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Formulation and evaluation of albendazole nanoparticle

Yerikala Ramesh, Koorapati Balasaradhi*, Kaki Rohan Abhilash

Department of Pharmaceutics, Ratnam Institute of Pharmacy, Pidathapolur (V & P), Muthukur (M), SPSR Nellore (Dt) - 524346, Andhra Pradesh, India.

ABSTRACT

Therefore, there is a need to develop alternative novel drug delivery formulations of albendazole to improve its intestinal absorption and also to reduce its side effects during regular therapy. The Albendazole nanoparticles were prepared by hot homogenization method under high magnetic stirring using stearic acid as lipid and poloxamer 188 was used as surfactant. Initial pre-formulation studies using FTIR spectroscopy reveals that there are no interactions between Albendazole and other excipients and hence they can be used for the preparation of nanoparticles. The entrapment efficiencies varied from a minimum of 43.56 ± 0.95 % to a maximum of 85.1 ±0.58% and it can be concluded that higher amount of lipid is necessary for obtaining a good entrapment efficiency. The drug content of albendazole nanoparticles for all formulation ranges from 65.8% to 98.1%. A spherical shape was observed for the particles and the particles had a smooth morphology when examined under SEM. *In vitro* release studies of the formulations carried out in pH 7.4 PBS showed that the total amount of drug is released for 9hrs with sustained effect. That the formulations showed a drastic increase in size when stored at room temperature where the size of particles increased from an initial to 343.7 ±7.9 nm at the end of 1 month to 898.1 ± 5.8 nm at the end of 2 months. Entrapment efficiency of the formulation was determined at each interval to ensure that the drug molecules didn't undergo any degradation during storage.

Keywords: Albendazole, Nanoparticles, Particle size, Entrapment efficiency.

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***Address for Correspondence:**

Koorapati Balasaradhi, Department of Pharmaceutics, Ratnam Institute of Pharmacy, Pidathapolur (V & P), Muthukur (M), SPSR Nellore (Dt) - 524346, Andhra Pradesh, India.

INTRODUCTION

Nanoparticles consist of a solid lipid matrix (at room and body temperatures) which are stabilized by surfactants and have a mean size of 50- 1000 nm and stabilized by surfactants [1]. The main advantages of Nanoparticles are a controlled release of the drug can be achieved, The stability of the drug can be improved, high amounts of drug loading can be achieved, hydrophilic and lipophilic drugs can be incorporated there is no toxicity of the various lipids used of organic solvents can be prevented**,** Preparation of large scale of Nanoparticles can be easily scaled up for industrial purpose [2]. Nanoparticle are solid colloidal particles ranging from 10 to 1000 nm (1.0 µm), in which the active principles (drug or biologically active material) are dissolved, entrapped, and/or to which the active principle is adsorbed or attached [3]. In recent years, significant effort has been devoted to develop nanotechnology for drug delivery, since it offers a suitable means of delivering small molecular weight drugs, as well as macromolecules such as proteins, peptides or genes to cells and tissues and prevents them against enzymatic degradation [4]. Therefore, there is a need to develop alternative novel drug delivery formulations of albendazole to improve its intestinal absorption and also to reduce its side effects during regular therapy. Already, approaches like albendazole suspensions, liposome's and polymeric nanoparticles were developed to improve the efficacy [5].

MATERIALS & METHODS

The Albendazole was purchased from Chimak Health Care, Himachal Pradesh, Excipients like Steric acid, Polaxamer 118 was procured from Sigma Aldrich chemicals Pvt. Ltd., USA, Bees wax, Dimethyl formamide, Sodium hydroxide, was procured from S.D. Fine Chem. Ltd. Mumbai. All other reagents used were of analytical grade.

Methodology:

FT-IR Studies: The purity of the drug was determined by subjecting Albendazole for IR analysis using Fourier Transform Infrared Spectroscopy (FT/IR 8400S (CE) Shimadzu spectrophotometer). The samples were prepared as KBr pellet method. Drug and potassium bromide are mixed in the ratio of 1:100 and a pellet is formed by compressing at 8 ton/mm^2 pressure [6]. The wavelength range was selected from 400 - 2000 cm^{-1} in Shimadzu FT-IR spectrophotometer. Similarly an IR peak is obtained for

physical mixture of Albendazole, Stearic acid, Bees wax, Polaxamer 188 and mixtures.

