IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 3 Ver. II (May.- Jun.2016), PP 10-18
www.iosrjournals.org

Development and in Vitro Evaluation of Ambroxol Hydrochloride Niosomes Using Thin Film Hydration Method

Parika Singh*[1] Mrs.Nitya Shanti[2] Basant Sharma[1] and Neha krishnarth[3]
[1]*Consern Pharmaceuticals, Ludhiana*
[2]*Sardar Bhagwan Singh post graduate institute of Pharmaceutical Sciences, Dehradun*
[3]*Dr.K.N.Modi Institute of Pharmaceutical Sciences, Modinagar*

Abstract: *Niosomes (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 main objective of research work provides sustained release of drug, maximum therapeutic effect for a long period of time, also decreases the frequency of dosing. Ambroxol HCl used as model drug, used in pulmonary bronchitis. Therefore formulation of Ambroxol HCl as niosomes would provide advantages of sustained release dosage forms. Niosome vesicles were prepared using thin layer evaporation method and were physico-chemically characterized. Ambroxol HCl was formulated with different non ionic surfactants such as Span20, Span60, Tween60 and Tween80 and Dicetyl phosphate (DCP) as charge inducing agent. The average diameter of the vesicles was found to be 4.16. The in vitro diffusion studies suggest that higher entrapment efficiency was found with slow release. Niosomes prepared using Span 20 (F10, F11, and F12) shown better entrapment than that was prepared using Span60 (F1, F2, F3). Tween60 (F7, F8, F9) also shown better entrapment efficiency than Tween 80 (F4, F5, F6).Tween 60 shown better entrapment than other formulations.*

Keywords: *Ambroxol Hydrochloride (AMB), Cholesterol, DCP, Niosomes, Span 20, Span60, Tween60, Tween80, Thin film hydration method.*

I. Introduction

Development of new drugs is difficult, expensive and rather time consuming in the process involving preclinical testing, investigational new drug application (IND), clinical trials, phase I, II, & III, new drug application (NDA) and FDA approval. Improving safety and efficacy of existing drugs has been attempted using different methods such as individualizing drug therapy, dose titration and therapeutic drug monitoring and, most importantly, delivering drugs at controlled rates at targeted sites [1]. Today, lipid and non-ionic surfactant based drug delivery systems have drawn much attention from researchers as potential carriers of various bioactive molecules that could be used for therapeutic applications. Several commercial liposome/niosome-based drugs have already been marketed with a great success. For example, liposomes and niosomes have been used to encapsulate colchicines [3], estradiol [4], tretinoin [5, 6], dithranol [7, 8] and enoxacin [9] for applications such as anticancer, anti-tubercular, antileishmanial, anti-inflammatory, hormonal drugs and oral vaccines [10- 17]. Different Novel approaches used for delivering these drugs include liposomes, microspheres, nanotechnology, micro-emulsions antibody-loaded drug delivery, magnetic microcapsules, implantable pumps and niosomes [18]. Non-ionic surfactant vesicles (NSVs or niosomes) result from the self assembly of hydrated surfactant monomers. They are similar in physical structure and form to the more widely studied phospholipids vesicles (liposomes) [22], consisting of single or multiple surfactant bilayers (lamellae) enclosing an aqueous core. A schematic diagram of a non-ionic surfactant vesicle is shown in Fig. Preliminary X-ray scattering data on unilamellar sorbitan monostearate (C18- sorbitan monoester)-cholesterol niosomes suggest a bilayer spacing of 15 nm and a bilayer thickness of 3.3-3.4 nm [23], the latter similar to the figure obtained for the bilayer thickness of phospholipid vesicles (3.4-3.9 nm) [24]. Although terminology suggests that distinctions exist between liposomes and niosomes, of which the basic unit of assembly is the amphiphile, their properties are largely similar and the differences between liposomes (phospholipid vesicles) and non-ionic surfactant vesicles are sometimes blurred as liposomes are often prepared incorporating a non-ionic surfactant component [25,26], while non-ionic surfactant vesicles may also be formulated with various ionic amphiphiles such as stearylamine and dicetylphosphate [27,28] to achieve greater protection against flocculation in vesicle suspensions. The removal of unentrapped solute from the vesicles is important as it may cause undesirable side effects and may add charge to niosomes leading to physical instability.

