ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preparation and Evaluation of Topical Liposome Containing Glucosamine Hydrochloride

Nadia A Hussein Al-Assady* and Akram Abdul Salam.

Dep. of Chemistry, College of Education Pure Science, University of Basrah, Iraq.

ABSTRACT

Chitin was prepared from the shells of local marine shrimps have been used, were they have been brought from the local market in the city of Basra. Then chitin was hydrolyzed by hydrochloric acid solutions. The production yield of glucosamine hydrochloride from chitin was optimized. Glucosamine hydrochloride was characterized by FT-IR , NMR and Mass spectra. Glucosamine liposomes were prepared by thin film hydration technique using soya lecithin and cholesterol in different weight ratios. They were evaluated for particle size, entrapment efficiency and *in vitro* drug release. The liposomal dispersion which showed an entrapment of (88,94)%and drug release of (74.32,85.93) % in 9hrs was optimized.

Keywords: topical liposome, glucosamine, chitin, marine shrimps.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Glucosamine (2-amino-2-deoxyglucose) is an amino sugar that occurs in acetylated and polymerized forms in chitin(1) (Figure 1). Chitin is mainly produced from cuticles of various crustaceans, principally crabs and shrimps(2,3). Glucosamine in the human body participates in the structure of cartilage and works to stimulate joint function and repair(4,5). It has been proven effective in numerous scientific trials for easing osteoarthritis pain, aiding in the rehabilitation of cartilage, renewing synovial fluid, and repairing joints that have been damaged from osteoarthritis (6, 7).

The preparation of glucosamine hydrochloride from chitin is a simple hydrolysis reaction. During this reaction, chitin is deacetylated and depolymerized to glucosamine hydrochloride in the presence of hydrochloric acid solution. Kamasastri and Prabhu prepared glucosamine from chitin by treating it with a large excess of concentrated hydrochloric acid (8,9).

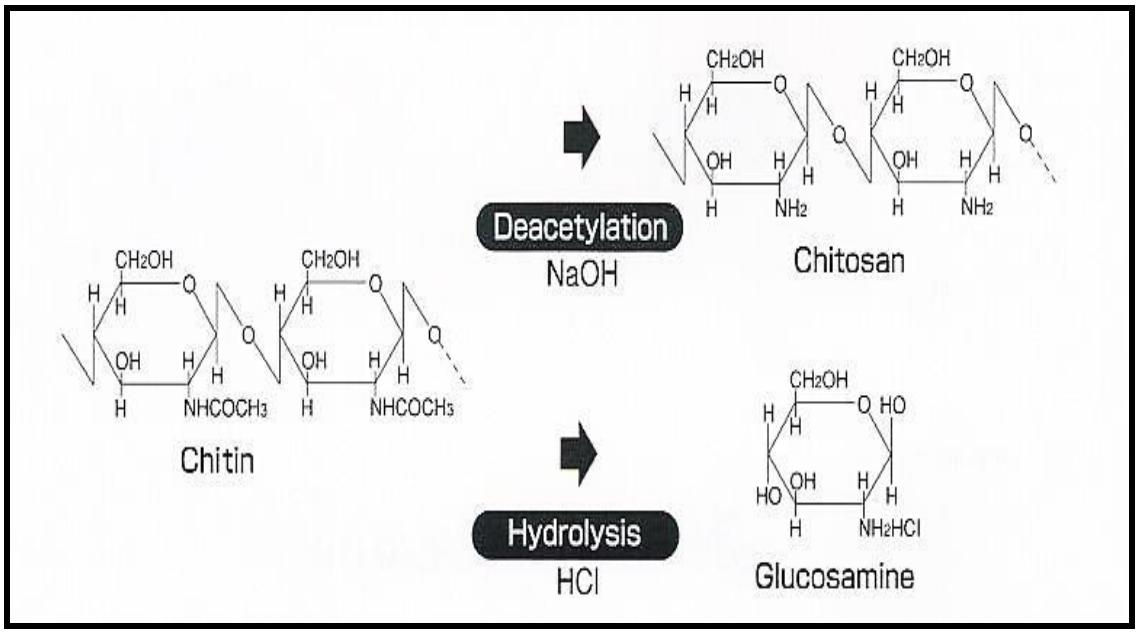


Figure (1): Chemical structures of chitin, glucosamine hydrochloride

Liposomes have been frequently studied as drug delivery carriers because they have the potential to achieve the goals of delivery (concentration, protection, evasion, targeting, and release) when properly produced. Before the liposomes can become useful carriers, they must be able to hold a therapeutically useful amount of drug. There are many possibilities for loading hydrophilic drugs and particles into the liposomes' internal volume. It is also possible to load hydrophobic drugs into the membrane structure(10-12).

Glucosamine hydrochloride is a hydrophilic molecules and water soluble. It is proposed that using these molecules in a topical liposomal formulation may show better moisturizing effect. The aim of the present study was to prepare and characterize liposomes containing glucosamine hydrochloride. The glucosamine hydrochloride was synthesized from shrimps. The particle size, size distribution, release characteristics, and drug diffusion were studied .

MATERIALS AND METHODS

Chemicals

Shrimps have supplied been brought from the local market in the city of Basra. Soya lecithin was purchased from Merck, Germany. Cholesterol was supplied by (B.D.H,England) . All of the solvents were of analytical grade. Muller-Hinton Agar was supplied by (Titan Biotech Co., Rajasthan, India). dialysis membrane,

12,000 -14,000 molecular weight cut off was purchased from Spectrum Laboratories Inc., USA. All other chemicals were of reagent grade.

Preparation Methods:

Extraction of chitin from Shells of Shrimp (13)

In this study, the shells of local marine shrimps have been used; they have been brought from the local market in the city of Basra. First the collected shrimp shells were washed with tap water, and then the samples were sun light dried for 24 hours, and further dried in oven at 80° C, then we crashed into fine powder. And treated (15g) with (125ml) boiled acidic water (10%HCl) in the (250ml) reaction vessel fitted with a condenser for one day then the product was filtered, washed with distilled water and dried at 50°C under vacuum . The powder was treated with (125ml) of (5%NaOH) solution for 12hrs at 80°C in water bath.

The product was filtered, dried and dissolved in (100ml) of (90%) formic acid at room temperature. Formic acid was removed by rotary evaporator at 90°C. The product was washed with distilled water (3times), dried and powdered.

Preparation Glucosamine Hydrochloride (14)

The process for Glucosamine Hydrochloride production was performed as follows: 10g of chitin and (150ml) of HCl conc, were introduced into (250 ml) reflux vessel, the mixture was kept at the given temperatures until the solid was completely dissolved. The resulting hydrolyzed was filtered to remove the solid particles present in solution, and it was left to crystallize at room temperature (25 ± 2°C by 25 days). In order to increase the crystallization rate, ethyl alcohol (15 mL, w = 95%) was added, and the Production of Glucosamine Hydrochloride from Crustacean Shell**.**

Preparation of Liposomes (15, 16)

Aqueous liposomal dispersions were prepared by conventional thin film hydration method. Different weight ratio of soya lecithin, and cholesterol were weighed and dissolved in chloroform and methanol mixture (2:1) in 125ml round bottom flask. A thin film was formed on evaporating organic solvent under vacuum using rotary evaporator at (45-60) °C. The dried lipid film was hydrated with (10ml) of phosphate buffer solution (pH 7.2) which containing drug. The dispersion was left undisturbed at room temperature for 2-3 hours to allow complete swelling of the lipid film and hence to obtain vesicular dispersion.