Formulation Development of Albendazole Nanoparticles: The Nanoparticles of Albendazole were prepared by hot homogenization method under high speed magnetic stirring with slight modifications. An accurately weighed quantity of lipid & wax was heated carefully on a water bath at 80 °C in order to form a melted phase of the lipid & Wax. To this melted lipid Albendazole was added and was heated until a clear homogeneous phase is formed [7]. Simultaneously, a weighed quantity of the surfactant was added to the water to form an aqueous phase which is also heated to 80°C. The hot lipid phase was dispersed in the surfactant solution and stirred on magnetic stirrer for 30 minutes continuously to form solid lipid nanoparticles of Albendazole.

Table 1 Composition of Albendazole Nanoparticles formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Albendazole (mg)	20	20	20	20	20	20	20	20
Stearic acid (mg)	150	150	100	100	150	150	110	110
Bees Wax (mg)	100	100	120	120	150	150	130	130
Polaxamer (mg)	200	200	200	200	200	200	200	200
Span 80 (ml)	0.5	0.75	1.0	1.25	0.5	0.75	1.0	1.25
Ethanol (ml)	10	10	101	10	10	10	10	10

Scanning Electron Microscopy: The prepared Nanoparticle formulation was examined for surface morphology and shape using scanning electron microscope [8]. The scanning electron microscopy was performed on Hitachi high technologies corporation-S4800 type II, Japan. The samples were dried thoroughly in vacuum desiccators before mounting on brass specimen studies.

Particle Size: The average mean diameters and size distribution of albendazole loaded nanoparticles was found out by photon correlation spectroscopy using a Zeta sizer (nano ZS90, Malvern Instruments) at 25°C. The samples were kept in polystyrene cuvette and the readings were noted at a 90° fixed angle [9].

Zeta Potential: The electrophoretic mobility (zeta potential) measurements of drug loaded nanoparticles were made using Zeta sizer (Nano ZS90, Malvern Instruments). The samples were placed in a polystyrene cuvette (at 25°C) and a Zeta dip cell was used to find out the potential [10].

Entrapment Efficiency: 2ml of the formulation was taken and ultra-centrifuged at 13, 000 rpm at 4°C for 90 minutes using Eppendrof centrifuge [11]. The supernatant was recovered using micro pipette and analyzed by UV method for free drug content.

Drug content: 50mg of Albendazole nanoparticles was crushed and suspended in water to extract the drug from the nanoparticles. After 24 h, the filtrate was assayed spectrophotometrically at 295 nm for drug content against water as blank [12].

***In Vitro* Release Studies:** The *in vitro* release studies was carried out using in pH 7.4 phosphate buffer by dialysis bag method with a molecular weight cut off of 12,000- 14,000 Da. Precisely 2 ml of the formulation was placed in the dialysis bag by sealing both the ends with the help of clips. The dialysis bag is dipped in a 50 ml dissolution medium maintained at 37± °C and stirred at 100 rpm using a magnetic stirrer. 2 ml of the buffer solution is removed at an interval of 1, 2, 3, 4, 6, 7, 8 and 10 hrs and is replaced by an equal amount of fresh buffer to maintain sink conditions [13]. The content of drug in the samples was determined by ultra-visible spectroscopy at λ_{max} of 295 nm.

Stability Studies as per ICH guidelines: Stability of formulations during storage includes the preservation of initial particle size and prevention of degradation reactions. Stability studies were carried out for freeze dried method. The samples were stored in room temperature (25 to 30 °C) and in refrigerator (3 to 5 °C) over a period of 2 months. Samples were evaluated at 0, 1 and 2 months for their particle size, entrapment efficiency and changes in their physical appearance [14].