II. Method Of Preparation

2.1 Determination of Melting point: An open end capillary tube was taken and one end is closed by fusion and the drug powder was filled into it. Then, the capillary was placed in silicon oil bath in a digital melting apparatus and the temperature at which the powder first starts melting was noted as the melting point.

2.2 Scanning Of Drug: The scanning of drug was performed by double beam UV Spectrophotometer to determine the ƛ max of drug, it was found to be 242.6 nm in pH-7.4 phosphate buffer and 0.1N Hydrochloric acid.

2.3 Preparation of Standard curve of Ambroxol Hydrochloride: In methanol: 25 mg of pure drug was accurately weighed using electronic balance and dissolved in 50 ml of methanol to produce a stock solution of 500 µg/ml. Subsequent dilutions were made from sub- stock to produce solutions of concentration 2-10µg/ml. These solutions were then scanned on a double beam UV spectrophotometer to obtain the maximum absorption wavelength and corresponding absorbance. All experiments were done in triplicates.
In water and buffers: Accurately weighed 10 mg of pure drug was dissolved in minimal quantity of buffer and diluted to 100 ml using different buffer solutions to produce a stock solution of 100µg/ml. From this stock solution, serial dilutions were made to produce successive concentrations of 2-10µg/ml. These solutions were then analysed for corresponding absorbances. All the experiments were done in triplicates.

2.4 Preparation of Niosomes The non-ionic surfactant vesicles were prepared by the conventional thin film hydration method. Accurately weighed quantities of drug, surfactant and cholesterol in different ratios were dissolved in chloroform in round bottom flask. Same ratio of DCP was added to each formulation as negative charge inducing agent. The chloroform was evaporated at 60^0c under reduced pressure using rotary flash evaporator. After chloroform evaporation the flask was kept under vacuum over night in a nitrogen atmosphere to remove residual solvent .Thin films were hydrated with 6ml of pH-7.4 phosphate buffer saline, and flask was kept rotating at 60^oc at various revolutions per min (rpm).Formulations were sonicated at 50 Hz in bath sonicator for 30 min [67].

2.5 The evaluation parameters are-

2.5.1 Particle size and shape analysis

Particle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. About 50 niosomes were measured individually, average was taken and their size distribution range and mean diameter were calculated. Further microphotographs of optimized niosomes were taken by using 9 megapixel Sony DSC-W110 digital cameras.

2.5.2 Entrapment Efficiency

Niosomes containing drug were separated by centrifugation. The niosomal dispersions were centrifuged at 9000 rpm for 30 minutes and decanted fluids were separated from sediment material. An aliquot of freshly niosomal dispersion after lysis with 50 %n-propanol was analyzed at 242.6 nm to calculate amount of entrapped drug. % Entrapment efficiency=Amount of drug entrapped*100 /Total amount of drug

