"Preparation, Characterization and Evaluation of Albendazole Loaded Solid Lipid Nanoparticles from Beeswax for Screening of its Anti-Helminthic and Anti-Cancer Properties"

A dissertation

Submitted in partial fulfilment of the requirement for the degree of

Masters of Science

In

Chemistry

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CERTIFICATE

This is to certify that the thesis entitled "Preparation, Characterization and Evaluation of Albendazole Loaded Solid Lipid Nanoparticles from Beeswax for Screening of its Anti-Helminthic and Anti-Cancer Properties" which is submitted by **Sunidhi Sharma**, in the partial fulfilment for the requirement for the award of degree of **Master of Science** in Chemistry to the **School of Chemistry and Biochemistry**, **Thapar Institute of Engineering and Technology, Patiala** is an authentic record of bonafide research work, carried out by her under my supervision.

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this thesis entitled "Preparation, Characterization and Evaluation of Albendazole Loaded Solid Lipid Nanoparticles from Beeswax for Screening of its Anti-Helminthic and Anti-Cancer Properties" in the partial fulfilment for the requirement for the award of degree of **Master of Science** in Chemistry to the **School of Chemistry and Biochemistry**, **Thapar Institute of Engineering and Technology, Patiala**, is my own work carried out during the period of November 2018 to July 2019 under the supervision of **Dr. Diptiman Choudhury** (Assistant Professor), School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala. No part of the matter embodied in this thesis has been submitted to any other university or institute for the award of any degree.

Date: July 15th, 2019

Place: TIET, Patiala

Sunidhi Sharma

This is to certify that the above statement made by the candidate is correct and true to best of my knowledge.

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Date: 15-07-2019

Sunidhi Sharma

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ABBREVIATIONS

Abz	Albendazole
BBB	Blood Brain Barrier
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulphoxide
DLS	Dynamic Light Scattering
FBS	Fetal bovine serum
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
PBS	Phosophate buffer saline
RPMI	Roswell Park Memorial Institute Medium
Rt	Room temperature
SLN	Solid Lipid Nanoparticle
SLN-Abz	Albendazole loaded Solid Lipid Nanoparticles

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ABSTRACT

SLNs are the non-toxic, biodegradable, nanospheres with a solid lipid matrix that are being employed for targeted drug delivery applications. These particles have shown sustained drug release pattern and are known to improve the aqueous solubility of hydrophobic drugs. In the present study, Albendazole drug, known for its poor water solubility, was loaded in SLN particles that were produced using the methanol extract derived from the beeswax and checked for its effects on the helminthic worm *Haemonchous contortus* and lung cancer cell line A549. In-vitro studies on A549 cell line was done to examine the cytotoxicity of these particles against the cancer cell line and it was found to be more toxic when Albendazole drug was loaded in the particles than in comparison to the SLN particles (without drug). DLS was used to determine the size of these particles and FT-IR and RAMAN spectrum were studied for confirmation of formation of SLN-Abz particles.

1. Introduction

Beeswax, also known as Cera Alba, is a non-toxic natural wax with anti-allergenic properties, prepared by the worker bees of *Apis* genus. It constitutes of four unsaturated acids (palmitoleic, oleic, linoleic, and linolenic) and two saturated fatty acids (palmitic and tetracosanoic)[1] among other hydrocarbons and wax esters. Beeswax is found to have a melting range of 62 to 64℃ and a flashpoint of 204.4℃.

Beeswax is known to have applications in candle making; as a stiffening agent in cosmetics, foods, and beverages; as thickeners and emulsifiers in manufacturing and as a fragrance in soaps and perfumes. Beeswax is also known for its anti-swelling effects and is used for lowering cholesterol; against ringworm, jock itch and fungal skin infections; reducing diaper rashes and relieving pain.

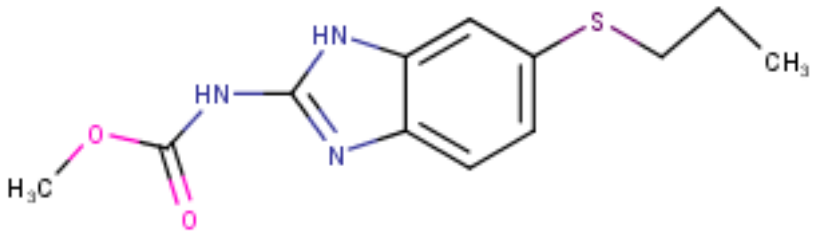
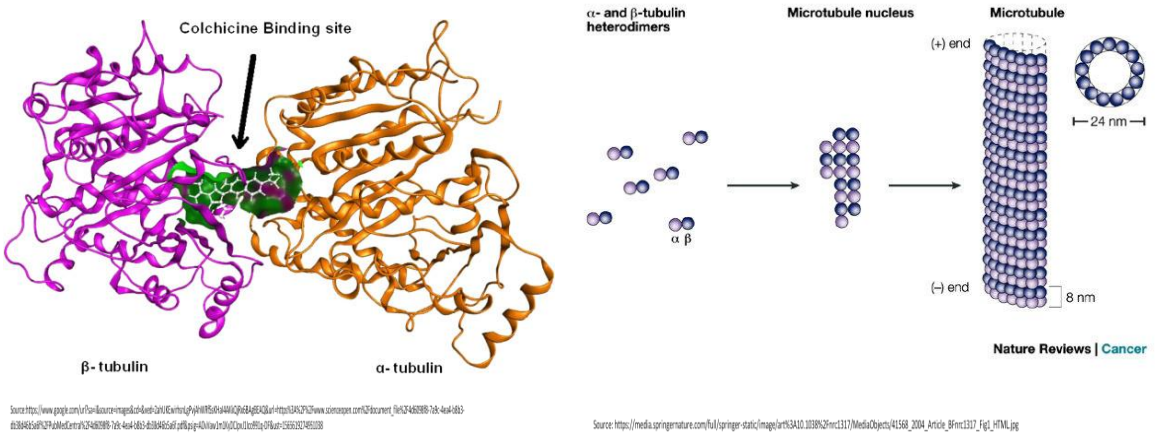


Figure 1: Albendazole molecule

Albendazole is a benzimidazole broad-spectrum, orally-administered, antihelminthic drug, used predominantly in the treatment of Echinococcosis, caused by a parasitic worm *Echinococcus* which is known to cause cysts in lung and liver. Methyl[5-(propylthio)-1-H-benzimidazol2-yl] carbamate, commonly known as albendazole, has the chemical formula of $C_{12}H_{15}N_3O_2S$ (**Figure 1**) and molecular weight of 265.35g/mol. It is a solid, colorless to yellowish crystal having a melting range of 208-210℃. It is practically water-insoluble, showing solubility of 0.016 mg/ml and therefore is administered with a fatty meal[2].

