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Research Article

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Study of niosomated cytarabine

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ABSTRACT

Cytarabin was nanoniosomated using non ionic surfactant through ether injection method. The percent entrapment efficiency was found to be 98%. This was calculated based on passing niosomated cytarabine onto column of Sephadex G-50 and the obtained fractions were pooled and ruptured in order to determine per cent of unentrapped cytarabine. The released data were fitted in to different popular kinetic models. The released of cytarabine from the niosome was followed by first order kinetic and Hixson Crowell model. The size of nano niosomes containing cytarabine was determined to be 347nm with the zeta potential of 12.7 mV. ThusIC_{50} values for the pure drug and nano niosomated formulations indicate the higher effectiveness of the cytarabinenoisomal formulation.

Key words: Nanoniosome, cytarabine ,first order kinetics, Hixson and Crowell modell

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INTRODUCTION

Nanotechnology has been applied to biomedical research in order to study its impact on diagnosis, prevention and treatment of various diseases. Thereby nanotechnologists are focusing to develop appropriate, safe and efficient drug vehicles at nano scale. Researches on nano drug delivery have been expanded during its start in 1970s where numerous products are available in the markets now or are on their ways to the markets [1]. Niosomes can be considered as a drug carrier at nano scale in nano medicine. Thus niosomes are non ionic surfactant vesicles employed to carry drugs into specific targets. Niosomes as drug vehicles are preferred to liposomes due to their simplicity, cost effectiveness, behaving like liposome (in vivo) and prepared from uncharged single chain surfactants [2]**.** However niosomes are non ionic surfactants that can either be multilamellar or unilamillar vesicles. Thus they are formed on hydration of non ionic surfactant film which gets hydrated to encapsulate/entrap the desired drug [3].They are biodegradable, biocompatible and non immunogenic in nature and exhibit flexibility in their structural characterization. In addition, handling and storage of niosomes require no special conditions. Furthermore, they can be used as a vehicle to deliver the drug to specific tissues, thereby reducing the required dose causing improvement in therapeutic effect of the drug and decreasing the side effects [4]. It is an essential to study the pattern of the drug release entrapped within the biodegradable, biocompatible and non immunogenicniosomal vesicles. Henceforth different kinetics models have been proposed such as statistical, dependent and independent models [5]. Therefore attempts were made to niosomatecytarabine or cytosine arabinoside a drug employed to treat white blood cells cancers such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma [6].It kills cancer cells by interfering with DNA synthesis [7]. It belongs to the family of nucleoside analogues and mimics the nucleoside in uptake and metabolism and gets incorporated into newly synthesized DNA resulting synthesis inhibition and chain termination [8]. In this article cytarabine was entrapped in niosomal vesicles .Thereafter, its

size, zeta potential, appearance, in vitro drug release and fitting the data into dependent model namely: zero order, first order kinetics, Higuchi and Hixson-Crowell models and finally evaluating its effect on MCF7 cells.

EXPERIMENTAL SECTION

Cytarabine (Cytarabine hydrochloride Taizhou Crene Biotechnology Co), span60 (sorbitan mono laurate), tween 80, cholesterol, MTT reagent (0.5 mg/ml) (Sigma, USA), acetonitrile (Merck), RPMI 1640 Medium (Invitrogen), MCF-7 cell line (National Cell Bank Department, Pasteur Institute of Iran). Other reagents used were of analytical grade.

Preparation of niosomes

Niosomal formulation of cytarabine was prepared by ether injection process. The principle of the method is based on injection of ether into an aqueous solution at 60ºC. In brief, the mixture of span 20 and cholesterol was dissolved in 15 ml of ether which was then added to 10mL of phosphate buffer (60 ºC) containing cytarabine slowly. The mixture was kept on magnetite stirrer at 100 rpm and 60 ºC. The temperature was kept constant at 60 ºC during the evaporation process. The niosomes of unilamillar and multilamellar containing cytarabine were formed.

Zeta potential measurement

In vivo performance of the niosomes relatively could be related to charges present on the surface of the vesicles. The stability of niosomal formulation is also related to the stability of niosomal formulation. Thus the zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system .The prepared formulation was suitably diluted in order to measure the zeta potential and the size of the vesicles by Zeta sizer (Malvern, Instruments Ltd, Worcestershire, Zen 3600 UK).

Scanning electron microscopy (SEM)

A drop of aqueous suspension of niosomatedcytarabine was spread on a slab and dried under vacuum. The sample was coated with a 20 nm thick gold layer in a cathode evaporator. The diameter of particles in each field was calculated by using JSM-5200 operation of scanning electron microscope (Tokyo, Japan) at 15 kV.