2.5.3 In vitro Release Studies

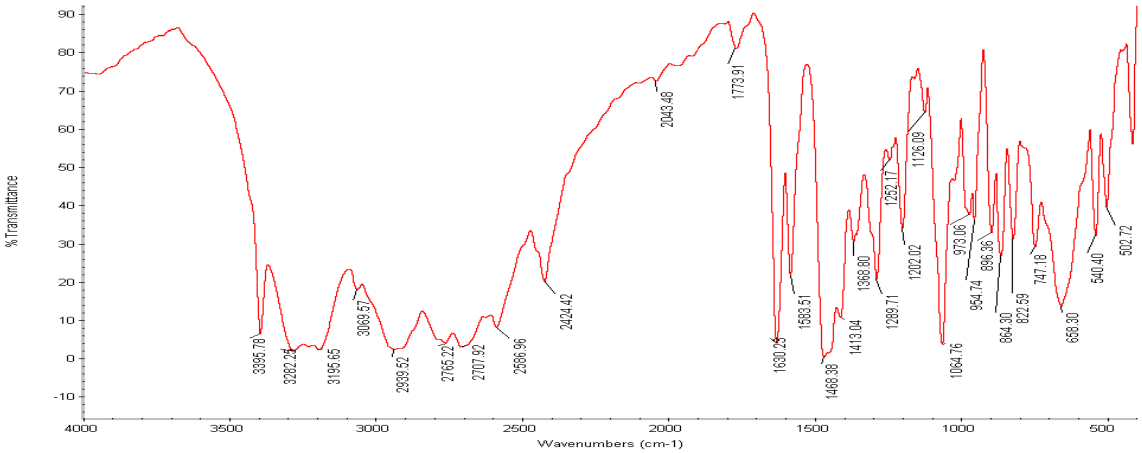
After the initial treatment of dialysis technique using modified Franz diffusion cell, 2ml of niosomal dispersion was placed inside. It was transferred to a beaker containing 30ml of pH-7.4 phosphate buffer. The assembly was stirred on a magnetic stirrer at 37^0 C. One ml samples were withdrawn at fixed intervals and replaced with equal volume of fresh media. The samples were withdrawn and analyzed for drug by UV spectrophotometer.

III. Results And Discussion

3.1 Preformulation Studies

3.1.1 Melting Point- The observed melting point was found to be236ºC. The reported melting point was 235-240^{o}c.

3.1.2 FTIR spectra of Drug



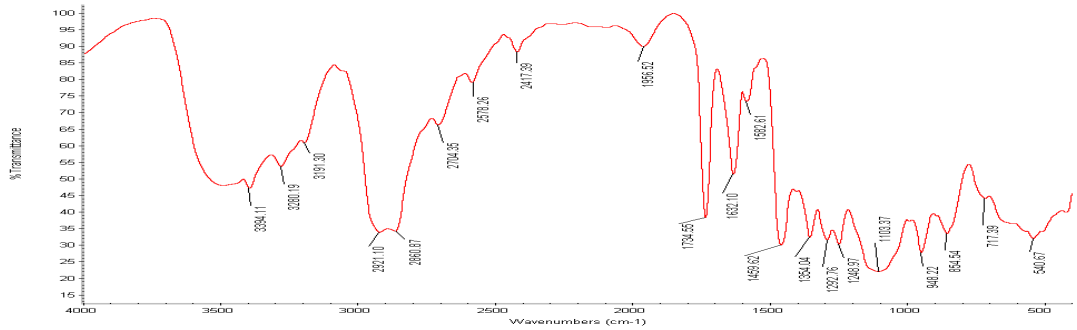
"Fig"1- FTIR spectra of Ambroxol Hydrochloride

Table1- Major FTIR band assignments of AMB HCl

Band position (cm^{-1})	**Assignment**
3395.78	1^0 amides, NH-str, H_2 bonded.
3282.25	2^0 amides, NH-str, H_2 bonded.
3195.65	NH-str, symmetric.
2939.52	Alkanes (CH-str)
2707.92	Carboxylic acid for (O-H str)
2586.96	

