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Preparation and *In-vitro* evaluation of proliposomes of Albendazole

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Abstract
Novel drug delivery system (NDDS) and nanoscience are widely seen as having a tremendous promise to revolutionize the scientific landscape in terms of research and applications. Among various approaches for development of NDDS for biomedical applications, carriers offer great advantages in drug delivery, sensing and imaging.
Proliposomes are used as alternatives for liposomes which are composed of water soluble porous powder as carrier phospholipids and drugs dissolved in organic solvent. Drug and phospholipid material is coated on carrier material to form free flowing granular material which shows better stability, greater solubility and shows controlled release. The proliposome approach was developed as a straight forward, reproducible, and reliable manufacturing technique for large-scale production of liposome dispersions. The technology is based upon the intrinsic property of hydrated membrane lipids to form vesicles on contact with water. It is designed particularly for the molecular dispersion and delivery of water-insoluble materials where association efficiencies approaching 100% can be achieved.

Keywords: Proliposomes, albendazole, phospholipid, liposomes

Introduction
Among the group of several NDDS, lipid Nano formulations have been paid great attention due to its biocompatibility and biodegradable nature. They have huge potential for delivery of drugs in several diseases. In the field of Nano medicine, they offer interesting benefits viz. enhancing drug efficacy, providing controlled and convenient drug release. The performance of lipid based formulations greatly depends upon the composition and structure of formulations. Most common lipid-based formulations include liposomes, solid lipid nanoparticles, nanostructured lipid carriers, nisosomes, proliposomes etc. [2].
Between, several colloidal particulate drug delivery systems, liposomes are very distinct when compared with conventional dosage forms, because the particles can act as the drug containing reservoirs and modify the particle composition or surface to adjust the drug release rate or the affinity for the target site. Almost from the time of their first report in 1960s by Banghamand co-workers, liposome has received great attention of researchers as prospective carriers for various bioactive molecules [3]. Liposomes have attracted much attention as drug carriers to improve the effect of treatment, reduce side effects, and improve stability by protecting drugs from degradation or transformation

Proliposomes
Are novel generation of carrier mediated drug delivery system having several advantages over conventional liposomes? It has shown better stability and ease of sterilization on large scale by preventing drug over loading. Maximum amount of drug encapsulation helps in more penetration of drug and producing a sustain release effect at the site of administration [10]. The concept of proliposomes was first proposed by Payne et.al in 1986, who defined proliposomes as dry, free flowing powders, granular product that immediately forms a liposomal dispersion on contact with water or a biological fluid in the body [10]. Because of the solid properties, the stability problems of liposome can be solved without influencing their intrinsic characteristics.

Advantages of proliposomes
1. Increase the dissolution of a poorly soluble drug
2. Increase lipophilicity
3. Improve the permeability
4. Improve intestinal uptake
5. Decrease hepatic first-pass metabolism
6. Improve gastric/intestinal stability of the encapsulated drug
7. Ease of translating into a desired dosage form

Table 1: Commercially Available Oral Lipid-based Products

Trade Name	Molecule	Therapeutic use	Company
Agenerase®	Amprenavir	HIV antiviral	Glaxo Smith Kline
Rocaltrol®	Calcitriol	Calcium regulator	Roche
Cipro®	Ciprofloxacin	Antibiotic	Bayer
Neoral®	Cyclosporin A/I	Immuno-suppressant	Novartis
Gengral®	Cyclosporin A/III	Immuno-suppressant	Abott
Accutane®	Isotretinoin	Anti-comedogenic	Roche
Kaletra®	Lopinavir and Ritonavir	HIV antiviral	Abott
Norvir®	Ritonavir	HIV antiviral	Abott
Lamprene®	Clofazamine	Treatment of leprosy	Alliance laboratories

Sustiva®	Efavirenz	HIV antiviral	Bristol-Meyers
Fenogal®	Finofibrate	Anti hyperlipproteinomic	Genus
Restandol®	Testosterone undecanoate	Hormone replacement therapy	Organon laboratories
Convulex®	Valporic acid	Anti-epileptic	Pharmacia
Juvela®	Tocopherolnicotinate	Hypertension, hyperlipidemia	Eisai Co.

Components used for the preparation of proliposomes

Water soluble carriers: Maltodextrin, Sorbitol, Microcrystalline Cellulose, Magnesium Aluminium Silicates, Mannitol [9].

