doi.org/10.36721/PJPS.2024.37.1.REG.043-052.1

Binary and ternary complex formation and characterization of artemisinin with sulfobutyl ether β-cyclodextrin and oleic acid

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Abstract: Drug-resistant malaria is a global risk to the modern world. Artremisinin (ART) is one of the drugs of choice against drug-resistant (malaria) which is practically insoluble in water. The objective of our study was to improve the solubility of artemisinin (ART) via development of binary complexes of ART with sulfobutylether β-cyclodextrins (SBE7 β-CD), sulfobutylether β-cyclodextrins (SBE7 β-CD) and oleic acid (ternary complexes). These are prepared in various drugs to excipients ratios by physical mixing (PM) and solvent evaporation (SE) methods. Characterizations were achieved by powder X-ray diffraction (PXRD), scanning electron microscopy (SEM) and attenuated total reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. The aqueous-solubility in binary complexes was 12-folds enhanced than ternary complexes. Dissolution of binary and ternary complexes of artemisinin in simulated gastric fluid (pH 1.6) was found highest and 35 times higher for ternary SECx. The crystallinity of artemisinin was decreased in physical mixtures (PMs) while SECx exhibited displaced angles. The attenuated-intensity of SECx showed least peak numbers with more displaced-angles. SEM images of PMs and SECx showed reduced particle size in binary and ternary systems as compared to pure drug-particles. ATR-FTIR spectra of binary and ternary complexes revealed bonding interactions among artemisinin, SBE7 β-CD and oleic acid.

Keywords: Artemisinin, sulfobutylether-βcyclodextrins, sulfobutylether-β-cyclodextrins-oleic acid, crystallinity, transform infrared – FTIR-spectra.

INTRODUCTION

Malaria is triggered by protozoal parasite and transmitted in human being through five different plasmodium parasites such as: *P. falciparum, P. malariae, P. Knowlesi, P. ovale* and *P. vivax.* The most common and lethal infection of malaria is usually associated to the *Plasmodium falciparum* (Mendis *et al.,* 2001; Mueller *et al.,* 2007). Malarial symptoms seemed like vomiting, shivering, fever, arthralgia (joint pain), anemia (hemolysis), convulsions and retinal damage (Beare *et al.,* 2006). Repeated prompt coldness followed by sweating and fever was perceived 4-6 hrs in typical situations. The most serious neural complication in cerebral malaria was seen in *P. falciparum* infection. Over the last 02 decades, the mortality rate is high due to inadequate care of persistent neurophysiological complaints after recovery of malaria (Idro *et al.,* 2005). Malaria with *P. falciparum* in non-immure person might be fatal; the prompt diagnosis and treatment is crucial in such cases. Parenteral therapy should be recommended for rapid recovery, after that patients switched to oral therapy pertaining to their conditions (Memish *et al.,* 2003). Drugs most commonly used as antimalarial agents; Artesunate/Amodiaquine, Artemether/lumefantrine, Artesunate/Sulfadoxine, Artesunate/Mefloquine, Quinine, Atovaquone/Proguanil, Chloroquine, Cotrifazid, Mefloquine, Primaquine, Sulfadoxine/Pyrimethamine and Hydroxychloroquine.

Artemisinin is an effective antimalarial drug which was isolated by Chinese scientists from "*Arfemisia annua*" (Herb) in 1972. By crystallization it was simply purified after extraction from plant (Woodrow *et al.,* 2005). It is found in 03 species of Artemisia. The first documentation of the compound of artemisinin was attained by Li Shizen as an antimalarial drug in his compendium (Materia Medica) in 1956. The top active antimalarial drug is the artemisinin products. The worldwide most commonly used derivatives are dihydroartemisinin, artemether, artesunate and arteether. These derivatives exhibited rapid activity, better potency and employed to treat severe and complicated cases of malaria (Tayyab *et al.,* 2013). As concerned the solubility of artemisinin, it is weakly soluble in oils and water, however, the native compounds such as; artemether, dihydroartemisin and arteether are oil soluble. While, Artelinic acid and sodium-artesunate are water soluble. The chloroquine resistant and cerebral malaria is quickly managed with β-arteether through blood schizonticidal potential. As artesunate is appropriately soluble in water, therefore injectable form can be made. Artemether is therapeutically effective in case of multi-drug resistant (*P. falciparum*) (Tayyab *et*

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al., 2013). Cyclodextrin (CD) in drug dosage form was primarily patented in 1953 with significant improvement of stability and aqueous-solubility (Davis and Brewster, 2004). The new 2-hydroxyprpyl derivative was consequent from α- and β-cyclodextrin. The sulfobutyleter obtained from β-cyclodextrin, maltosyl and glucosyl (pronged derivatives) were also derived from β-cyclodextrin (Hashimoto, 1991). Pharmaceutical importance of cyclodextrin including; improvement of bioavailability, solubility and stability. In addition, mask of taste/odor and hemolysis drop is also exhibited in cyclodextrin. Cyclodextrins are truly cyclomalto-oligosaccharides contained 6-8 gluco-pyranose units which characterized as; α-Cycoldextrin, β-Cycoldextrin and also γ-Cycoldextrin. Aqueous solubility and stability of hydrophobic compounds increased by complex formation with different cyclodextrins (Usuda *et al.,* 2000). The sodium salt of sulfobutyl-ether β-cyclodextrin was prepared semi-synthetically in Hungary by "Cyclolab Ltd., H-1097, Budapest" and particularly raised the aqueous-solubility of compounds without presenting any toxic effects (Luke *et al.,* 2010; Messner *et al.,* 2011).

Oleic acid is pale to yellowish color oily liquid with characteristic odor, skin penetrating and emulsifying characteristics. It comprises of octadecenoic acid with some saturated and unsaturated acids in various quantities. The aim of the study was to improve the solubility property of artemisinin by the formation of binary and ternary complexes with oleic acid and sulfobutyl ether 7β-cyclodextrin. That ultimately modified the physicochemical properties (bioavailability and dissolution) of artemisinin.