RESULTS AND DISCUSSION

Drug excipients compatibility studies

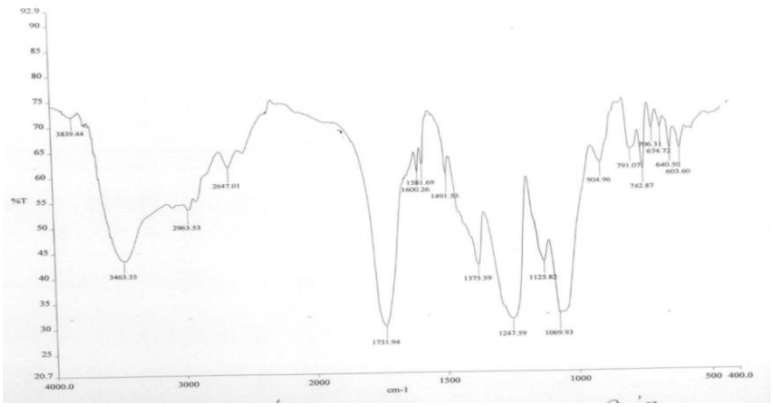

Figure 1: FT IR Spectra of Albendazole

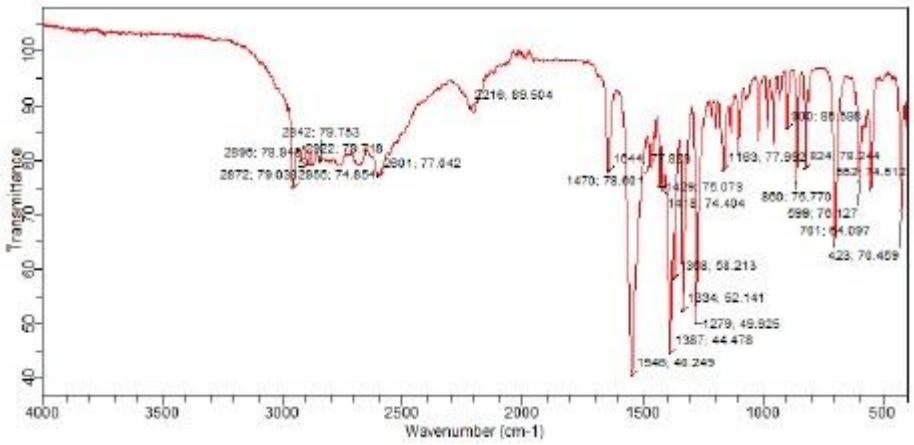

Figure 2: FTIR spectra of Stearic acid

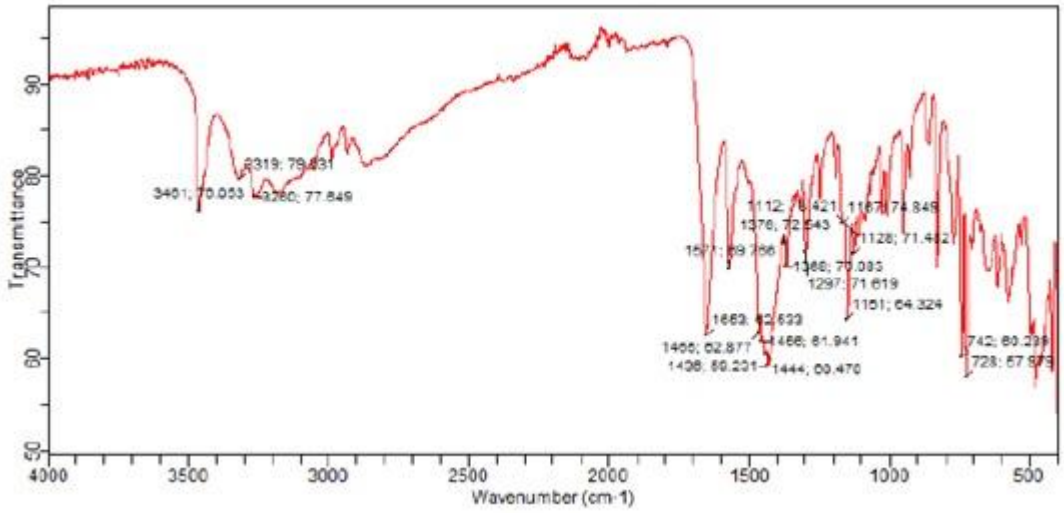

Figure 3: FTIR spectra of Bees wax

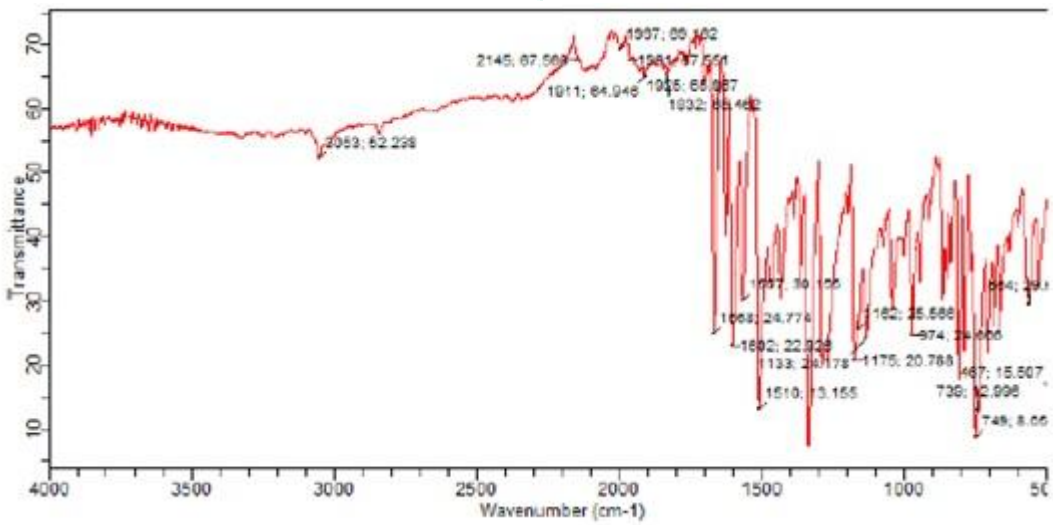

Figure 4: FTIR spectra of Poloxamer 188

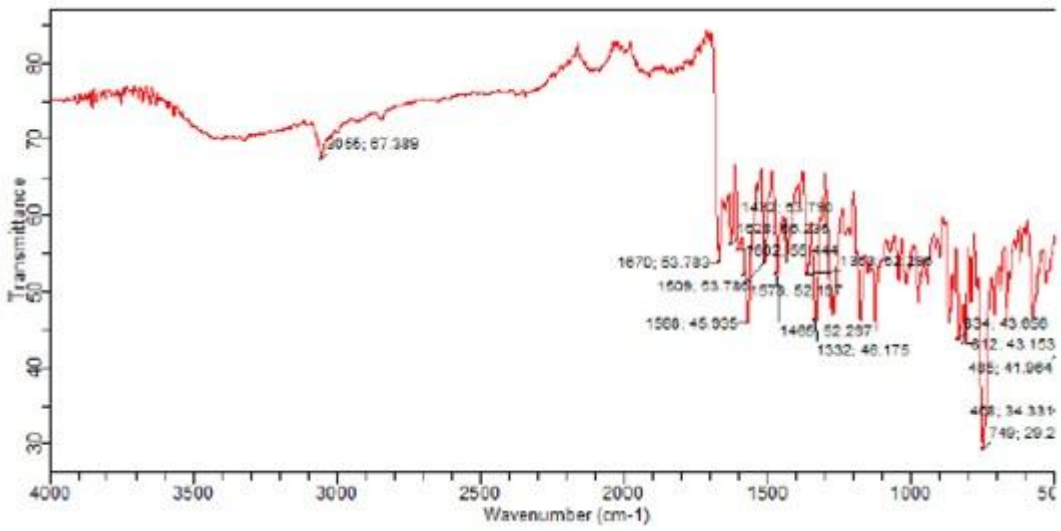

Figure 5: FTIR spectra of drug and polymer mixture

Table 2: FTIR Interpretation drug and polymer mixture

Albendazole			
S. No.	**Interpretation**	**IR absorption bands (cm^{-1})**	
		Characteristic peak	**Observed peak**
1.	C–Cl stretch	850–550	742.87
2.	C–N stretch	1250–1020	1069.93
3.	C–N stretch	1250–1020	1247.59
4.	C=O stretch	1740–1720	1731.94
5.	O–H stretch	3500–3200	3463.35
Stearic acid			
1.	C-H Bend	1650-1580	1644
2.	C-C Stretch	1600-1585	1546
3.	C-H Stretch	3000-2850	2955, 2895
Bees Wax			
1.	C-Br Stretch	690-515(m)	641.88
2.	O-H Bend	950-910(m)	633.87
3.	C-O Stretch	1320-1000(m)	1297.71
4.	C-H Bend	1470-1450(m)	1456.61
Eudragit RS 100			
1.	(s)= C-H bend	1000-650	745
2.	(s, b) N-H wag	900-675	754
3.	(s) C-O stretch	1320-1000	1056
Poloxamer 188			
1.	C–O stretch	1320–1000	1175
2.	C–H wag	1300–1150	1133
3.	C–C stretch	1500–1400	1423
4.	N–H bend	1650–1580	1602
Drug + Polymer Mixture			
1.	O-H stretch	3300-2500	3055,2955
2	C-O Stretch	1320-1000	1128
3	N-H Bend	1650-1580	1628
4	N-O Stretch	1550-1475	1509

