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PREPARATION AND EVALUATION OF ARTEMISININ-FREEZEDRIED POWDERS WITH ENHANCED BIOAVAILABILITY

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ABSTRACT

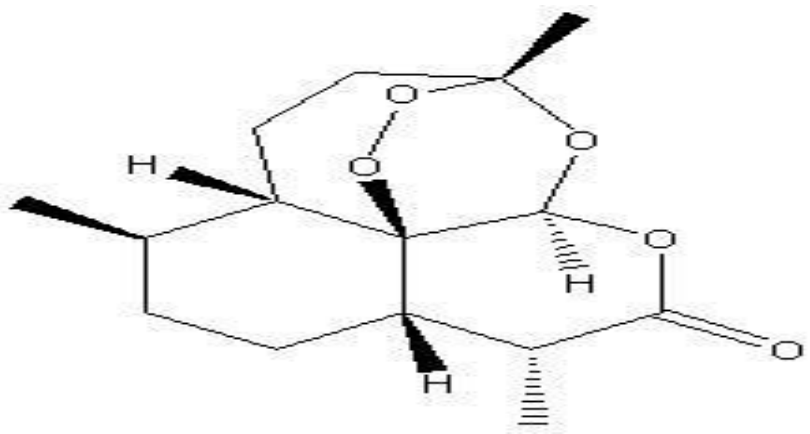
Artemisinin (ART) is an oral antimalarial agent with poor aqueous solubilityand low oral bioavailability. The present study describes the preparation of artemisinin freeze dried powder using different carriers (polyvinyl pyrolidone K-25 (PVP K-25), hydroxyl propyl cellulose (HPC) and dextrin) designed to enhance the solubility of artemisinin. and hence oral bioavailability of artemisinin, a poorly water-soluble Drug. Artemisinin freeze dried powders were prepared by dissolving different carriers (PVP K-25, HPC and dextrin) in water followed by the addition of artemisinin at a ratio of 1:4. The resultant products were evaluated using the solubility and dissolution studies, differential scanning calorimeter (DSC) and scanning electron microscopic (SEM). Relative bioavailability studies were conducted in Sprague-Dawely rats using the optimized formulation against the reference suspension. The in vitro studies showed that the aqueous solubility of artemisinin has increased significantly for the preparation containing artemisinin-dextrin at a ratio of 1:4. Further, the effect of incorporation of different co-carriers (citric acid or mannitol) to artemisinin-dextrin freeze-dried powder at different ratio was evaluated. There was a significant increase in the solubility and dissolution rate of artemisinin was obtained with the artemisinin-dextrin-citric acid freeze-dried powder at a ratio of 1:3:1. In addition, there was statistically significant increase in the oral bioavailability

of freeze dried artemisinin-dextrin-citric acid mixture compared to reference suspension. The rate and extent of absorption were enhanced by 3.4 folds. In conclusion freeze, dried product of artemisinin was able to increase the solubility, dissolution and therefore, the oral bioavailability.

KEYWORDS: Artemisinin, freeze dried, solubility, dissolution, oral bioavailability.

INTRODUCTION

Artemisinin is the name given to the active principle of qinghaosu, an extract of the Chinese medicinal plant qinghaosuor green Artemisia (*Artemisia annua* L.). The herb *Artemisia annua* L. has been used for many centuries in Chinese traditional medicine for treatment of fever and malaria.[1,2] In 1972 Chinese researchers isolated artemisinin from *Artemisia annua* L., (sweet wormwood) and its structure was elucidated in 1979 as shown below.



The chemical structure of artemisinin

Artemisinin and its derivative have several advantages over existing antimalarial drugs. First, there is little or no cross-resistance with other antimalarial drugs. Second, they clear the peripheral blood of parasites more rapidly than the other available drugs. Finally, resistance to artemisinin and its derivatives have not yet developed despite widespread clinical use.[3] It has been used successfully in several thousand malaria patients in China, including those with both chloroquine-resistant strains of *Plasmodium falciparum* and produced rapid recovery in 141 cases of cerebral malaria.[4] In particular, artemisinin is the only drug that remains activetowards*P. falciparum*in contrast to other drugsthat have developed mildly to complete resistance. The demise of chloroquine, sulfadoxine, pyrimethamine and other antimalarial drugs resistance has highlighted the important role of artemisinin in the current antimalarial armamentarium.

However, the major drawback of this compound is the low aqueous solubility, resulting in poor and erratic absorption upon oral administration. This together with its short half-life and high first pass metabolism might lead to incomplete clearance of parasites resulting in recrudescence.[5]

To overcome the problem of poorly solubility and dissolution of artemisinin, various approaches have been reported.[6] Among the other approaches is freeze drying (lyophilization) technique, which is used widely in the pharmaceutical industry. Tachibana and Nakuma were among the first to dissolve both the drug and the carrier in the common solvent and then evaporate the solvent to produce a solid solution.[7] Jaccard and Leyder employed freeze drying technique in making oral pharmaceutical preparation and found that the product have increased absorption and bioavailability with poorly water soluble drugs like spironolactone, nicerogline and trolendymein in comparison to their respective conventional formulations.[8] Betageri and Makarla (1995) reported that lyophilized solid dispersions of glyburide-PEGs had the maximum effect on the rate and extent of dissolution of glyburide compared to their physical mixtures. The results clearly suggested that the inclusion of a carrier together with suitable lyophilization process can have a positive influence on poorly water-soluble drugs.

The use of freeze drying techniques to enhance the solubility, the dissolution rate and bioavailability of poorly water soluble drugs (nimesulide[9], nifedipine[10], griseofulvin[11] and loviride.[12]

As none of the studies have reported artemisinin freeze dried product. The aim of the present study was to enhance the aqueous solubility and the dissolution rate of artemisinin and hence,it's oral bioavailability using freeze drying technique.