3.1.3 FTIR spectra of Drug-Excipients Interactions

3.1.4 AMB HCl and Tween-80 mixture.

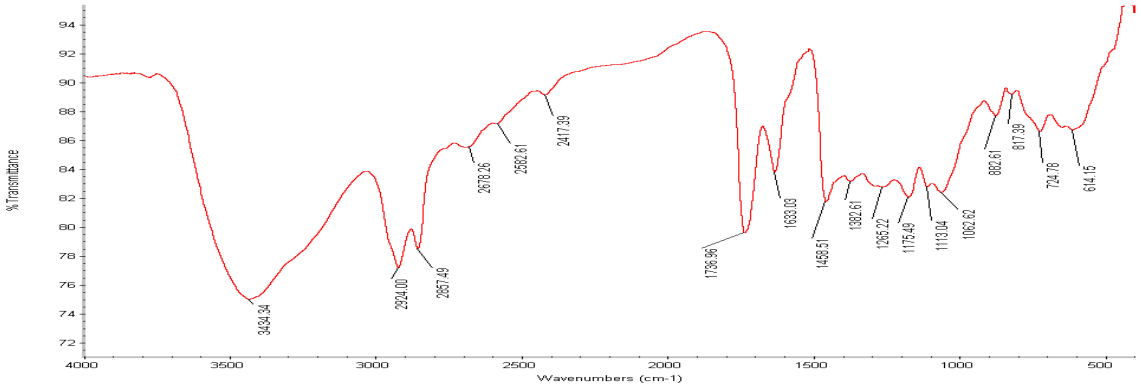


"Fig" 2-FTIR spectra of AMB HCl and Tween-80 mixture

Table2- Major FTIR band assignments of AMB HCl and Tween-80 mixture

Band position (cm^{-1})	**Assignment**
3394.11	1^0 amides, NH-str, H_2 bonded.
3280.19	2^0 amides, NH-str, H_2 bonded.
3191.30	NH-str, symmetric.
2921.10	Alkanes (CH-str)
2860.87	
2704.35	Carboxylic acid for (O-H str)
2578.26	
2417.39	

3.1.5 AMB HCl and Span-20 mixture.

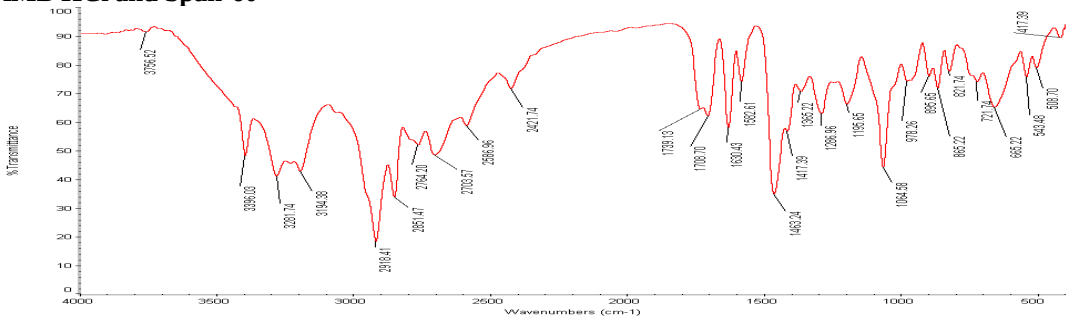


"Fig"3- FTIR spectra of AMB HCl and Span-20 mixture

Table3- Major FTIR band assignments of AMB HCl and Span-20 mixture

Band position (cm^{-1})	Assignment
3434.34	1^0 amides, NH-str, H_2 bonded.
2924	Alkanes (CH-str)
2657.49	Carboxylic acid (OH-str)
2657.49	
2678.26	
2582.61	
2417.39	

3.1.6 AMB HCl and Span-60

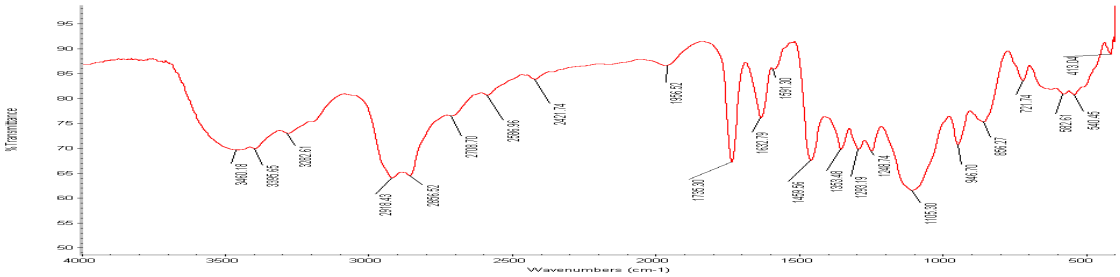


"Fig"4- FTIR spectra of AMB HCl and Span-60 mixture

Table4- Major FTIR band assignments of AMB HCl and Span-60 mixture

Band position (cm^{-1})	Assignment
3396.03	1^0 amides, NH-str, H_2 bonded.
3281.74	2^0 amides, NH-str, H_2 bonded.
3194.38	NH-str (symmetric)
2918.41	Alkanes (CH-str)
2851.47	
2764.20	Aldehyde (CH-str)
2703.57	Carboxylic acid (OH-str)
2586.96	
2421.74	