Albendazole's mechanism of action is that it inhibits the polymerization of β-tubulin into microtubules by attaching to the colchicines-sensitive site of β-tubulin (**Figure 2**). This causes a reduction in the amount microtubules in the intestinal cells which in turn reduces their absorptive function. Due to this, the uptake of glucose by the parasites is affected and the glycogen storage is also depleted, hence resulting in insufficient energy for the ATP production. This causes the death of the parasite[3][4].

But nowadays the parasitic resistance to this drug in some helminths has been found out and the cause is thought to be caused by the changes in the β-tubulin protein which causes reduced binding of the β-tubulin with the drug.



(a) **(b)**

Figure 2: (a) Colchicine binding site on β-tubulin where albendazole comes and binds and inhibits the polymerization into microtubule. **(b)** Polymerization of α- and β- tubulin heterodimers to form microtubule.

So, to increase the effectiveness of the drug, different ways have been employed from times to times and one among that way is loading the drug in a solid lipid nanoparticle. Solid lipid nanoparticles, SLNs, are the submicron particles that are used as drug delivery carriers for the hydrophobic molecules that have poor absorption in the GIT.

Albendazole drug was loaded into a matrix of fatty acids that were obtained from methanol extract of beeswax by extraction using the soxhlet apparatus. Poloxamer 407, because of its amphiphilic nature, was used as cross-linker to increase the water solubility. The hydrophobic part of Poloxamer 407 surrounds the fatty acids, which have entrapped albendazole molecules, leaving the hydrophilic part outside which makes the particle more water-soluble.

These particles were characterized using (i) DLS technique, which told us about the particle size; (ii) FT-IR and RAMAN, which told us about the functional groups present and how the albendazole have been entrapped in nanoparticles; (iii) FE-SEM, which gave us the magnified images of the formed particles; (iv) Release Kinetics, drug release was observed from the particles at the physiological pH of 7.4 and physiological temperature of 37℃.

These particles were tested for their anti-helminthic and anticancer properties, both of which have already been reported for the albendazole drug. For the testing of anti-helminthic properties, *Haemonchous contortus* was used. It is a Nematode parasite, commonly known by the name of barber's pole worm, belonging to the richostrongyloidea

family (**Figure 3 (a)**). It has a cylindrical body with the reddish appearance and is found in tropical and subtropical areas [5]. It has a high propensity for anti-helminthic drug resistance so it's better to develop diagnostic methods that provide better effectiveness.

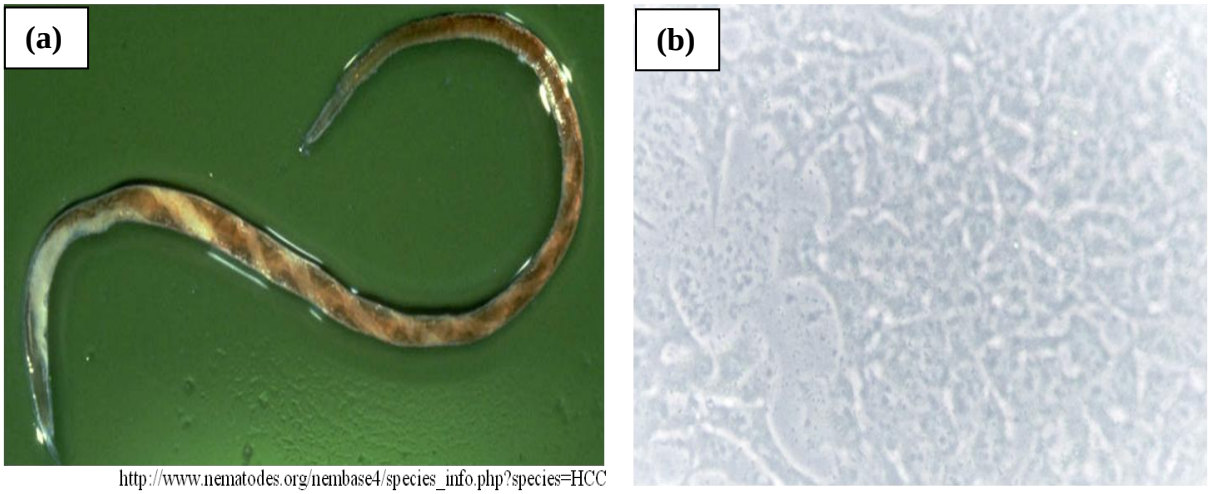


Figure 3: (a) *Haemonchous contortus* worm **(b)** A549 cell line

Albendazole loaded SLNs have also been checked for the anti-cancer activity by working on lung cancer cell line. The A549 cell line has been originated from explants culture of lung carcinomatous tissue that was collected from a 58-year-old Caucasian male.[6] These cells were grown in 60mm cell culture plates at 37°C, 5% CO_2 and humidified condition in an incubator, in DMEM medium that is supplemented with FBS (**Figure 3 (b)**). All the procedures were carried out in laminar flow cabinet under sterile conditions and all the materials were discarded according to the suitable method for particular biological waste (i.e. by autoclaving)[7].

MTT assay was performed on these cells and it was observed that more cells were dead when treated with SLN-Abz particles than in control and in without drug-loaded SLN particles.

2. Literature Review

Nanoparticles are the microscopic particles covering a range up to 100 nm. Nanoparticle research is the most studied branch with its uses in various fields like optical, manufacturing and materials, electronic field and biomedical. Nanotechnology is being currently used in diagnostic techniques, antibacterial medications, wound therapy, cell repair, and drug distribution systems[8]. Nanoparticles have been used excessively to create drug delivery systems as these can be easily manipulated and can be used to make targeted, control and sustained drug release. Various modes of administration can be used like oral, nasal, injection, etc. In these nanocarriers, a bioactive material is entrapped in a carrier molecule[9].

Different types of nanocarrier systems are being used like carbohydrate-based, protein-based or lipid-based. Of these, lipid-based drug delivery is recent and is known to have an advantage of better encapsulation efficiency, better solubility, and low toxicity[10]. Four types of lipid-based nanocarriers are being used, namely solid lipid nanoparticle (SLN), nanostructure lipid carrier (NLC), nanoliposome and nanoemulsion.

Different formulation techniques for the formation of solid and semi-solid lipid-based excipients that have been in use are Capsule filling, Spray Cooling, Adsorption on the solid carrier, Spray Drying, , melt extrusion, supercritical fluid based methods, melt granulation, and solid lipid nanoparticles. Of these SLNs and NLCs are the submicron particles having a size range of 50-1000nm. These have been utilized mainly in controlled drug-release applications in oral route, intravenous route or tropical route. Surfactants like polysorbate 80 or poloxamers have been used in SLNs. In-vivo evaluations performed on the rats have shown that the oral bioavailability has grown up to 5-fold as both surfactants have shown to moderately inhibit the P-gp[11][12][13].