SUPPLEMENTARY MATERIALS

Table 1: Composition details of artemisinin: dextrin: citric acid/mannitol used for the preparation of freeze dried powder

Ratio	Artemisinin (mg)	Dextrin (mg)	Citric Acid or Mannitol (mg)
1:3:1	100	300	100
1: 2.75: 1.25	100	275	125
1: 3.25 : 0.75	100	325	75

Table 2 Solubility of plain artemisinin in pure Artemisinin and artemisinin carrier at a ratio of drug to carrier 1:4.Mean±SD, n=3

Preparation	Solubility (µg/ml)
Pure artemisinin	10.50±0.03
Freeze dried of artemisinin - Dextrin	79.55±0.05
Freeze dried of artemisinin - PVP K-25	23.45±0.03

Table 3.Individual Numerical Values of C_{max}, $AUC_{0-\infty}$, and T_{max}obtainedwith the two formulations

Subjects	Artemisinin Suspension			Freeze Dried Suspension		
	C_{max}(ng/ml)	AUC $_{0-\infty}$ (ng. hr/ml)	T_{max} (hr)	C_{max}(ng/ml)	AUC $_{0-\infty}$ (ng. hr/ml)	T_{max} (hr)
1	70.20	431.75	1	285.50	1287.27	1
2	78.50	443.48	1	255.50	1255.39	1
3	81.50	429.14	1	317.50	1375.63	2
4	74.50	358.55	1	309.50	1615.92	1
5	85.60	470.33	1	315.20	1990.90	1
6	75.50	478.06	2	296.80	1665.85	1
7	73.50	433.64	2	234.60	1120.55	1
8	80.50	491.61	2	296.50	1720.45	1
Mean	77.47	442.07	1.38	288.88	1503.77	1.13
SD	4.66	38.52	0.48	27.68	272.20	0.33

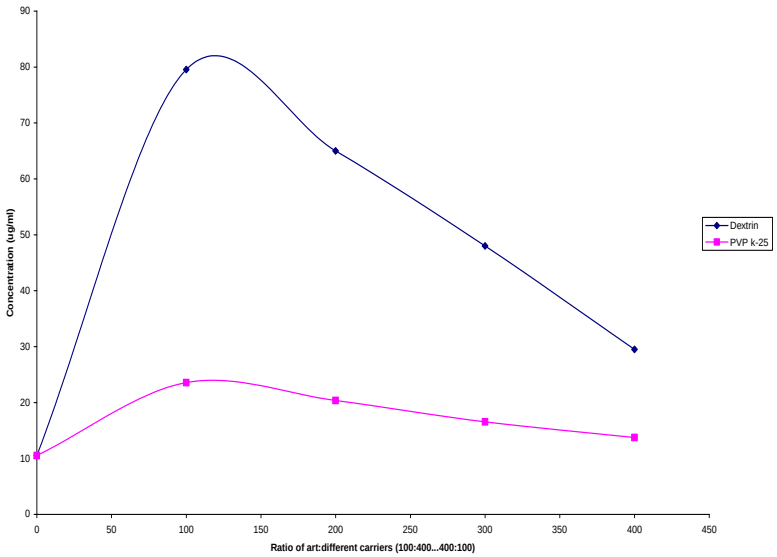


Fig .1 Comparative solubility profiles of artemisinin in different carriers (dextrin/PVP K-25) prepared using water as a solvent

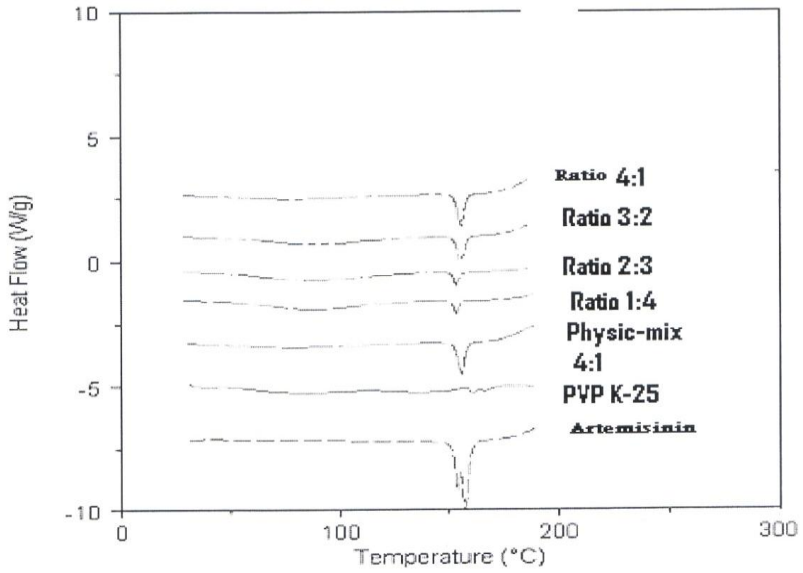


Fig. 2 Thermograms of artemisinin and artemisinin-PVP K-25 at different ratio prepared using water as solvent