Artemisinin itself has little solubility but extremely better absorbed from gut wall after oral administration. The dissolution potential of artemisinins' complexes with large amount of sodium dioctyl-sulfo succinate was progressive than least quantity of this composite. However, complexes with magnesium-stearate (1%) had reverse effect on dissolution rate (Tayyab *et al.,* 2013). The most dynamic use of cyclodextrins in pharmaceutical preparations is to develop the solubility, stability and bioavailability of drug moiety. The efficiency of binary and ternary complex formulations (drug/cyclodextrin) boosted by combination with some organic acids or bases. The superior solubility of some drugs such as; prostaglandins, steroids, pro-drugs and anti-cancers were attained through cyclodextrin complexes (Kumar *et al.,* 2013; Nguyen *et al.,* 2021). In addition, formulations of CDs have primarily reduced the denaturation of peptides and proteins (Rasheed, 2008; Conceicao *et al.,* 2018). Stability and stabilizing consequences of CDs linked to the functional group of drug molecule, such as; photostability improved by a complex of CDs with trimeprazine photostability (Lutka and Koziara, 2000).

When we talk about binary and ternary complexes, the number of –OH group on CDs, mark them soluble in water. The recognized order of water solubility of α-CD (13%), β-CD (02%) and γ-CD (26%). The least solubility of β-CD showed the existence of inner –H bonding complex between resulting –OH groups (Davis and Brewster, 2004). The increase of solubility-curve of artemisinin complex with CD and order of stability as per obvious constant were presented as α-CD < γ- CD < β-CD. It was concluded that enhancement of artemisinin's solubility has occurred by the addition of CDs (Usuda *et al.,* 2000; Circioban *et al.,* 2018). The poor bioavailability of Dihydroartemisinin through oral route is due to its least aqueous solubility, which improved by the incorporation of Dihydroartemisinin in DHA-PVPK30 (50 times) and DHA-HPβCD (84 times) (Ansari *et al.,* 2011).

The incredible rise of aqueous solubility of pharmaceuticals has perceived by complexation with sulfoalkylether-β-cyclodextrin. The reported most valuable derivative of sulfoalkyl was Sulfobutyl ether-β-cyclodextrin-7-sodium salt (SBE7-β-CD). Various techniques have employed to develop the carbamazepine and SBE7-β-CD complex and found greater protection against all epileptic episodes comparative to carbamazepine in non-complex formulations (Jain *et al.,* 2011; Shukla *et al.,* 2020; Ueda *et al.,* 1998; Stella and Rajewski, 2020).

MATERIALS AND METHODS

Sulfobutylether 7β-cyclodextrin (China), Artemisinin (China), Methanol (Merck-Germany), Hydrochloric acid (Merck-Germany), Oleic acid (Switzerland), Isobutyl alcohol (MERCK-Germany), De-ionized Water (Waterman Karachi, Pakistan), Potassium bromide FTIR Grade (Fisher chemicals-USA), Silica Gel Beads (China), other all solvents and substances were of analytical grades.

Instruments

UV/VIS PERKIN ELMER Spectrometer (Lambda 25, USA), Lyophilizer, LABCONCO (England), Attenuated Total-Reflectance Fourier-Transform Infrared (ART-FTIR) PERKIN ELMER Spectrometer (USA), Buchi Rotary Evaporator (Switzerland), USP II-DIGITECK Dissolution Apparatus (Pakistan), Calorimeter for Differential Scanning, TA Instruments (USA), pH Meter, JENCO (USA), Vortex Mixer (Model: 250VM, HIWASHIN TECH. CO.), Centrifuge machine, HETTICH (GERMANY) and Rotary Tablet Compression Machine (Model: ZP-19, China).

Methodology

Artemisinin's calibration curve

Stock solution of artemisinin was prepared by dissolving 500 mg of powder in methanol (50 ml) in volumetric flask. Dilutions of various strengths were prepared from

this stock solution, such as; 10, 20, 30, 40, 50, 60, 80 and 100 µg per ml. One ml of each dilution was transferred to separate 08 test tubes and added 0.2% NaOH (5ml) in each test tube and heated on water bath (50˚C) for 30 min. The absorbance at 293nm was determined using UV/visible spectrophotometer.

Preparation of samples

The binary complexes of pure artemisinin and carriers molecules such as; sulfobutyl ether β-cyclodextrins and oleic acid were prepared in ratios like 1:1, 1:2.5, 1:4 and 1:9, while ternary complexes were mad as 1:1:1, 1:1:0.27, 1:4:1, 1:3.6: 3.6, 1:2.5:0.27 and 1:9:1. Physical Mixing and Solvent Evaporation techniques were employed for the preparation of both complexes.

Preparation of Complexes by physically mixing

For binary complex formation, the specified quantities as per ratio (1:1, 1:2.5, 1:4 and 1:9) of artemisinin and sulfobutyl ether β-cyclodextrins were mixed gently for 5 – 7 min in pestle and mortar. Passed these homogenous mixtures through a sieve (180 µm mesh) and stored in amber color bottles in glass desiccator (25℃). The ternary complexes were prepared in similar fashion using quantified ratio (1:1:1, 1:1:0.27, 1:4:1, 1:3.6:3.6, 1:2.5:0.27 and 1:9:1) of artemisinin, sulfobutylether-β-cyclodextrins and oleic acid.

Preparation by Solvent Evaporation

As per binary complexes, the designed amounts of artemisinin were dissolved in methanol (suitable volume) and sulfobutyl ether β-cyclodextrins in Deionized water. Solutions were gently mixed separately in 100 ml beakers with magnetic stirrer till clear homogenous solutions were attained. Both solutions of itemized ratios were mixed in 1000 ml round bottom flask and shacked (150rpm) at room temp for 04 hrs. Then solvent was removed using Buchi rotary-evaporator at less than 38℃. Mixtures were shifted to petri dishes and kept overnight in oven at 37^0C for the further removal of solvent. These mixtures were dried, grinded, passed through a sieve and tagged as SECx (Solvent Evaporation Complex). Finally, these complexes were stored in desiccator keeping in amber color bottle at 25^0C.

In case of ternary complexes, specified pre-weighed quantities of artemisinin, sulfobutyl ether β-cyclodextrins and oleic acid were dissolved in methanol, Deionized water and isobutyl alcohol respectively. The further adopted procedure of assembling ternary complexes was the same as applied in binary method.

Equilibrium Solubility Test

Accurately weighed (0.4 g) each sample and dissolved in 10 ml of deionized water in conical flask (50 ml). Flasks were shaken on vortex mixer for 1-2 min at 1400 rpm and then incubated (37℃) for 07 days in shaking incubator at 150 rpm. After centrifugation (6000 rpm) of solutions for 15 min, supernatant liquid was gradually poured in to test tubes. Using deionized water, the upper clear-layer (01 ml) was diluted to 05 ml. Diluted solutions (01 ml) were poured into test tubes and added 05 ml of 0.2% of NaOH solution and finally heated at 50℃ on water bath for 30 min. After cooling, samples were examined using UV/Visible spectrophotometer at 293 nm. For the authentication of degradation, a control test was performed with pure artemisinin and equilibrium-solubility of artemisinin was determined.