3.1.7 AMB HCl and Tween-60 mixture.



"Fig"5- FTIR spectra of AMB HCl and Tween-60 mixture

Table5- Major FTIR band assignments of AMB HCl and Tween-60 mixture

Band position (cm^{-1})	Assignment
3460.18	1^0 amides, NH-str, H_2 bonded.
3395.65	
3282.61	2^0 amides, NH-str, H_2 bonded.
2918.43	Alkanes (CH-str)
2856.52	
2708.70	Carboxylic acid (OH-str)
2586.96	
2421.74	

Table6- Thermo analytical data of Drug and Drug-Surfactants physical mixtures

	DSC		
Sample	**T_{onset}(fusion)^{0}c**	**T_{peak}(fusion)^{0}c**	**Enthalpy(fusion) mJ/mg**
Drug	225	244	83.6
Drug+ Tween 80	230	241	18.8
Drug +Span60	225	232	29.2

The DSC has shown 2 endothermic peak at 244^{0c} and 346^0c. The first peak corresponding to m.p. and heat of fusion ΔH =83.6mj/mg and IInd peak was related to decomposition and evaporation of drug. In the drug-excipients physical mixture, the melting endotherm of drug was well preserved with little changes in terms of sharpening, broadening shifting towards a lower temperature. These minor changes in endotherm peak of drug due to reduction in purity of each component, but not due to incompatibility. Crystallization peak was observed with physical mixture of surfactant at 400-405^0c

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3.2 Standard Plot of Ambroxol Hydrochloride in pH 7.4 phosphate buffer

10mg of drug was dissolved in 100 ml of pH 7.4 phosphate buffer, serial dilutions were then prepared to make a final concentration of 2-10µg/ml. The absorbances were then measured at λ max 242.6 nm.

Table7- Standard Plot in pH 7.4 phosphate buffer

S.No.	Concentration (µg/ml)	Absorbance*
1.	2	0.041±0.001
2.	4	0.1±0.01
3.	6	0.15±0.02
4.	8	0.207±.02
5.	10	0.215±.02

* Value represent mean± SD (n=3)

3.3 Standard Plot of Ambroxol Hydrochloride in pH-7.4phosphate buffer

The drug solution was scanned in pH 7.4 phosphate buffer and λ max was determined to be 242.6 nm and standard plot was constructed. The regression value was found to be 0.9981.

3.4 Standard Plot of Ambroxol Hydrochloride in 0.1N HCl

10mg of drug was dissolved in 100 ml of 0.1 N HCl serial dilutions were then prepared to make a final concentration of 2-10µg/ml. The absorbance was then measured at λ max 242.6 nm.

Table8- Standard Plot of AMB HCl in 0.1 N HCl

S. No.	Concentration (µg/ml)	Absorbance*
1.	2	0.126±0.001
2.	4	0.285±0.001
3.	6	0.452±0.001
4.	8	0.592±0.001
5.	10	0.732±0.001

*Value represents Mean± SD (n=3).

3.5 Standard plot of Ambroxol Hydrochloride in 0.1N HCl

The drug solution was scanned in 0.1N HCl and λ max was determined to be 242.6 nm and standard plot was constructed. The regression value was found to be 0.9987.

