and Controlled Drug Delivery, Novel Carrier Systems”, First Reprint, CBS Publishers & Distributors, New Delhi, 39.
5. Fordham JN, Kirwan J, Cason J, Currey HL. Prolonged reduction in polymorphonucler adhesion following oral colchicine. Ann Rheum Dis 1981;40:605–8.
6. Ferron GM, Rochdi M, Jusko WJ, Scherrmann JM. Oral absorption characteristics and pharmacokinetics of colchicine in healthy volunteers after single multiple doses. J Clin Pharmacol 1996;36:874–83.
7. Rochdi M, Sabouraud A, Girre V, Venet R, Schermann JM. Pharmacokinetics and absolute bioavailability of colchicine after i.v. and oral administration in healthy human volunteers and elderly subjects. Eur J Clin Pharmacol 1994;46:351–4.
8. Tateishi T, Soucek P, Caraco Y, Guencerich FP, Wood AJ. Colchicine biotransformation by human liver-microsomes. Identification of CYP3A4 as the major isoform responsible for colchicine demethylation. Biochem Pharmacol 1997;53:111–6.
9. Dvorak Z, Modriansky M, Pichard-Garcia L, Balaguer P, Vilarem MJ, Ulrichová J et al. Colchicine down-regulates cytochrome P450, 2B6, 2C8, 2C9, and 3A4 in human hepatocytes by affecting their glucocorticoid receptor-mediated regulation.Mol Pharmacol 2003;64:160–9.
10. Leighton JA, Bay MK, Maldonado AL, Schenker S, Speeg KV. Colchicine clearance is impaired in alcoholic cirrhosis. Hepatology 1991;14:1013–5.
11. Rudi J, Raedsch R, Gerteis C, Schlenker T, Plachlky J, Walter-Sack I, et al. Plasma kinetics and biliary excretion of colchicine in patients with chronic liver disease after oral administration of a single dose and after long-term treatment. Scand J Gastroenterol 1994;14:1013–5.
12. Speeg KV. Effect of cyclosporine on colchicine secretion by the kidney multidrug transported studied in vivo. Pharmacology 1992;261:50–5.
13. Ben-Chetrit E, Schermann JM, Levy M. Colchicine in breast milk of patients with familial Mediterranean fever. Arthritis Rheum 1996;39:1213–7.
14. Lidar M, Livneh A. Familial Mediterranean fever: clinical, molecular and management advancements. Netherlands J Med 2007;65:318–24.
15. Maisch B, Seferovic PM, Ristic AD, Erbel R, Rienmüller R, Adler Y, et al. Task Force on the diagnosis management of pericardial disease of the European Society of Cardiology. Guidelines on the Diagnosis and management of pericardial diseases Eur Heart J 2004;25:587–610.
16. Imazio M, Demichelis B, Parrini I, Cecchi E, Demarie G, Ghisio A, et al. Management, risk factors, and outcomes in recurrent pericarditis. Am J Cardio 2005;96:736–9.
17. Sussman JS, Brozena SSC, Skop N, Korecka M, Shaw LM. Accidental intravenous colchicine poisoning. Ther Drug Monit 2004;26:688–92.
18. Centers for Disease Control and Prevention (CDC. Deaths from intravenous colchicine resulting from a compounding pharmacy error – Oregon

and Washington, 2007. MMWR Morb Mortal Wkly Rep 2007, 56; 2007. p. 1050–2.

