



## Solubility and bioavailability enhancement of albendazole by complexing with hydroxy propyl $\beta$ cyclodextrin

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### ABSTRACT

The solubility and dissolution properties of any drug are vital determinants of its oral bioavailability. The effect of hydroxyl propyl beta cyclodextrin, on the dissolution, bioavailability was evaluated using a commercial suspension as reference product. Both formulations were orally administered in mice. Plasma samples were taken at different times, and drug concentration was assayed by HPLC. Due to the rapid metabolism of ABZ its main metabolite albendazole-sulphoxide (ABZSO), which also has anthelmintic activity, was assayed. The binary system containing Albendazole and hydroxyl propyl beta cyclodextrin is prepared by kneading method and complex formed is characterized using scanning electron microscope (SEM), DTA, FTIR. And this binary mixture is formulated into suspension form and optimization of was done by selecting different concentration of hydroxypropyl beta cyclodextrin and the formulation is evaluated for sedimentation volume, redispersibility, pH measurement, viscosity measurement and drug content estimation at various time intervals for 3 months to find out the effect of hydroxy propyl beta cyclodextrin on the stability of Albendazole suspension. In vitro dissolution study, ex vivo study, histopathological studies, in vitro anthelmintic activity, and zeta potential compared with two marketed formulations Zental and noworm and release rate of ABZ:HP $\beta$ CD suspension were markedly higher compared with marketed formulation due to increase in the wetting properties of the drug. A bioavailability study on Albino wistar rat was done, bioavailability results shows that ABZ:HP $\beta$ CD complexes had faster absorption than a conventional ABZ formulation.

**Keywords:** Albendazole-sulphoxide (ABZSO), Bioavailability, HPLC, In-vivo study, Kneading method, Zeta potential,

### INTRODUCTION

Albendazole (ABZ), methyl [5-(propylthio)-1-H-benzimidazol-2yl] carbamate, is a wide spectrum anthelmintic drug used for human and animal infections. When administered orally, albendazole undergoes first pass metabolism and quickly bio transformed into its active metabolite albendazole-sulphoxide (ABZSO), which is then oxidised to the inactive form albendazole-sulphone (ABZSO<sub>2</sub>). Albendazole is the drug of choice for the treatment of echinococcosis, hydrated cysts and neurocysticercosis, but it is well known that this drug is poorly and erratically absorbed from gastro-intestinal tract and it has low oral bioavailability, which is related to its low water solubility. Its poor water solubility is the major disadvantage for the use of albendazole in the treatment of systemic helminthiasis [1]. As the albendazole therapy is important in systemic cestode infections, particularly in inoperable or disseminated cases of hydatidosis and neurocysticercosis, different efforts have been made to improve ABZ solubility. Furthermore lack of water solubility reduces flexibility for ABZ formulations and administration. Therefore, the overcome of poor aqueous solubility of ABZ is an important goal. One of the possible ways to overcome this problem is to alter the physical properties of the drug by forming a complex with hydroxyl propyl beta cyclodextrin (HP $\beta$ CD) [2]. Cyclodextrins are structurally related natural products they belong to the family of

cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity, and are formed by the bacterial digestion of cellulose, and this CDS contain (1, 4)-linked D-glucopyranose units. This D-glucopyranose has chair conformation and due to this CDS are cone shaped. The most common type of CDS are alpha, beta and gamma CDS formed by 6, 7&8glucose units. But these CDS have limited solubility and various derivatives that are mainly used in pharmaceutical field. Among various derivatives hydroxyl propyl beta cyclodextrin is widely used because of its amorphous nature and is used to increase the solubility, bioavailability, and dissolution rate of poorly soluble drugs [3,4] Albendazole the current drug candidate chosen for the study ,is a poor water soluble drug known to demonstrate dissolution limited absorption. ABZ is commercially available in tablet and capsule and choice of suspension instead of tablet is frequently governed by patient acceptance. Although tablets and capsules are usually given to adults, children are easily treated with adequately flavoured suspensions [5] In this work a hydroxyl propyl beta cyclodextrin complexed suspension is formulated and objective is to determine whether HPβCD improves the solubility and dissolution of albendazole. The aim of the study is to compare the bioavailability characteristics of HPβCD-ABZ suspension to a marketed formulation.

## EXPERIMENTAL SECTION

### Materials and Methods

Albendazole which is obtained as gift sample from Cipla Ltd, Mumbai and hydroxyl propyl beta cyclodextrin from Yarrow Chem. Products, Mumbai. And all other chemicals used in this study are of analytical reagent grade.

### Formulation of Drug-Cyclodextrin Inclusion Complexes [6]

#### *Preparation of binary mixture of Albendazole /Hydroxyl propyl β cyclodextrin*

Various techniques for the preparation of drug-cyclodextrin complexes include co-precipitation, slurry complexation (kneading method), damp mixing, paste complexation, heating, dry mixing, neutralization, freeze drying, and slugging methods. In this work kneading method is used for the preparation of binary mixture; ABZ-HPβCD

#### *Complexation with Hydroxyl propyl β cyclodextrin by kneading technique*

The Albendazole and hydroxyl propyl β cyclodextrin (1:0.5, 1:1, and 1:1.5) were prepared by kneading method. In this method hydroxyl propyl β cyclodextrin is taken in a glass mortar, little water was added mixed to obtain homogenous paste. The drug was added slowly while grinding. The mixture was ground for 1hour and during this process approximate quantity of water was added to maintain suitable consistency. The paste was dried in hot air oven at 40°C for 48hours. The dried complex was further passed through 60#mesh and packed in closed container.