Phospholipids

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl ethanolamine (cephalin) – PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG).

Steroids: Cholesterol and its derivatives

Solvents: ether, chloroform, methanol, ethanol

Experimental Work

Methods of proliposome preparation

Process: Proliposomes containing Albendazole were prepared by film deposition carrier method, and the composition was characterized in Table 1. In brief, accurately weighed amounts of lipid mixture comprising of HSPC and cholesterol at several molar ratios (1:2, 1:4, 1:6, and 1:8 respectively) and drug (200 mg) were dissolved in 20 mL of solvent mixture containing ethanol and methanol (9:1). The resultant solution was transferred into a 250 mL round bottomed flask, and spray dried mannitol (250 mg) was added to form slurry. The flask was attached to a rotary flash evaporator (perfit, India), and the organic solvent was evaporated under reduced pressure at a temperature of 45 ± 2 ºC. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature so as to gain dry, free-flowing product. The obtained proliposome powders were sieved with a 60 mesh screen and stored in a tightly closed container at cool condition for further evaluation.

Characterization of proliposomes

- Optical microscopy
- Vesicle size analysis
- Entrapment efficiency EE% = Theortical drug content / Practical drug content×100
- FTIR spectra of final formulation
- Transmission electron microscopy
- In vitro release study
- In-vitro drug release kinetic study

Result and Discussion

1. Preformulation results

1.1 Organoleptic Properties

Table 2: Organoleptic Properties of Albendazole

Sr. no.	Properties	Inferences
1	Colour	Colorless crystals
2	Odor	Odorless
3	Form	Crystalline
4	Taste	Bitter

1.2 Melting point of drug

Table 3: Melting point of Albendazole

Drug	Observed melting point (°C)	Reference melting point (°C)
Albendazole	208.66±0.577	208-210

(Value is expressed as mean ± SD; n = 3)

1.3 UV-VIS spectra

UV absorption maxima (λ_{max}) of Albendazole in Methanolic glacial acetic acid was determined by using UV-visible spectrophotometer. Drug exhibited maxima at 235nm.

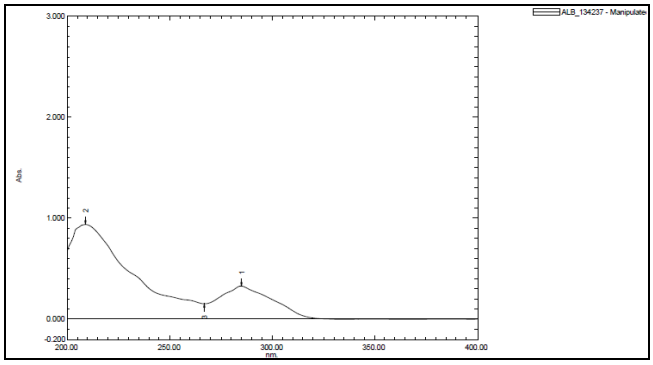

Fig 1: Absorption maxima (λ_{max}) of Albendazole in Methanolic glacial acetic

Table 4: Calibration curve of Albendazole in Methanolic glacial acetic acid (λ_{max} = 235 nm)

S.No	Concentration (µg/ml)	Absorbance (mean ±SD)
1	1	0.137±0.0017320
2	2	0.247±0.0051961
3	3	0.360±0.01692
4	4	0.463±0.001732
5	5	0.579±0.009018
6	6	0.67±0.011135
7	7	0.771±0.00888
8	8	0.894±0.002645
9	9	0.981±0.00577

(All values are expressed as mean ± SD; n = 3)

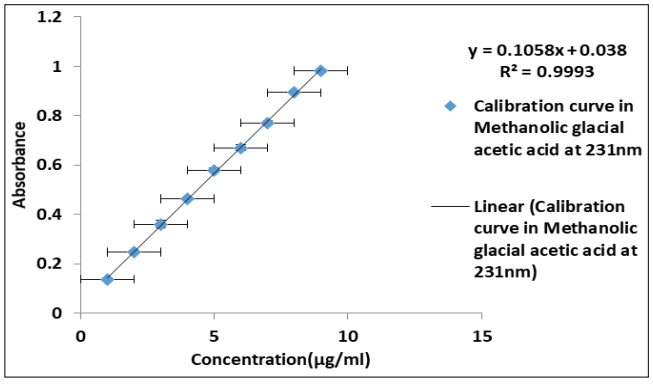

Fig 2: Graph of standard calibration curve of Albendazole in Methanolic glacial acetic acid