3.6 In-vitro release studies

The evaluations for drug release for the prepared formulations was performed and the results of the formulations giving better drug release from all the prepared formulations obtained are as follows-

Table9- Comparative Data of drug permeated for the prepared formulations

TIME (Hr)	% CDR OF PREPARED FORMULATIONS						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
0.25	8.13	8.60	8.25	6.27	15.69	5.23	12.44
0.5	10.5	12.3	11.08	9.04	20.63	7.03	16.92
0.75	13.86	18.13	14.47	11.66	24.79	10.28	18.29
1	15.47	24.06	17.60	14.94	27.21	13.40	20.37
1.5	20.13	28.65	25.48	17.74	29.1	16.03	25.19
2	22.96	32.45	31.39	20.49	31.37	19.32	28.28
2.5	24.24	37.04	36.77	23.90	34.62	22.93	30.99
3	28.44	40.35	40.33	28.10	37.71	25.95	34.22
3.5	31.01	47.46	43.38	32.65	40.64	29.74	36.48
4	34.93	52.09	46.03	36.98	43.17	33.17	40.88
4.5	38.82	55.90	49.55	41.42	46.67	36.68	44.23
5	42.12	62.23	54.29	45.15	49.55	40.62	47.31
5.5	45.02	68.60	58.11	49.79	52.72	44.20	49.74
6	49.14	73.97	62.58	53.61	56.19	46.93	52.92
6.5	52.20	77.59	65.99	57.74	59.03	49.94	56.50
7	59	80.10	70.27	61.03	62.26	52.08	60.05
7.5	59.79	83.56	74.75	65.19	65.55	55.06	62.38
8	62.13	87.41	78.15	68.39	70.87	57.75	65.67
Time (hr)	**%CDR**						
	F8	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**
0	0	0	0	0	0	0	0
0.25	12.44	14.3	30.34	32.32	17.90	2.44	2.79
0.5	15.76	17.91	32.29	35.26	21.75	4.73	5.32
0.75	16.97	22.45	35.54	39.65	23.97	6.31	8.17
1	18.55	24.44	38.17	41.37	26.01	8.41	9.25
1.5	21.56	25.89	39.80	48.33	28.78	11.02	10.7
2	24.07	27.14	43.77	49.78	31.39	13.69	12.9
2.5	29.09	28.98	46.67	53.44	38.13	16.91	15.72
3	34.60	33.3	48.93	56.72	40.87	20.33	17.71
3.5	38.28	36.57	51.69	59.70	43.55	22.45	20.56
4	43.68	39.92	53.68	62.72	46.87	25.09	23.13
4.5	48.65	43.46	56.62	65.80	50.72	28.26	24.73
5	52.46	45.69	58.91	69.62	53.60	30.59	26.94
5.5	56.50	48.88	62.04	72.22	57.94	33.31	30.6
6	61.12	50.75	65.11	74.74	61.32	36.21	32.95
6.5	63.40	54.37	68.23	78.56	65.35	38.64	35.24
7	67.96	57.62	71.51	81.87	69.92	41.49	38.74
7.5	72.01	61.62	74.16	85.11	74.36	44.79	40.7
8	77.25	66.05	76.60	88.50	79.00	47.71	43.27

3.7 Comparative graph of different ratios of Span60 -%CDR vs Time (hr)

It was shown from the experimental work that F2 formulation showed better release than the F1 &F3.As the concentration of surfactant increases, release also increases. F3 also shown better release than F1 due to increase in cholesterol concentration.Cholestrol alter the fluidity chain in bilayer.

3.8 Comparative graph of different ratios of Tween 80 - %CDR vs Time (hr)

It was shown from the experimental work that F5 formulation shown better release than F4 because of increased concentration of surfactant.F6 formulation shown less release than F4 because in Tween 80 as the concentration of cholesterol increased, entrapment decreased.Tween60 shown better release than Tween80 because longer the alkyl chain, lesser the drug release.

3.9 Comparative graph of different ratios of Tween 60- %CDR vs Time (hr)

It was shown from the experimental work that F8 formulation shown better release than the F7 &F9.As the concentration of surfactant increases, release also increases. F9 also shown better release than F7 due to increase in cholesterol concentration.Cholestrol alter the fluidity chain in bilayer.