### Characterization of binary mixture of ABZ-HPβCD

#### **Fourier transforms infrared (FTIR) spectroscopy**[7,8]

Fourier transform IR spectra were recorded for albendazole, HPβCD, and kneaded mixture. Samples were prepared in KBr disc (2mg sample in 200mg KBr). The scanning range was 400-4000cm<sup>-1</sup>, resolution was 4cm<sup>-1</sup>. Any change in the chemical composition after combining with the excipient were investigated with IR spectral analysis

#### **Differential thermal analysis (DTA)** [9,10]

DTA has been reported as a method to characterize the binary mixture. DTA thermo grams were recorded for albendazole, HPβCD, physical mixture, and kneaded mixture. The studies were performed by recording the heat flow rate from 23<sup>o</sup>-450<sup>o</sup>, at a rate of 10°C/min.

### SEM

Scanning electron microscopy was used for analysing albendazole powder, HPβCD, and kneaded mixture using JOEL Analytical Scanning Electron Microscope (JOEL –JSM 6490 LA) at an accelerating voltage of 15 ke V

## FORMULATION AND EVALUATION STUDIES

### Preparation of oral suspensions

Suspensions containing 40mg/ml were prepared in about 100ml of purified water, the required amount of suspending agent (sodiumCMC) was added and kept overnight for proper hydration. This solution was used as the vehicle in the preparation of the suspensions. Accurately weighed quantity of the drug was distributed in the vehicle. Tween 80 was added to above dispersion. The slurry concentrate of the drug was mixed gently for 15min. Other ingredients like sodium benzoate, citric acid, sucrose, sorbitol, colouring agent and flavouring agent were added and the volume was made up with water. The prepared suspensions were homogenized and transferred to the final containers. The composition of HPβCD-ABZ suspension is given in Table1.

**Table 1: Composition of different formulations containing HP $\beta$ CD-ABZ Suspension**

INGREDIENTS	F1 (control) gm	F2 (1:0.5) gm	F3 (1:1) gm	F4 (1:1) gm	F5 (1:1.5) gm
Albendazole	4	4	4	4	4
HP $\beta$ CD	-	2	4	4	6
Sodium CMC	1	1	1	0.75	1
Tween 80	1	1	1	1	1
Sodium benzoate	0.8	0.8	0.8	0.8	0.8
Citric acid	0.25	0.25	0.25	0.25	0.25
Sucrose	30	30	30	30	30
Sorbitol	20	20	20	20	20
Pineapple flavour	0.30	0.30	0.30	0.30	0.30
Tartarazine colour	0.0001	0.0001	0.0001	0.0001	0.0001
Purified Water	q.s	q.s	q.s	q.s	q.s
Total (ml)	100 ml	100 ml	100ml	100 ml	100 ml

**EVALUTION OF HP $\beta$ CD-ABZ SUSPENSION [11,12]****1. Sedimentation Volume**

Sedimentation volume (F) is nothing but ratio of the final volume of sediment (Vu) to the original volume of sediment (V0) before settling. 50 ml of each suspension were transferred to 50 ml measuring cylinder and the volume of sediment formed was noted after 24hr, 72hr, 1wk, 2wk, 1month, 2month and 3 months.

The sedimentation volume was calculated using the formula:

$$F = V_u / V_0$$

**2. Redispersibility**

The bottles containing suspension were held up right between fingers and rotated clockwise upside down through 180° in a semicircular path and back in the anti-clockwise direction (one cycle). This process was repeated continuously until sediment was completely redispersed.

**3. pH measurement**

The pH of the prepared suspension was measured by using pH meter by calibrating with standard buffers (pH 4.2 and pH 9.0).

**4. Viscosity measurement**

The suspension of the prepared suspension was measured by Brookfield viscometer (Model: DV1 Prime Rheometer) using spindle S-61.

**5. Particle size measurement**

The particle size of albendazole in the prepared suspensions was measured by optical microscopy. The size of 100 particles was measured and the average particle size was determined.

**6. Drug content estimation**

Suspension equivalent to 100 mg of Albendazole was accurately weighed or measured and transferred to 100 ml volumetric flask and dissolved in 20 ml 0.1N HCL. Then the solution was shaken for 20 min. The resulting solution was further diluted to 100 ml with 0.1N HCL. 1 ml of the above solution was pipette out into 100 ml volumetric flask and made up to the mark with 0.1N HCL. The absorbance was measured at 229 nm against the blank. The amount of the drug in a sample was calculated from the calibration curve.

**7. In vitro dissolution studies [13]**

Drug release study of HP $\beta$ CD complexed Albendazole suspension was carried out in USP XXIII dissolution test apparatus-II (Veego digital tablet dissolution test apparatus, model VDA-8D) and the dissolution medium was 0.1N HCL (pH 1.2). The volume of dissolution medium was 900ml, and it was maintained at 37 $\pm$ 0.5°C and stirred at a paddle speed of 50 rpm. Three millilitres were collected at time interval of 5, 20, 40, 70, 90, 110, and 120 minutes. The withdrawal samples were replaced by equal amounts of the dissolution medium to maintain a constant volume. The samples extracted at the time intervals were analysed spectrophotometrically at 229nm for determination of the albendazole content using the calibration curve. Each formulation was tested in triplicate and the mean values are calculated.