Table 5: Result of regression analysis of UV method for estimation of Albendazole

Statistical parameters	Results
λ max	235 nm
Regression equation ** Y=mx+C	Y=0.1058x-0.038
Slope (b)	0.1058
Intercept (C)	0.038
Correlation coefficient (r^2)	0.9993

1.4 Partition coefficient of drug

Table 6: Partition coefficient determination of Albendazole

1		
Albendazole	**Theoretical value**	**Practical value**
	3.07	3.49±0.722

1.5 Solubility studies of Albendazole in solvent

Table 7: Solubility studies of Albendazole for different solvents

Sr. No.	Solvent	Solubility (mg/ml)
1	Methanol	5.198±0.00945
2	Diethyl ether	0.0475±0.0001
3	Acetone	0.249±0.00144
4	1N HCL	75.77±0.23786
5	DCM	4.26±0.0144
6	Ethanol	5.712±0.00016
7	Distilled water	0.0137±0.00016

(* Each value is average of three independent determinations)

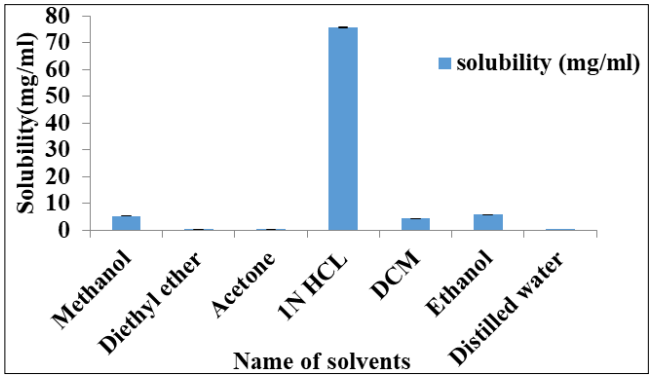

Fig 3: Solubility study of albendazole in different solvents

Discussion: Albendazole is highly soluble in 1N HCL (75.77 mg/ml).

1.6 FTIR Compatibility

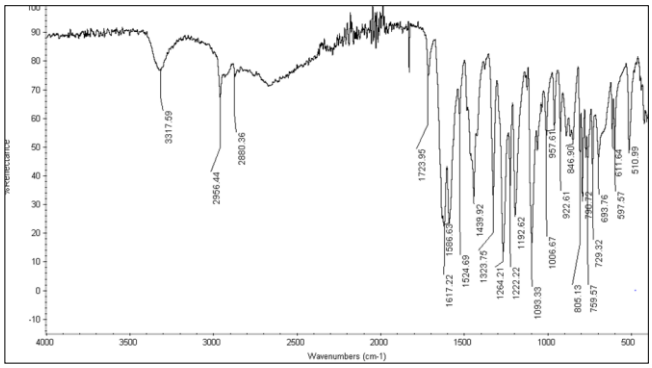

Fig 4: FTIR spectra of Albendazole

Table 8: Interpretation of FTIR spectra of Albendazole

Functional group	Reference peak (cm^{-1})	Observed peak (cm^{-1})
-C-H stretching	2850-3000	2956.44, 2880.36
-O-H stretching	3200-3600	3317.59
-C-Cl stretching	1000-1400	1006.67, 1033.33
-C=C stretching	2210-2260	2308.33, 2352.58
-C-N stretching	1080-1360	1222.22, 1264.21, 1192.62, 1323.75, 1331323
C=C alkene	1600-1700	1617.22, 1723.95
Carboxylic acids(O–H stretch)	1524.68	1524.69

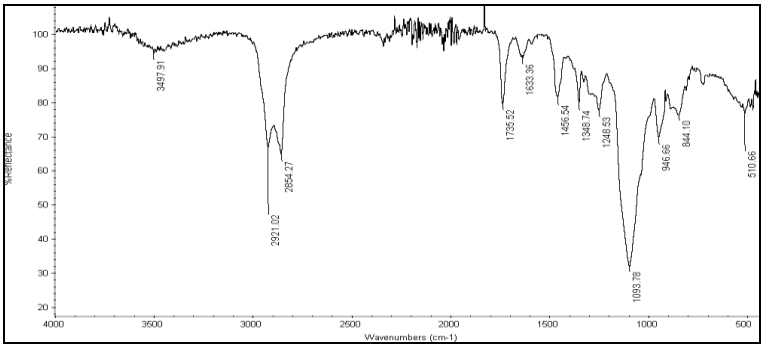

Fig 5: FTIR spectra of Physical mixture

Table 9: Interpretation of FTIR spectra of Physical mixture

Functional group	Reference peak (cm^{-1})	Observed peak (cm^{-1})
-C-H stretching	2956.44, 2880.36	2921.02, 2854.27
-O-H stretching	3317.59	3497.91
-C-Cl stretching	1006.67, 1033.33	1093.78