Formulation of albendazole nanoparticles: They were prepared by hot homogenization method under high speed magnetic stirring using stearic acid as lipid, Bees wax act as a wax and poloxamer 188 as surfactant. On a whole 8 formulations were prepared by changing the different ratios of lipids &Wax. The percentage of surfactant used ranging from 0.5, 0.75, 1.0 & 1.25%).

Determination of particle shape and surface morphology by using scanning electron microscopy: Scanning electron microscopy image revealed that the particles have a smooth texture and a spherical morphology as shown in figure 6.

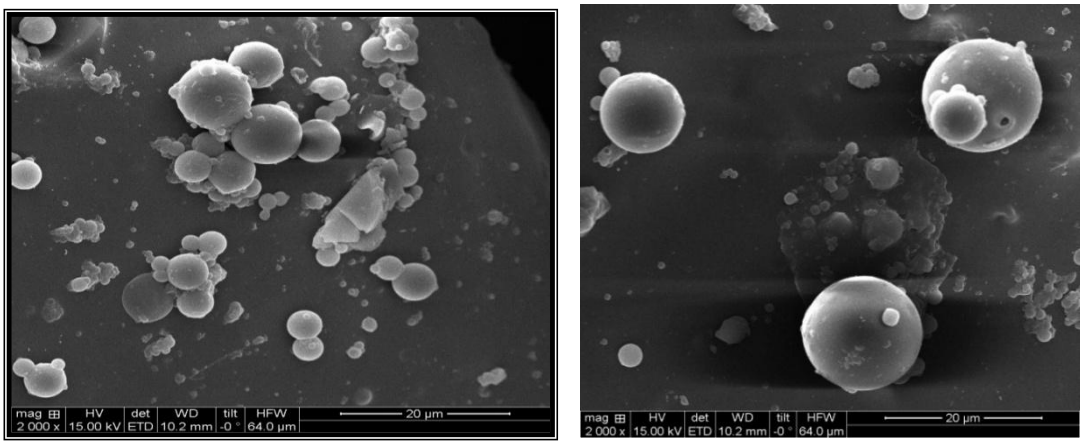

Figure 6: Scanning Electron Microscope image of the Nanoparticles

Determination of particle size by photon correlation spectroscopy: The Zeta average diameters of the formulations are mentioned in table 3. The values show that the size of the nanoparticles for all formulations ranges from 110.6 ±1.5 nm to 400.9 ± 2.4nm. The effect of various concentrations of lipids and surfactants on the size of the particles was studied.

Determination of zeta potential: The Zeta potential of all the formulations varied between -0.04 ±1.6mV and -9.15 ±1.1 mV and is mentioned in table 3. Poloxamer being a non-ionic surfactant was not able to induce potential on the surface of the nanoparticles and the partial negative observed was due to the charge induced by lipid and and drug.

Determination of entrapment efficiency: The encapsulation efficiencies mentioned in table 3 reveals that the in all the formulations the drug is moderately encapsulated and the values varied between a minimum of 43.56 ± 0.95 to 85.1 ± 0.58%. Albendazole being a hydrophobic drug has shown moderate entrapment in the stearic acid

Drug content: The Drug content mentioned in table 3 reveals that the in all the formulations the drug is moderately encapsulated and the values varied between a minimum of 65.8 % to 98.1% for formulation.