Amphotericin B loaded lipid drug particles are sold under the name of Abelcet (Sigma-Tau) and are of size ranging 2-5µm. Loaded SLNs have also been used for their applications as UV absorbers. Cetyl palmitate nanodispersions and 3,4,5-trimethoxybenzoylchitin-loaded SLNs have been found to show a threefold increase in UV protection. And an enhanced effect was seen on the addition of tocopherol. Etomidate and tetracain have shown spontaneous release and clobetasole propionate and prednisolone have initially shown an initial burst release and then prolonged release from the solid lipid matrix[14][15].

The mean particle size for Compritol based SLN formed using high-pressure hot homogenization technique was found to be about 185nm and for Percirol was found to be 210nm[16]. Freitas and Müller had found that the gelation was increased by high temperature, shear stress, and light. This causes the crystallization of lipid particles, which in turn adopts the β-modification and this causes the increase in particle surface[11].

Prednisolone has been found to have a loading rate of 50-56% when prepared using hot homogenization technique and the lipophilic glucocorticoid diester prednicarbate was found to be completely associated when loaded to SLNs. Clobetasol propionate completely associates to SLNs when prepared using the solvent diffusion method. The chemical stability of retinol, tocopherol, and coenzyme Q10 was found to improve when incorporated in SLNs as compared to an aqueous dispersion[17]. Silver nanoparticles and its interaction with insulin has shown great wound healing property[18].

Biocompatible lipids like stearic acid, Compritol®888 ATO, cetyl palmitate, Precirol® ATO5, cetyl alcohol, trimyristin (Dynasan®114), glyceryl monostearate, tristearin (Dynasan®118), Imwitor®900, etc have been used for the formation of SLNs and are physiologically tolerant when used in-vivo[19][23]. These can be fabricated using non-toxic organic solvents. Hydroxypropyl-β-cyclodextrin and compritol ATO888 SLNs were published for targeted diclofenac sodium delivery into the colon. In-vitro and ex-vivo tests illustrated that targeted DDS can be regarded as a safe and biocompatible colon delivery system[20][21][23]. Zara et al and Yang et al. have reported targeted delivery of drugs into the brain using SLNs. These have been used for enhancing the efficacy of drug targeting to the brain as they can restrain the efflux functions of the brain capillary endothelial cells and therefore help in crossing the BBB carrying the molecules into the brain[22].

Recently, more focus has been shifted on using SLNs for therapeutic purposes like in cancer chemotherapy and brain drug delivery.

3. Materials and Methods

3.1 Reagents and Chemicals Used:

The chemicals and solvents used were purchased from commercial suppliers and were used without any other further purification. Ethanol; Chloroform; Albendazole; Hydrochloric acid; Poloxamer 407; PBS pH 7.4; Beeswax, RPMI Media; DMEM Media; MTT; DMSO; Saline Solution.

3.2 Synthesis of SLN and SLN-ABZ:

Albendazole (ABZ) loaded solid lipid nanoparticles were fabricated using the double emulsion technique (Zhang et al 2006). For this, the ethanol extract was extracted from beeswax in a soxhlet apparatus. These extracts were then dried in hot air oven till all the ethanol gets evaporated. Dried sample (50mg) was weighed and dissolved in chloroform. ABZ dissolved in 0.1M HCl solution (0.5ml) was mixed in the lipid emulsion solution and homogenized for 1 minute on Spinix. This primary emulsion formed was mixed with 2% Poloxamer 407 solution (20ml) and then homogenized for another 1 minute. The solvent was evaporated and concentrated to 10ml using Rotaevaporator and at last, were left for air drying till all the water evaporates. (**Figure 4)**

And for the preparation of SLN particles (without drug), dried extract (50mg) was weighed and dissolved in chloroform and homogenized for 1 minute on Spinix. This was mixed with 2% Poloxamer 407 solution (20ml) and then homogenized for another 1 minute. The solvent was evaporated and concentrated to 10ml using Rotaevaporator and at last, were left for air drying till all the water evaporates.

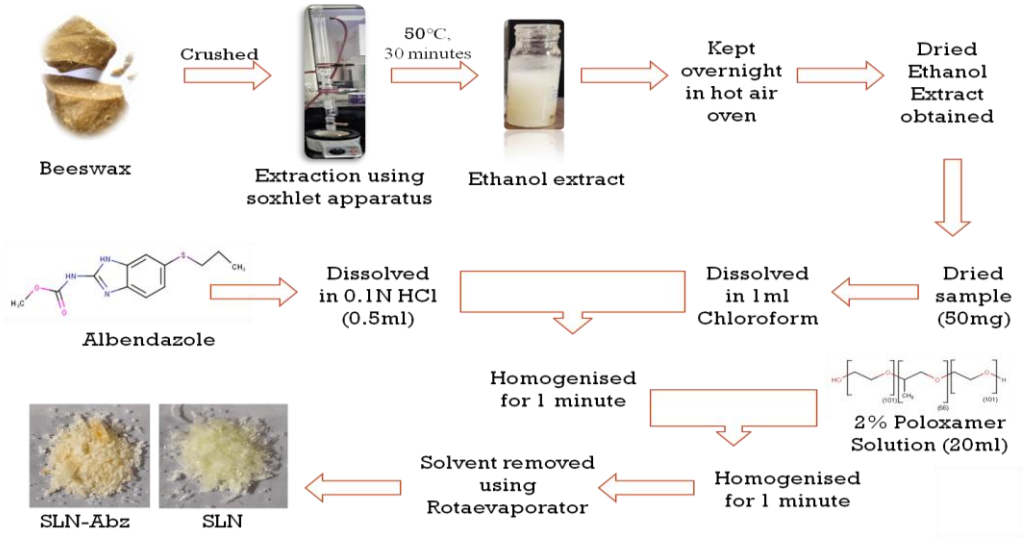


Figure 4: Synthesis of SLN (without drug) and SLN-Abz particles

4. Characterization

4.1 Analysis of Particle Size:

The particle size of SLN-Abz was observed using a DLS (Nanobook 90 plus, Brookhaven, USA). The samples were diluted in deionized water and then poured in a glass cuvette and checked for the particle size.

4.2 Morphological Characterization:

The morphology of the SLN-Abz was examined using Field Emission Scanning Electron Microscopy (FE-SEM). Approximately 20µL of the aqueous solution of dispersed nanoparticles was drop casted on the silicon wafer and was dried in a hot air oven at 37℃. Dried samples were gold coated for 20 minutes and then the images taken in FE-SEM (SEM, Nova Nano SEM-450, FEI).

4.3 RAMAN:

The RAMAN spectra for SLN-Abz, SLN and ABZ were measured using Raman Horiba LabRam HR evolution at rt. Solid-state laser of 785nm was used in a range of 100-2000cm^{-1}. The samples were air-dried at 37℃ overnight and then placed on a glass slide for the measurement of the spectra. The obtained spectrum was converted to a .txt file for further processing and analysis.