Entrapment efficiency

The entrapment efficiency of cytarabine loaded niosome was studied by column chromatography using Sephadex G-50 and phosphate buffer as an eluent. The flow rate was adjusted to 1mLmin^{-1} and fractions of 1.5 ml were collected. The fractions containing niosomalcytarabine were collected and pooled. They were ruptured as reported by Azizi and Norouzian [9] and their cytarabine content was measured at λ280 nm. Thus the entrapment efficiency was calculated as follows:

$$\frac{\text{Total drug used(mg)- unentrapped drug(mg)}}{\text{Total drug used}} \times 100$$

Kinetic studies

Data of the cytarabine release from niosomes were analyzed for release kinetics. Drug release kinetics from nanovesicles is analyzed by zero-order kinetics, first order kinetics, Higuchi and Hixson-Crowell models.

Zero order kinetics model follows the equation as $(Q_t/Q_0) = k_0t$, where, k_0 is the zero order rate constant; Q denotes the amount of letrozole released at time t and Q_0 denotes the initial amount of letrozole.

First order kinetics model follows the equation as $\log (Q_t/Q_0) = -k_1t / 2.303$, where $k_1$is the first order rate constant.
Higuchi model, $Qt/Q_0= k_h\, t^{1/2}$ Q denotes amount of letrozole released at time t Q_0 denotes the amount of letrozole in nanoniosome initially, k_h is the Higuchi matrix release kinetics.

Hixson and Crowell model:
$Q_0^{1/3}-Q_t^{1/3}=\kappa t$, where Q_0 is the initial amount of drug in the niosomes, Q_t is the remaining amount of drug in the niosomes at time t, and κ (kappa) is a constant incorporating the surface-volume relation. The data were fitted into the models (zero and first orders, Higuchi and Hixson-Crowell) as reported by Suvikanta et al [5] and the graphical representations were plotted in order to obtain correlation coefficient (R^2).

In vitro **release study**

Cytarabine release rate of release from niosomes is specified by membrane diffusion technique. The noisome suspension equivalent to 1 mg of cytarabine and niosomated cytarabine was poured into a dialysis bags (cut off 12000Da, sigma) separately. The dialysis bags were immersed inside a container containing 250 ml of phosphate buffer, pH 7.4, and placed on the magnetic stirrer (37 ºC, 120 rpm) separately. At certain intervals, 2 ml of phosphate buffer was taken and replaced with an equal volume of the buffer. The ODs of samples were separately measured spectrophotometrically at λ 280nm.

Evaluation of cellular cytotoxicity

Assessment of cytotoxicity on MCF-7cells was performed using MTT assay. The cells were cultured at dilution rate of 1×10^4 cells per well in DMEM medium containing 10% fetal bovine serum and 1% Penicillin/ Streptomycin antibiotics under 10% carbon dioxide at 37° C. After 24 hours of cells culturing, the supernatant was poured off and the cells with pharmaceutical formulations of pure cytarabine and niosomated cytarabine were treated at 0.15, 0.075, 0.0375, 0.0187, 0.0094, 0.0046, 0.0023 and 0.0011 micro-molar concentrations. After 48 hours of incubation, the culture media with pharmaceutical formulations were removed and100 µL of PBS, 0.5 mg/ml MTT solution with pH equal to 4.7 was added to each well and incubation was carried out for 3 hours. The MTT solution was then removed, and 200 µL of isopropanol was added to each well and stirred to dissolve the formed Formazan crystals. In the next stage, absorption was read at λ 570nm using ELISA reader (BioTek Instruments, VTU.SA).The cell viability rate was obtained from the ratio of treated cells absorption with different formulations of the drug to the absorption of control cells, and the results were evaluated using the Pharm program. The IC50 values were reported for each of the samples.