-C-N stretching	1222.22, 1264.21, 1192.62 1323.75 1222.22, 1264.21, 1192.62, 1323.75 1331323	1248.53, 1348.74
-C=C alkene	1617.22, 1723.95	1633.36, 1735.52
Carboxylic acids(O–H) stretch)	1524.69	1456.54

Discussion: From the physical mixtures of drug and excipients there were no major shifting as well as no loss of any functional peaks between the spectra of drug and its physical mixtures. Hence, it was confirmed that there were no interaction between the drug and excipients used**.**

2. Development of Powder proliposomes

Table 10: Different compositions of proliposome formulation

Formulation Code	Drug: HSPC:Mannitol (µmol)	Drug of (mg)	HSPC (mg)	Cholesterol (mg)	Mannitol (mg)	% Entrapment
A1	1:02:01	200	20	-	250	61.45±0.190
A2	1:04:01	200	40	-	250	87.99±0.170
A3	1:06:01	200	60	-	250	67.58±0.047
A4	1:08:01	200	80	-	250	58.28±0.027

After the screening of different soya lecithin concentration and mannitol with drug and it was found that the maximum % Entrapment showed in 1:04:01 ratio. So, we observed the best result in above A2 formulation, this ratio further proceed for next formulation optimization.

Table 11: Final compositions of proliposomal formulations

S. No.	Formulation Code	Drug: HSPC:Mannitol (µmol)	Drug of (mg)	HSPC (mg)	Cholesterol (mg)	Mannitol (mg)
1	A5	1:4:1:1	200	40	10	250
2	A6	1:4:2:1	200	40	20	250
3	A7	1:4:3:1	200	40	30	250
4	A8	1:4:4:1	200	40	40	250

3. Characterization of proliposomes

3.1 Optical microscopy

The prepared Albendazole loaded proliposomal vesicles were considered fewer than 100x magnifications to observe the formation of Albendazole loaded vesicles. The liposomes are unilamellar spherical vesicles with smooth surface.

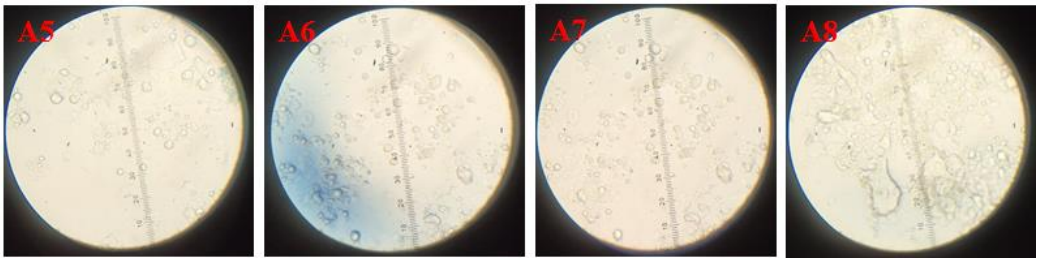

Fig 6: Optical microscopy of different proliposomal formulation

3.2 Particle and Zeta seizer of vesicles

The zeta potential value of the prepared Albendazole loaded proliposome was – 14.1 ± 1.30 mV.