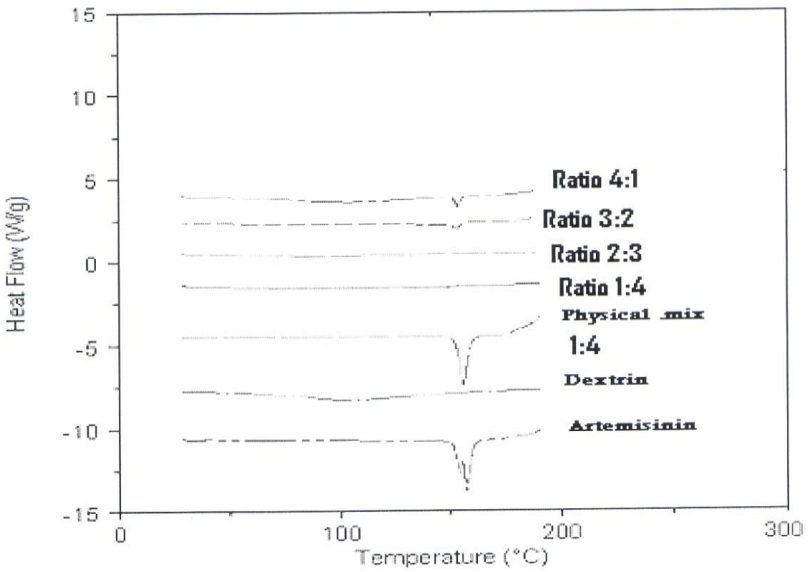
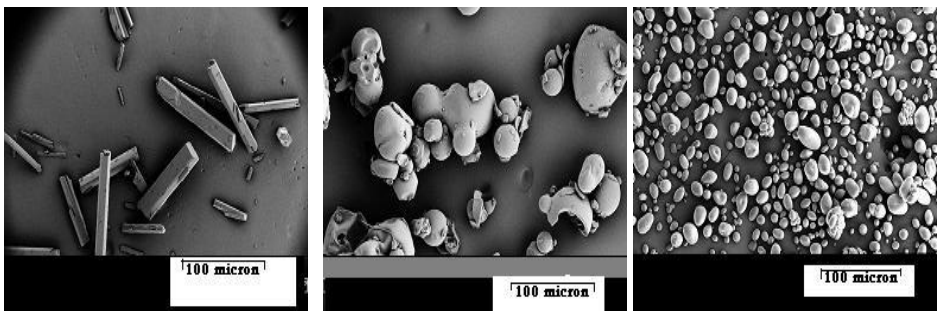
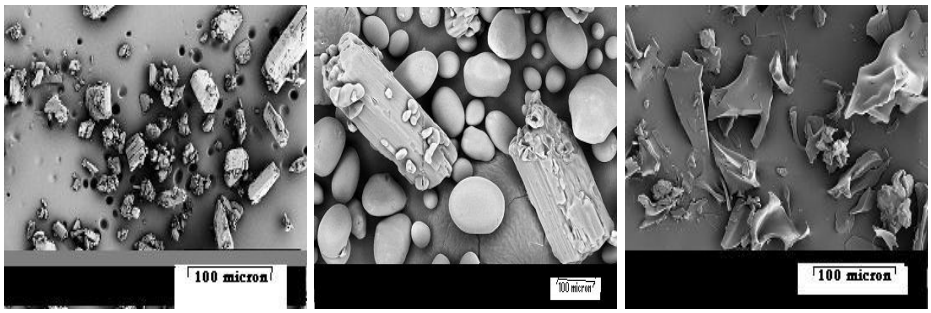


Fig 3 Thermograms of artemisinin and artemisinin-dextrin at different ratios

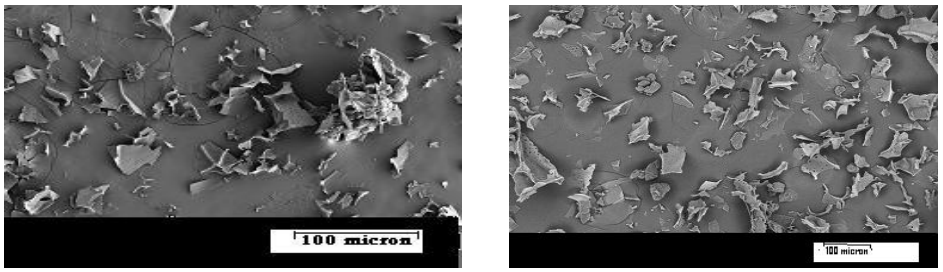


Artemisinin **PVP K-25** **Dextrin**

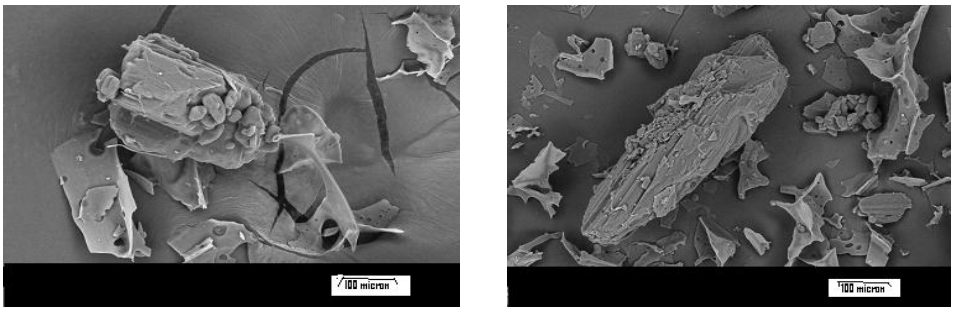


PM (ART-PVP K-25) PM (ART-Dextrin) ART-PVP K-25 FD

Fig 4 Micrographs of artemisinin, carriers, physical mixture (PM) of artemisinin-different carriers and freeze dried (FD) product of artemisinin-PVP K-25 prepared at a ratio of 1:4 (drug : carrier)



ART-Dextrin (1:4) FD ART-Dextrin (2:3)FD



ART-Dextrin (3:2) FD ART-Dextrin (4:1) FD

Fig.5 Micrographicof artemisinin-different freeze dire powder at different ratios

