

# Preparation and *In Vitro* Characterization of Artemisinin Freeze-Dried Powders with Various Carriers

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## Abstract

The present study was conducted to investigate the enhancing effect of different polymeric carriers on solubility and hence oral bioavailability of artemisinin, a poorly water-soluble drug. Freeze-dried powder preparations of artemisinin and various carriers (polyvinyl pyrrolidone K-25 (PVP K-25), Hydroxypropyl Cellulose (HPC) and dextrin) were obtained 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 solubility and dissolution studies, Differential Scanning Calorimetry (DSC) and Scanning Electron microscopy (SEM). These *in vitro* studies showed that the aqueous solubility of artemisinin was significantly increased 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 ratios was evaluated. 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.

**Keywords:** Artemisinin; HPC; PVP K-25; Dextrin; Solubility

## Introduction

Artemisinin (ART) is the active principle isolated from the Chinese medicinal herb *Artemisia annua* [1]. ART and its derivatives have gained increasing attention as antimalarial drugs due to their low toxicity and high efficacy against malarial parasites, including the cases caused by multidrug resistant and cerebral strains. It is one of the few antimalarials that remain effective against multi drug-resistant strains of *Plasmodium falciparum* [2]. It is a fast acting blood schizonticide with short parasite clearance time, leading to rapid symptomatic relief of malarial infections [3].

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 [4].

To overcome the problem of poor solubility and dissolution of artemisinin, various approaches have been reported. One of these approaches is the freeze-drying (lyophilization) technique, which is widely used in the pharmaceutical industry. Tachibana and Nakuma [5] were among the first to dissolve both the drug and the carrier in common solvent and then evaporate the solvent to produce a solid mixture. Jaccard and Leyder [6] employed freeze-drying technique in making oral pharmaceutical preparations and found that the products have increased absorption and bioavailability with poorly water

soluble drugs like spironolactone, nicergoline and trolendymein in comparison to their respective conventional formulations. Betageri and Makarla reported that lyophilized solid dispersions of glybenclamide-PEGs had the maximum effect on the rate and extent of dissolution of glybenclamide compared to their physical mixtures. The results clearly suggested that inclusion of a carrier together with suitable lyophilization process can have a positive influence on poorly water-soluble drugs.

Van Eerdenbrugh et al. [7] reported that the dissolution rate and absorption properties of lovirode administered as sucrose co-freeze-dried nano-powder were improved significantly. The use of freeze-drying techniques to enhance the solubility, dissolution rate and bioavailability of poorly water-soluble drugs (nimesulide, nifedipine, griseofulvin and lovirode) has been demonstrated by Shoikri et al. [8], Iman & Mona [9] and Van Eerdenbrugh et al. [7], respectively.

The aim of the present study was to enhance the aqueous solubility and the dissolution rate of artemisinin using freeze-drying techniques.

## Materials

Artemisinin was obtained from Kuming Pharmaceutical Corporation (Kuming, Germany). Dextrin, citric acid and mannitol were obtained from BDH Chemicals (Poole, England). All others chemicals and reagents used were either analytical or HPLC grades.

## Methods

### Preparation of artemisinin freeze-dried powders with different carriers

Freeze-dried powders containing different proportions of artemisinin to Polyvinyl Pyrrolidone (PVP K-25), Hydroxylpropyl Cellulose (HPC) and dextrin, were prepared using 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 at 70 – 80°C and stirring. Dextrin is not soluble in most common solvents, but it is highly soluble in hot water.

Another set of experiments were 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 addition of artemisinin with constant heating at 60°C - 80°C. Various ratios of ART: dextrin: citric acid / mannitol were prepared; the composition details are shown in Table 1.

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

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

### Determination of artemisinin solubility in the prepared freeze-dried powders

The solubility of artemisinin in the above prepared freeze-dried powders was determined. An excess amount of pure artemisinin and freeze-dried powder were separately added into 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 filtrates were then suitably diluted and treated prior to analysis by High Performance Liquid Chromatography (HPLC) using an Ultraviolet (UV) detector operated at a wavelength of 260 nm. The chromatographic separation was performed using a Genesis C18 column (150 x 4.6 mm) (Genesis, UK). The mobile phase was 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 [10].

### Differential scanning calorimetry (DSC) studies

Thermal analysis using DSC has proven to be a useful tool in characterizing the freeze-dried powders [11,12]. Differential scanning calorimetric studies were performed for all the freeze-dried powders

with a 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, and 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-mentioned freeze-dried powders 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 powders of artemisinin-dextrin-citric acid, artemisinin-dextrin-mannitol, and their corresponding Physical Mixtures (PM) were evaluated using the paddle method with a USP 24 dissolution test-apparatus (Sotax AT7, Basel, Switzerland).

The test was performed in 900 ml of distilled water as the dissolution medium under non-sink condition. The temperature was maintained at maintained at 25°C ± 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 powders 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. Samples 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 filters (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 analysis by the HPLC method. For each sample, the dissolution test was run in triplicate.