**8. In vitro anthelmintic activity [14]**

The assay was performed in vitro, using adult earthworm (*Eisenia fetida*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being for preliminary evaluation of anthelmintic

activity. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into four groups of six earthworms in each. Six earthworms approximately equal size were placed in each petri dish containing 10ml of test solution (optimized formulation) and two standard drug formulations (ZENTEL AND NOWORM) and one group was treated as control at room temperature. The time taken for complete paralysis and death was recorded. The mean paralysis time and mean lethal time for each compound was calculated (each reading taken in triplicate). The time taken for worms to become motionless was noted as paralysis time. To ascertain death, each worm was frequently applied with external stimuli which stimulates and induce movement in the earthworms, if alive.

### 9. Zeta potential

The zeta potential of the optimized formulation and the marketed formulation was determined by Zetasizer.

### 10. *Ex vivo* permeation studies: Everted intestinal sac of chicks: [15,16]

Male white Leghorn chicks weighing between 500 and 600 g were bought from the local market. For isolation of everted intestine, the chicks were slaughtered, a median incision of the abdomen was performed, and the small intestine was freed. The lumen was carefully cleared from mucus by rinsing with a pH 7.4 buffer solution (Krebs–Ringer solution). An intestinal segment of the first 6-cm length was removed and transferred to oxygenated Krebs–Ringer solution. It was washed thoroughly with warm Krebs–Ringer solution. The proximal extremity of the intestine was turned back and ligated on a glass rod to form an everted bag. The intestinal segment of about 6cm in length were tied at one end and the sacs were filled with 5ml of kerbs-Ringer Bicarbonate buffer(receiver compartment) and the other end was lignated with needle for sampling. At time equal to zero, the everted sac was submerged in 30ml of experimental test suspension (donar compartment).The donar compartment is saturated with 5% carbon dioxide and 95% oxygen gas in  $37\pm 0.1$  centi-degree water bath. The whole sample solution from receiver compartment was taken every 10min for 1hr, then equivalent fresh KR buffer was added to serve as the serosal solution at the time intervals and incubation was continued. The process of absorption study must be away from light. Each experiment was performed six times and proper dilutions of sample are done with KR buffer solutions and absorbance is measured using UV Spectrophotometry.

### 11. Histopathology for Viability of Intestinal Cells [16]

The chicken ileum sample after 1hr permeation study was stretched on a piece of X-ray and preserved using 10% Formalin neutral buffered. (pH7.4-7.6).

### Preparation of chicken ileum for histological studies

The prepared chicken ileum were treated with various formulations like, physiological salt solution, optimised formulation and marketed formulation ,kept in 10%buffered formalin solution. The specimens were cut into section vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing and stained with hematoxylin and eosin. These samples were observed under light microscope and compared with control sample. The changes in the ileum after treatment were evaluated.

### 12. Stability Study [17]

The main objective of stability study is to evaluate stability of optimized formulation at different temperature and humidity conditions. Optimized batch has been placed for 3 months at  $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ , RH 75%  $\pm 5\%$ . At the end of each week, the formulation were evaluated for, sedimentation ratio, viscosity, *in-vitro* release, pH particle size, and % drug content.

### 13. *IN-VIVO* PHARMACOKINETIC STUDY [18]

#### Study design using Wistar albino rats

The protocol for the animal study in prescribed proforma B was submitted to the Institutional Animal Ethics Committee (IAEC) of the Amrita Institute of Medical Sciences, Kochi. The protocol was approved by the IAEC Approval No. IAEC/2011/1/1(Appendix-1).Wistar albino rats (MALE) weighing  $280\pm 20$  g were fasted overnight and divided in to three groups. Group I received a vehicle as control and group II received albendazole suspension as standard (marketed) and group III received optimized formulation of Hydroxyl propyl beta cyclodextrin complexed albendazole suspension as test. Blood samples were collected at intervals of 15min, 30min, 1 h followed by 2, 4 and 6hr, and 8hr by retro orbital puncture method and blood samples were collected on to the heparinized tubes, and RBCs will be allowed to settle by centrifugation at 5000 r/min for 35 min. The supernatant will be collected as plasma and will be analyzed by HPLC. The pharmacokinetic parameters for Albendazole following oral administration will be determined from the plasma concentration-time data. Pharmacokinetic study design is given in table 2.

Table.2.Study design for pharmacokinetic study			
Group	No of animal	Treatment plan	Drug, dose, route
I (Control)	6	Administer vehicle alone	Vehicle, 0.5ml/kg, oral
II (standard)	6	Administer ABZ suspension (marketed) and observation will be done for 0-8 hr	ABZ suspension (marketed),40mg/kg, oral
III (Test)	6	Administer HPβCD : ABZ suspension and observation will be done for 0-8 hr	HPβCD :ABZ suspension,40mg/kg, oral

### Extraction procedure

In a 1.5ml eppendorf polypropylene tube, plasma aliquots of 250  $\mu$ l is taken and the sample was extracted with 4mL of ether–dichloromethane–chloroform (60:30:10, v/v/v) by vortex mixing for 5min. After centrifugation at 3000 rpm, the upper organic layer was removed and evaporated to dryness at 40 °C under a nitrogen stream. The residue was reconstituted with 50  $\mu$ l of methanol–water–chloroform (70:30:5, v/v/v), shaken on a vortex and a 20  $\mu$ l aliquot was injected onto the HPLC system. The pharmacokinetic parameters of albendazole following oral administration were determined from the plasma concentration time data.