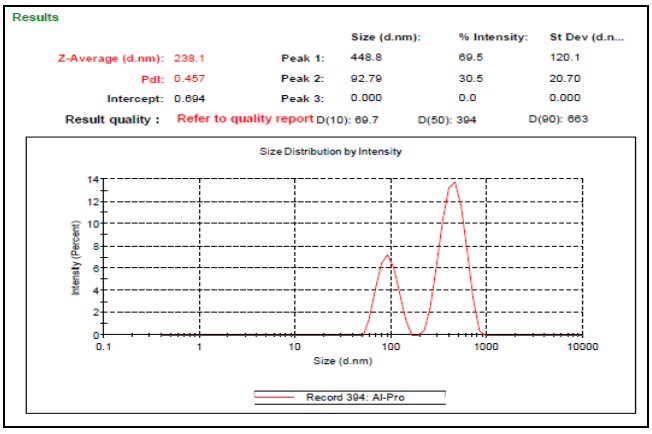

Fig 7: Particle size of A7 final formulation

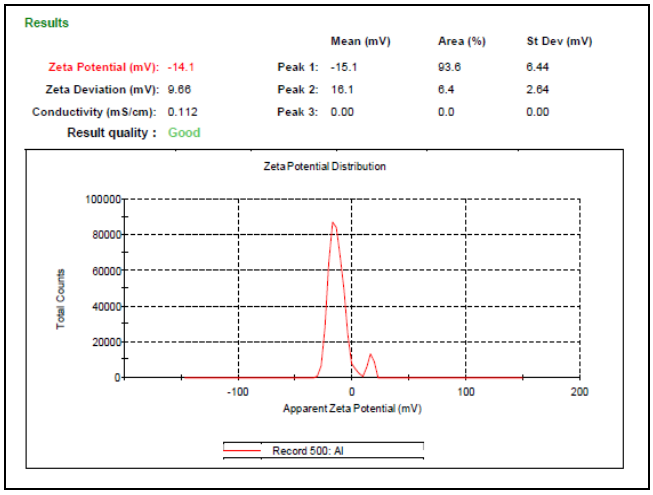

Fig 8: Zeta potential of A7 final formulation

3.3 Albendazole loaded Proliposome Entrapment efficiency (EE %)

Table 12: Entrapment Efficiency of different Proliposomal formulations

Formulation Code	% EE
A5	85.12±0.118
A6	86.48±0.047
A7	93.10±0.047
A8	78.65±0.242

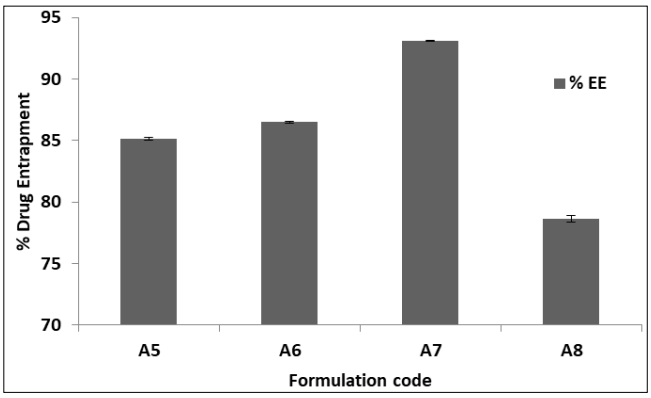

Fig 9: % Entrapment Efficiency of different Proliposomal formulations

3.4 FTIR spectra of final formulation

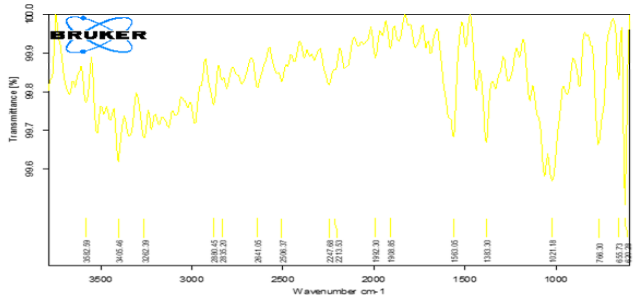

Fig 10: FTIR spectrum of Final formulation (A7)

Table 13: Interpretation of FTIR of Final formulation

Functional group (vibrational)	Reference peak (cm^{-1})	Observed peak (cm^{-1})
C-H stretching	2850-3000	2880.45
-O-H stretching	3200-3600	3262.39, 3405, 36.82.59
-C-Cl stretching	1000-1400	1021.18
-C-N stretching	1080-1360	1383.30
Aromatic C=C Bending	1700-1500 (m, m)	1563.05
Aromatic C-H Bending	860 - 680	766.30, 655.73

Discussion: The FTIR spectrum of the best-selected formula of the prepared Albendazole self-dispersible dry Proliposomal powder (A7) indicates there is no interaction between the drug Albendazole and excipients of this formula.