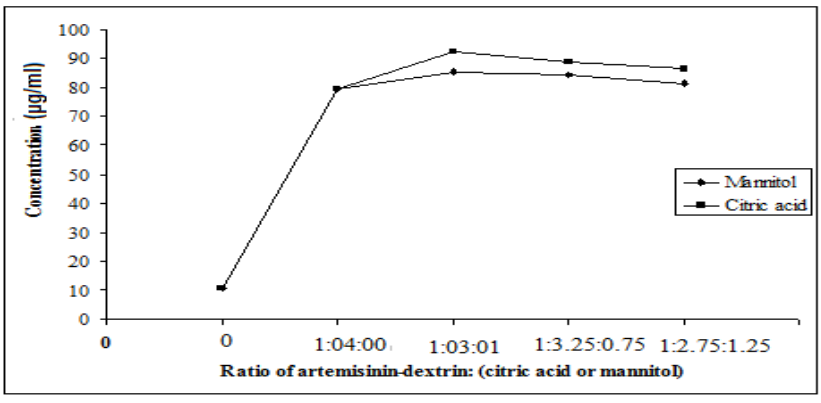
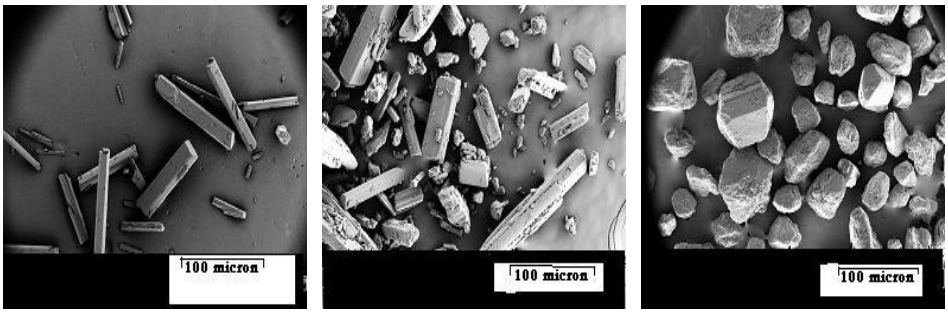
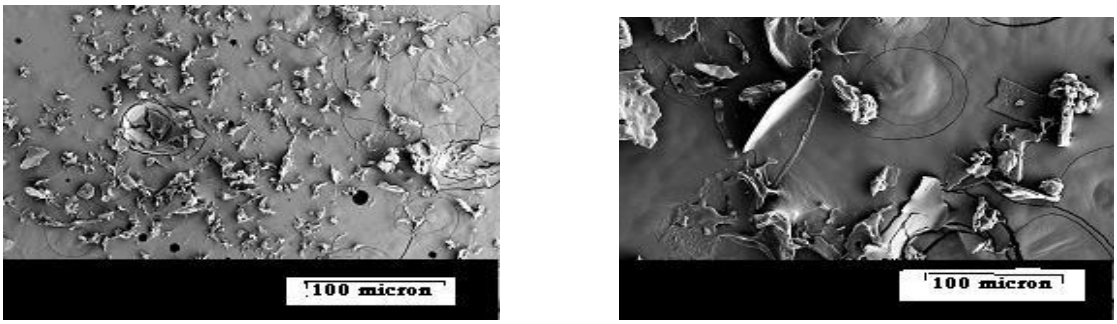


Fig 6 Artemisinin solubility in ART:dextrin:citric acid/mannitol freez driedpowder



Artemisinin **Mannitol** **Citric Acid**



Artemisinin-Dextrin –CA (FD) (1:3:1) **Artemisinin-Dextrin-Mannitol (FD) (1:3:1)**

Fig .7 Micrographs of pure artemisinin, mannitol, citric acid and freeze dried product of artemisinin-dextrin-citric acid / mannitol

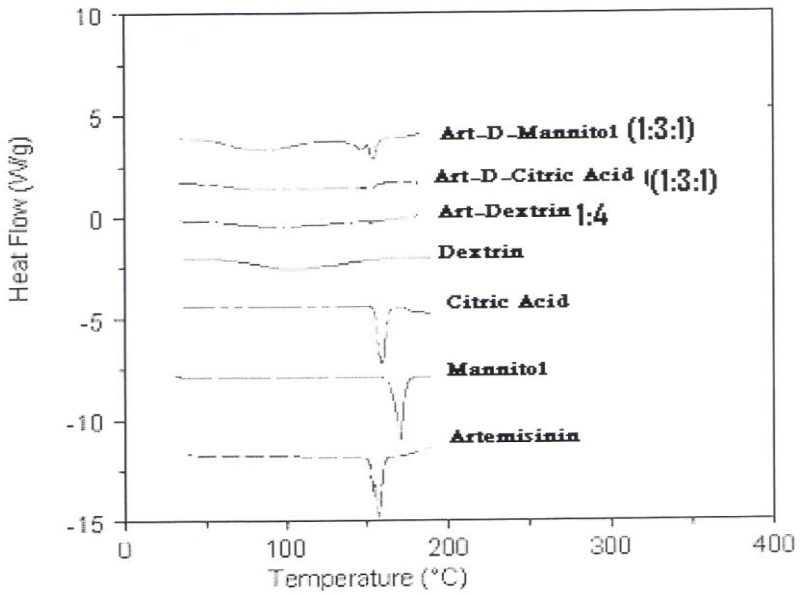


Fig 8 Thermograms of artemisinin and artemisinin-dextrin (D)-mannitol / citric acid at ratio of 1:3:1