## RESULTS AND DISCUSSION

### Characterization of binary mixture of ABZ-HPβCD

#### FTIR Spectroscopy

Drug-excipient compatibility studies were also confirmed by FTIR and the spectra of the drug(ABZ),HPβCD, and ABZ-HPβCD physical mixture are shown in figure 1.The FTIR studies from the spectra confirmed the absence of any chemical incompatibility between the drug and the hydroxyl propyl beta cyclodextrin. The spectrum of ABZ showed N-H stretching vibration at 3336  $\text{cm}^{-1}$ , bending vibration at 1525-1630  $\text{cm}^{-1}$  and aliphatic C-H at 2958  $\text{cm}^{-1}$ , stretching of alkane at 2959  $\text{cm}^{-1}$ , coo bending of ketone at 1710 $\text{cm}^{-1}$ .The spectrum of ABZ has NH stretching vibration at 3336  $\text{cm}^{-1}$  due to carbamate. The spectrum of HPβCD is characterized by intense bands at 3,300-3500  $\text{cm}^{-1}$  due to O-H stretching vibration. The vibrations of the —CH and CH<sub>2</sub> groups appears in the 2,800-3,000  $\text{cm}^{-1}$  region, CH stretching at 1164.15  $\text{cm}^{-1}$  and H-O-H bending at 1647  $\text{cm}^{-1}$ .The kneaded mixture showed superimposed spectra of ABZ and HPβCD which proves the compatibility of excipients with the ABZ.

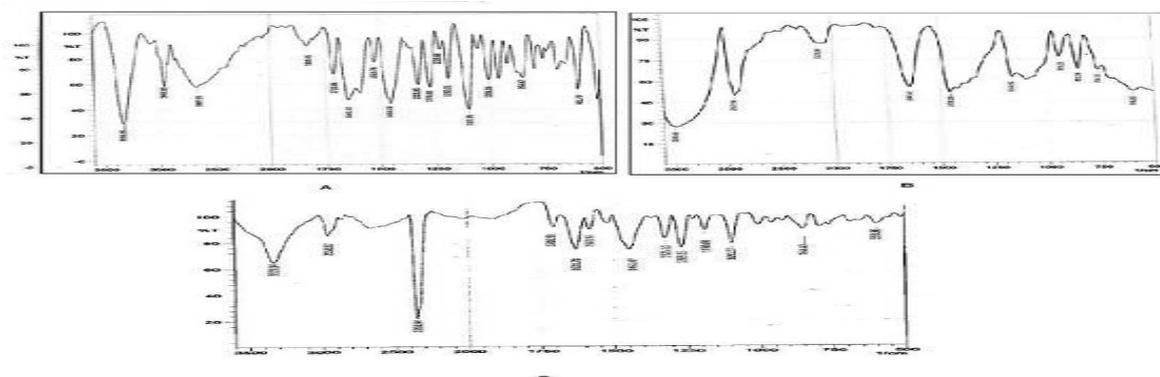


FIGURE 1 : FTIR SPECTRUM OF A)Albendazole B)HPBETA CD C)ABZ-HP BETA CD DTA ANALYSIS

DTA of drug, HPβCD, physical mixture, kneaded mixture are shown in figure 2.DTA of Albendazole exhibits an endothermic peak at 204<sup>0C</sup>.The sharp endothermic peak at this region is due to the melting of crystalline Albendazole. From DTA it was confirmed that the drug is crystalline in nature and has a melting point 203<sup>0C</sup> and followed by endothermic peak there is an exothermic peak at 348<sup>0C</sup>.The DTA curve of HPβCD shows its thermal decomposition which begins around 280<sup>0C</sup> and peaks are exothermic in nature. In the ABZ:HPβCD obtained by physical mixing, the DTA technique shows the endothermic peak corresponding to the melting of ABZ. The presence of this peak indicates that a true inclusion complex has not been achieved. Nevertheless, in the ABZ:HPβCD system obtained by kneading method, the endothermic peak corresponding to the melting of ABZ is not present. A new peak appears at temperature between 320<sup>0C</sup> and 380<sup>0C</sup> that can be attributed to melting of true inclusion complex.