3.5 Transmission electron microscopy

Transmission electron micrographs revealed that most of the vesicles are well well-known, spherical and discreet with sharp borders having large inner aqueous space after hydration of proliposomes.

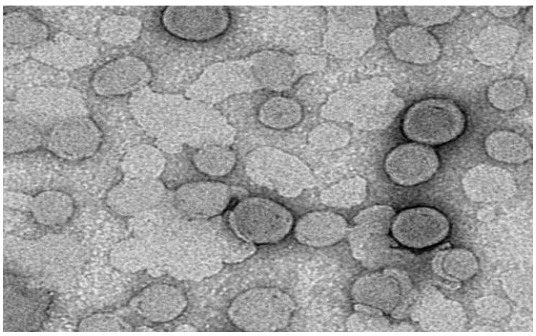

Fig 7.11: Tem images of final formulation (86)

3.6 *In-vitro* release study

Table 14: Comparison between final formulation and pure drug

Time (hr)	% Drug release of A7	% Drug release of pure drug
0.5	1.04±0.777	1.34±0.177
1	6.03±0.199	3.56±0.199
2	10.79±0.047	5±0.047
3	16.35±0.027	6.74±0.027
4	19.64±0.191	7.76±0.152
5	24.44±0.191	10.27±0.055
6	36.69±0.047	12.61±0.082
7	41.09±0.027	13.12±0.072
8	45.45±1.910	13.01±0.304
10	53.62±0.273	13.23±0.047
12	61.02±0.473	13.07±0.109
24	72.3±0.473	13.2±0.098

Discussion: The % dissolution of Albendazole from proliposomes was found to be 3.0–3.5 times higher than the pure drug (control). We found A7 proliposomal formulation show maximum % drug release compare to pure drug of Albendazole.

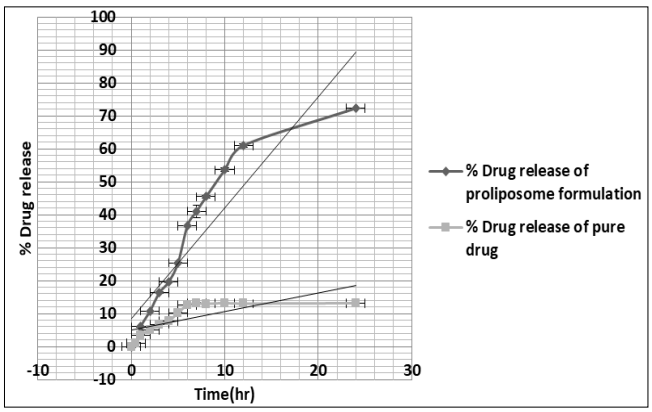

Fig 12: Comparison between final formulation (A7) and pure drug of Albendazole

3.7 In-vitro drug release kinetic study

To understand the mechanism by which the drug was released from the proliposome powder A7 formulation, various release kinetics model including zero order, first order, Higuchi and Korsmeyer-Peppas model were applied as shown in Fig 13-16.