Tablets Preparation

Mixed 250g lactose and 200g starch to acquire homogenous mixture. Starch slurry was prepared by adding 50g starch powder to 100ml of deionized water. Boiled around 250ml water in separate beaker and prepared slurry was poured into the beaker to achieve a paste. This paste was mixed with a mixture of lactose and starch. Mixed thoroughly for 20-30 min, dried in oven at 70℃ for 02hrs and then granules were made by passing through a sieve of 16# mesh. The quality of granules was accomplished to find less than 1% moisture contents and then stored in well closed container.

Tablets were produced with granules, 5% disintegrent (primogel), 0.01% magnesium stearate as lubricant and samples of designated quantities prepared by physical and solvent evaporation mixtures equivalent to 40mg of the artemisinin. The compression was done using tablet rotary press to form 500mg tablets having binary and ternary complexes with various ratios of artemisinin and polymers. Tablets were labeled and stored in brown glass bottles for the evaluation of dissolution tests.

Dissolution Studies

USP-II Paddle method (100rpm) in 1000ml bio-relevant condition was employed for the determination of drug release. As artemisinin is a neutral compound, the best suitable medium used for dissolution was demi water as an alternative for selected buffer (Tayyab *et al.,* 2013). Tablets comprising active drug, excipients and polymers in specified ratio were added in 900ml of Fasted Simulated Gastric Fluid (FSGF) in apparatus at 37^0C. 10 ml sample was drawn with replacement of fresh same quantity of FSGF at programmed time intervals, such as; 5min, 15min, 30min, 60min., 90min, 120min, 180min and 240min. The evaluations of samples (artemisinin) were accomplished as per recommendation of procedures at 293nm using spectrophotometer (UV/Visible).

Characterization Methods

Fourier Transform Infrared Spectroscopy

Different types of spectra were determined with FTIR spectrophotometer between 4000–400cm^{-1}, while resolution was put as 1cm^{-1}. Samples of artemisinin were studied by the formation KBr pellets.

Table 1: Artemisinin Solubility in Binary and Ternary Complexes

ART : Polymer Ratio	Pure Artemisinin (µg/ml)	Physical Mixtures (µg/ml)	Solvent Evaporation Complexes (µg/ml)
ART:SBE β-CD (1:1)	104.10	117.977	155.056
ART:SBE β-CD (1:4)	104.10	130.337	158.426
ART:SBE β-CD (1:2.5)	104.10	133.707	120.224
ART:SBE β-CD (1:9)	104.10	123.595	131.460
ART:SBE β-CD:OA (1:1:1)	104.10	335.955	295.505
ART:SBE β-CD:OA (1:1:0.27)	104.10	255.056	247.191
ART:SBE β-CD:OA (1:4:1)	104.10	257.303	281.438
ART:SBE β-CD:OA (1:3.6:3.6)	104.10	300.001	332.584
ART:SBE β-CD:OA (1:2.5:0.27)	104.10	239.325	235.955
ART:SBE β-CD:OA (1:9:1)	104.10	229.213	220.224

The perceived universal order of solubility reduction was to be SECx > PMs (Munir *et al.,* 2022). The improvement of ART with SBE7β-CD complex solubility was acknowledged better artemisinin wettability in hydro-phobic cyclodextrins.

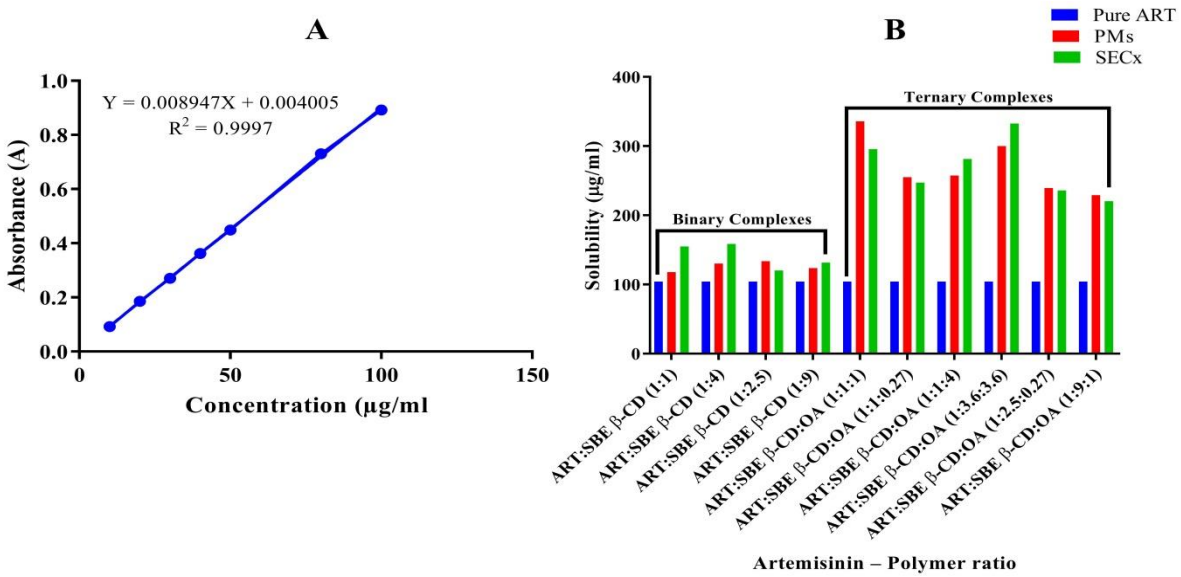

Fig. 1: (fig. 1A) ART standard calibration curve, (fig. 1B) artemisinin solubility in deionized H_2O at 37°C by various PMs and SECx preparations