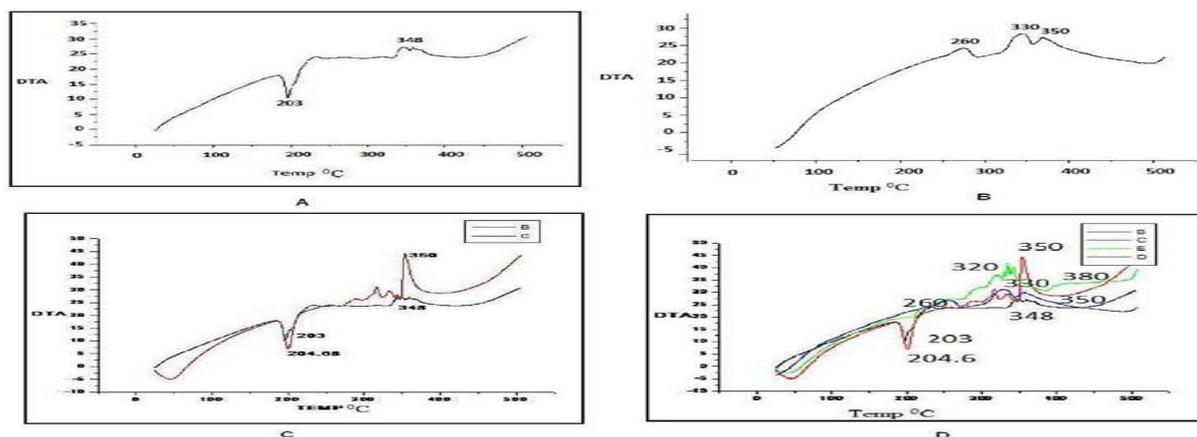


FIGURE 2 : DTA OF a)Albendazole b)HP BETA CD C)Physical mixture D)KNEADED MIXTURE

### SEM ANALYSIS

The SEM microphotographs of pure ABZ, HPβCD and Kneaded mixture (ABZ: HPβCD) are shown in Figure 3. After complexing with HPβCD the irregular crystalline shape of ABZ is changed into amorphous form.

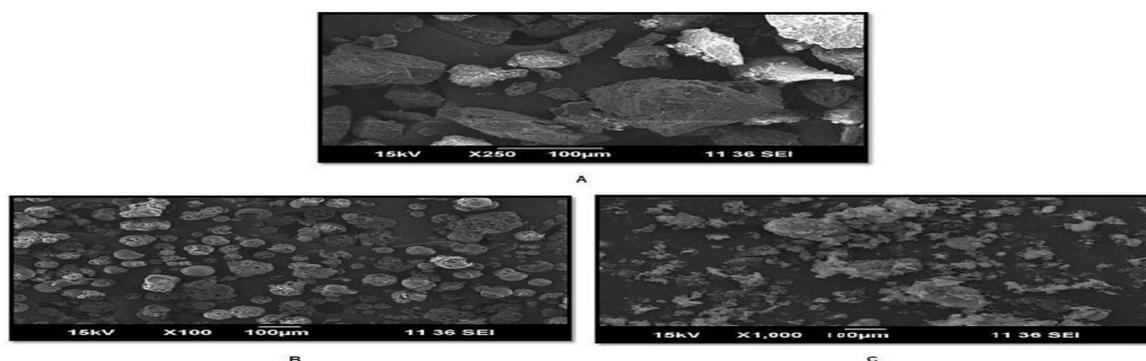


FIGURE 3: SEM OF A)ALBENDAZOLE B)HP BETA CD C)Kneaded mixture ABZ-HP BETA CD

### EVALUATION OF ABZ-HPβCD SUSPENSION

Suspension F1 was formulated employing drug and suspending agent, whereas suspension F2, F3, F4, and F5 were formulated by employing drug, suspending agent and hydroxyl propyl β-cyclodextrin. The drug and hydroxyl propyl β-cyclodextrin complexes in F2, F3, F4 and F5 were in the ratio 1:0.5, 1:1, 1:1, 1:1.5 respectively. These formulations were evaluated for various quality parameters to determine their stability such as sedimentation volume, redispersibility, particle size, pH and drug content for 3 months time in regular intervals. All formulations contain sodium CMC as the suspending agent. These formulations were compared with two marketed product (Zentel and Noworm) to find out which formulations is more stable in terms of sedimentation, particle size, redispersibility, pH, and drug content. And optimized formulation is compared with marketed formulation on the basis of *in-vitro* release study, *in-vitro* anthelmintic activity, zeta potential, *ex-vivo* study and *in-vivo* study.

#### Sedimentation volume:

The sedimentation volume in the case of F1 was found to be 1 at the end of 24 hrs whereas it was around 1 in the case of suspensions containing different ratios of hydroxyl propyl β-cyclodextrin and with aging (after 3 months) it was found that, sedimentation is more in the case of F1 formulation (control) and the marketed product Zentel and Noworm, and the formulation containing different concentration of hydroxyl propyl β cyclodextrins showed less sedimentation, and its given in Figure 4. Thus highest sedimentation ratio was seen with the suspension containing highest ratio of hydroxyl propyl β cyclodextrin(1:1.5). This indicates that suspensions containing hydroxyl propyl β cyclodextrins exhibited more stability than those without hydroxyl propyl β cyclodextrin.

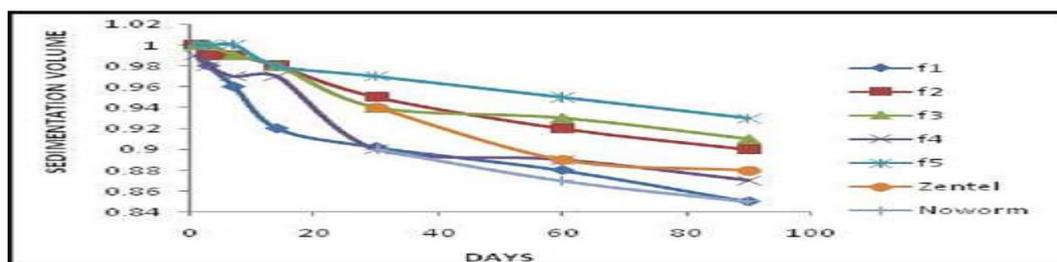


FIGURE 4: Sedimentation volume for different formulation for 3 months

### Redispersibility

Good redispersibility was seen in the case of Albendazole suspension containing different ratios of hydroxyl propyl  $\beta$  cyclodextrins within a 3-4 cycles during storage (F2, F3, F4, and F5). On the contrary, it took 6-7 cycles in case of F1, Zentel and Noworm. The above conclusion in sedimentation volume holds true here also.