Table 1: The weight ratios used in the preparation of the liposomes

Formulation Code	Drug(mg)	Soya lecithin(mg)	Cholesterol (mg)
FL1	30	100	10
FL2	30	100	20
FL3	30	100	30
FL4	30	100	40
FL5	30	100	50
FL6	30	100	60
FL7	30	80	30
FL8	30	90	30
FL9	30	100	30
FL10	30	200	30
FL11	30	300	30
FL12	30	400	30

ISSN: 0975-8585

FL13	30	500	30

Analytical Methods

Characterization of Prepared Glucosamine

FTIR spectrum of glucosamine hydrochloride were recorded using a FTIR (model 4100 type A, Perkin-Elmer, Norwak, CT, USA) spectrometer using KBr pellets (400–4.000 cm−1) .

FT-IR spectrum of Glucosamine hydrochloride

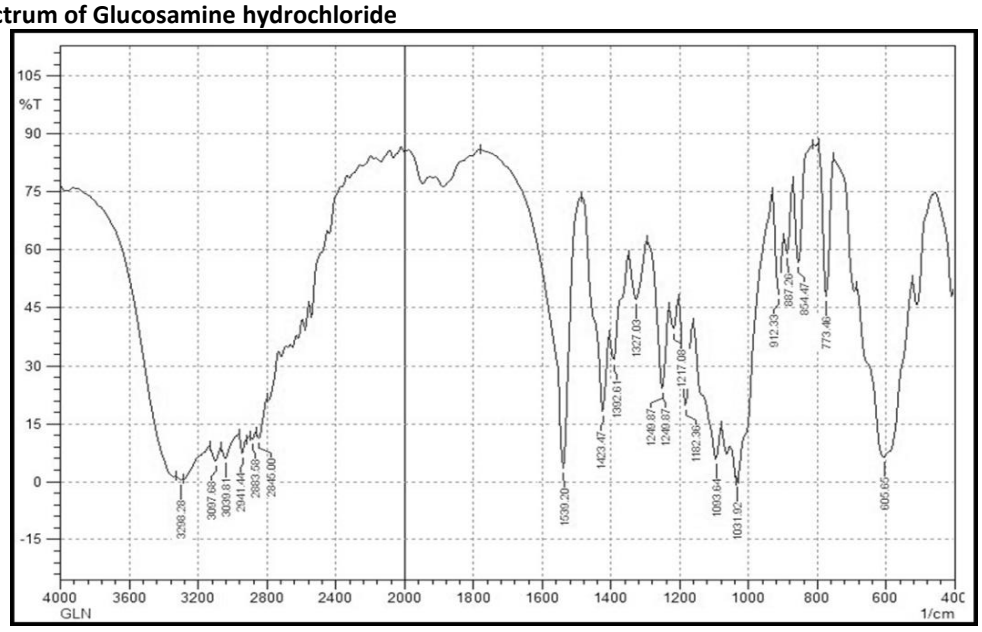


Figure 2: FTIR of Glucosamine Hydrochloride

The FT-IR spectrum of Glucosamine hydrochloride produced (Fig. 2) exhibits an intense band at 3370-3300 cm^{-1} associated with the O–H and N–H stretching, a NH_2 scissoring band at 1615 cm^{-1} and at 1094 cm^{-1} due to secondary alcohol –OH.

Mass Spectra

The exhibit peaks, and the base of beaks of the Glucosamine Hydrochloride is dependent on the derivatives of acetylene.