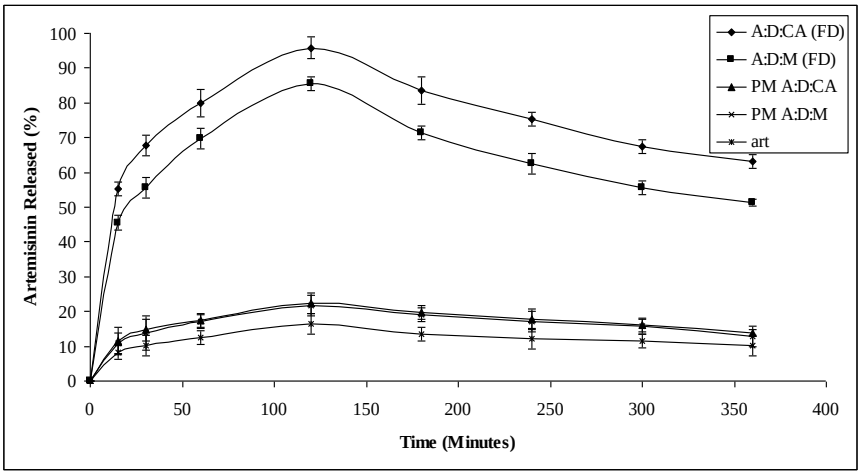


Fig 9 Comparative dissolution profile of artemisinin from freeze dried product and physical mixture (PM) of artemisinin-dextrin-citric acid / mannitol (A:D:CA/M) at ratio 1:3:1

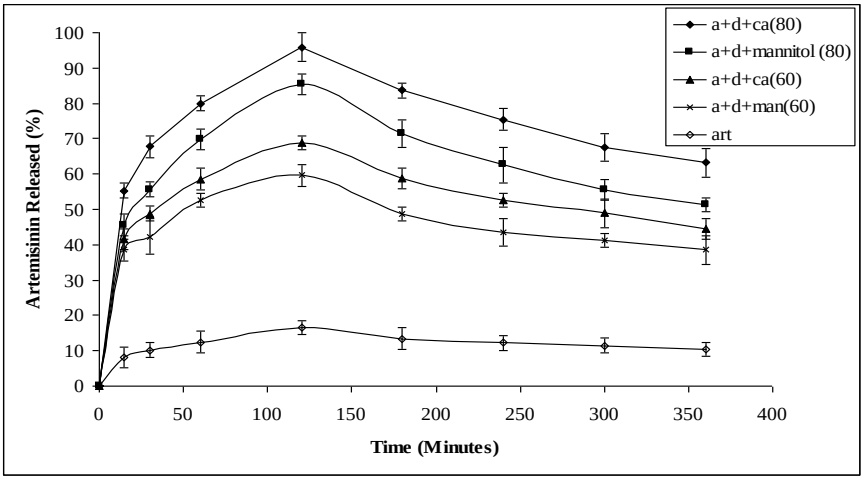


Fig 10 Comparative dissolution profile of artemisinin from pure artemisinin and in artemisinin-dextrin-citric acid / mannitol freeze dried product (A-D-CA/M) at ratio of 1:3:1 using water at 80°C and 60°C

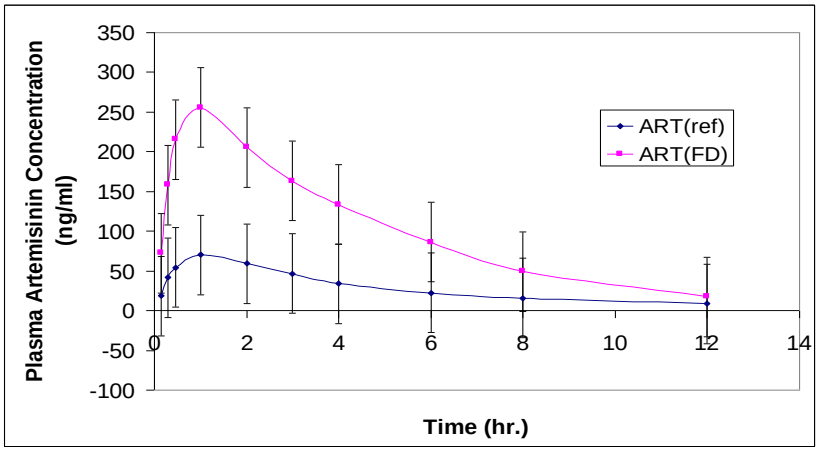


Fig 11. Mean plasma artemisinin concentration versus time profiles after dosing with artemisinin freeze-dried suspension or artemisinin suspension (10mg/Kg) (mean ± SEM, N=8)

Preparation of Artemisinin Freeze Dried Powders in Different Carriers

The freeze drying process could be completed in 24 hours. After 24 hours, the freeze-dried powder residues appeared as a porous light and fluffy mass, except for the preparation containing HPC, which was very sticky in nature and difficult to be collected and processed and hence was excluded from further study.

MATERIALS AND METHODS

Materials

Artemisinin was obtained from Kuming Pharmaceutical Corporation (Kuming, Germany). Dextrin, citric acid, and mannitol were obtained from BDH Chemicals (Poole, England). Amlodipine was obtained from Sigma Chemical (LousUSA). Acetonitrile (ACN), formic acid and methyl tertiary butyl ether (MTBE) were purchased from J.T Baker (USA).All others chemicals and reagents used were either analytical or HPLC grades.

Preparation of Artemisinin-(different carriers) Freeze Dried Powders

Freeze dried powders containing different proportions of artemisinin to Polyvinyl Pyrrolidone (PVP K-25), hydroxypropyl cellulose (HPC) and dextrin, were prepared using these ratios of drug to carrier of 1:4, 2:3, 3:2 and 4:1. They were prepared by first dissolving each carrier in distilled water followed by the addition of artemisinin with constant heating at60/80°C and stirring.