19. Riley K. FDA approves colchicine for acute gout, Mediterranean fever. Agency also provides new information to physicians regarding safe use of drug. Media Inquiries 2009;301:796–4674 Consumers Inquiries 888-INFO-FDA.
20. Kesselheim AS, Solomon DH. Incentives for drug development – the curious case of colchicine. New Engl J Med 2010;362:2045–8.
21. Ben-Chetrit E, Levy M. Colchicine prophylaxis in familial Mediterranean fever: reappraisal after 15 years. Semin Arthritis Rheum 1991;20:241–6.
22. Yurdakui S, Tuzun CMY, Ozyzgan Y, Hamuryudan V, Senocak OM, Yazici H. A double-blind trial of colchicine in Behçet's syndrome. Arthritis Rheum 2001;44:2686–92.
23. Zemer D, Livneh A, Danon YL, Pras M, Sohar E. Long term colchicine treatment in children with familial Mediterranean fever. Arthritis Rheum 1991:34973–7.
24. Nakamura A, Matsuda M, Tazakawa K, Shimojima Y, Ikeda S. Successful with infliximab and low-dose methotrexate in a Japanese patient with familia Mediterranean fever. Intern Med 2007;46:1247–9.
25. Aikawa NE, Gonçalves, Silva CA, Gonçalves C, Bonfá E, de Carvalho JF. Late response to anti-TNF-alpha therapy in refractory mucocutaneous lesions of Behçet's disease; Dec 11 2009 [Epub ahead of print].
26. Alpay N, Sumnu A, Caliskan Y, Yazici H, Türkmen A, Gül A. Efficacy of anakinra treatment in a patient with colchicine-resistant familial Mediterranean fever. Rheumatol Int Apr 13 2010, doi:10.1007/00296–010–1474–6.
27. Goldkind SF, Sahin L, Gallauresi B. Enrolling pregnant women in research – lessons from the H1N1 influenza pandemic. New Engl J Med 2010;362:2241–3.
28. Douglas G, King B. Colchicine inhibits human trophoblasts differentiation in vitro.Placenta 1993;14:187–201.
29. Rabinovitch O, Zemer D, Kukia E, Sohar E, Mashiach S. Colchicine treatment in conception and pregnancy: two hundred thirty-one pregnancies in patients with familial Mediterranean fever. Am J Reprod Immunol 1992;28:245–6.
30. Adler Y, Finkelstein Y, Guindo J, Rodrigues de la Serna AM, Schoenfeld Y, Bayes Genis A, et al. Colchicine treatment for recurrent pericarditis. A decade of experience. Circulation 1998;97:2183–5.
31. Iacobuzio-Donahue CA, Lee EL, Abraham SC, Yardley JH, Wu TT. Colchicine toxicity: distinct morphologic findings in gastrointestinal biopsies. Am J Surg Pathol 2001;25:1067–73.
32. Al-Darajj WI, Al-Mahmoud RM, Ilvas M. Gastric changes following colchicines therapy in patients with FMF. Dig Dis Sci 2008;53:2079–82.
33. Baker SK, Goodwin S, Sur M, Tarnoposky MA. Cytoskeletal myotoxicity from simvastatin and colchicine. Clin Neuropharmacol 2002;25:266–8.
34. Satturwar, PM; Fulzele, SV; Nande, NS and Khandare, JN (2002), “Formulation and evaluation of ketoconazole niosomes”, Indian Journal of Pharmaceutical Sciences, Vol.64, 155-8.
35. Niel E, Shermann JM. Colchicine today. Joint Bone Spine 2006;73:672–8..
36. Van Winden ECA, Zuidam NJ, Crommelin DJA. Strategies for large scale production and optimized stability of pharmaceutical liposomes developed for parenteral use. In: Lasic DD, Papahadjopoulos D, eds. Medical Applications of Liposomes. Amsterdam: Elsevier; 1998:567-604.
37. Shahiwala, A and Misra, A (2002), “Studies in topical application of niosomally entrapped nimesulide”, J. Pharm. Sci., 5 (3), 220-225.

38. Sheena, IP; Singh, UV; Kamath, R; Umadevi, P and Udupa, N (1998), “Niosomal withaferin with better antitumor efficacy”, Indian Journal of Pharm. Sciences, 60 (1),45-48.
39. “Indian Pharmacopoeia” (1996), Govt. of India, Ministry of Health & Family Welfare, 4th Ed., Vol. II, A-144.
40. Florey, K (2001), “Analytical Profile of Drug Substances and Exicipients”, Academic Press ,Milliford, New Jersey, 28,199-240.
41. Azmin, MN; Florence, AT; Handajani, VR; Stuart, JFB; Vanlerberghe, G and Wittakar, JS (1985), “The effect of nonionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice”, J. Pharm. Pharmacol., 37,237-242.
42. Deamer, D and Bhangham, AD (1976), “Biochem. Biphys. Acta, , 443, 629-634. Yekta Ozer, Atilla Hinal A; Bouwstra, Joke A (1991) Eur. J. Pharm. Biopharm., 37(2), 75-79.
43. Ruckmani, K; Jayakar, B and Ghosal, SK (2000), “Non-ionic Surfactant Vesicles of Cytarabine HCl for Effective Treatment of Leukemias”, Drug Development and Industrial Pharmacy, 26(2), 217-222.
44. Wan, LSC and Lee, PFS (1974), Can. J. Pharm. Sci., 9, 82.
45. Kulkarni, SB; Betageri, GV and Singh, M (1995), “Factors affecting microencapsulation of drugs in liposomes”, Jour. of Microencapsulation, 12, 229-246.
46. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm. 1998;172:33-70.
47. 47.Uchegbu IF, ed. Synthetic Surfactant Vesicles: Niosomes and Other Non-Phospholipid Vesicular Systems, Drug Targeting and Delivery, Volume 11. Amsterdam: Harwood Academic Publishers; 2000.
48. 48.Arunothayanun P, Turton JA, Uchegbu IF, Florence AT. Preparation and in vitro/in vivo evaluation of luteinizing hormone releasing hormone (LHRH) loaded polyhedral and spherical/tubular niosomes. J Pharm Sci. 1999;88(1):34
49. 49.Kristmundsdottir T, Gudmundsson OS,Ingvarsdottir K. Release of Diltiazem from Eudragit microparticles prepared by spray drying.Int J Pharm. 137:159-65 (1996).
50. 50.gowda.DV,khan MS, nagandra R-spary dried indapamide microparticle for controlled release –a novel approach. International Journal of Pharma and Bio Sciences, Vol.1/Issue-4/Oct-Dec.2010
51. Hu C, Rhodes DG. Proniosomes: a novel drug carrier preparation. Int Pharm. 1999;185(1):23-35.

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