ISSN: 0975-8585

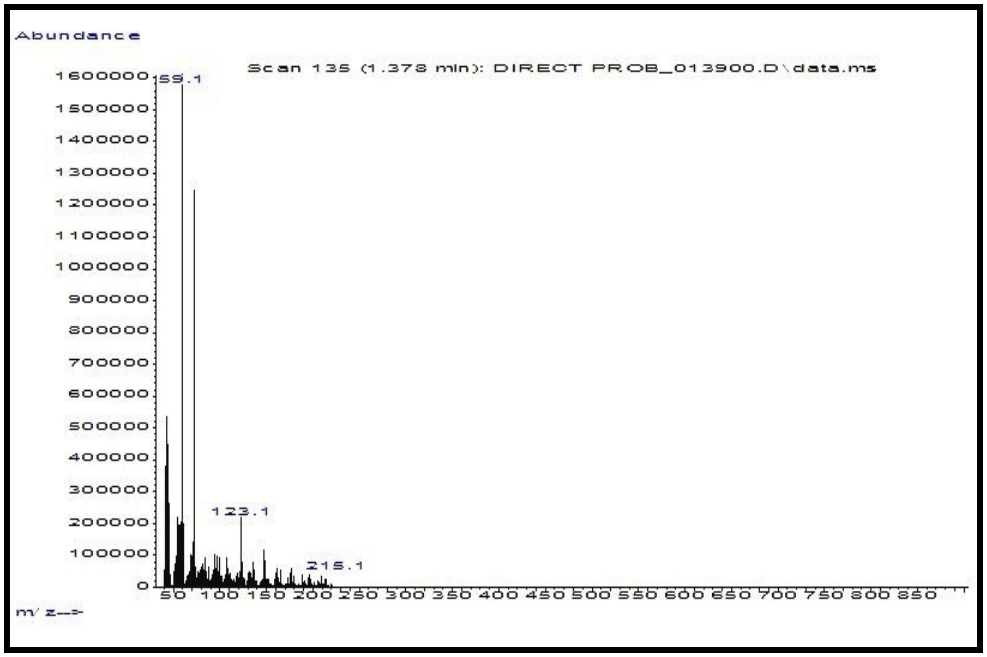


Figure 3: **Mass Spectrum of Glucosamine Hydrochloride**

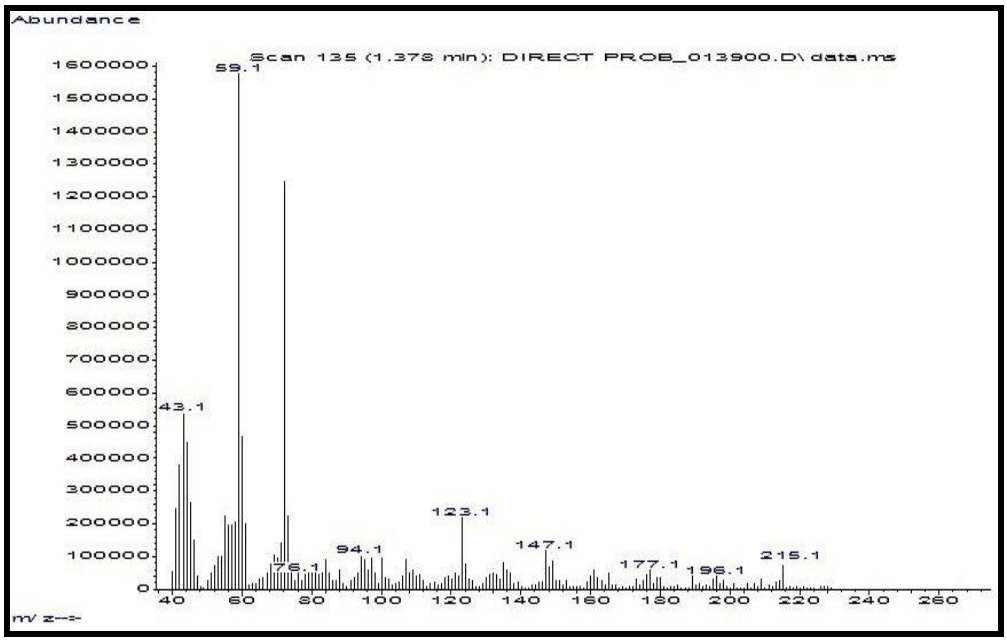


Figure 4: Spectrum resulting from fragmentation in the GLCN

ISSN: 0975-8585

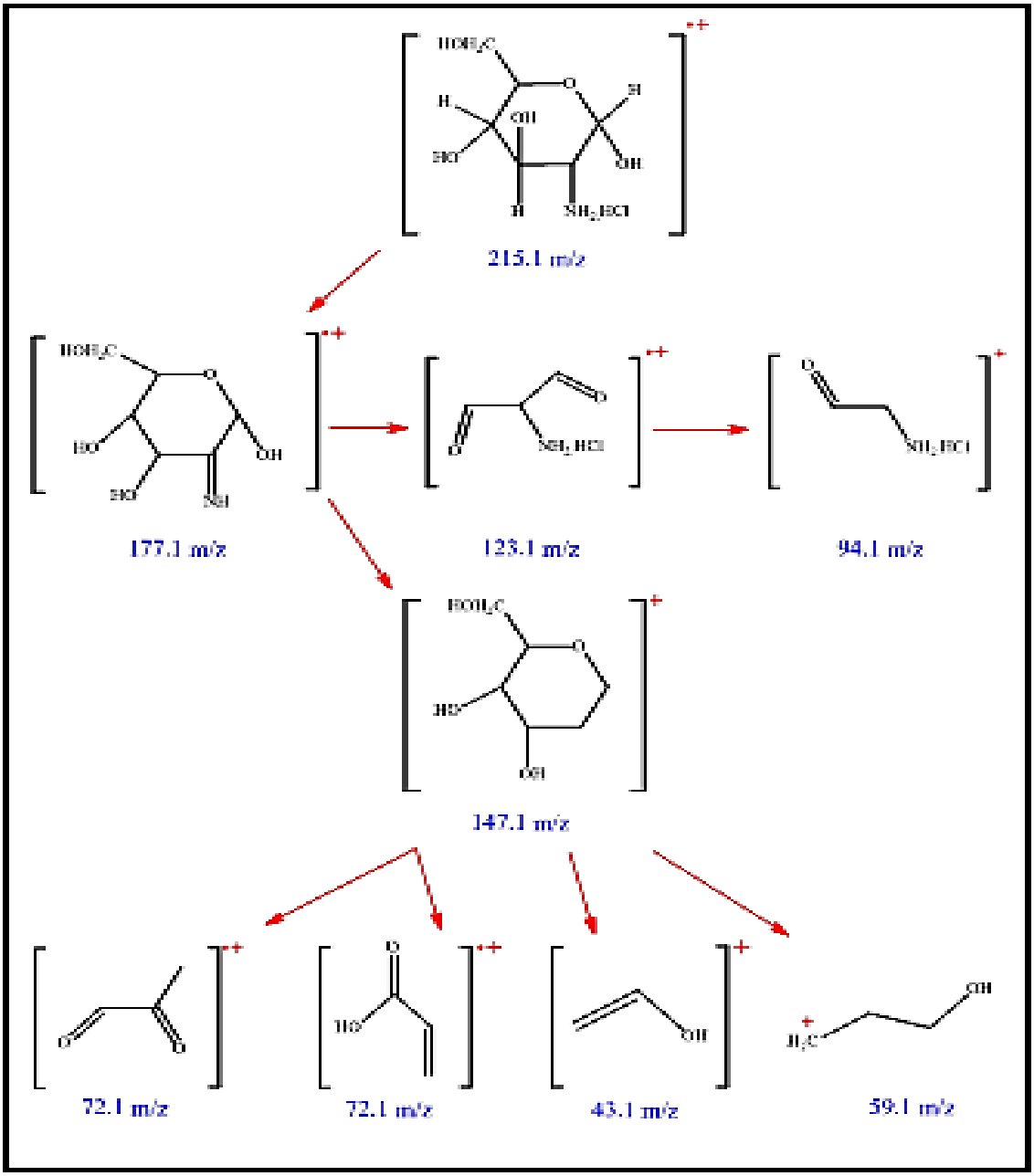


Figure 5: Proposed mechanism for the formation of glucosamine degradation

[1]HNMR Spectra

Glucosamine Hydrochloride was determined by 1H-NMR(Bruker2000) (Figure 6) and table (2)