Another set of experiment was done by adding co-carriers citric acid or mannitol into the artemisinin-dextrin mixture. They were prepared by dissolving dextrin with citric acid / mannitol in hot distilled water followed by the addition of artemisinin with constant heating at 60°C/80°C. Various ratios of ART: dextrin:citric acid/mannitol was prepared, the composition details are shown in Table 1.

All the above mixtures were prepared and freeze at -53°C for 24 hours before lyophilization. The frozen mixtures were transferred to the freeze dryer(Labocono freeze dryer, Labocono Corporation USA) under the pressure of 8-10 mm Hg and condensed at -75°C for a period of 24 hours. The lyophilized powders were stored in a desiccator at a room temperature prior to their characterization by SEM, DSC and solubility determination, and dissolution studies.

Determination of Artemisinin Solubility in the Prepared Freeze Dried Powders

The solubility of artemisinin in the above-prepare freeze-dried powders was determined. An excess amount of pure artemisinin and freeze dried powder were separately added to flasks containing 20 ml of distilled water. All the samples were shaken vigorously at 30°C for 24 hours; 5 ml samples were collected from each flask and filtered through a membrane filter (0.2 µm). The filtrate were then suitably diluted and treated prior to an analysis by high-performance liquid chromatography (HPLC) using ultraviolet UV detector operated at a wavelength 260nm. The chromatographic separation was performed using a Genesis C_{18} column (150 x 4.6 mm) (Genesis, UK). The mobile phase composed of a mixture of 0.01M disodium hydrogen phosphate adjusted to pH 6.5 and acetonitrile (75:25, v/v). The HPLC analysis was based on the method reported by Zhao & Zeng.[22]

Differential Scanning Calorimetry (DSC) Studies

Thermal analysis using DSC has proven to be a useful tool for characterizing the freeze-dried powders.[23,24] Differential scanning calorimetric studies were performed on all the freeze dried powders with TA instrument model 2010 differential scanning calorimeter (DE, USA). Each sample (10 mg of powder in aluminum pans) was scanned from 25 to 200°C at a rate of 10°C per minute; the data were analyzed using the Universal analysis software (TA instrument, USA).

Scanning Electron Microscopy (SEM) Studies

The images of artemisinin and all the above freeze-dried powder were obtained by using a scanning electron microscope, and the micrographs were taken at a magnification of 500X.

Dissolution Study

The in vitro dissolution studies of pure artemisinin, freeze-dried product of artemisinin-dextrin-citric acid, artemisinin-dextrin-mannitol and their corresponding physical mixture (PM) were evaluated using the paddle method of the USP 24 dissolution test apparatus (Sotax AT7, Bassel, Switzerland).

The test was performed in 900 ml of distilled water as the dissolution medium under non-sink condition. The temperature was maintained at 25.0 ±0.5 °C while the paddle rotation speed was set at 100 rpm. The temperature of 25°C was chosen because at a higher temperature artemisinin would recrystallize out when the samples are exposed to room temperature after collection. A weight of 250 mg of pure artemisinin was used for each vessel. An equivalent

weight of 250 mg of artemisinin was also used for freeze-dried products of artemisinin-dextrin-citric acid or mannitol and their corresponding physical mixture.

All powders were sieved through a 300 µm laboratory test sieve (Endecotts Ltd., England) prior to the dissolution studies.Sample of 5 ml were withdrawn at various designated time intervals of 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes; using an automatic fraction collector (SDX Fractional Collector, Sadex, Malaysia). The samples were filtered through 0.2 µm syringe membrane filter (Whatman, UK). The initial portion of the filtrate was discarded and the subsequent portion collected was subjected to appropriate dilution with distilled water prior to an analysis by HPLC method. For each sample, the dissolution test was run in triplicate.

Animal Studies

The Ethics Committee on Animal Studies, UniversitiSains Malaysia, reviewed and approved the animal study procedure for the experiment performed. The experiment was carried out using 8 adult male, Sprague-Dawley rats weighing 265 to 320 g (mean = 292.5, SD = 28.5), according to a 2-period, 2-sequence crossover design, with two weeks washout period.The rats were randomly divided into two groups of 4 rats each. The animals were fasted for 12 hours prior to the start of the study. After drug administration, no food was allowed for a further 6 hours, but free access to water was maintained throughout the study. A suspension of artemisinin was prepared for both freeze dried artemisinin and reference drug powder in 0.5% (w/v) sodium carboxymethyl cellulose aqueous solution to a concentration of 10mg/ml each. After fasting the rats for 12 hours prior to the study, the animals were dosed with 10mg/kg body weight of either test or reference suspensions based on randomization schedule.

Blood samples of approximately 0.7 ml were collected from the tail vein into heparinized microcentrifuge tubes at 0 hour before dosing, 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 8 and 12 hours post-administration.The tail was clipped at the beginning of each phase of the experiment, subsequently the clogged blood was removed using cotton wool soaked with heparin 100 IU (Patton & Gilford, 1981; Ling *et al*., 2006). The blood samples were then centrifuged for 15 minutes at 12800g. The aliquot of plasma obtained from each blood sample was transferred into a new microcentrifuge tube. All samples were stored at -20°C until analysis**.**

LC-MS/MS assay for plasma samples

The instrumentation comprised of Quattro-micro tandem mass spectrometer with Z-spray atmospheric pressure ionization (API) source (Micromass, Manchester, UK) using electrospray ionization (ESI) operated in positive mode. Chromatography was performed on an Alliance 2695 separation module (Waters, M.A, USA). The delivery system consisted of an autosampler and a column heater. The chromatographic separation was obtained using an X-Terra MS C_8encapped (5µm) (150 x 2.1 mm) analytical column (Water, USA). The method consist a simple liquid-liquid extraction with methyl tertiary butyl ether (MTBE) with subsequent evaporation of the supernatant to dryness followed by the analysis of the reconstituted samples by LC-MS/MS using amlodipine as internal standard.