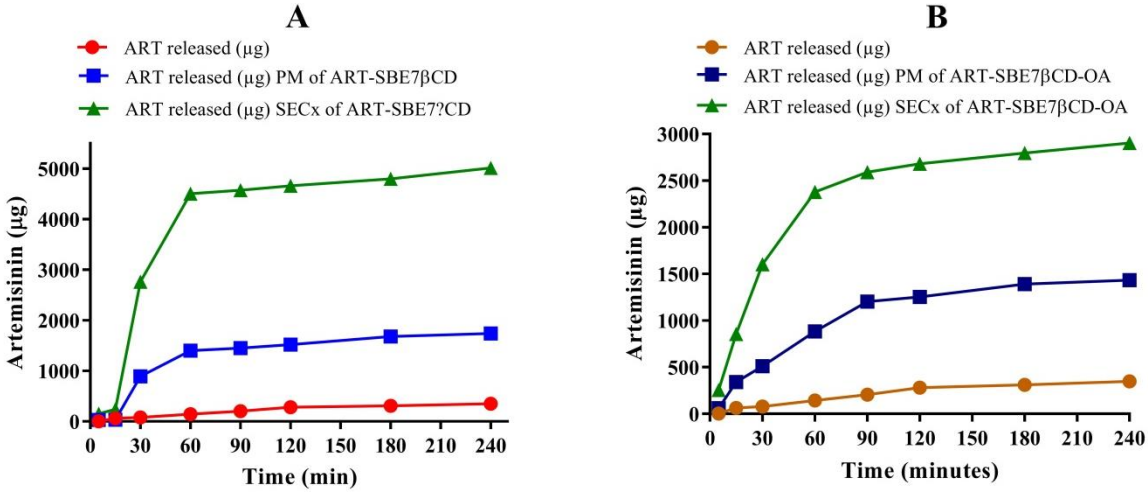

Fig. 2: (fig. 2A) Dissolution profile of binary complexes of artemisinin : SBE7-β/CD, (fig. 2B) dissolution profile of ternary complexes of artemisinin : SBE7-β/CD : Oleic acid

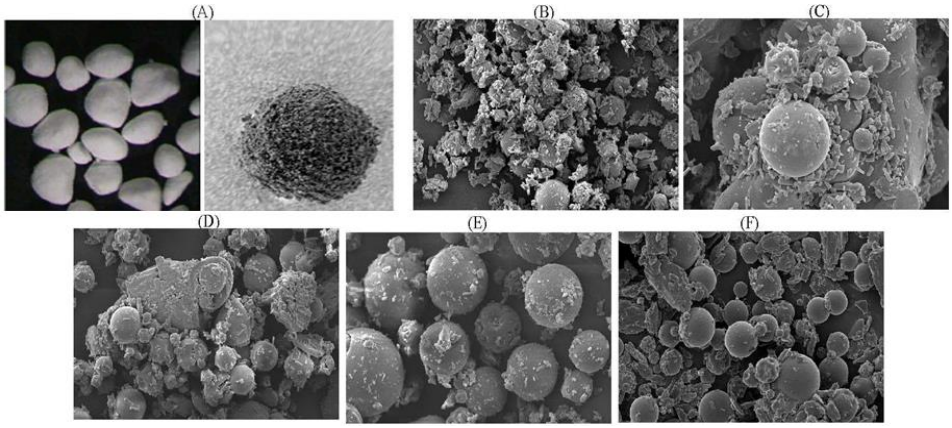

Fig. 3: (fig. 3A) SEM analysis of Pure artemisinin, (fig. 3B) PM of ART-SBE7β/CD (1:1), (fig. 3C) PM of ART-SBE7β/CD (1:2.5), (fig. 3D) PM of ART-SBE7β/CD (1:4), (fig. 3E) PM of ART-SBE7β/CD (1:9), (fig. 3F) ART-SBE7β/CD-OA PM (1:1:0.27)

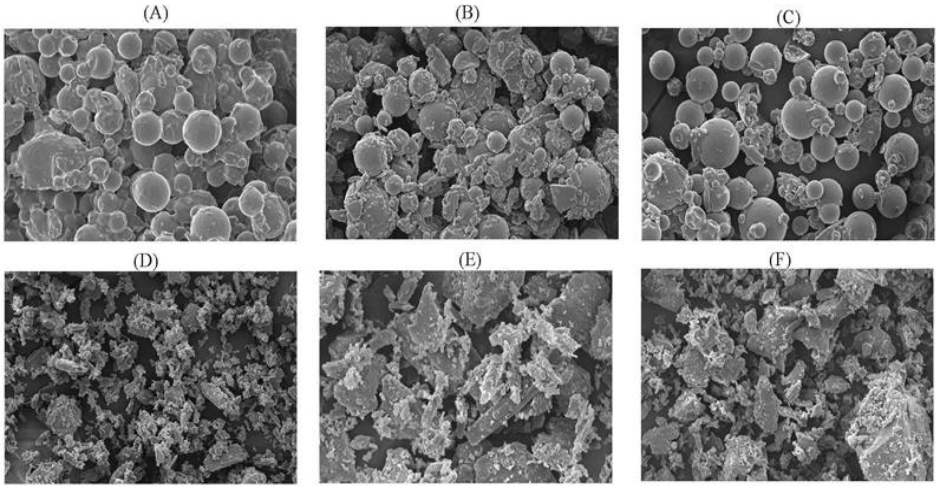

Fig. 4: (fig. 4A) SEM Analysis of ART-SBE7β/CD-Oleic acid Physical Mixture (1:4:1), (fig. 4B) ART-SBE7β/CD-Oleic acid Physical Mixture (1:2.5:0.27), (fig. 4C) ART-SBE7β/CD-Oleic acid Physical Mixture (1:9:1), (fig. 4D) ART-SBE7β/CD SECx (1: 1), (fig. 4E) ART-SBE7β/CD SECx (1: 2.5), (fig. 4F) ART-SBE7β/CD SECx (1: 4)

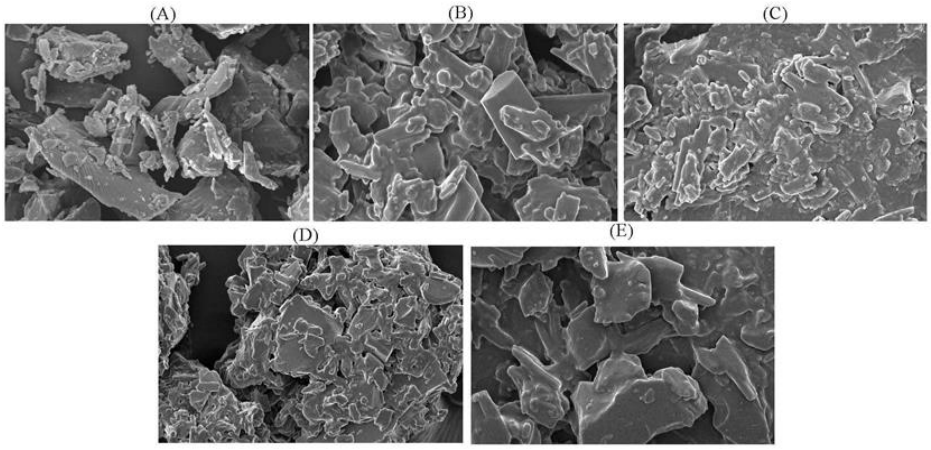

Fig. 5: (fig. 5A) SEM analysis of ART-SBE7β/CD SECx (1:9), (fig. 5B) ART-SBE7β/CD-OA SECx (1:1:0.27), (fig. 5C) ART-SBE7β/CD-OA SECx (1:4:1), (fig. 5D) ART-SBE7β/CD-OA SECx (1:2.5:0.27), (fig. 5E) ART-SBE7β/CD-OA SECx (1:9:1)