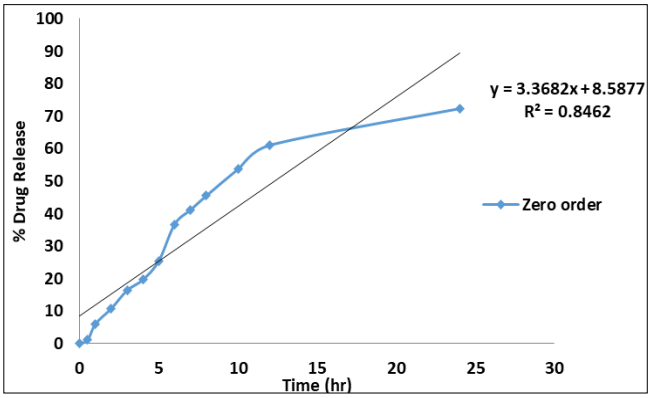

Fig 13: Zero order release kinetics of optimized A7 formulations

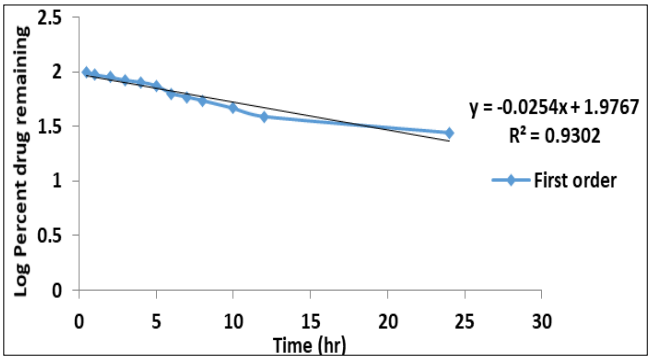

Fig 14: First order release kinetics of optimized A7 formulation

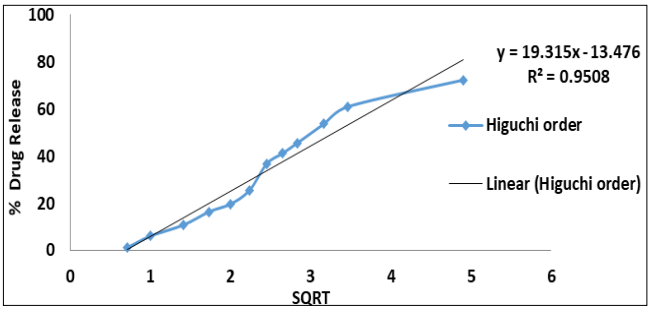

Fig 15: Higuchi model release kinetics of optimized A7 formulation

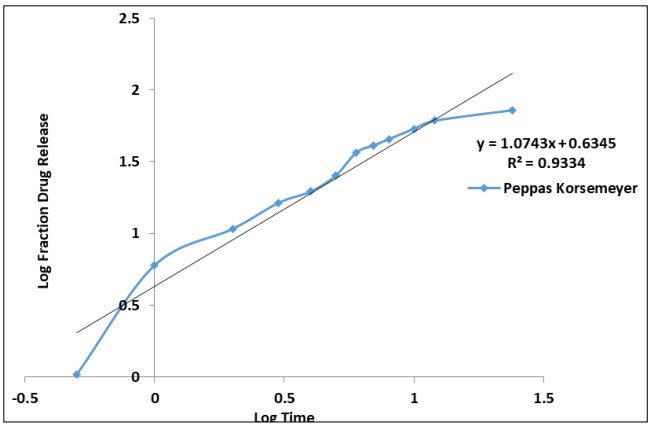

Fig 16: Korsemayer-peppas release kinetics of optimized A7 formulation

Table 15: Kinetic equation parameter of A7 Formulation

Formulation name	Zero order		First order		Higuchi		Peppas	
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0
	0.8462	3.368	0.9302	-0.0254	0.9508	19.315	0.9334	1.0743

8. Summary and Conclusion

On physicochemical evaluation, melting point of Albendazole was found to be 208.66 ± 0.577°C. On UV spectrophotometer analysis absorption maxima was found to be 235 nm in Methanolic glacial acetic acid. Albendazole highly soluble in 1N HCL (75.77 mg/ml), slightly soluble in DCM (4.26 mg/ml), Ethanol (5.71 mg/ml), and Methanol (5.19 mg/ml) and practically insoluble in Distilled water (0.0137 mg/ml), diethyl ether (0.047mg/ml), and drug is less soluble in rest of solvents. The partition coefficient of Albendazole in n-octanol: water was found to be 3.49±0.722 this indicated that the drug is lipophilic in nature. On FTIR spectroscopy analysis there was no interaction between drug and polymer.

To find an appropriate range for each parameter, a set of preliminary screening was conducted with the help of % Entrapment. Which found that Albendazole Proliposome could be produced in the solvent evaporation method when different concentration of soya lecithin: mannitol cholesterol with drug were about 40 mg, 250 mg, 30 mg, and 200 mg (1:4:3:1), respectively. Then, to identify the effect of soya lecithin and cholesterol parameters both on % Entrapment and particle size/ zeta potential, was found 238.1nm and PDI was 0.457, was – 14.1 ± 1.30 mV, The TEM micrographs reveled that A7 formulation of Albendazole loaded proliposome were formed uniform powder.(103, 104)

The Albendazole loaded proliposome formulations varies, and A7 finalized on basis of % entrapment efficiency, and % drug release on the basis of se were found, 93.10±0.047 and 72.3±0.473. The % drug release observed maximum in A7 as compare to pure drug. It was found that the in vitro drug release of A7 was best explained by Higuichi as the plot showed the highest linearity. The value of R^2 found to be 0.950 highest for the higuichi order.

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