ISSN: 0975-8585

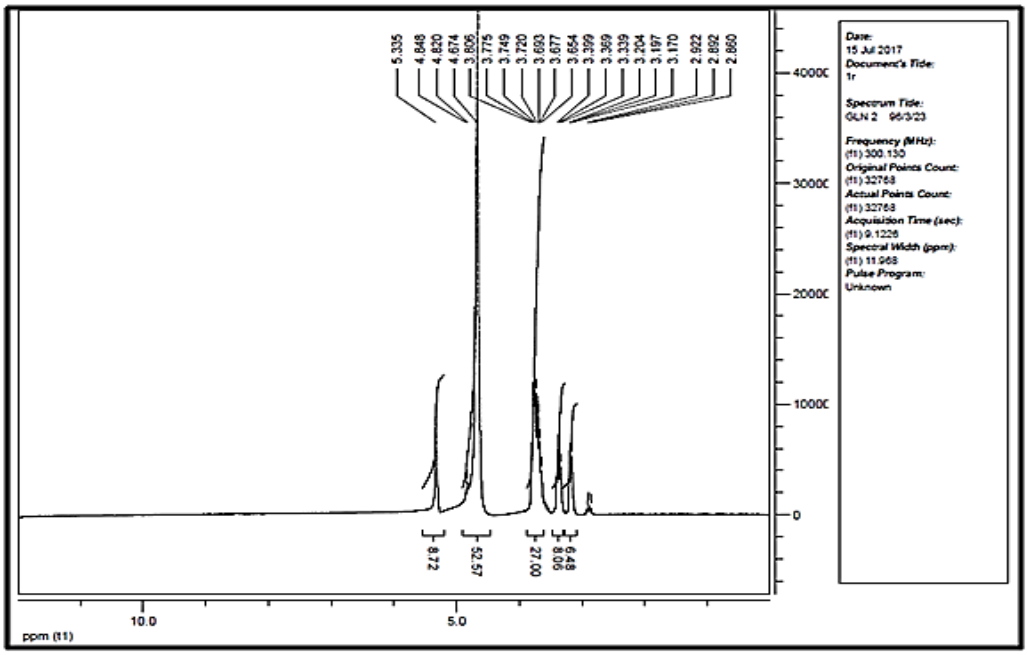


Figure 6: chemical shifts of the ^{1}H NMR signals

Table 2: chemical shifts of the 1H NMR signals

Protons site	Group	Signal shape	Number of protons	Chemical Shift (ppm)
C3	C-H	Triplet	1	3.197
C2	C-H	Triplet	1	3.369
C4 C5 C6	C-H C-H C-H	Triplet Quartet Doublet	1 1 2	3.654-3.806
C2 C1,C3,C4,C6 C2	N-H O-H H-Cl	Singlet Singlet Singlet	2 4 1	4.848 4.674 4.848
C1	C-H	Singlet	1	5.335

RESULTS AND DISCUSSION

Characterization Liposomes

Optical Microscopy

The vesicles were observed under optical microscope(Carl Zeiss) was found to be discrete and spherical in shape. The images(shown in Fig 7) clearly indicated the discrete structures of liposomes vesicles.

ISSN: 0975-8585

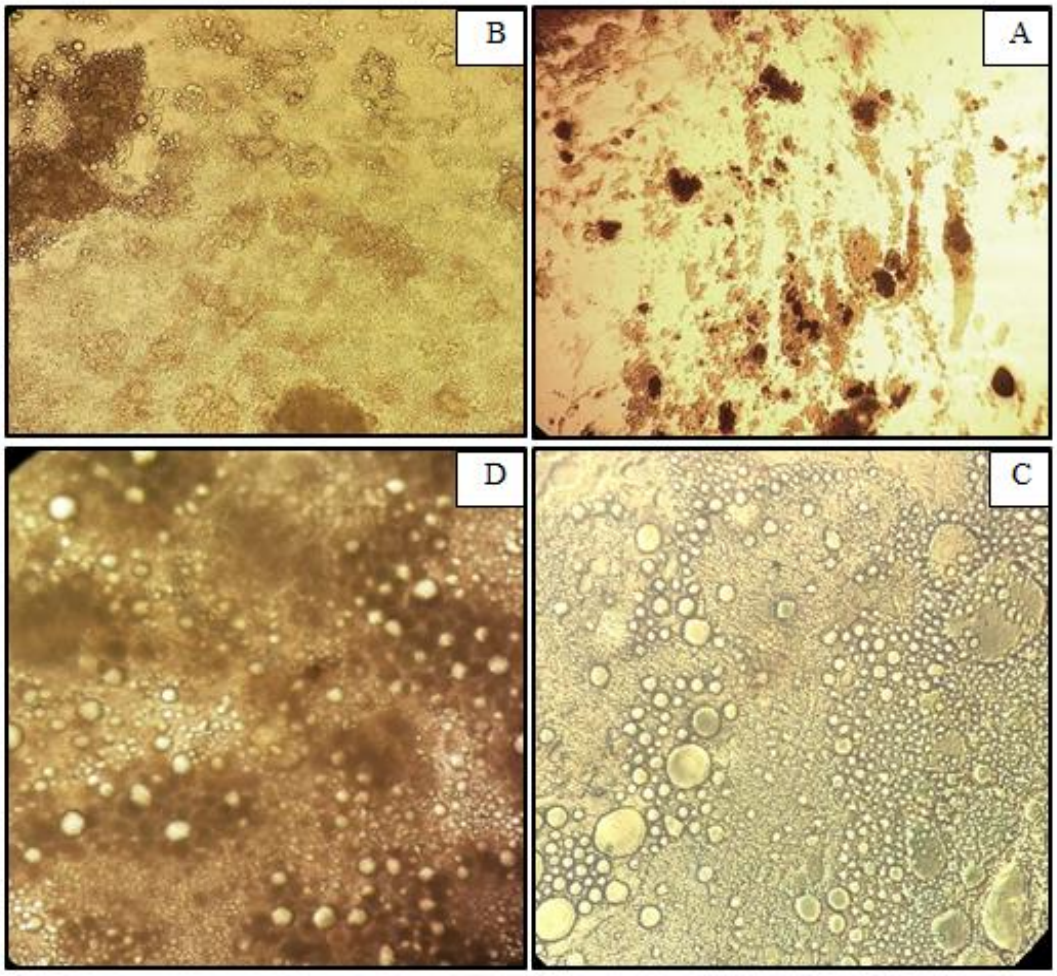
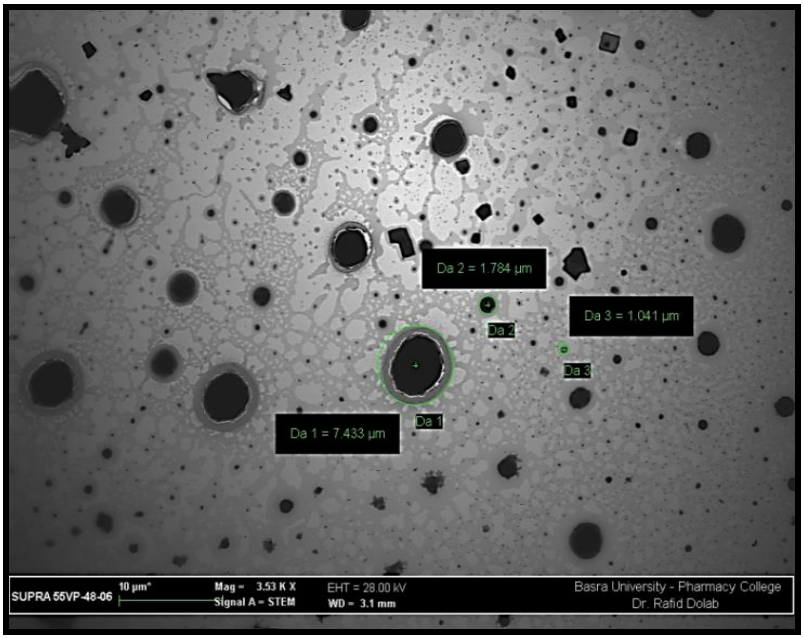


Figure 7: Liposome in Optical Microscope

Transmission electron microscopy (TEM)



ISSN: 0975-8585

Figure 8: Liposome in Transmission electron microscope at(µm)

Drug-loaded liposomes were observed by TEM(Carl Zeiss) using a JEM 1010®, Jeol (USA). Liposomal dispersion (500 µL) was diluted with phosphate buffer and stabilized with glutaraldehyde 2.75%. The samples were prepared by placing the diluted liposomes onto a 400-mesh grid coated with carbon film. TEM images were analyzed using the soft-imaging software ImageJ® (measurement included 100 liposomes).