Table 3: Evaluations of Albendazole Nanoparticles

Formulation code	Zeta Size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment Efficiency (%)	Drug content %)
ANF1	110 ± 1.5	0.215 ±0.23	-4.71 ±1.3	43.56 ±0.95	65.8
ANF2	211 ±1.23	0.304±0.4	-1.16 ±1.7	54.61 ±1.2	69.2
ANF3	227 ± 2.3	0.355 ±0.12	-4.29 ±1.9	47.91 ±1.1	90.3
ANF4	**249 ± 0.59**	**0.215±1.4**	**-0.261 ±1.2**	**85.12 ±0.58**	**98.1**
ANF5	332 ± 1.41	0.407 ±1.9	-9.15 ±1.1	45.72 ±0.69	75.2
ANF6	367±0.99	0.521 ±1.3	-0.04 ±1.6	56.34 ±0.55	85.2
ANF7	400 ±2.4	0.02 ±1.1	-4.32 ±2.3	48.01 ±1.2	88.5
ANF8	108 ±0.94	0.656 ±1.8	-7.83 ±0.98	64.9 ± 1.4	78.2

***In Vitro* drug release studies:** In this section, the *in vitro* drug release studies have been carried out in pH 7.4 buffer (simulated intestinal pH) using a Dialysis bag which allows only the drug released from the nanoparticles to pass across the membrane. The drug solution was taken in the Dialysis bag which is placed in the buffer and samples are withdrawn at regular intervals of time for measuring drug concentration. All the formulations release till 10hrs which extend the therapeutic activity in the disease site. The release has followed biphasic pattern where 30-40% of the drug was released in first 1 hr and the remaining got released till 9 hrs.

Table 4: *In vitro* release release studies of albendazole nanoparticles

Time (Hrs)	% of Drug release							
	ANF1	ANF2	ANF3	ANF4	ANF5	ANF6	ANF7	ANF8
1 Hrs	5.54	3.06	5.96	6.23	11.58	13.14	14.64	7.68
2 Hrs	19.62	16.96	16.24	17.06	15.42	16.98	29.32	12.36
3 Hrs	27.96	24.46	23.59	24.80	19.32	23.16	33.16	24.64
4 Hrs	38.68	31.58	32.64	38.64	23.16	33.94	37.72	39.32
5 Hrs	41.58	45.42	49.32	46.38	37.06	46.31	47.86	43.16
6 Hrs	55.42	57.06	50.91	50.22	47.08	58.64	52.48	55.32
7 Hrs	67.06	64.8	74.81	74.12	59.42	60.98	65.60	68.64
8 Hrs	70.19	72.48	81.10	81.86	62.64	71.11	78.62	74.19
9 Hrs	88.64	80.22	89.12	94.92	70.26	76.56	87.28	89.86