### Particle size measurement:

There was no significant change in particle size in all five formulations (F1, F2, F3, F4 and F5) at the end of 24 hours. But on ageing, the particle size marginally increased in the case of F1 and in Zentel and Noworm whereas in all other cases the size was decreased, its shown in Table. The reason for increased particle size may be due to crystal growth whereas in other cases, as hydroxyl propyl beta cyclodextrin solubilises the drug particles, the size may be reduced with time.

### Drug content

Assay of drug shown 95% to 101% albendazole content indicates that the drug content remains within the standard limit during 3 months.

### pH

The pH value of F1 was found to be 7.5 and in case of zentel and noworm value is 6.7 and 6.9, but when drug complexed with HP $\beta$ CD the values were found to be less ranging from 5.7-6.4 and its given in table. But on ageing, the values remained more or less constant in all the formulations. This indicates that though complexation results in lowering of pH value but there is no chemical change that results on aging. In the case of basic amine drugs, increased solubility can be achieved by decreasing the pH value below the pKa of the amine group and pH value can be minimized by adding cyclodextrins like HP $\beta$ CD to achieve the same solubility at a pH value closer to physiologic pH.

### Viscosity

Viscosity of formulations F1, F2, F3, F4, F5, Zentel and Noworm is evaluated for 30<sup>th</sup> day, 60<sup>th</sup> day and 90<sup>th</sup> days. On ageing the viscosity of F1, Zentel and noworm showed significant change whereas in the case of F3 and F4 the values were decreased and in the case of F5 change in viscosity is less indicating that F5 formulations is more stable compared to other formulations.

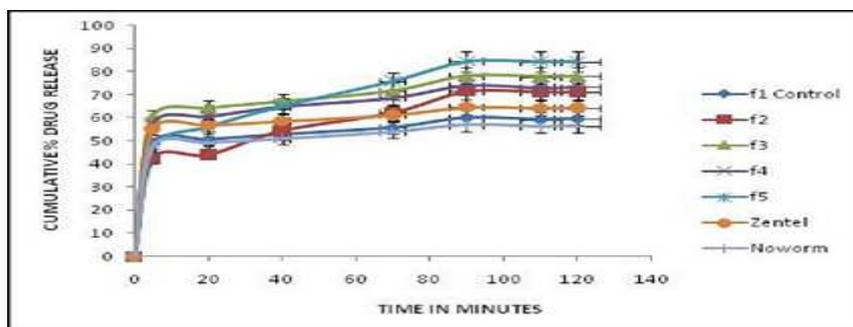


FIGURE 5: Cumulative percentage release of different formulations

### *In vitro* dissolution studies:

The release time profile of Albendazole from different formulation and marketed formulation are shown in Figure 5. The release kinetics of drug was found to be first order. Higuchi's diffusion model gave a better and best fit of release data indicating diffusion dominating as the plots showed maximum linearity with  $R^2$  value of 0.980 for F5. The korsmeyer Peppas model was found to be linear with the correlation coefficient value of 0.967 and "n" value

for F5 was 0.197. Since the P-value is found to be less than 0.05, there is a statistically significant difference between the means of the 7 variables at the 95.0% confidence level and given in table 3 and kinetic data modelling of different formulation of ABZ is given in table 4.

#### In vitro anthelmintic activity:

The anthelmintic screening of the optimized formulation (F5) is done with marketed product Zentel and Noworm and F5 formulation showed an excellent activity than other two marketed product. A closer inspection of data from Figure 6, indicate that F5 formulation showed better paralytic activity than standard ie, Zentel and Noworm, and with respect to the death of worms the optimized formulation F5 showed an excellent anthelmintic activity than marketed products.

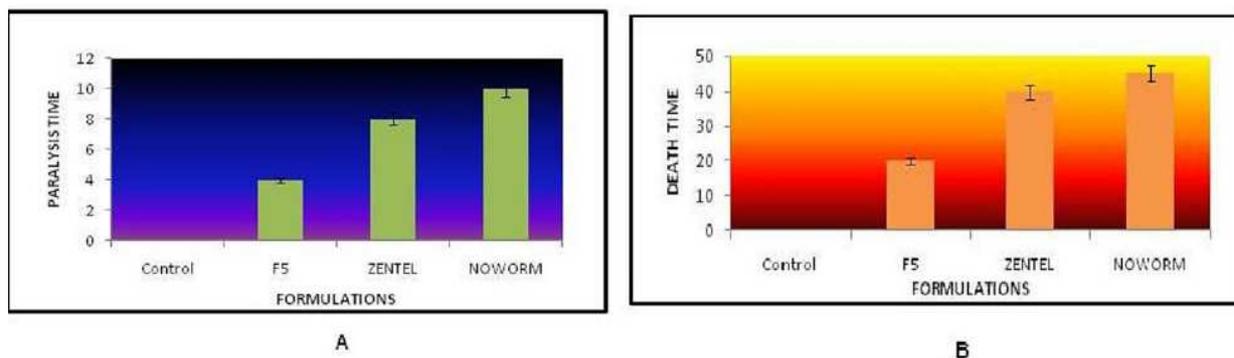


Figure 6 : Anthelmintic activity of formulations

#### Zeta potential

Zeta potential of optimized formulation (F5) and marketed formulations are given in table .Zeta potential are commonly used to assess the stability of colloidal systems. Particles with zeta potential more positive than +30 mV or more negative than -30Mv are normally considered stable. Among the evaluated suspension optimized formulation (F5) exhibited maximum zeta potential.