4.4 FT-IR:

The FT-IR spectrum for SLN-Abz, SLN and Abz were measured using Agilent Technologies Cary 800 Series FTIR Spectrophotometer at rt. Air-dried samples were placed on the sample holder and scanned in the range of 200-4000 cm^{-1}. The spectrum was observed and the file was converted to .txt for further processing and analysis.

4.5 Release kinetics:

To study the release kinetics of SLN-Abz particles, around 50mg of the particles were taken in PBS buffer (pH 7.4) and the system was maintained at 37℃ and a spin of 130-150 rpm was applied. Samples of around 1mL were collected at regular intervals of 30 minutes and checked for its concentration using UV-visible spectroscopy at 295nm. The absorption values were later plotted against time (in minutes) and noted for the trend of drug release.

5. Applications

5.1 Effect of SLN-Abz on *Haemonchous contorts* (parasitic worm):

Goat abomasum samples were collected from the slaughterhouse and *Haemonchous contortus* (parasitic worms) were picked out from the same. Worms were washed 2-3 times with 1X PBS buffer (pH 7.4). 5 Male and 5 Female worms were identified and were added in a petri dish. The stock solution of particles having concentration 1mg/mL was made. RPMI media (2ml) was added along with the compound of concentration 2µM, 5 µM, 10 µM, 20 µM, 50 µM, 100 µM and 200 µM in each petri plate. The albendazole drug solution was added as a control in 50 µM, 200 µM, 1mM, and 2mM concentrations. Observations were done for paralysis and death time by placing the worm on a glass slide under the compound microscope. If the worm showed no movement then a drop of warm saline solution was touched with the worm. If the worm moved, that means that the worm is paralyzed and if the worm doesn't show any movement that means the worm has died.

5.2 Effect of SLN-Abz on A549 (cancer cell line):

MTT assay was employed to determine the effect of SLN-Abz on A549 lung cancer cell line. The cells were cultured in DMEM media in 96-well plate and when confluent, they were incubated overnight with the SLN (without drug) and SLN-Abz particles. The effect of different concentrations like 5µM, 10 µM, 20 µM, and 100 µM was checked. The incubation of cells was done at 37℃ at 5% CO2 concentration and a humid environment. After 24 hours, washing with PBS (pH 7.4) was done and MTT (10 µL) was added to DMEM media (100 µL) and was incubated for 3 hours. Then around 100µL of DMSO was added after removing media and placed in the dark for about 15 minutes and then checked for the absorbance values in an ELISA plate reader at 570nm.

6. Results and Discussions

6.1 Analysis of Particle Size:

The particle size of without drug solid lipid nanoparticles (SLN) and albendazole loaded solid lipid nanoparticles (SLN-Abz) were estimated using DLS (**Figure 5**) and were observed to be in the range of 165 ± 97 nm and 257 ± 94 nm respectively.

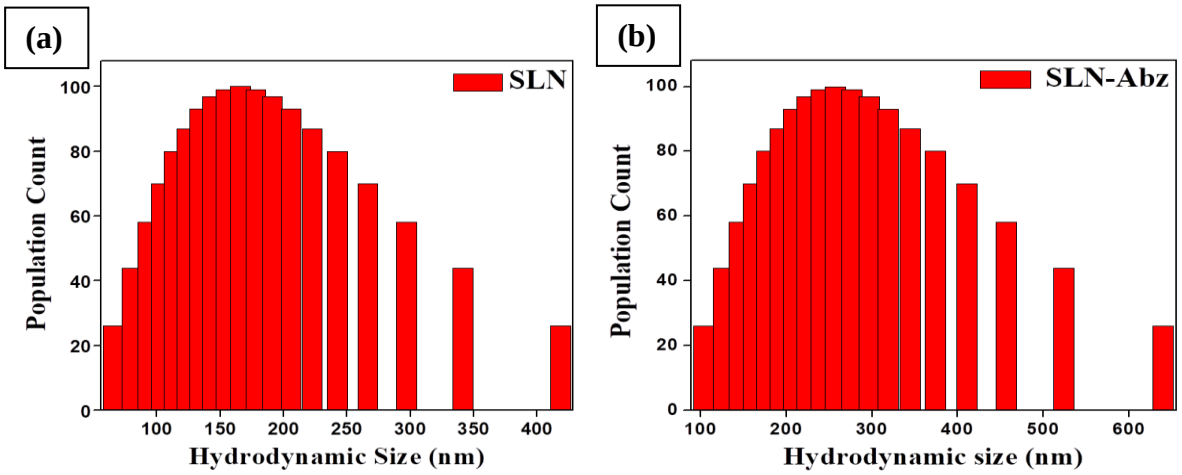


Figure 5: DLS data of (a) Without drug SLN particles and (b) SLN-Abz.

6.2 Morphological Characterization:

The Field Emission Scanning Electron Microscopy (FE-SEM) images of SLN-Abz (**Figure 6**) showed cuboidal shaped particles, where maximum particles were of size ranging from 100nm to 200nm. These particles were found to be porous with pore size of about 3nm. Particle depth was found showing that these particles are 3D in nature.

6.3 RAMAN:

The RAMAN spectra of SLN, SLN-Abz and Abz particles were observed for a range of 600 to 2000 cm^{-1} (**Figure 7**) and it was found that the spectra for the SLN and SLN-Abz molecules was almost the same and the spectra of SLN-Abz had little to no similarities to the spectra of Abz molecule. This showed that the Albendazole molecule is completely entrapped in the nanoparticles. Albendazole molecule showed a characteristic peak for the C-N symmetric stretching at 1540 cm^{-1} and C-S aliphatic stretching peak at 680-760 cm^{-1}. Another sharp characteristic peak of albendazole molecule was found to be at 1266 cm^{-1} for the Amide III. SLN and SLN-Abz molecules showed normal stretching and bending frequencies at 1480 cm^{-1}, 1140-1280 cm^{-1}, 1060 cm^{-1} and 840-920 cm^{-1} (**Table 1**)[24][25][26].