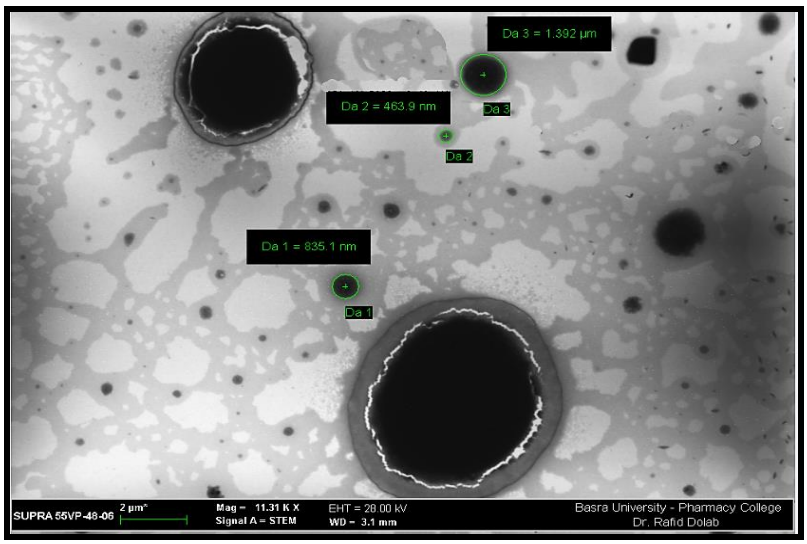


Figure 9: Liposome in Transmission electron microscopeat (nm)

Drug Content, Entrapment Efficacy and Percentage Drug Release of Liposomes

The glucosamine liposomal formulations were subjected to the estimation of drug content. The drug content was in the range of 94.78 % w/w to 101.81 % w/w for the liposome formulations. (as shown in Table 3)

Table 3: Percentage Drug Release of Liposomes

Formulation Code	Percentage Yield %
FL1	65
FL2	74
FL3	88
FL4	75
FL5	61
FL6	52
FL7	63
FL8	82
FL9	88
FL10	94
FL11	85
FL12	71
FL13	54

Invitro Drug Release Studies (17-19)

ISSN: 0975-8585

The glucosamine Liposomes were subjected to in vitro diffusion studies and the results indicated that the formulation FL3 showed highest in vitro drug release (% 74.32) (as shown in Fig 10, Table 4). The formulation FL6 showed lowest in vitro drug release of % 50.94. The diffusion constants of formulations F8 and F1 were 0.029 and 0.009 respectively (as shown in Table 3). The formulation F8 showed greater diffusion constant and F1 showed lesser diffusion constant.

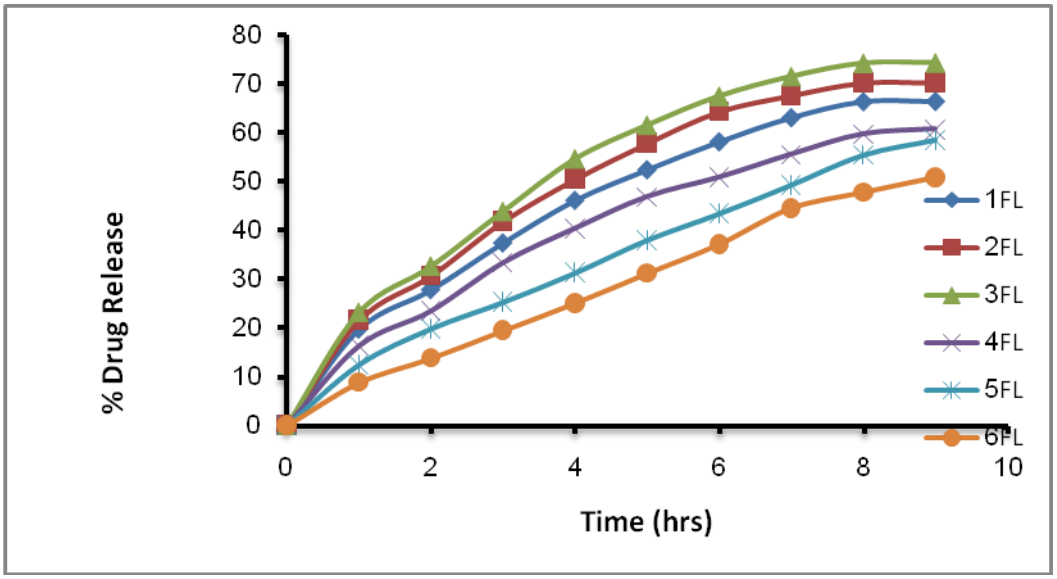


Figure 10: In vitro percentage drug release of liposomes prepared by different cholesterol concentrations

Table 4: In vitro percentage drug release of liposomes prepared by different cholesterol concentrations

FL6	FL5	FL4	FL3	FL2	FL1	Time(hour)
0	0	0	0	0	0	**0**
8.83	12.47	16.36	23.17	21.51	19.71	**1**
13.83	19.82	23.51	32.64	30.65	27.79	**2**
19.49	25.24	33.35	43.86	41.76	37.35	**3**
25.13	31.23	40.41	54.63	50.42	46.06	**4**
31.22	37.92	46.87	61.54	57.75	52.32	**5**
37.21	43.32	50.85	67.49	64.27	58.04	**6**
44.61	49.18	55.54	71.51	67.58	62.99	**7**
47.87	55.31	59.68	74.24	70.18	66.27	**8**
50.94	58.39	60.71	74.32	70.21	66.32	**9**