#### Ex vivo permeation studies

The cumulative amount of drug ABZ, permeated per unit area across chicken ileum was plotted as a function of time and steady state flux was calculated from the slope of linear portion. Ex-vivo comparison study with time in hrs on x axis and amount of drug permeated on y axis is shown in figure7. Since the P-value is found to be less than 0.05, there is a statistically significant difference between the means of the 4 variables at the 95.0% confidence level. The statistical analysis by one-way ANOVA showed that the difference in the permeation pattern of optimized formulation, marketed formulations, and control are statistically significant and given in table 3 and flux and enhancement ratio given in table 4.

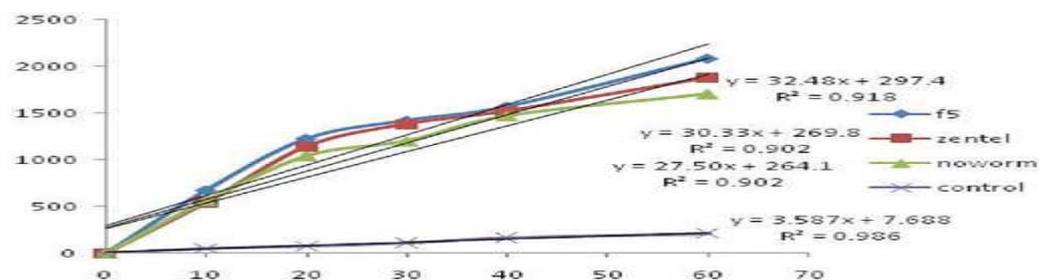


Figure 7 : Ex-vivo permeation study of formulations

Table 3: Statically analysis of Ex-vivo permeation study

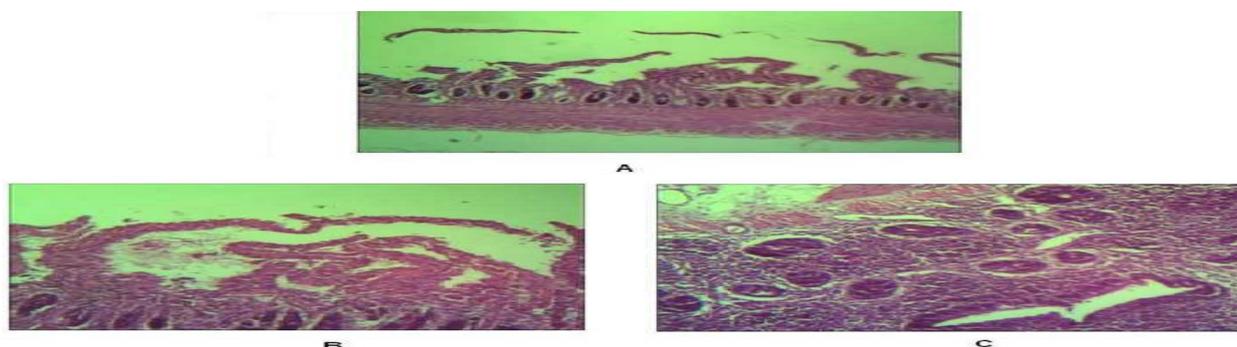
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4376323	3	1458774	4.14004	0.01954	3.09839
Within Groups	7047150	20	352358			
Total	11423473	23				

**Table 4: Flux & Enhancement Ratio from ex-vivo permeation study**

Formulation	Flux ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )	Enhancement Ratio
Optimized	32.48	4.41
Zentel	30.33	4.1
Noworm	27.50	3.7

### Histological Studies for Viability of Intestinal Cells

The histology of the chicken ileum treated with normal saline, marketed formulation and optimized ABZ: HP $\beta$ CD complexed suspension is represented in the Figure 8. In histological finding of chicken ileum treated with normal saline; section shows small intestinal mucosa with preserved villi and columnar cell lining. Smooth muscle layer is also noted. Lamia propia showing lymphoid aggregates, indicating vital cells in the group of chicken intestinal epithelium. In the case of histological finding of marketed formulation section shows small intestinal mucosa with mild flattening of mucosal surface. Villi are not prominent cell death is not seen. Mild oedema of lamina propria is noted. And in the case of optimized formulation section shows small intestinal musosa with preserved villi and intestinal glands, with intact nucleus and rest of intestinal layers are normal and cell death is not seen, indicating vital cells in the group of chicken intestinal epithelium.



**Figure 8 : Histopathology of ileum A)Treated with normal saline B)With marketed formulation C)With optimized formulation**

### Stability Study

The optimized formulation F5 were put on short term stability by packing in HDPE containers in stability chamber at  $400\text{ C} \pm 20\text{ C}$ , RH  $75\% \pm 5\%$  for the period of 3 months.

### Physical Parameters

The physical parameters of suspension kept for stability study were evaluated and compared with the initial values for any significant changes. Results of which is shown in table 8. In the stability study done for 3 months it was noted that the surface was free of any kind of microbial or fungal growth or bad odour. No change in the appearance of suspension and it shows not less than 96% drug content, which shows that there are no significant changes in drug content. The results obtained from stability studies for 3 months showed that all physical parameters are within the specified limit.