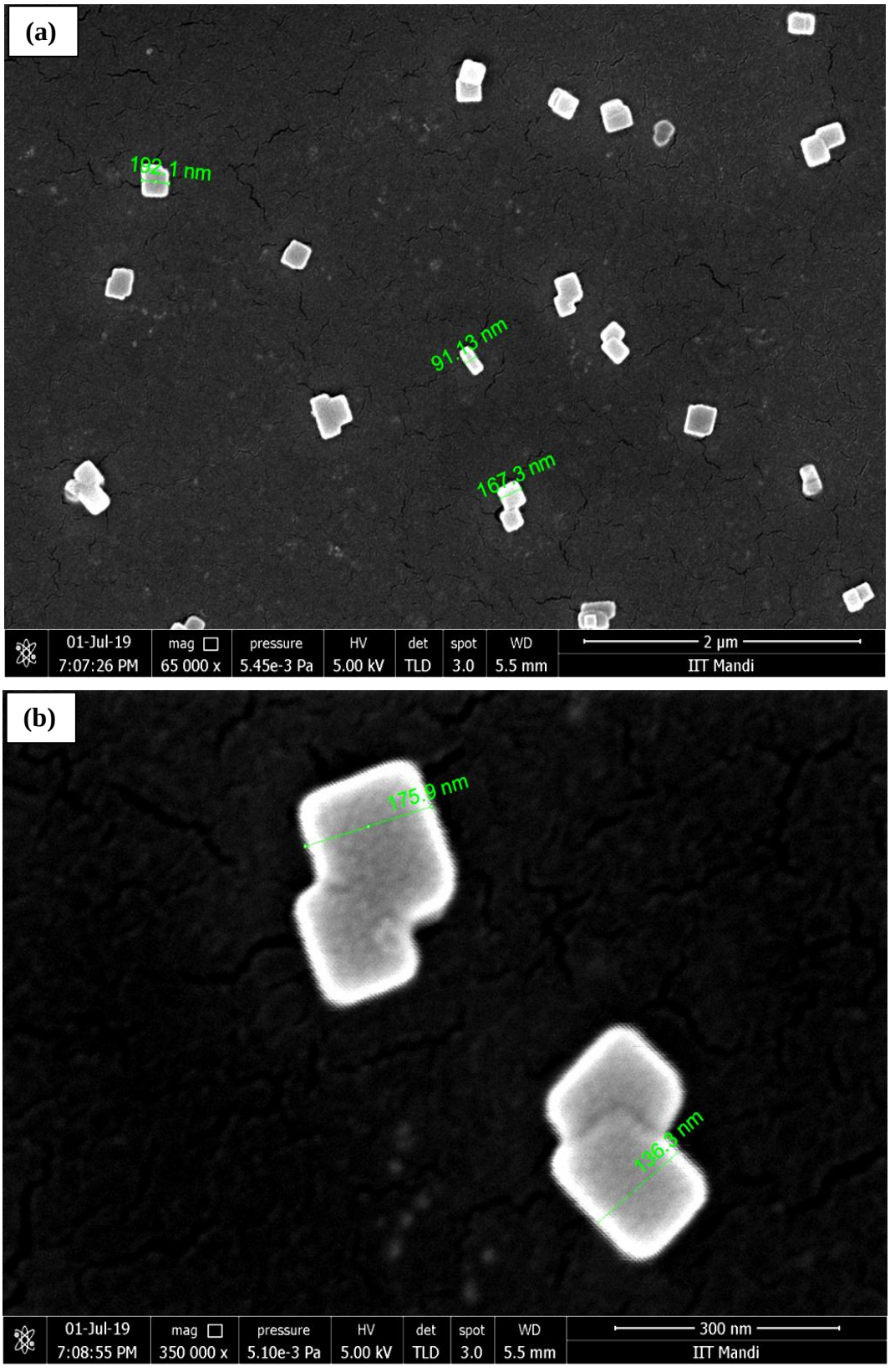


Figure 6: FE-SEM images of SLN-Abz particles **(a)** Scale of 2µm **(b)** Scale of 300nm (continued...)

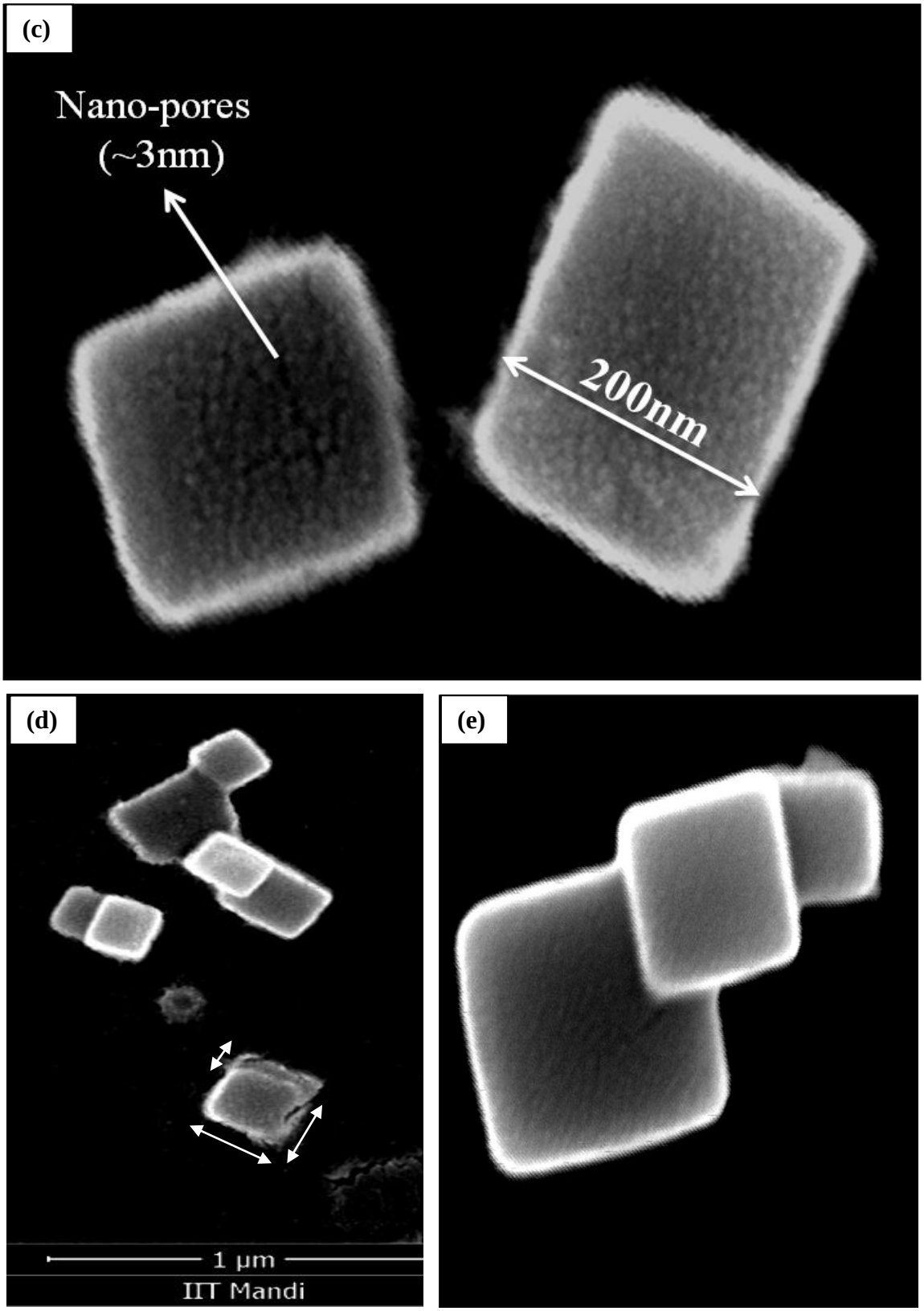


Figure 6: FE-SEM images of SLN-Abz particles **(c)** Nano-pores in SLN-Abz particles **(d)** Particles showing 3D nature **(e)** SLN-Abz particles.