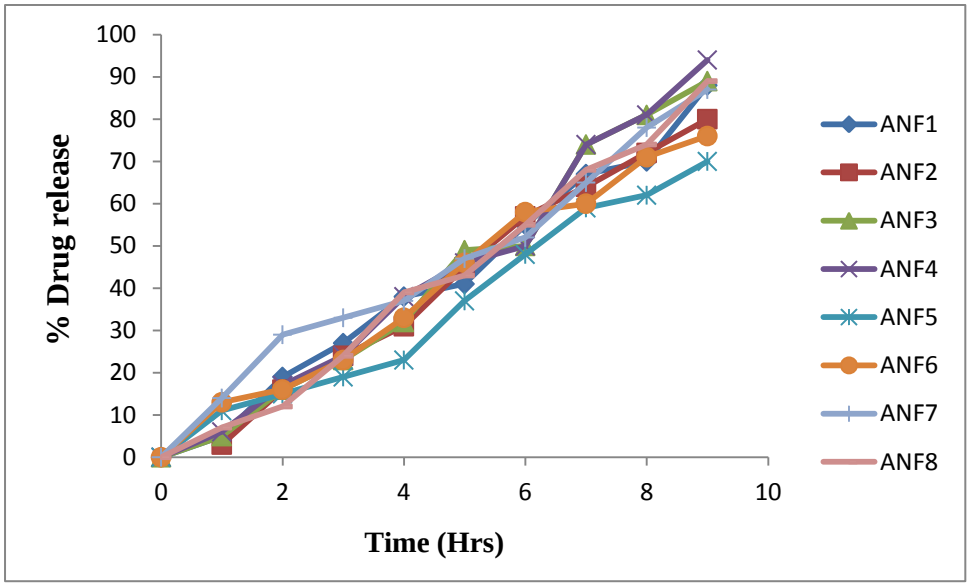

Figure 7: *In Vitro* Release Release Studies of Albendazole Nanoparticles

Table 5: Release kinetics of Albendazole Nanoparticles ANF1 TO ANF5

Model	ANF 1		ANF 2		ANF 3		**ANF 4**		ANF 5	
	R^2	m	R^2	m	R^2	m	**R^2**	**m**	R^2	m
Zero order	0.655	69.4	0.939	1123	0.007	15.93	**0.202**	**72.88**	0.928	1414
First order	0.494	0.061	0.540	0.067	0.257	0.038	**0.352**	**0.044**	0.438	0.062
Higuchi's Matrix	0.516	4508	0.767	7420	0.023	212.0	**0.189**	**515.5**	0.803	9618
Korsmeyer-Peppar	0.835	2.354	0.884	2.545	0.572	1.709	**0.663**	**1.813**	0.806	2.517

Table 6: Release kinetics of Albendazole Nanoparticles ANF6 TO ANF8

Model	ANF 6		ANF 7		ANF 8	
	R^2	M	R^2	m	R^2	M
Zero order	0.917	15.49	0.949	154.4	0.937	1593
First order	0.481	0.052	0.465	0.051	0.399	0.061
Higuchi's Matrix	0.798	1057	0.848	1067	0.899	11409
Korsmeyer-Peppas	0.835	2.032	0.827	2.033	0.785	2.560

Stability Studies: Smaller particles tend to aggregate faster during transport, storage and dispersion. Stability studies were carried out on freeze dried at room temperature (20 - 25°C) and refrigerator (3 to 5 °C) over a period of 2 months. Particle size, appearance of the formulation, drug release and FT-IR were evaluated to confirm the stability of the formulation. When stored at room temperature, the formulations showed instability by formation of larger floccules and when dispersed in water the solution turned into heavier particles. Whereas, the formulation when stored in refrigerated condition was stable with no visible floccules formation. Hence, it is concluded that the formulations should be stored in refrigerated conditions. When stored at room temperature where the size of particles increased from an initial to 343.7 ±7.9 nm at the end of 1 month to 898.1 ± 5.8 nm at the end of 2 months. The Entrapment Efficiency of formulation didn't show any change during the 60 days of storage in both refrigerated condition and room temperature. The entrapment efficiency is mentioned in table 8 & 9.

Table 8: Particle sizes of the formulations during stability studies

Stability Condition	**Average Particle size of DS15 (nm)**		
	0 days	**1 month**	**2 months**
Room temperature (25°C)	249 ± 0.59	343.7 ±7.9	899.8 ± 5.8
Refrigerated temperature (3 to 5 °C)	249 ± 0.59	255 ± 0.19	280 ± 0.39

Table 9: Entrapment efficiency of formulation on storage condition

Storage condition	**Day 30**	**Day 60**
Room temperature	73.1 ±0.58%	72.29 ±3.2%
Refrigerated temperature	75.1 ±0.58%	76.53±3.6%

SUMMARY & CONCLUSION

The present work was proposed to prepare and optimize Albendazole loaded Nanoparticle to improve its bioavailability and reduce its side effects which are caused when given conventionally. Initial pre-formulation studies using FTIR spectroscopy reveals that there are no interactions between Albendazole and other excipients and hence they can be used for the preparation of nanoparticles. Albendazole nanoparticles were prepared by hot homogenization method under high magnetic stirring using stearic acid as lipid and poloxamer 188 was used as surfactant. On whole 8 batches of formulations were prepared by varying the amount of lipid and surfactant and were evaluated for various parameters like particle size, shape, morphology, physical state, entrapment efficiency, Drug content and *in vitro* drug release. Particle size was measured using Malvern Zeta sizer and the size range of the particles was found to be in the range of 110.6 ±1.5 nm to 400.9 ± 2.4nm. Increase in size of nanoparticles was observed when we gradually increase the lipid content. The different concentrations of surfactant were evaluated for optimum one required for the preparation of stabilized solid lipid nanoparticles. The entrapment efficiencies varied from a minimum of 43.56 ± 0.95 % to a maximum of 85.1 ±0.58% and it can be concluded that higher amount of lipid is necessary for obtaining a good entrapment efficiency. The drug content of albendazole nanoparticles for all formulation ranges from 65.8% to 98.1%. Albendazole, being a hydrophobic drug has moderate entrapment efficiency. A spherical shape was observed for the particles and the particles had a smooth morphology when examined under SEM. In vitro release studies of the formulations carried out in pH 7.4 PBS showed that the total amount of drug is released for 9hrs with sustained effect. Taking the particle size, entrapment efficiency and in vitro release into account F4 was found out to be the best formulation which has a particle size of 249 ± 0.59 nm and entrapment efficiency of 85.1 ±0.58%. The drug content was release from 98.1%. Further, F4 is one among the few formulations which showed a drug release up to 9hrs, perform with release order kinetics like zero order, first order, Higuchi matrix, korsmeyer peppas it must best fit the model was zero order kinetics. The stability studies are performed with appearance of the formulation will stored at room temperature, the formulations showed instability by formation of larger floccules and when dispersed in water the solution turned into heavier particles. Whereas, the formulation when stored in refrigerated condition was stable with no visible floccules formation. The particle size of the formulation is evaluated by photon correlation spectroscopy and the particle size. That the formulations showed a drastic increase in size when stored at room temperature where the size of particles increased from an initial to 343.7 ±7.9 nm at the end of 1 month to 898.1 ± 5.8 nm at the end of 2 months. Entrapment efficiency of the formulation was determined at each interval to ensure that the drug molecules didn't undergo any degradation during storage.