RESULTS AND DISCUSSION

Non ionic surfactants are non/less toxic, imparting stability, less hemolytic, less cell surface irritant, permeability enhancer, wetting and solubilizing agents[10-12] and P-glycoprotein inhibitors [13]. Due to the aforementioned properties drug such as doxorubicin and daunorubicin [14]steroids such as hydrocortisone and dexamethasone [15] , HIV protease inhibitors such as ritonavir and saquinavir [16,17], have been niosomated. Niosomes of doxorubicin prepared from C16 monoalkyl glycerol ether with and without cholesterol were reported by Uchegbu et al [18]. Cytarabine was niosomated through ether injection technique. This method was used by Deamer and Bangham in 1976 as reported by Arora and Jain [19]. It is similar to ethanol injection method; but it differs from ethanol injection method in many ways. It involves injection of immiscible organic solution containing surfactant or surfactant-cholesterol or surfactant cholesterol-diacetyl phosphate or surfactant cholesterol- drug solution mixture very slowly into an aqueous phase through a narrow needle at vaporization temperature of organic solvent. Vaporization of ether leads to the formation of single layered vesicles (SLVs). Namrata et al [20] reported formulation of niosomal aceclofenac through ether injection method employing cholesterol and Span 60 s vesicle constituting components. Aggarwal et al niosomated acetazolamide by different techniques such as reverse phase evaporation, ether injection and film hydration [21]. The entrapment efficiency of acetazolamide was determined to be about 36% (ether injection method) while we found the entrapment efficiency of 98% by column chromatography using Sephadex G-50 .The nano vesicle containing cytarabine was eluted in the void volume of the column as a sharp peak and the unentrapped cytarabine appeared as a second peak. The first peak was pooled and ruptured in order to determine the amount of entrapped drug as reported earlier [9]. Furthermore the zeta potential and size of the prepared niosomal cytarabine were determined to be 12.7 mV and 347 nm respectively (fig. 1). Zeta potential is an important physicochemical parameter, which can influence the stability of niosomal preparation. Extremely positive or negative zeta potential values cause larger repulsive forces, while electrostatic repulsion between particles with the same electric charge prevents aggregation of the spheres [9]. Ideally, the optimum value of zeta potential should lie between +25 and - 25 mv. Outside this range, the niosomes may not remain stable for a long period of time and this could adversely affect parameters such as entrapment efficiency and sustained release. A higher zeta potential indicates higher kinetic energy and tends to move particles towards agglomeration[22]. Scanning electron micrograph of nanoniosomal preparation of cytarabine showed their crystallinity (fig.2). The data obtained from drug release study through dynamic membrane technique was applied to popular mathematical models [23, 24] (fig 3). Table 1 depicts the regression coefficient values indicating the release of cytarabine follows first order kinetic where the concentration depends on time and obeys Hixson-Crowell model. Thus it indicates that the surface area and diameter of the vesicles are changed with time. Thus IC_{50} values for the pure drug and nanoniosomated formulations indicate the higher effectiveness of the cytarabinenoisomal formulation. In

conclusion, as compared to conventional formulation, the drugs carried by vehicles such as niosomes at nano scale can increase the effectiveness and decrease the side effects of the drug under medication.

Figure 3. In vitro release study of niosomated cytarabine and free cytarabine

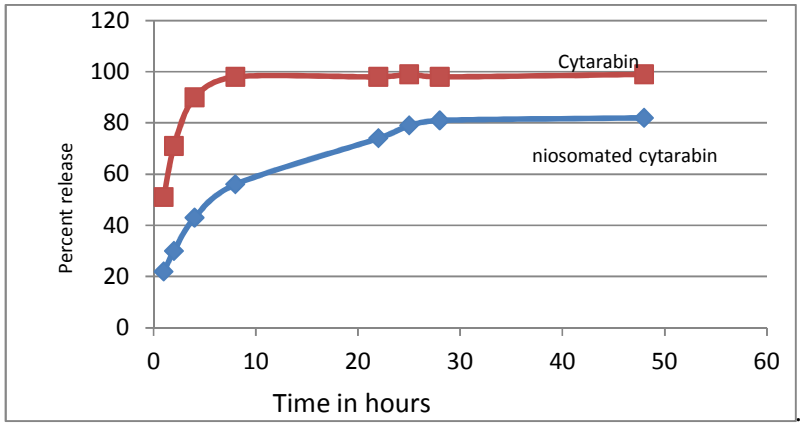


Table 1 Regression coefficient of niosomated cytarabine fitted into different models

Model	Zero order	First order	Higuchi	Hixson and Crowell
Regression coefficient	0.42	0.72	0.95	0.98

Figure 1 Size distribution of niosomated cytarabine

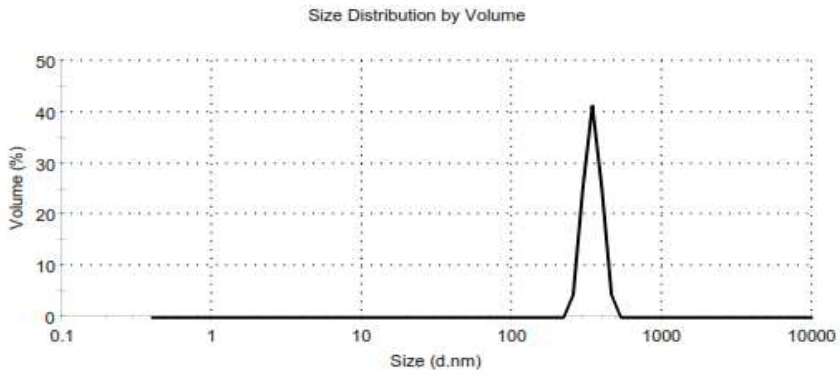
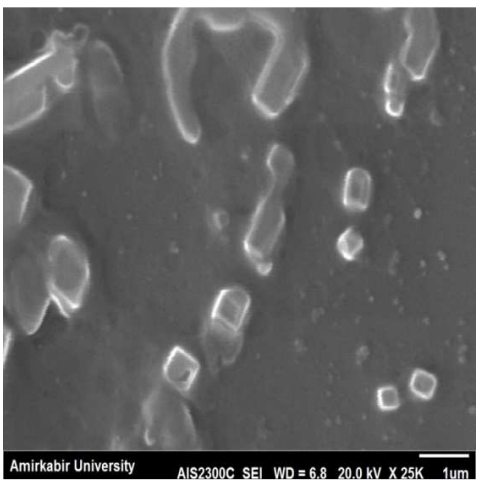