ISSN: 0975-8585

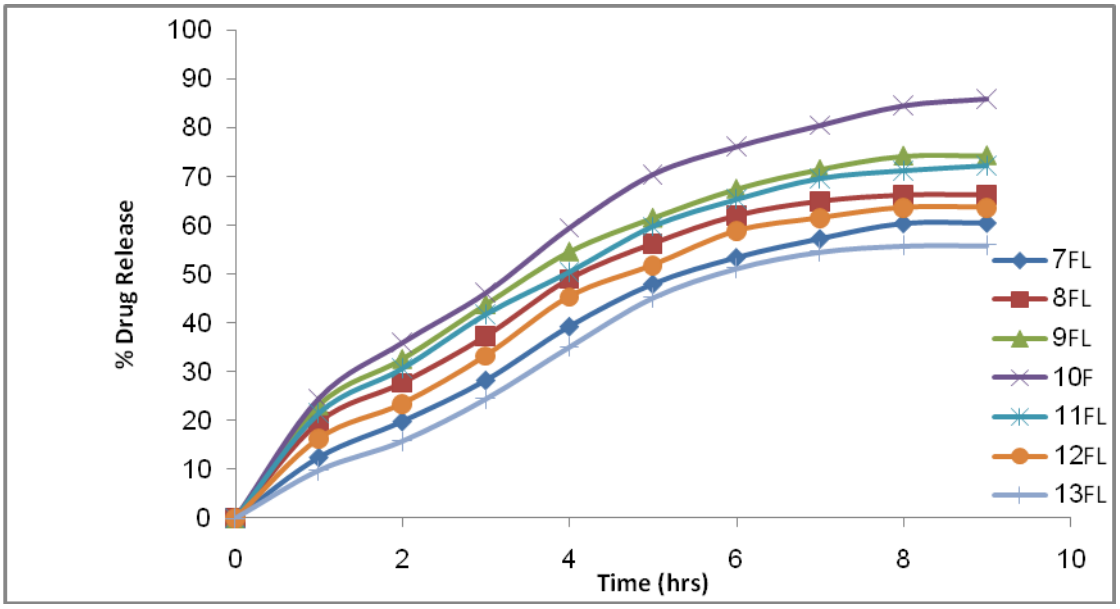


Figure 11: In vitro percentage drug release of liposomes prepared by different lecithin concentrations

Table 5: In vitro percentage drug release of liposomes prepared by different lecithin concentrations

FL13	FL12	FL11	F10	FL9	FL8	FL7	Time(hour)
0	0	0	0	0	0	0	**0**
9.83	16.36	21.51	24.53	23.17	19.71	12.47	**1**
15.83	23.51	30.65	35.96	32.64	27.79	19.82	**2**
24.49	33.35	41.76	46.17	43.86	37.35	28.24	**3**
35.13	45.41	50.42	59.48	54.63	49.06	39.23	**4**
45.22	51.87	59.75	70.43	61.54	56.32	47.92	**5**
51.21	58.85	65.27	76.12	67.49	62.04	53.32	**6**
54.61	61.54	69.58	80.49	71.51	64.99	57.18	**7**
55.87	63.68	71.18	84.52	74.24	66.27	60.31	**8**
55.94	63.71	72.21	85.93	74.32	66.32	60.39	**9**

Kinetics of Drug Release(20,21)

The slopes and the regression coefficient of determinations are listed in (Table 5). The coefficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. Additional evidence for the diffusion controlled mechanism was obtained by fitting the Korsmeyer–Peppas equation to the release data. The diffusion exponent n value was found to be in range of (0.014 to 0.985) for different glucosamine liposomes compositions, indicating Fickian diffusion for (less 0.5) and Non- Fickian diffusion for (above 0.5) of drug through liposomes.

ISSN: 0975-8585

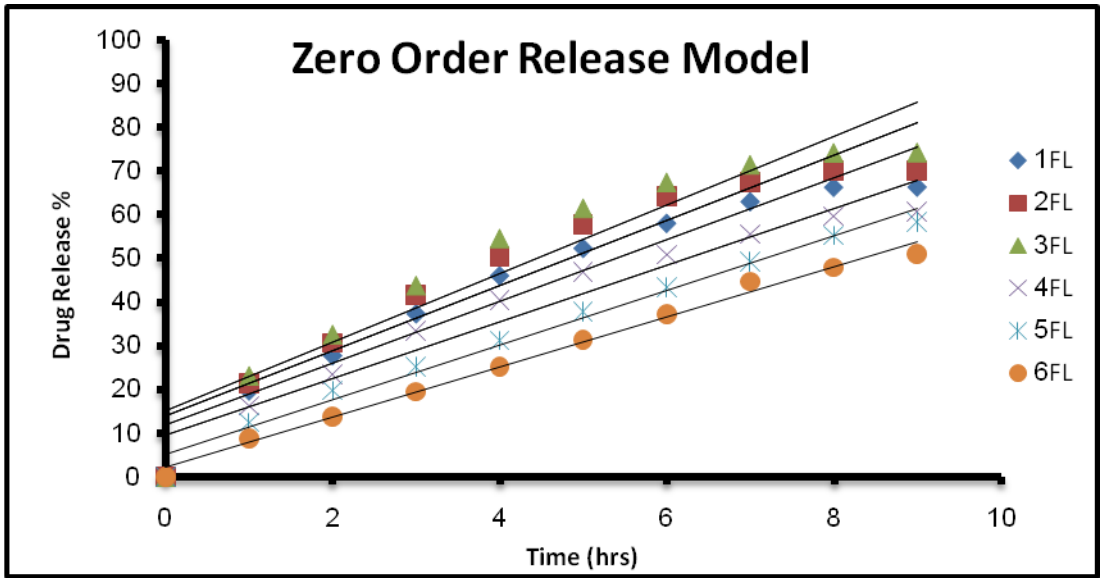


Figure 12: kinetics of freeing the drug from the zero order of the liposomes when the concentration of cholesterol is changed

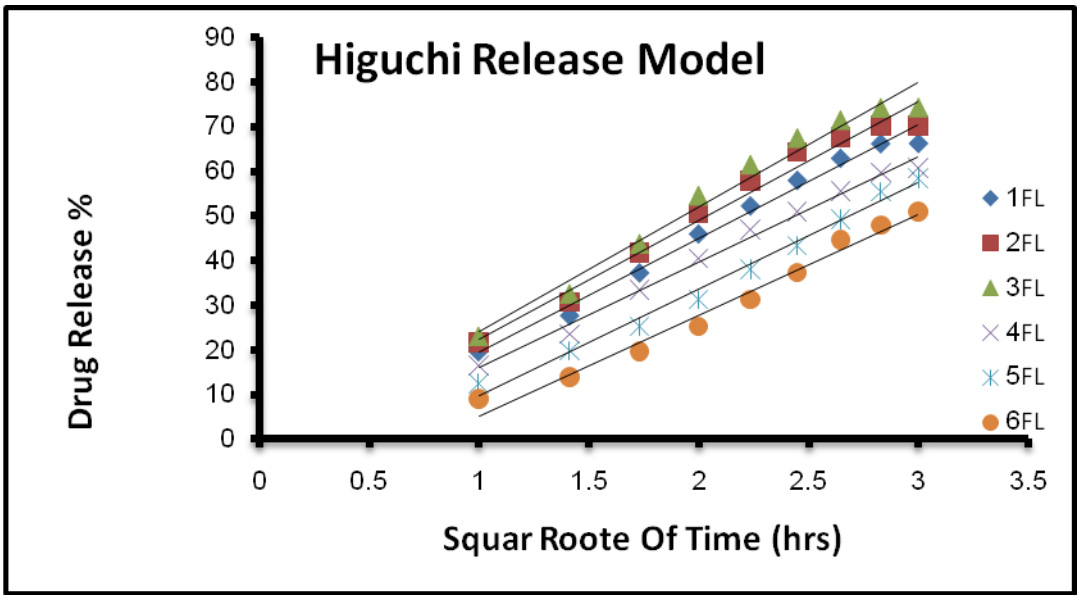


Figure 13: Kinetics of drug release according to the Higuchi equation of liposomes when cholesterol concentration is changed