REFERENCES

1. Muller R H, Wissing S A and Kayser. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Del Rev 2004; 56:1257-1272.
2. Wolfgang M, Karsten M. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Del Rev 2001; 47:165-196.
3. Muller RH, Radtke M, Wissing SA, Solid lipid nanoparticles and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv. Drug Deliv. Rev. 2002; 54:131-155.
4. R.H. Muller, K. Mader, S. Gohla, Solid lipid nanoparticles for controlled drug delivery a review of the state of the art, Eur. J. Pharm. Bio harm. 2000; 50: 161– 177.
5. Cavalli R, Caputo O, Carlotti ME, Trotta M, Scarnecchia C, M.R. Gas co, Sterilization and freeze-drying of drug-free and drug-loaded solid lipid nanoparticles, Int. J. Pharm. 1997; 148:47-54.
6. Vijaya Suman Reddy D, Ramesh Y, Parameshwar K, Jhansi reddy K, Formulation and Evaluation of Mouth Dissolving Tablets of Metoprolol Tartrate by New Coprocessed Technique, Research journal of Pharmaceutical, Biological & Chemical Science, 2011; 2(3):385-390.
7. Puglia C, Samad A, Ali A, Aqil M, Sharma M, Mishra AK. Gelrite-based *in-vitro* gelation ophthalmic drug delivery system of gatifloxacin. J Disp Sci Tech, 2010; 29:89-96.
8. Ramesh Y, Abhirami B, Gnana Sri K, Kaveri S, Neha Sulthana SK, Sravya A.S.L.S.M., Sujatha K, Formulation And Evaluation Of Oxymetazoline Hydrochloride Nasal Gels, Journal of Drug Delivery & Therapeutics, 2018; 8(6):49-57.
9. Radomska, Bonferoni MC, Rossi S, Ferrari F, Güneri T, Caramella C. Cyclosporine A loaded SLNs: evaluation of cellular uptake and corneal cytotoxicity. Int J Pharm, 2007; 364:76-86
10. Pignatello R, Du YZ, Yuan H, Ye YQ, Zeng S. Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system. Colloids Surf B Biointerfaces, 2006; 45:167-173
11. Puglia C, Samad A, Ali A, Aqil M, Sharma M, Mishra AK. Gelrite-based in-vitro gelation ophthalmic drug delivery system of gatifloxacin. J Disp Sci Tech, 2010; 29:89-96
12. Viswanatha Reddy M, Saravana Kumar K, Ramesh Y, Chanukya Kumar G, Venkateswarlu I, Venkatesh R, Preparation And Evaluation Of Quetiapine Fumarate Microspheres, Journal Of Pharmacy Research, 2011; 4(11):41-43.
13. Casadei M.A, Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine- based mixed micelles: preparation and characterization. Int J Pharm, 2010; 340:153-162.
14. Ramesh Y, Sireesha V, Transdermal Patch of Ramipril Loaded Chitosan Nanoparticles Dispersed in Carbopol Gel, Journal of Drug Delivery & Therapeutics, 2017; 7(6):56-65.