Figure 2 Scanning electron micrograph of niosomatedcytarabin



REFERENCES

[1]Yiyao L; Hirokazu M; N Michihiro, *Colloids and Surfaces B: Biointerfaces*, **2007**; 58: 180–187.
[2]Thakur V; Arora S; Prashar B; P Vishal, *International Journal of Pharmaceutical and Chemical Sciences*, **2012**; 1(3): 981-993.
[3]Navya MN; Parthiban S; Adlin JJN; A Vikneswari, *International Journal of Advanced Pharmaceutics*, **2015**; 5(1): 9-14.
[4]Anchal S; P Pravin, *Journal of Applied Pharmaceutical Science*, **2012**; 2(6):20-32.
[5]Suvakantha D; Padala N M; Lilakanta N; C Prasanta, *Acta Poloniae Pharmaceutica-Drug Research*, **2010**; Vol. 67 (3): 217-223.
[6] Wang WS; Tzeng CH; Chiou TJ; Liu JH; Hsieh RK; Yen CC; PM Chen, *JpnJ Clin Oncol*., **1997**; 27 (3): 154–7.
[7]Ogbomo H; Michaelis M, Klassert D; Doerr HW; J Cinatl, *Neoplasia*, **2008**;10 (12): 1402–10.
[8]Galmarini CM; Mackey JR; C Dumontet, *Leukemia*, **2001**; 15(6): 875-890.
[9]Azizi S; D Norouzian, *Journal of Chemical and Pharmaceutical Research*, **2015**; 7(2): 423-427.
[10]Jiao J, *Adv Drug Deliv Rev*, **2008**; 60: 1663–73.
[11]Zografi G, Interfacial phenomena. In: Gennaro AR, editor, Remington: the science and practice of pharmacy, 17thEdition, Mark Publishing, Pennsylvania,**1995**; 241–51.
[12]Hall DG, Thermodynamics of micelle formation. In: Schick MJ, Editor, nonionic surfactants: physical chemistry, surfactant science series, Marcel Dekker, New York,**1987;** 233–96.
[13]Zhang S; ME Morris. Efflux transporters in drug excretion In: Wang B, Siahaan T, Soltero R, editors,drug delivery: principles and applications, John Wiley & Sons, New Jersey, **2005**; 381–98.
[14]Shtil AA; Grinchuk TM; L Tee, *Int J Oncol.*, **2000**; 17: 387–92.
[15]Ueda K; Okamura N; M Hirai, *J Biol Chem.*, **1992**; 267: 24248–52.
[16]Perloff MD; Von Moltke LL; Marchand JE; DJ Greenblatt, *J Pharm Sci*., **2001**; 90: 1829–37.
[17]Huisman MT; Smith JW; Wiltshire HR; Beijnen JH; AH Schinkel, *J Pharmacol Exp Ther.*, **2003**; 304: 596–602.
[18]Uchegbu IF; Double JA; Kelland LR; Turton JA; AT Florence, *J Drug Target*, **1996**; 3: 399–409.
[19]Arora R; CP Jain, *Asian J Pharm.*, **2007**; 1(1): 29-39.
[20]Namrata M; Vinamrata S; Anu K; Vivek C; S Gunjan, *Journal of Scientific and Innovative Research,***2014**; 3(3): 337-341.
[21]Aggarwal D; Alka G; P KIndu, *Journal of Pharmacy and Pharmacology*, **2004**, 56:1-9.
[22]Gyanendra S; Harinath D; SK Saraf; AS Shubhini, *Tropical Journal of Pharmaceutical Research,* **2011**; 10 (2): 203-210
[23]Arun RC; Raj PSK; KK Satish, *Journal of Pharmacy and Pharmaceutical Science*, **2012**; 4(4): 398-404.
[24]Benoy BB; Biswanth SA; M Arup, *Acta Pharm.*, **2006**; 56: 417–429.