ISSN: 0975-8585

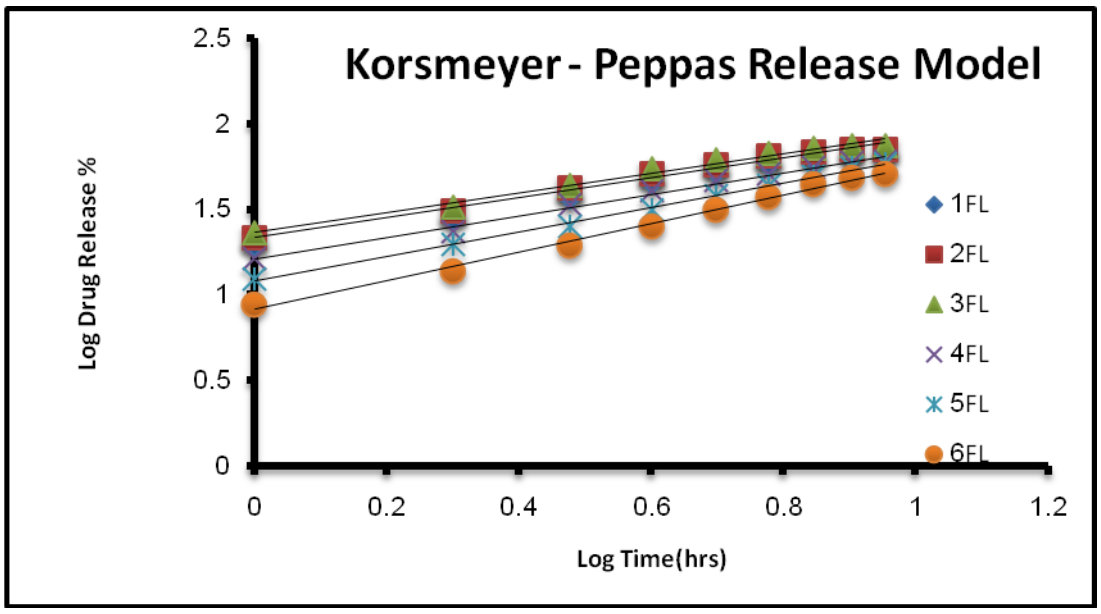


Figure 14: Kinetics of drug release according to the Korsmeyer equation of liposomes when cholesterol concentration is changed

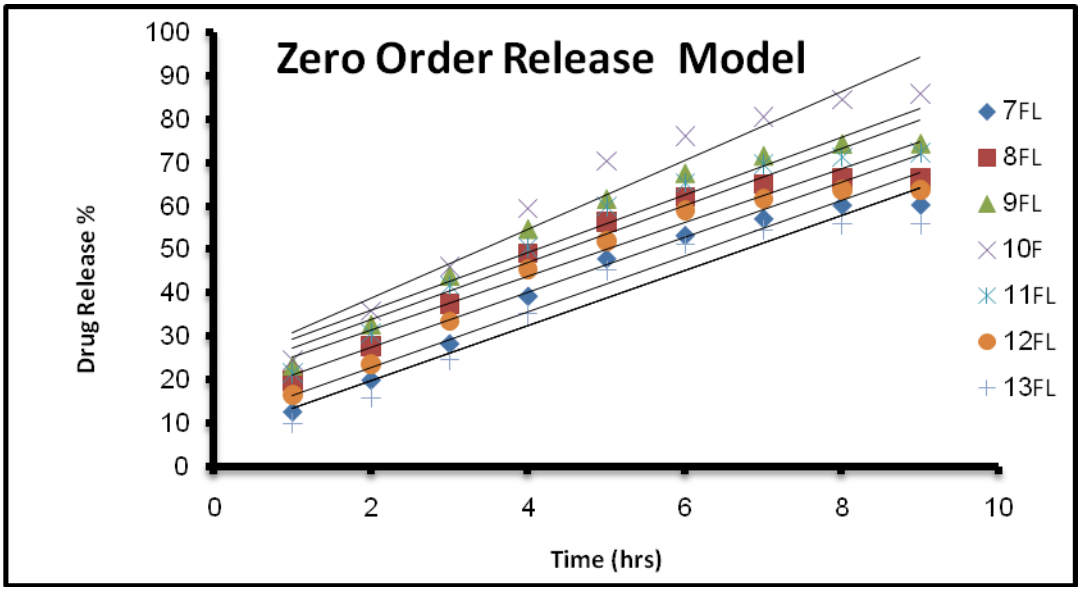


Figure 15: Kinetics of drug release from the zero order of the liposomes when the concentration of lecithin is changed

ISSN: 0975-8585

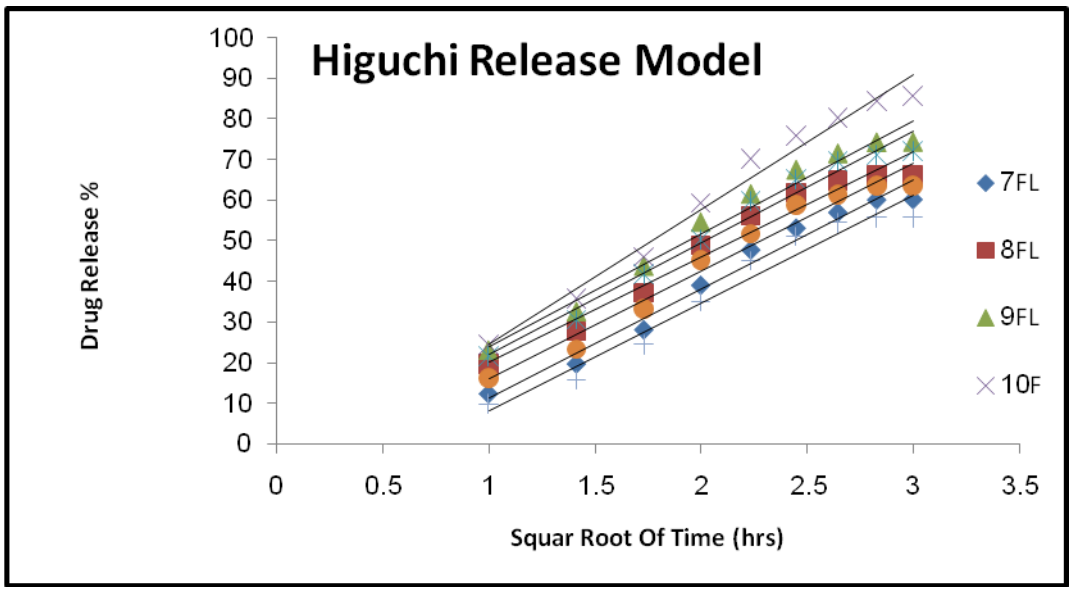


Figure 16: Kinetics of drug release from the Higuchi equation of the liposomes when the concentration of lecithin is changed