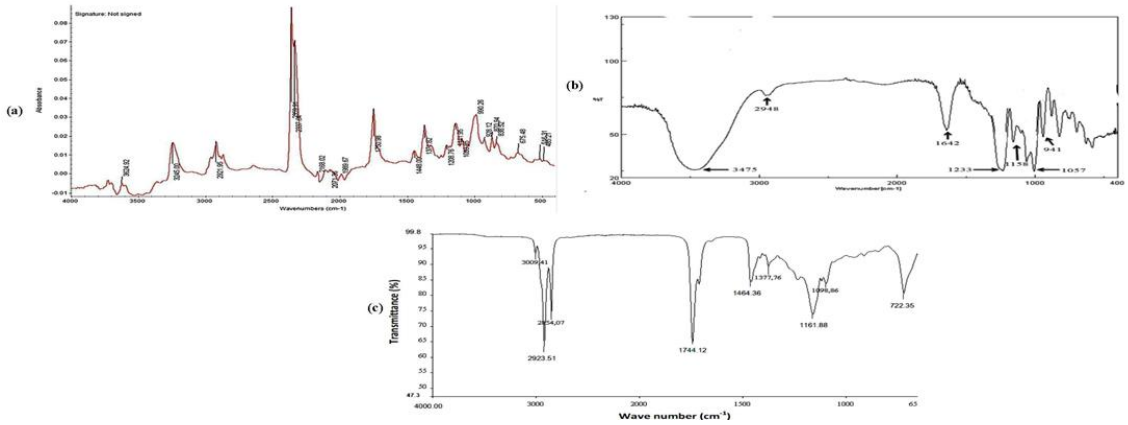

Fig. 6: (fig. 6a**)** Pure artemisinin ATR-FTIR spectra, (fig. 6b**)** Pure SBE7 β/CD, ATR-FTIR spectra, (fig. 6c**)** Pure Oleic acid ATR-FTIR spectra

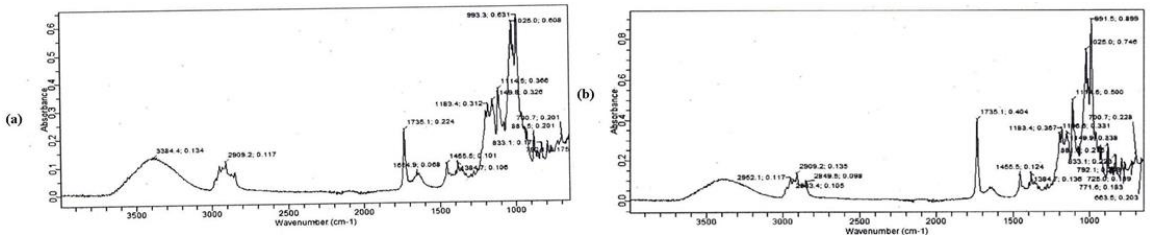

Fig. 7: (fog. 7a**):** ART-SBE7β/CD ATR-FTIR spectra, PM (1: 4), (fig. 7b**):** ART-SBE7β/CD ATR-FTIR spectra, SEC (1:4)

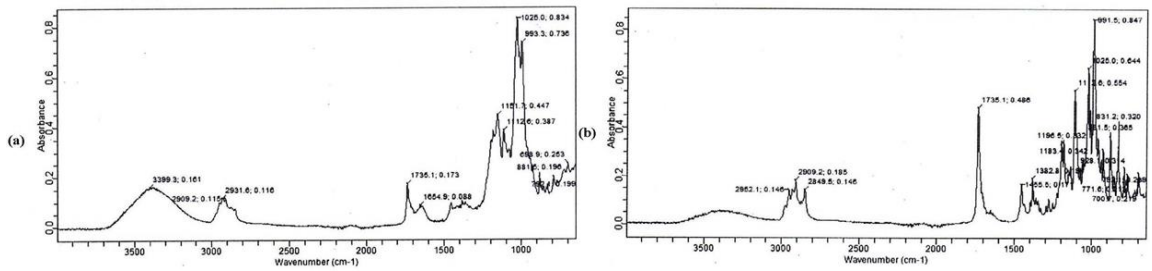

Fig. 8: (fig. 8a**)** ATR-FTIR spectra of ART-SBE7β/CD-Oleic acid PMs (1:4:1), (fig. 8b**):** ATR-FTIR spectra of ART-SBE7β/CD-Oleic acid SECx (1:4:1)

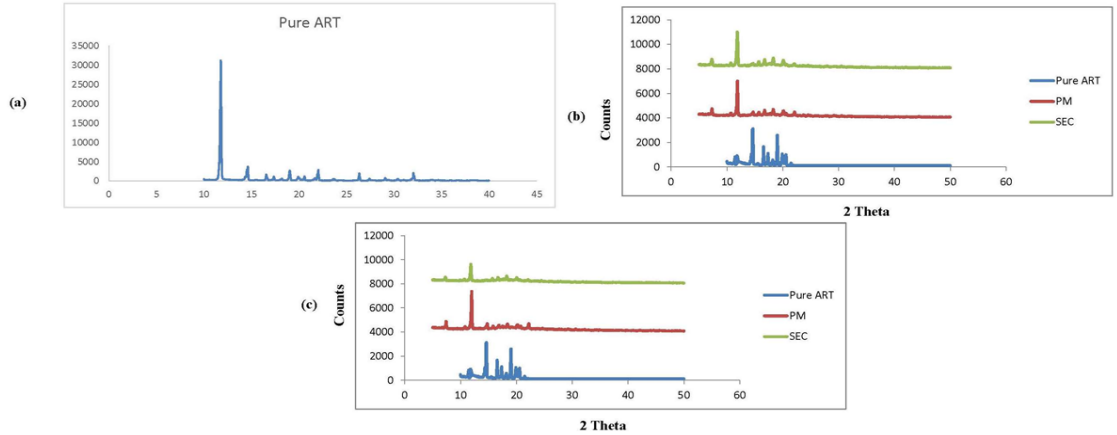

Fig. 9: (fig. 9a**)** XRD Pattern Pure ART, (fig. 9b**)** XRD Pattern ART-SBE7/CD (1:1), **(c)** XRD Configuration of ART-SBE7β/CD (1:4)