### Dissolution Study

Dissolution study was performed at the end of 7<sup>th</sup> day, 15<sup>th</sup> day, 30<sup>th</sup> day, 60<sup>th</sup> day and 90<sup>th</sup> day and compared with the initial dissolution data for any significant changes. Based upon above stability study carried out for 3 months, it was concluded that the optimized formulations is stable under ambient conditions.

### IN-VIVO STUDY

Drug content in plasma was analysed by HPLC method for marketed formulation (Zentel) and test group (optimized formulation).

### Calculation of pharmacokinetic parameters

In our experimental conditions in Albino wistar rat plasma, unmodified ABZ was never detected due to a strong liver first pass effect. The main two metabolites are the S-oxidation compounds, namely the sulfoxide (ABZ:SO) and the sulphone (ABZ :SO<sub>2</sub>). In our bioavailability studies instead of albendazole, the active metabolite ABZ: SO was evaluated. The Figure 9 shows the plasma levels of ABZ: SO after single dose (40mg/kg) of ABZ:HP $\beta$ CD suspension and zentel suspension. The pharmacokinetic parameters are given in table 5. The increase in bioavailability from ABZ:HP $\beta$ CD suspension in relation to reference suspension (zentel) maybe due to increase in ABZ solubility due to the presence of HP $\beta$ CD. Statistically significant difference were obtained with one-way

ANOVA comparative statistical study between both formulations with ( $p < 0.05$ ). Time for maximum plasma concentration ( $T_{max}$ ) obtained was 4hr for standard (zental) and 2hr for ABZ:HP $\beta$ CD suspension.  $(AUC)^{0-\infty}$ ,  $C_{max}$ ,  $T_{max}$ , AUMC,  $K_a$ ,  $K_e$  shows significant difference between two formulation. All the pharmacokinetic parameters obtained were evaluated statically. The data were tested by one-way analysis of variance (ANOVA) and at 95% confidence interval, P value found to be less than 0.05 were considered significant. So from this data it is clear that a comparatively larger amount of drug was absorbed into plasma from ABZ:HP $\beta$ CD suspension than the standard.

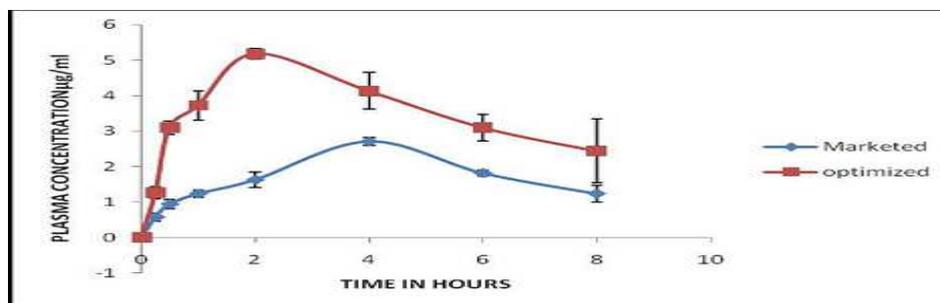


Figure 9: Plasma levels of ABZSO versus time (h)

Table 5: Pharmacokinetic Parameter of ABZ-SO after an oral dose of 40 mg/kg of different ABZ Formulation		
Kinetic Parameter	ABZ-Test	ABZ-Standard
$C_{max}$ ( $\mu\text{g/ml}$ )	5.18	2.71
$T_{max}$ (Hours)	2	4
$(AUC)^{(0-\infty)}$ ( $\mu\text{g.h/ml}$ )	48.29	15.7
$(AUMC)^{(0-\infty)}$ ( $\mu\text{g.h}^2/\text{ml}$ )	427.6	113.69
$T_{1/2}$ (Hours)	5.5	3.43
$K_e$ ( $\text{Hours}^{-1}$ )	0.126	0.202
$K_a$ ( $\text{Hours}^{-1}$ )	1.09	0.451
MRT(Hours)	8.85	7.24

## CONCLUSION

Albendazole is the drug of choice for the treatment of echinococcosis, hydrated cysts and neurocysticercosis, but it is well known that this drug is poorly and erratically absorbed from gastro-intestinal tract and it has low oral bioavailability, which is related to its low water solubility. From the findings of in-vitro studies, it can be concluded that solubility enhancement of Albendazole was successfully achieved by using HP $\beta$ CD. The ABZ:HP $\beta$ CD complexed suspension developed in the present studies (formulation F5) using R value of 0.97 possess a better in-vitro release and other parameters was also found to be stable, and in-vivo studies are conducted. In-vivo study results showed that the ABZ: HP $\beta$ CD complexed suspension have greater bioavailability than marketed formulation, which was statistically significant with P value less than 0.05. Pharmacokinetic parameters such as AUC, AUMC  $T_{max}$ ,  $K_a$ ,  $K_e$ ,  $C_{max}$ , MRT and  $t_{1/2}$  were evaluated for the ABZ:HP $\beta$ CD complexed suspension and conventional formulation (marketed formulation). On the basis of the findings, it is concluded that the 50% increase in bioavailability attained by the formulation can definitely reduce the existing higher dose of the drug thus reducing the GI disturbance.

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