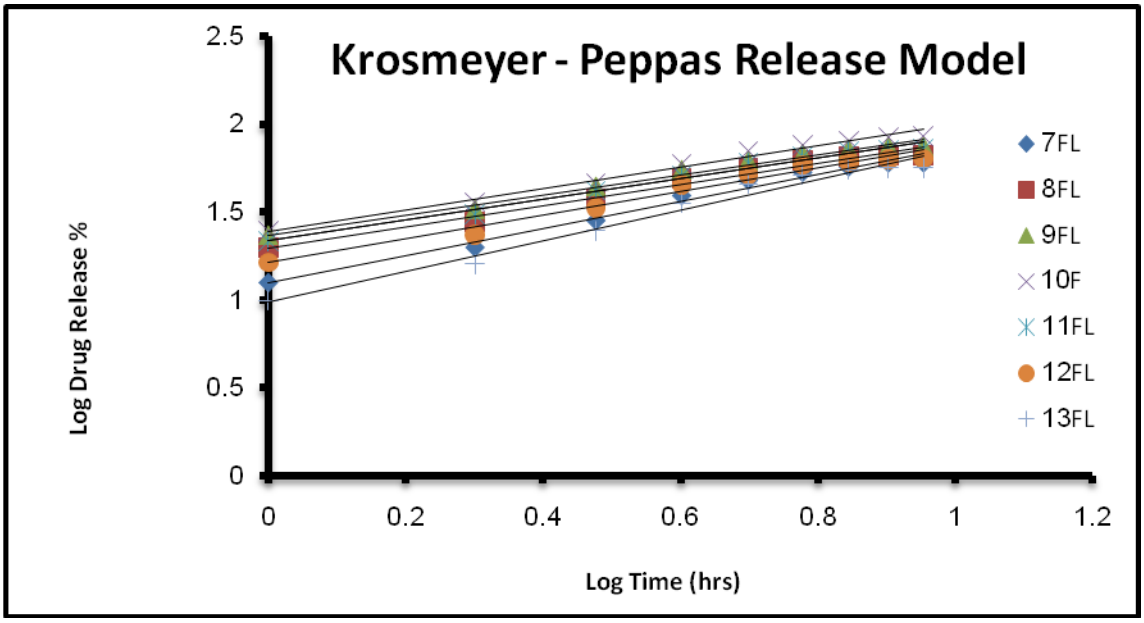


Figure 17: Kinetics of drug release from the Korsmeyer equation of the liposomes when the concentration of lecithin is changed

Table 5: Correlation coefficient values for different Liposomes formulations

Formulation	Zero order	Higuchi model	Peppas model	
			R^2	'n' value
F1	0.951	0.987	0.991	1.291
F2	0.929	0.978	0.986	1.337
F3	0.926	0.976	0.984	1.368
F4	0.960	0.992	0.992	1.212
F5	0.996	0.988	0.997	1.08
F6	0.993	0.980	0.994	0.914
F7	0.941	0.976	0.983	1.096

F8	**0.919**	**0.962**	**0.975**	**1.295**
F9	**0.926**	**0.976**	**0.984**	**1.368**
F10	**0.939**	**0.981**	**0.987**	**1.39**
F11	**0.931**	**0.977**	**0.985**	**1.334**
F12	**0.921**	**0.967**	**0.976**	**1.21**
F13	**0.925**	**0.962**	**0.974**	**0.988**

REFERENCES

[1] Clegg, D. O., and Jackson, Ch. G. 2005. P. M., Blackman, M. R., Cragg, G., Levine, M., Moss, J., and White, J. D. New York: Marcel Dekker.

[2] Xu, Y., Gallert, C. and Winter, J. 2008. Appl. Microbiol.Biotechnol. 79: 687-97.

[3] S.B. Carter, S.E. Nokes, C.L. Crofcheck, Letters in Applied Microbiology 47 (2004) 945–949.

[4] Pujalte JM, Llavore EP, Ylescupidez FR. Double-blind clinical. Curr Med Res Opin 1980;7:110–4.

[5] Pipino F, Franchin F. "Double-blind" clinical study of a nonsteroid anti-inflammatory drug: glucametacine. Minerva Med 1980;71:2845–50. In Italian.

[6] Chiusaroli, R., Piepoli, T., Zanelli, T., Ballanti, P., Lanza, M., Rovati, L. C. & Caselli, G. (2011). Hindawi Publishing Corporation International Journal of Rheumatology, doi:10.1155/2011/939265.

[7] Luo, J., Hu, Y. S., Wu, Y., and Fan, W. K. 2005. Chin. J. Clini. Rehabil. 9: 70-2.

[8] Novikov, V. Y. 2004. "Acid Hydrolysis of Chitin and Chitosan." Russ. J. Appli. Chem. 77: 484-7.

[9] Li, J.-l., Zhou, Y., Zhang, M., and Zhang, H.-b. 2007. "Optimization of." Anhui Med. Pharm. J. 11 (1) 11-2.

[10] Priyanka R Kulkarni, Jaydeep D Yadav, Kumar A Vaidya. Int J. Curr Pharm Res, 3(2), 2011, 10-18.

[11] Bangham A.D, Standish M.M, Watkins J.C. Journal of Molecular Biology 1965;13 238-252.

[12] Chein Y M. Novel drug delivery systems, New York, Marcel Dekker Inc, 2ndedition, 50, 1992,1-2.

[13] Z. Hossain Asaduzzaman ,J. Sc. Ind .Res. ,40(3-4),163-168,(2005)

[14] Anderson, J. W., Nicolosi, R. J., and Borzelleca, J. F. (2005)." Food Chem. Toxicol. 43: 187-201.

[15] Argan, N. S. L., Harikumar and Nirmala, (2012). International Journal of Research in Pharmacy and Chemistry, 2(2).

[16] Mansoori, M. A., Jawade, S., Agrawal, S., & Khan, M. I. (2012). IJPI's Journal of Pharmaceutics and Cosmetology, 2(10), 23-29.

[17] Mitkari, B. V., Korde, S. A., Mahadik, K. R., & Kokare, C. R. (2010). Indian Journal of Pharmaceutical Education and Research, 44(4), 324-329.

[18] B. R. Srinivas Murthy*1, K.Vishakha2, P. Prakash1, D. Jamal Basha3, T. Govardhan1, Muni Sai Prathap1, International Journal of Research in Pharmaceutical and Nano Sciences. 4(3), (2015), 162-174.

[19] Nadia A Hussein Al-Assady* and Anssam K Numa.Research Journal of Pharmaceutical, Biological and Chemical Sciences . 2014 5(4) 295-312.

[20] Nadia A.Hussein Al-Assady , Eman Saad, Inaam M. N. Alrubayae, Journal of Basrah Researches ((Sciences)) 2013, 39. (3),114-131

[21] Nadia A.Hussein Al-Assady,Sroor W. Alkanani.Journal of International Academic Research for Multidisciplinary 2016, 4(3), 84-103.