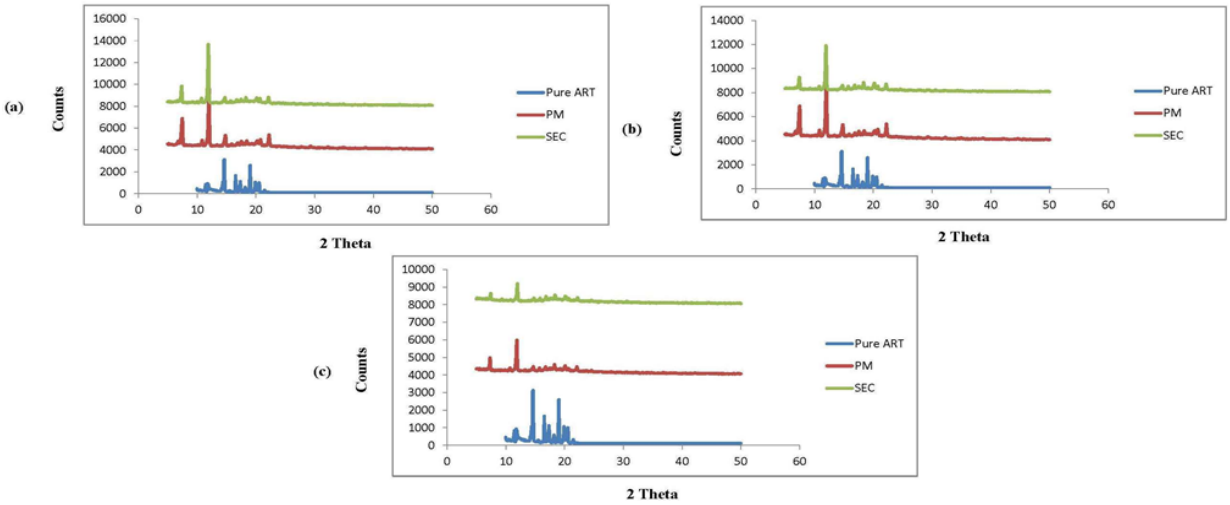

Fig. 10: (fig. 10a) XRD of ART-SBE7β/CD-OA (1:1:1), (fig. 10b**)** XRD reports of ART-SBE7β/CD-OA (1:1:0.27), (fig. 10c**)** XRD pattern of ART-SBE7β/CD-OA (1:2.5:0.27)

Electron Microscopy

The morphological characteristics of a pure drug, blend and complexes is confidently scanned using electron microscopy. The increase of 5KV was applied for the scanning of various samples. The resulting images were additionally examined under different magnifications, such as; x2500, x1500, x1000 and x350.

X-Ray Diffraction

CuK-α radiations were directly passed through the samples and determined among the angle range of 5° and 50°. The collection of outlines was observed with the provision of 40KV volt and 30mA current. Data processing was accomplished by using Jade 6.0.XRD (model) having appropriate stride width of 0.04°.

RESULTS

Equilibrium Solubility Studies

Calibration curve of artemisinin

The absorbance of solutions having artemisinin strengths such as; 10µg, 20µg, 30µg, 40µg, 50µg, 60µg, 80µg and 100µg per milliliter was determined after making dilutions with NaOH (0.2%) using UV/VIS spectrophotometer at 293nm. Regarding the equilibrium solubility studies of samples, 0.4g of every sample was transferred to conical flask (50ml) containing 10ml deionized-water. Flasks were vortexed at 1400rpm for 02min, then incubated (shaking) for 07 days at 37˚C with 150rpm. The supernatants were carefully transferred in clean test tubes after centrifugation (6000rpm) of mixtures for 15min. Diluted 01ml of upper clean layer of mixtures to 05ml with de-ionized water. Then mixed 01ml of diluted solutions with 0.2% NaOH (05 ml) and heated on water bath (50˚C) for 30min. After cooling, all these samples were analyzed spectroscopicaly (UV/Visible) at 293nm. The authenticity of samples was determined by comparing the control with pure artemisinin.

Artemisinin Solubility in Binary and also in Ternary Complexes

The solubility of pure artemising in deionized water was found 104.10µg/ml. The highest content of ART was found in solvent-evaporation mixture as compared to physical complexes and pure ART. The physical mixtures of ART-SBE7β/CD (Binary complexes) showed the enriched solubility profiles (118µg/ml) and solvent evaporation complex (SEC) in same binary complex exhibited 155µg/ml solubility in water, which showed ART binary complexes were 12 folds improved (table 1). In ternary complexes of oleic acid polymers (ART-SBE7β/CD-OA) ratio from 1:4:1 to 1:9:1.

Dissolution of Artemisinin in Fasted-Stated-Simulated-Gastric-Fluid (FaSSGF)

Dissolution studies were performed for the evaluation of pharmaceutical performance of both artemisinin binary and ternary complexes in the presence of excipients. A suitable medium (FaSSGF) having 1.6 pH was employed for dissolution procedures. The quantity of pure ART released from the tablets (500mg) after particular time intervals of 05min, 15min, 30min, 60 min, 90min, 120min, 180min and 240min were 2.71, 3.6, 62.5, 78.2, 144.5, 205.7, 281.3, 311.5 and 348.6µg/ml respectively. The dissolution profiles of ART-binary and ART-ternary complexes of PMs and SECx have been studied under the same conditions, which clearly revealed the significant progress of dissolution times on comparative to the pure artemisinin. PMs of binary complexes (ART-SBE7βCD) exhibited 4.22 times better dissolution profile, while SECx presented 12 times superior data when compared with pure ART (fig. 2A). The dissolution outlines of PMs and SECx of ternary complexes (ART-SBE7βCD-OA)

exposed 5.2 and 8.6 times healthier results respectively comparative to pure ART (fig. 2B).

Scanning of Electron Microscopy (SEM)

Extremely adjustable scanning electron microscopic technique was employed to deduce and recognize the nature and surface-topography of binary (ART-SBE7β/CD) (fig. 3A-3F) and ternary (ART-SBE7β/CD-OA) complexes (fig 4A-4F).