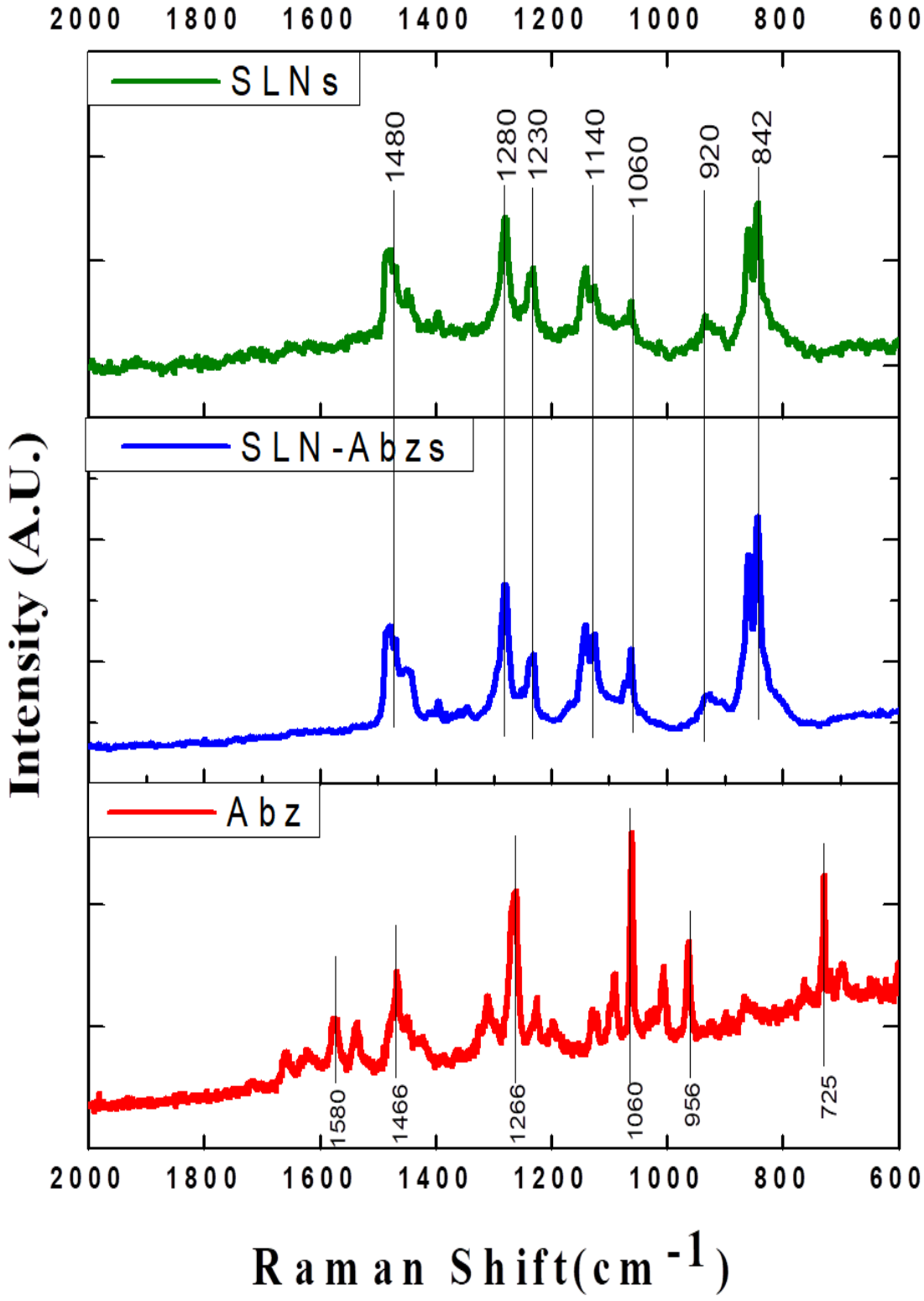


Figure 7: RAMAN spectra for SLN (without drug), SLN-Abz and Abz.

	SLN	SLN-Abz	Abz
υ(C-C) Aromatic ring chain vibration	-	-	1580
C-N Symm Stretching	-	-	1540
δ(CH2) δ(CH3) asym	1480	1480	1466
Aromatic ring	-	-	1301
Amide III	-	-	1266
υ(CC) aliphatic chain vibrations	1280 1230 1140	1280 1230 1140	1215 1190 1124
υ(C-O-C) asym	1060	1060	1080 1060 1003
υ(C-O-C)	920	920 842	965
υ(C-S) aliphatic	-	-	761 725 681

Table 1: RAMAN data for SLN particles without drug, Albendazole loaded SLN and Albendazole (cm^{-1}). (υ- Stretching; δ- Bending)