3.10 Comparative graph of different ratios of Span 20- %CDR vs Time (hr)

It was shown from the experimental work that F11 formulation shown better release than the F10 &F12. As the concentration of cholesterol increases, release also increases.Cholestrol alter the fluidity chain in bilayer. F12 also shown better release than F10 due to increase in surfactant concentration. From figure 4.16 and figure 4.13, Span 60 shown better release than Span 20.

3.11 Comparative graph of different ratios of Span 60- %CDR vs Time (hr)

It was shown from the experimental work that F3 formulation showed better release than F13 formulation because DCP influence the release of formulation.

3.12 Comparative graph of different ratios of Tween 60- %CDR vs Time (hr)

It was shown from the experimental work that F8 formulation showed better release than F14 formulation because DCP influence the release of formulation.

The evaluations for flux for the prepared formulations was performed and the results of the formulations giving better flux from all the prepared formulations obtained are as follows-

Table10- Comparative Data of flux for the prepared formulations

Time (hr)	Flux (µg/cm²/hr)						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
0.25	16.19	26.07	25.01	19.02	47.56	15.85	37.7
0.5	10.45	18.75	16.80	13.70	31.27	10.65	25.64
0.75	9.19	18.31	14.61	11.78	25.04	10.39	18.47
1	7.69	18.22	13.33	11.32	20.61	10.15	15.43
1.5	6.67	14.47	12.87	8.95	14.69	8.10	12.72
2	5.71	12.29	11.89	7.76	11.88	7.32	10.71
2.5	4.82	11.22	11.14	7.24	10.49	6.95	9.39
3	4.71	10.19	10.18	7.09	9.52	6.55	8.64
3.5	4.40	10.27	9.39	7.06	8.79	6.43	7.89
4	4.34	9.86	8.71	7.00	8.17	6.28	7.74
4.5	4.29	9.41	8.34	6.97	7.85	6.17	7.44
5	4.19	9.42	8.22	6.84	7.5	6.15	7.16
5.5	4.07	9.45	8.00	6.85	7.26	6.08	6.85
6	4.07	9.34	7.90	6.76	7.09	5.92	6.68
6.5	3.99	9.04	7.69	6.73	6.88	5.82	6.58
7	3.97	8.66	7.60	6.60	6.73	5.63	6.49
7.5	3.96	8.44	7.55	6.58	6.62	5.56	6.30
8	3.86	8.27	7.40	6.47	6.71	5.46	6.21
Time (hr)						**Flux (µg/cm²/hr)**	
	F8	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**
0	0	0	0	0	0	0	0
0.25	37.70	43.34	91.966	97.95	54.26	7.39	8.45
0.5	23.88	27.14	48.92	53.42	32.96	7.17	8.06
0.75	17.14	22.68	35.90	40.05	24.21	6.37	8.25
1	14.05	18.51	28.91	31.34	19.70	6.37	7
1.5	10.89	13.08	20.10	24.41	14.53	5.56	5.4
2	9.12	10.28	16.58	18.85	11.89	5.18	4.88
2.5	8.81	8.78	14.14	16.19	11.55	5.12	4.76
3	8.73	8.4	12.35	14.32	10.32	5.13	4.47
3.5	8.28	7.91	11.18	12.92	9.42	4.86	4.45
4	8.27	7.56	10.16	11.8	8.87	4.75	4.38
4.5	8.19	7.31	9.532	11.07	8.53	4.75	4.16
5	7.94	6.92	8.92	10.54	8.12	4.63	4.08
5.5	7.78	6.73	8.54	9.94	7.98	4.58	4.21
6	7.71	6.4	8.22	9.43	7.74	4.57	4.16
6.5	7.39	6.33	7.95	9.15	7.61	4.50	4.1
7	7.35	6.23	7.740	8.86	7.56	4.49	4.19
7.5	7.27	6.22	7.49	8.59	7.51	4.52	4.11
8	7.31	6.25	7.25	8.38	7.48	4.51	4.09