SEM revealed the crystalline pattern of complexes and in case of PMs which exhibited typical crystals with apparent exterior cavities holding unchanged SBE7β/CD compound. The combined structure of ART-SBE7β/CD-OA illuminated less number of crystals, because most of them are not exposed due to the trapping of crystals inside the SBE7β/CD moiety. Particle size was extremely reduced in case of complexes prepared by solvent-evaporation, while their outlines were modified due to the formation of scales. Consequently, photomicrographs acquired from scanning electron microscopy, elucidated the ART complex that modified the crystals' morphology and dimension among a variety of samples of PMs and SECx (fig. 5A-5E).

Total-Reflectance-Attenuated plus Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Pure Artemisinin Spectra

The typical vibrations of pure ART presented the spectrum at 2921cm^{-1} for C-O stretching, 2337cm^{-1} for C-H stretching, 870cm^{-1} for O-O stretching and 928cm^{-1} for O-O-C stretching that illustrated the features of O-O-C entity. The final interpreted data showed the existence of 1, 2, 4- tri oxane ring (fig. 6a).

Spectra of pure Sulfobutylether7β/Cyclodextrin

The extensive peak of Sulfobutylether 7β/cyclodextrin (pure) presented at 3475cm^{-1} due to the O-H stretching-vibrations and high peak at 2948cm^{-1} characterized the existence of C-H stretchings. The characteristic peak of SBE7 β/CD (fingerprint region) showed the absorbance at 1158cm^{-1} which confined the existence of C=O stretching vib (fig. 6b).

The pure spectra of Oleic acid

The ATR-FTIR spectrum of Oleic acid (pure) showed the stretching vibrations of CH2 and CH at 1464cm^{-1} and 1744cm^{-1} respectively. While, stretchings of C=C and COOH occurred at 2854cm^{-1} and 2923.5cm^{-1} (fig. 6c).

The Spectrum of Artemisinin-SBE7β-cyclodextrin complex

The ATR-FTIR spectrum of binary complex (ART-SBE7β/CD) with 1:4 ratio, showed a particular peak (ART) at 2921cm^{-1} (O-H) which in PMs and SECx shifted towards the blue-shift 2905cm^{-1} and 2909cm^{-1} respectively. The red-shift presentation for C-H (2849cm^{-1}) was shown for all binary complexes. The C-O (1025cm^{-1}) was shifted to 1111cm^{-1} (PMs) and 1112cm^{-1} (SECx) in red-shift modes. Stretchings of O-O-C (993cm^{-1}) for both PMs and SECx were also shifted to 881cm^{-1} and 792 cm^{-1} respectively. In PMs, O-O stretching band was disappeared as red-shift towards the 881cm^{-1} (SECx). In addition, C-H and O-H stretching bands were no more observable in all binary-complexes, though (C=O) stretching was evacuated to 1735cm^{-1} in PMs while unchanged in SECx (fig. 7a,b).

Artemsinin-SBE7β-cyclodextrin-Oleic acid (Ternary complex) ART-FTIR spectra

ART-SBE7β/CD-OA Spectrum of ternary complex (1:4:1) was visible at 2945cm^{-1} for O–H stretching-vib which indicated the hypochromic shift at 3372cm^{-1}. At 2905cm^{-1} signal (C–H) was blue-shifted to 2849cm^{-1} and 2844cm^{-1} in PMs as well as SECx correspondingly. C–O stretching-vibrations produced band at 990cm^{-1} that was red-shifted to 880cm^{-1} and 881cm^{-1} in both physical and SE mixtures respectively. Red-shift was observed from the stretchings of O-O-C from 847cm^{-1} to 828cm^{-1} in physical mixtures, however, in SECx, blue-shifted was to 820cm^{-1}. Due to the stretching of O-O at 812cm^{-1} shifted to 808cm^{-1} in PMs while no change was seen in SECx. The stretching bands were noticed from C-H and O-H in SBE7β/CD complex, while, C=O bands were unchanged in formulations. Even though, in oleic acid, C-H and O-H vibrations were observed at 2923cm^{-1} and 1744cm^{-1} respectively in all preparations. CH_2 stretching bands were tansfered to some extent (1112cm^{-1}) in PMs and 1111cm^{-1} in SECx. In SECx (C=O stretchings) red-shifted was observed to 1472cm^{-1} whereas it unaffected in PMs (fig. 8a,b).

Sharp peaks of pure ART have looked at 3245cm^{-1}, 2921cm^{-1}, 2337cm^{-1}, 1475cm^{-1} and 928cm^{-1} of O-H, C–O, C–H in 7-membered ring, O-O, O-O-C stretching-band indicated the characteristic moiety of O-O-C respectively which convinced the existence of 1,2,4-trioxane ring.

X-Ray Diffraction Results (X.R.D.)

Pure Artemisinin

The description of diffraction peaks of pure ART demonstrated with maximum intensity as 17562, 20460, 22772, 28821, 31252 and 30478 counts/sec at 11.68°θ, 11.70°θ, 11.73°θ, 11.75°θ, 11.77°θ and 11.79°θ respectively (fig. 9a).

Binary complexes of ART-SBE7β/CD

X-ray diffraction studies of physical mixtures of ART-SBE7β/CD (1:1) presented peaks at 7.3°θ with (694) intensity, 10.7°θ (391), 11.85°θ (2686), 14.7°θ (444), 15.70°θ (450), 16.75°θ (575), 17.4°θ (341), 17.75°θ (362), 18.3°θ (644), 20.1°θ (564) , 20.65°θ (359), 22.10°θ (456), 23.75°θ (240), 24.55°θ (260), 28.0°θ (196) and 30.55°θ (180). While, XRD characteristics peaks at 7.3°θ (747), 10.70°θ (469), 11.85°θ (2838), 14.60°θ (441),

15.70°θ (580), 16.75°θ (727), 17.40°θ (417), 17.75°θ (441), 18.30°θ (839), 20.10°θ 679), 20.65°θ (370), 22.10°θ (424), 24.55°θ (295), 28.0°θ (245) and 30.50°θ (233) exhibited by solvent evaporation binary complexes of ART-SBE7β/CD (1:1) (fig. 9b).

XRD data presented by PMs of ART-SBE7β/CD (1:4) binary complexes, 6.7°θ (383), 7.4°θ (774), 10.85°θ (438), 12.00°θ (2730), 74.80°θ (627), 15.80°θ (475), 16.85°θ (550), 17.50°θ (482), 18.40°θ (644), 20.20°θ (588), 20.75°θ (506) and 22.20°θ (651). While, results of SECx ((1:4)) of same mixture revealed low peaks 5.15°θ with intensity (335), 6.60°θ (325), 7.30°θ (526), 9.95°θ (311), 10.70°θ (375), 11.85°θ (1411), 14.65°θ (320), 15.7°θ (414), 16.10°θ (296), 16.70°θ (498), 17.35°θ (363), 17.75°θ (397), 18.30°θ (603), 20.10°θ (485), 20.65°θ (336), 21.55°θ (265), 22.10°θ (323) and 23.25°θ (249) (fig. 9c).