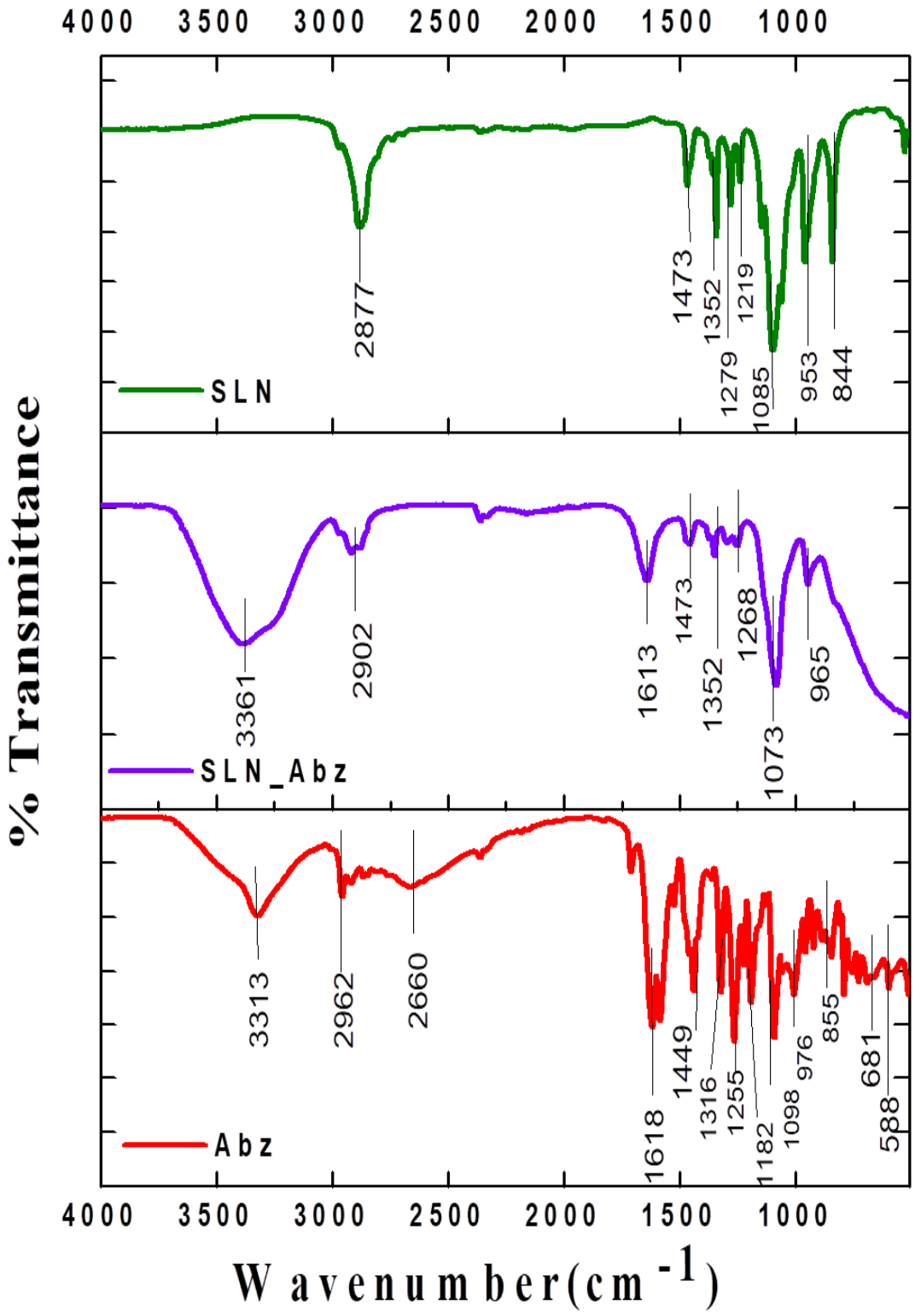


Figure 8: FT-IR spectra for SLN (without drug), SLN-Abz and Abz.

Remarks	SLN	SLN-Abz	Abz
N-H Stretching	-	3361	3313
C-H Stretching	- 2877	2902 -	2962 2660
C=N Stretching	-	1613	1618
C-H Scissoring	1473	1473	-
C-H Bending	- 1352	- 1352	1449 -
N-H stretching	-	-	1316
C-O Stretching	1279 1219	1268 -	- 1255
C-O Asymmetric Stretching	1085	1073	1182
Amine C-N stretch	-	-	1098
Fingerprint region	953 844 - 529	965 - - -	976 855 681 588

Table 2: FT-IR data for SLN particles without drug, Albendazole loaded SLN and Albendazole.

6.4 FT-IR:

The FT-IR spectra (**Figure 8**) clearly showed that the characteristic peaks for Albendazole and SLN particles were present in SLN-Abz molecules, proving that the albendazole has loaded to solid lipid nanoparticles. The most characteristic peak was of N-H stretching in albendazole molecule, which was a broad peak, was found at 3310-3360 cm^{-1} in SLN-Abz and Abz particles. Another peak for C=N stretching due to the benzimidazole molecule was found at 1613-1618 cm^{-1} in both the particles. N-H stretching peak and Amine C-N stretching were found at 1316 cm^{-1} and 1098 cm^{-1} respectively in Abz molecule and all the peaks found in SLN particles were found in SLN-Abz molecule (**Table 2**)[25][27][28].

6.5 Release kinetics and Drug encapsulation:

The release kinetics study of SLN-Abz particles was done at the physiological pH of 7.4 and physiological temperature of 37℃ and showed a sustained release of the drug for 3 hours (**Figure 9**). The absorbance values was noted for 0, 30, 60, 90, 120, 150, 180 minutes and were found out to be 0.072, 0.108, 0.176, 0.266, 0.328, 0.481 and 0.482 respectively. These values were measured at 295nm wavelength. The drug release from the solid lipid nanoparticles was found to increase steadily and the entire drug was released by the end of 3 hours. 0.388mM concentration of drug was found to be entrapped in SLN-Abz particles and it can be concluded that for improved drug release kinetics, more of the drug loading can be done and better or increased percentage of the cross-linking agent can be utilized for better drug encapsulation.

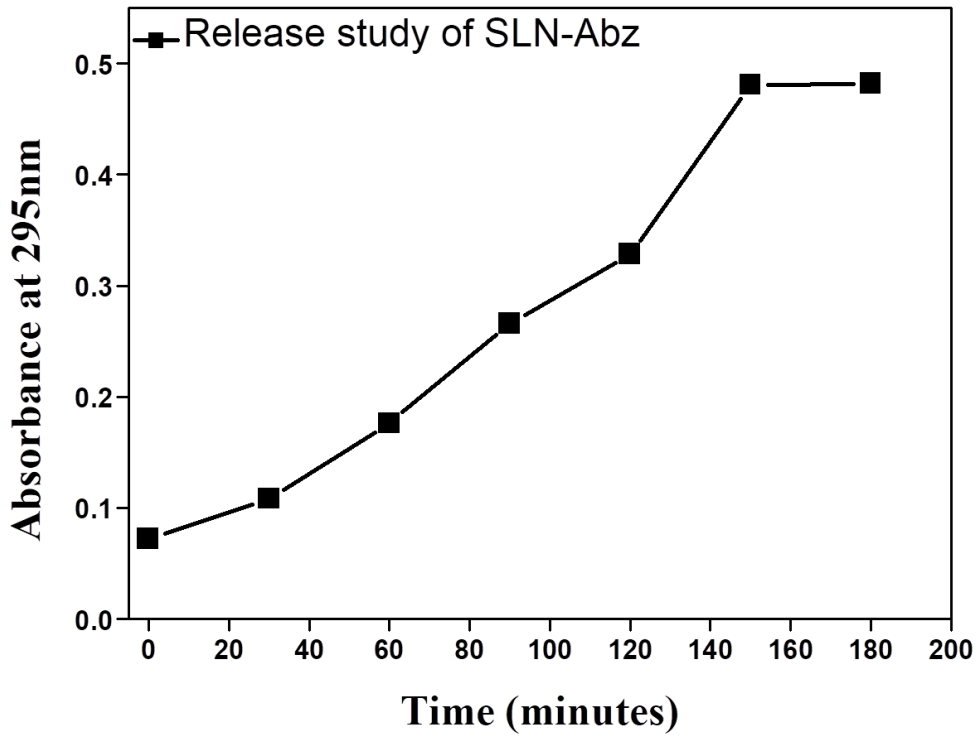


Figure 9: Plot for the release kinetics of SLN-Abz particles for a period of 3 hours.