3.13 Vesicle size determination

51 eye-piece division = 5 stage micrometer division

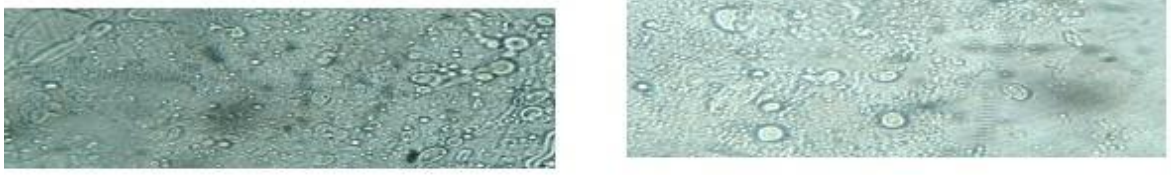
Each eye-piece division = 5/51×10 µm = 0.98 µm

Table11- Comparative data of vesicles size of various formulations

Size range µm	**Mean Size µm (d)**	**Number of vesicles in each size range (n)**				**Cumulative percent number of globules**				**Weight size (n*d)**			
		F2	**F5**	**F8**	**F12**	**F2**	**F5**	**F8**	**F12**	**F2**	**F5**	**F8**	**F12**
0-2	1	17	4	8	7	34	4	16	14	17	4	8	7
2-4	3	9	7	10	8	18	21	20	16	27	21	30	24
4-6	5	8	15	20	10	16	75	40	20	40	75	100	50
6-8	7	10	9	5	11	20	63	10	22	70	63	35	77
8-10	9	6	14	7	13	12	126	14	26	54	126	63	117
Σ(n) = 50						*Σ(n×d)*				208	289	236	276

$d_{average}=\Sigma(n\times d)/\Sigma(n)$			
F2	**F5**	**F8**	**F12**
4.16	5.78	4.72	5.5

Comparative Graph of the average vesicle size distribution
ImageA&B Niosomes under 400x Magnification with eye piece



On subjecting the Niosomes to 400x magnification, the Niosomes were clearly visible as shown in figure (figure 4.27, 4.28).The average diameter of Niosomes was approximately 4.16, however the vesicle size of the smallest. However the above images clearly showed the presence of Niosomes.

Table12- Entrapment Efficiency-Entrapment Efficiency of different formulations are

Formulations	F1	F2	F3	F4	F5	F6	F7
EE	58.8	77.13	70.15	71	80	59.7	74

Table13- Entrapment Efficiency of Different formulations

Formulations	F8	F9	F10	F11	F12	F13	F14
EE	83.3	69.14	70.63	76	74.8	69	70

It was found that from the experimental work, Span 20 showed better entrapment than Span60. Span60 (F1, F2, F3) showed better entrapment due to solid nature, hydrophobicity, phase transition temperature.F2 formulation shown large entrapment due to increase in cholesterol content. EE of Tween 60 (F7, F8, F9) showed larger than Tween80 (F4, F5, F6).Longer the alkyl chain, less drug entrapped.Tween 60 was shown better entrapment efficiency than other formulations.

IV. Conclusion

From the results of melting point, scanning report, solubility studies, FTIR spectra it can be concluded that the drug is pure with no impurities. AMB HCl was successfully encapsulated in niosomes by thin film hydration technique, which showed that it was appropriate technique to load AMB into lipid vesicles. In case of Tween 80, EE of F6 also decreased due to large amount of Cholesterol content.

Tween 60 shown better entrapment efficiency than Tween 80 due to longer the alkyl chain, lesser drug entrapped. Span 60 shown better entrapment efficiency than Span 20, but some time dissimilar the results. Tween 60 was shown better entrapment than other formulations. Niosomes formulated with different surfactant entrapped large drug, addition of DCP sustained the drug release for long time.

Thus niosomes of Ambroxol hydrochloride offered advantages over the other drug carriers with respect to biocompatibility, ease of preparation and their capacity to carry large amount of drugs. The results reveal that Niosomes are comparatively stable at lower temperature.

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