The method was linear (0.999) over the artemisinin concentration range of 7.8 – 2000 ng/ml in plasma. The method has a lower limit quantification of 3.9 ng/ml for artemisinin in the plasma. The Intra- and theinter-day accuracy were measured to be within 94-104.2%, and precision (CV) were all less than 5%. The extraction recovery means for internal standard and all artemisinin concentration used 82-85%.

Data and Pharmacokinetic Analysis

Pharmacokinetic analysis was performed using three parameters, namely, maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}) and total area under the plasmaconcentration curve ($AUC_{0-\infty}$) estimated from the individual plasma drug concentration data.TheC_{max} and T_{max}were obtained directly from the drug plasma concentration curves.[25] The total area under the plasma concentration-time curve was obtained by adding the area from time zero to the last measurable concentration (AUC_{0-t}) to the area of the last measurable concentration to infinity ($AUC_{t-\infty}$). The former was calculated using trapezoidal rule whereas the latter was calculated by dividing the last measurable concentration by the apparent elimination rate constant (K_e) which was estimated from the terminal slope of the individual plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression.[26] The apparent elimination half-life ($t_{1/2}$) was calculated from the equation $t_{1/2}$ = Ln (2) / K_e.

Statistical Analysis

The $AUC_{0-\infty}$, C_{max} and T_{max} values of the two preparations were analyzed statistically using an analysis of variance (ANOVA) procedure appropriate for a 2-way crossover study design, which allows effects due to subjects, periods, sequence and treatments to be

distinguished.[27] On the other hand, $AUC_{0-\infty}$ and C_{max} values were logarithmically transformed prior to analysis. On the contrary, the T_{max} values of the two preparations were analyzed using the Wilcoxon Signed Rank Test for paired samples. A statistically significant difference was considered at $p < 0.05$.

Determination of Artemisinin Solubility

Fig. 1 shows the solubility of artemisinin in the artemisinin-carrier freeze-dried powders with different ratios of artemisinin to dextrin or PVP K-25. It can be observed that the solubility of artemisinin was influenced by the type and amount of carrier used. Dextrin was able to increase the solubility of artemisinin to a greater extent than PVP K-25 at all drug to carrier ratios used. The above results showed that not only the type of the carriers used was important, but also the amount used can influence the drug solubility.[13] It is evident that at a ratio of 100: 400 drug to thecarrier the solubility of artemisinin was optimum. The solubility of artemisinin in both carriers at a ratio of100: 400 is shown in Table 2.

Differential Scanning Calorimeter (DSC) Studies

Fig. 2 and 3 represent the DSC thermograms of pure artemisinin, PVP K-25, dextrin, aphysical mixture of artemisinin-PVP K-25 / artemisinin-dextrin and freeze dried product of artemisinin-PVP K-25 / artemisinin-dextrin respectively.From Fig.2, it can be observed that the characteristic peak of artemisinin was clearly visible in the physical mixture and freeze dried product of artemisinin-PVP K-25 even in those powder with a high amount of PVP K-25 (1:4), indicating that there was no interaction between PVP K-25 and artemisinin. These results indicate that artemisinin was still in the crystalline form.

From the DSC thermograms in Fig. 3, it can be observed that there was an endothermic peak at 157.7°C for artemisinin, while for dextrin, an endothermic peak was observed at 75°C-125°C indicating the presences of residual moisture in dextrin.In case of physical mixture of artemisinin-dextrin, the endothermic peak of artemisinin was intact with a negligible decrease in enthalpy of melting from 63.1 to 61.50J/g, indicating that there was no interaction between artemisinin and dextrin.

In the case of afreeze-dried product of artemisinin-dextrin, it was observed that at a ratio of 1:4 and 2:3 the characteristic peak of artemisinin in the thermogram was absent. This indicates that artemisinin was no longer present in the crystalline form but was converted into the amorphous state.[14] The disappearance of the thermal features of the drug in the DSC

thermogram indicated that the drug and the dextrin interacted with each other.[8] Te Wierk et al has reported similar finding., in the freeze drying of diazepam with maltodextrin.[15]

While at ratios of 3:2 and 4:1 of artemisinin-dextrin, it can be observed that a small peak of artemisinin was recorded in the DSC thermogram indicating the presence of artemisinin still in the crystalline form.

Scanning Electron Microscopy (SEM) Studies

Fig. 4 illustrated the SEM micrographs of artemisinin, PVP K-25, dextrin, their physical mixture and froze dried product of artemisinin-PVP K-25 / dextrin. From the micrographs, it can be noted that the dextrin existed as small oval shaped particles. While in the physical mixture of artemisinin-dextrin the characteristic crystals of artemisinin were apparent. The freeze dried product of artemisinin-PVP K-25 also showed the characteristic of artemisinin crystals but were smaller in size. On the contrary, the freeze dried product of artemisinin-dextrin, appeared in the form of irregular particles in which the original morphology of both components were not visible in the ratio 1:4 and 2:3. However, when the ratio of artemisinin to dextrin was increased, (3:2) and (4:1) the characteristic crystals of artemisinin were clearly visible in Fig 5. Thus, based on the results obtained from the above mentioned, DSC, SEM and solubility studies, the artemisinin-dextrin freeze dried product at the ratio of 1:4 was selected for further investigation.