Ternary complexes of ART-SBE7β/CD-Oleic acid

XRD pattern of PMs of ART-SBE7β/CD-OA (1:1:1) displayed peaks 6.75°θ at 769 intensity, 7.45°θ (2862), 10.85°θ (830), 12.00°θ (5618), 14.80°θ (1306), 15.85°θ (555), 16.85°θ (632), 17.55°θ (737), 18.45°θ (805), 20.25°θ (836), 20.80°θ (913), 22.25°θ (1299), 32.25°θ (285) and 34.90°θ (257). XRD data of SECx of same ternary complexes showed 6.65°θ with intensity of 536, 7.35°θ (1748), 10.05°θ (418), 10.75°θ (707), 11.90°θ (5235), 14.75°θ (741), 15.75°θ (496), 16.80°θ (579), 17.50°θ (597), 18.30°θ (725), 20.15°θ (741), 20.70°θ (666), 22.15°θ (792) and 32.20°θ (229) (fig. 10a).

XRD results of PMs of ART-SBE7β/CD-OA (1:1:0.27) elucidated the peaks 6.7°θ having intensity (674), 7.4°θ (3286), 10.10°θ (461), 10.8°θ (827), 11.95°θ (6417), 14.80°θ (1143), 15.80°θ (531), 16.85°θ (602), 17.50°θ (672), 18.40°θ (766), 20.20°θ (759), 20.75°θ (779), 22.20°θ (1106), 29.20°θ (308) and 32.20°θ (263). XRD arrangements of SECx of same ratio of ART-SBE7β/CD-OA exhibited peaks 6.7°θ with 426 intensity, 7.45°θ (1198), 9.20°θ (353), 10.15°θ (327), 10.80°θ (510), 11.95°θ (3729), 14.75°θ (590), 15.75°θ (481), 16.85°θ (584), 17.5°θ (487), 18.35°θ (785), 19.15°θ (403), 20.20°θ (765), 20.75°θ (533), 22.20°θ (690), 24.6°θ (296) and 29.25°θ (239) (fig. 10b).

XRD results of PMs of ART-SBEβ/CD-OA (1:2.5:0.27) showed peaks 5.55°θ having intensity (353), 6.10°θ (324), 6.60°θ (346), 7.3°θ (885), 10.7°θ (380), 11.85°θ (1820), 13.25°θ (273), 14.70°θ (429), 15.7°θ (364), 16.75°θ (439), 17.45°θ (372), 17.75°θ (366), 18.30°θ (548), 20.10°θ (495), 20.65°θ (360), 22.10°θ (441), 24.60°θ (233), 30.55°θ (172), 32.15°θ (152) and 36.850°θ (131). XRD patterns of SECx of ART-SBEβ/CD-OA showed peaks 5.15°θ with intensity (372), 5.50°θ (368), 7.40°θ (581), 9.35°θ (295), 10.80°θ (279), 11.95°θ (1077), 14.80°θ (347), 15.25°θ (236), 15.80°θ (351), 16.85°θ (427), 17.20°θ (299), 17.60°θ (355), 18.40°θ (515), 20.15°θ (467), 20.70°θ (332), 22.20°θ (369), 23.20°θ (221), 23.70°θ (223), 24.65°θ (247) and 30.60°θ (176) (fig. 10c).

DISCUSSION

Statistical data (one way ANOVA) presented that dissolution rates of binary and ternary complexes were found significance when compared with weakly-soluble pure ART preparations. Results achieved from the formulations in current studies were significant in bio-relevant media, while consequent results will probably prove the reliable *in-vivo* action of drug formulation. In concern, it is highlighted that the combinations (ART, SBE7-β-CD and Oleic acid solvent evaporation complexes) were found the most outstanding mixtures of all the binary as well as ternary complexes in our study.

Samples' equilibrium solubility (artemisinin) was analyzed using UV/Visible Spectrophotometer. A standard calibration curve of pure artemisinin in methanol against absorbance was determined showing linear behavior at 293nm (fig. 1A). The resulting standard curve was employed as model for the calculations of ART-concentration in physical mixtures, solvent-evaporation mixtures as well as in freeze dried mixtures of ART‟s binary & ternary complexes.

As concerned the binary and Ternary Complexes of artemisinin, improvement of solubility with PMs (257.3µg/ml - 229.2µg/ml) and SECs (281.4µg/ml-220µg/ml) respectively at 37˚C that was 15-fold increase as compared to pure ART (104.10µg/ml) (fig. 1B).

The ATR-FTIR spectra of binary (ART-SBE7β/CD) and ternary complexes (ART-SBE7β/CD-Oleic acid) revealed the blue/red shift of different functional groups and proved the presence of host-guest interactions in our binary and ternary complexes.

The artemisinin crystallinity was decreased in physical mixtures of PMs and SECx showed displaced angles. The peak numbers decreased with attenuated-intensity of SECx along low intensity and more displaced-angles. The particle size has reduced in SEM images of PMs and SECx in binary and ternary system when compared with pure drug particle size.

CONCLUSION

Artemisinin showed the strong interactions among sulfobutyl-ether 7-β-cyclodextrin (SBE7-β-CD) and Oleic acid. It revealed boosted aqueous solubility characteristics with increasing excipient padding. The ATR-FTIR spectra confirmed the antimalarial activity of artemisinin was retained in almost all binary/ternary complexes (ART-SBE7β/CD and ART-SBE7β/CD-OA), due to the

existence of un-shifted O-O bond and presence of 1,2,4-trioxane ring which specified the existence of endo-peroxide moiety. Because of the excipients and ART interactions, band shifting and broadening occurred in (ATR-FTIR) spectrum by the functional groups, unlike the spectrum of pure artemisinin, SBE7β/CD and Oleic acid.

The improved ART dissolution rate is the result of complexes formation among the ART, SBE7β/CD and Oleic acid as well as because of alteration of crystalline drug to different complexes. When the ratio of SBE7β-CD and Oleic acid to the ART was increased, it illustrates enhanced pharmaceutical properties of artemisinin. Complexes with drug carrier in the proportion of 1:4:1 comprising ART-SBE7β/CD-Oleic acid exhibited the maximum dissolution profile as well as solubility in solvent evaporation.

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