6.6 Effect of SLN-Abz on *Haemonchous contorts* (parasitic worm):

For the anti-helminthic testing of SLN-Abz particles, *Haemonchous contortus* worms were employed. 5 males and 5 females each were utilized for testing. As a control, Saline, PBS (pH 7.4) and RPMI media were used and no deaths were observed for 24 hours. In case of albendazole (Abz) alone, worms had started showing paralysis at 6 hours and deaths were observed after 12 hours for the 1mM and 2mM concentrations and no death were observed for the lower concentrations till 24 hours. While for the SLN-Abz, even the lower concentrations like 5µM showed paralysis at 6 hours and death at 12 hours. After 24 hours, almost all the worms were found dead, showing better outcomes than in the case of albendazole alone (**Table 3**).

		0 hrs	3hrs	6hrs	12hrs	24hrs
Saline		-	-	-	-	1M (P)
PBS		-	-	-	-	-
RPMI Media		-	-	-	-	2M (P)
SLN-Abz	2µM	-	-	-	2M(P)	3M (P)
	5µM	-	-	2M(P)	5M(P) 2F(P)	3M (D) + 2M (P) 1F(D) + 4F(P)
	10µM	-	-	1M(P)	5M(P) 3F(P)	5M(D) 2F(D) + 3F(P)
	20µM	-	-	2M(P)	3M(P) 2F(P)	5M(D) 5F(D)
	50µM	-	-	3M(P)	5M(P) 1F(P)	5M(D) 5F(D)
	100µM	-	-	3M(P)	5M(P) 2F(P)	5M(D) 5F(D)
	200µM	-	2M (P)	5M(P) 2F(P)	2M(D) + 3M(P) 5F(P)	5M(D) 5F(D)
Abz	50µM	-	-	-	1M(P)	3M (P) 2F (P)
	200µM	-	-	-	-	3M(P)
	1mM	-	-	2M(P) 1F(P)	5M(D) 2F(P)	5M(D) 5F(D)
	2mM	-	-	4M(P) 2F(P)	5M(D) 2F(D) + 3(F)	5M(D) 5F(D)

Table 3: Paralysis (P) and death (D) time for male (M) and female (F) worms when treated with Saline, PBS buffer, RPMI media, SLN-Abz nanopaticles and Albendazole.

6.7 Effect of SLN-Abz on A549 (cancer cell line):

Cell viability of A549 cell line was evaluated for drug-loaded (SLN-Abz) and without drug loaded (SLN) nanoparticles using MTT assay. 1mg/ml stock solution was prepared and different concentrations of 5µM, 10 µM, 20 µM, and 100 µM were used for the 24 hours analysis and cell toxicity was observed by the SLN-Abz particles and no noticeable change was observed for the SLN particles (**Figure 10**). This result shows that cell death was due to the loaded drug Albendazole and not due to the SLN particles.

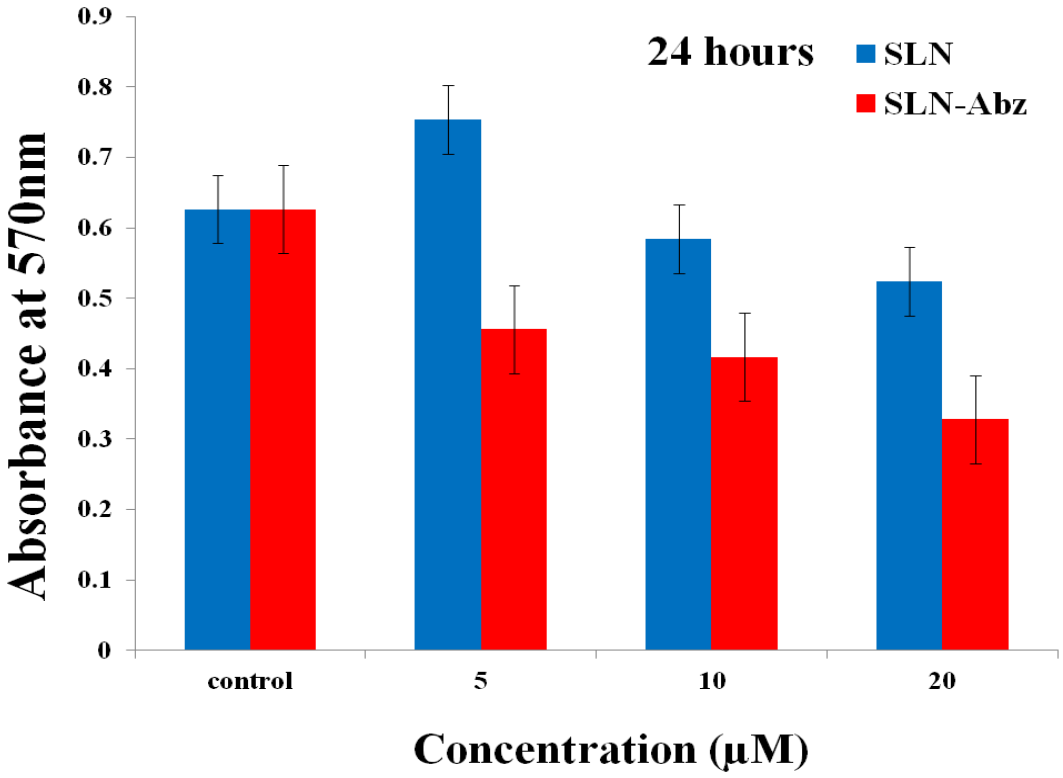


Figure 10: Cell viability of A549 lung cancer cell line in the presence of SLN and. SLN-Abz particles.

7 Conclusion

Using beeswax as starting material, Albendazole loaded solid lipid nanoparticles were made for oral drug delivery applications to enhance the solubility problems of albendazole molecule. Fatty acids were extracted using ethanol as a solvent and used for SLN preparation by the double emulsion technique. Yellowish square-shaped particles were obtained which had shown enhanced drug release properties and effects against Haemonchous contortus and A549 cancer cell line. Even lower concentrations of 10µM SLN-Abz had shown better results than 200 µM concentration of albendazole drug. This showed a better effect of the drug when it was loaded on SLN. For the cytotoxicity assay, toxicity was shown by SLN-Abz molecules and not by SLN. This shows that the effect was solely due to the albendazole drug and not due to the SLN particles. So these particles can be used as suitable carrier systems for efficient drug delivery through the oral route. The formation of drug-loaded particles was confirmed using FT-IR and RAMAN techniques and the particle size was confirmed using DLS. In future, different hydrophobic drugs can be loaded and studied for their enhanced solubility and better drug delivery applications. SLN-Abz can further be tested on normal as well as different cancer cell lines for analyzing their effects.

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