Study of Effect of Addition of Cryprotectant

Molpeceres et al. and Ozaki and Hayashi reported that for freeze dried products, theaddition of cryoprotectant was essential for the maintenance of the initial formulation characteristics.[16,17] So, in the present study to enhance the solubility of the freeze dried artemisinin-dextrin powder, Cryoprotectants were added.

Chacón used mannitol and other cryoprotectants to enhance the stability of the freeze dried products of cyclosporine loaded poly (D, L-lactideglycolide).[18] Table 1 shows the formula used for the preparation of the freeze dried product of artemisinin-dextrin using different ratios of two cryoprotectants, mannitol or citric acid.

From Fig 6, it can be observed that both citric acid and mannitol at various ratios increased the solubility of artemisinin-dextrin freeze dried products. Each carrier was incorporated into the mixture of artemisinin-dextrin in water and then were freeze dried. The freeze dried

powder containing citric acid was able to increase the solubility of artemisinin significantly more than the one with mannitol.

The increase in solubility was well supported by SEM and DSC studies as shown in Fig 7 and Fig 8 respectively. From Fig. **7**, it can be observed that the artemisinin freeze dried powder containing either citric acid (CA) or mannitol were present as irregular particles in which the original morphology of all components disappeared in the case of citric acid, tiny aggregate of amorphous small pieces of irregular size were present compared to mannitol.For this reason reduced particle size resulted in an increase in surface area responsible for enhancing the solubility of the drug.[19]

Fig. 8 represent the DSC curves of pure artemisinin, dextrin, citric acid, mannitol, and artemisinin-dextrin, freeze-dried powder containing citric acid / mannitol at a ratio of 100:300: 100. From the thermogram, it is clear that the endothermic peaks at 154.7°C, 165°C and 156°C corresponding to the melting points of pure artemisinin, mannitol, and citric acid respectively were observed. The DSC thermogram for the freeze dried products artemisinin-dextrin and artemisinin-dextrin-citric acid showed no characteristic endothermic peak. The disappearance of the specific peak of the drug indicates that the drug and the carrier interacted with each other.[8] Because the DSC curves of the freeze dried product of artemisinin-dextrin-mannitol recorded a peak corresponding to artemisinin melting enthalpy.This indicates that artemisinin and the carrier did not interact with each other and artemisinin is still in its crystalline form.

Dissolution Studies

Fig 9 shows the dissolution profile of freeze dried powders of artemisinin-dextrin citric acid / mannitol and their physical mixtures at aratio of 100:300: 100 and pure artemisinin. From the Figure, it is evident that the dissolution of freeze dried powders was faster compared to either aphysical mixture or drug alone. From Fig 10, it can be observed that the freeze dried product of artemisinin-dextrin-citric acid prepared at 80°C displayed faster rates than freeze dried product prepared at 60°C and freeze-dried product of artemisinin-dextrin-mannitol prepared at 60°C and 80°C. The above results were in line with findings reported by Corrigan that the physiochemical characteristics of the drug are affected by the method of its preparation[13], the type and properties of the polymer used.

The high rate and the extent of dissolution of freeze dried powder of artemisinin-dextrin may be attributed to the hydrophilic effect of dextrin which can reduce both the hydrophobicity of artemisinin as well as interfacial tension between artemisinin and the dissolution medium, so the particle size is reduced to molecular size. Thus faster dissolution rate can be achieved.

Animal Studies

Fig 11 shows the mean plasma concentration versus time profiles of artemisinin obtained with freeze-dried formulation and artemisinin suspension. It is apparent from the plots that thefreeze-dried suspension showed a marked increase in plasma drug levels compared to artemisinin suspension, indicating a higher rate and extent of artemisinin absorption.

The individual numerical values of C_{max}, $AUC_{0-\infty}$, and T_{max}, obtained after oral administration of the two formulations are presented in Table 3. It can be seen from the table that the mean C_{max} value of the artemisinin in the freeze-dried suspension was approximately 3.8-fold higher than that of artemisinin reference suspension. This difference in the mean C_{max} values was found to be statistically significant ($p < 0.05$). Similarly, the mean $AUC_{0-\infty}$ value of artemisinin freeze dried formula was approximately 3.4-fold higher than that of artemisinin suspension. Thus, the difference in the mean $AUC_{0-\infty}$ values was found to be statistically significant ($p < 0.05$).

The mean Tmax value for the artemisinin in the freeze-dried suspension was 1.13 hours compared to 1.38 hours obtained for artemisinin reference suspension. The difference between the mean T_{max} values was found to be statistically insignificant ($p > .05$).

According to Fick's firstlaw, the rate of drug diffusion through the intestinal wall is directly proportional to the concentration gradient of the drug in the gut and the blood. Hence, increased drug absorption is expected from a formulation with higher drug solubility and dissolution rate.

In addition enhanced artemisinin absorption or bioavailability from the freeze-dried suspension can be ascribed to saturation of the hepatic enzymes involved in thefirst-pass metabolism of artemisinin.[3,20] Ashton *et al*. demonstrated that the enzymes involved are saturable.[21] This rapid absorption of artemisinin freeze-dried suspension may cause enzyme saturation during hepatic first-pass metabolism, which may result in a higher fraction of unmetabolized drug entering the systemic circulation compared to artemisinin reference

suspension which has a slower absorption rate and hence may not be sufficient to saturate the enzymes involved.

CONCLUSION

From the above results, it can be concluded that freeze drying technique could be employed to prepare artemisinin-dextrin-citric acid freeze dried product, which was capable of increasing the solubility, dissolution rate and oral bioavailability of artemisinin.

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