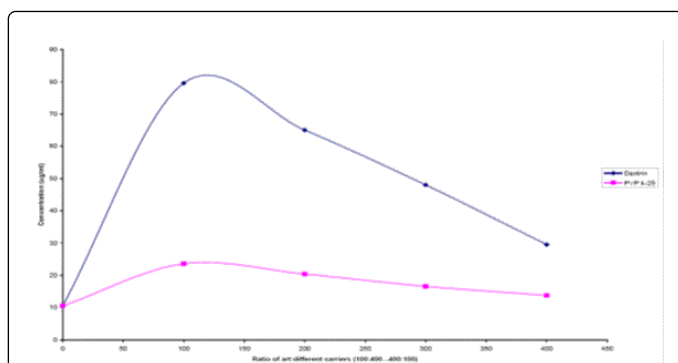
## Results and Discussion

### 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, difficult to be collected and processed, and hence was excluded from further study.

### Determination of artemisinin solubility

Figure 1 shows the solubility of artemisinin in the artemisinin-carrier freeze-dried powders with different ratios of artemisinin to dextrin or PVP K-25.



**Figure 1:** Comparative solubility profiles of artemisinin in different carriers (Dextrin/PVP K-25) prepared using water as a solvent.

It can be noticed 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-mentioned 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 4:1 drug to carrier the solubility of artemisinin was optimum. The solubility of artemisinin in both carriers at a ratio of 4:1 is shown in Table 2.

Preparation	Solubility (µg/ml)
	Mean±SD, n=3
Pure artemisinin	10.50±0.03
Freeze-dried powder of artemisinin - Dextrin	79.55±0.05
Freeze-dried powder of artemisinin - PVP K-25	23.45±0.03

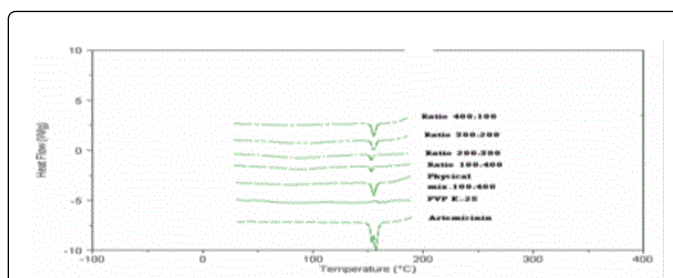
**Table 2:** Solubility of artemisinin in pure artemisinin and artemisinin carrier at a ratio of drug to carrier 1:4.

### Differential scanning calorimeter (DSC) studies

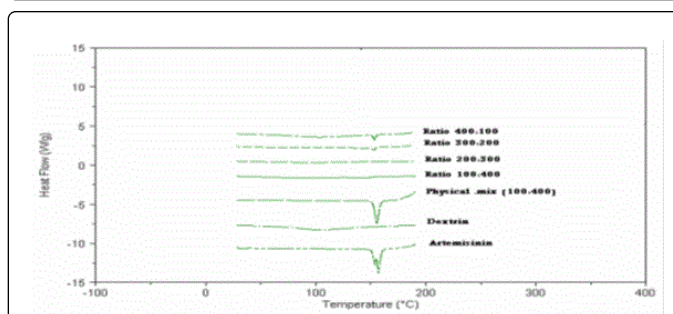
Figure 2 and 3 represent the DSC thermograms of pure artemisinin, PVP K-25, dextrin, physical mixture of artemisinin-PVP K-25 / artemisinin-dextrin, and freeze-dried product of artemisinin-PVP K-25 / artemisinin-dextrin, respectively.

In Figure 2, it can be noticed 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 powders 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 shown in Figure 3, it can be noticed 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 presence 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.



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



**Figure 3:** Thermograms of artemisinin and artemisinin-dextrin at different ratios.

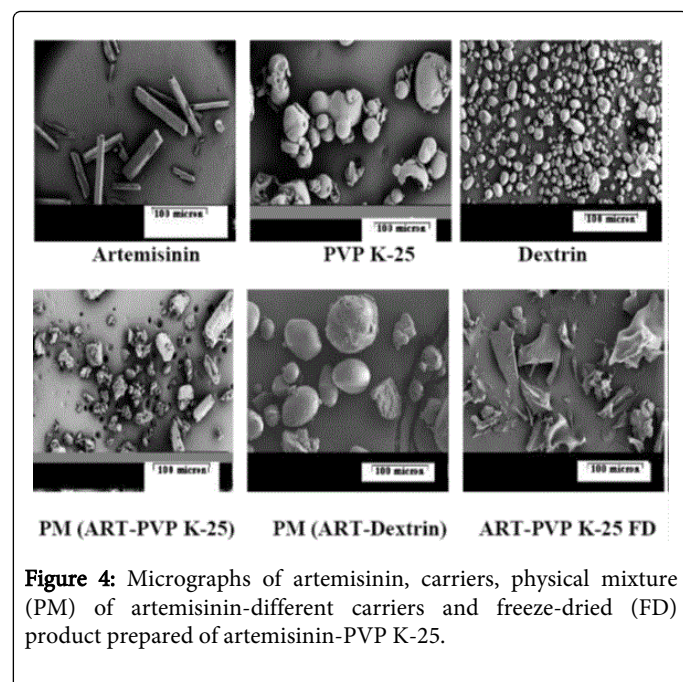
In the case of freeze-dried product of artemisinin-dextrin, it was noticed that at a ratio of 1:4 and 2:3, the characteristic peak of artemisinin in the thermogram was absent. This indicates that artemisinin was no longer present in the crystalline form but was converted into the amorphous state [14]. The disappearance of the thermal features of the drug in the DSC thermogram indicated that the drug and the dextrin interacted with each other [15]. Similar findings have been reported by Te Wierk et al. [16] in the freeze-drying powder of diazepam with amylopectin.

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

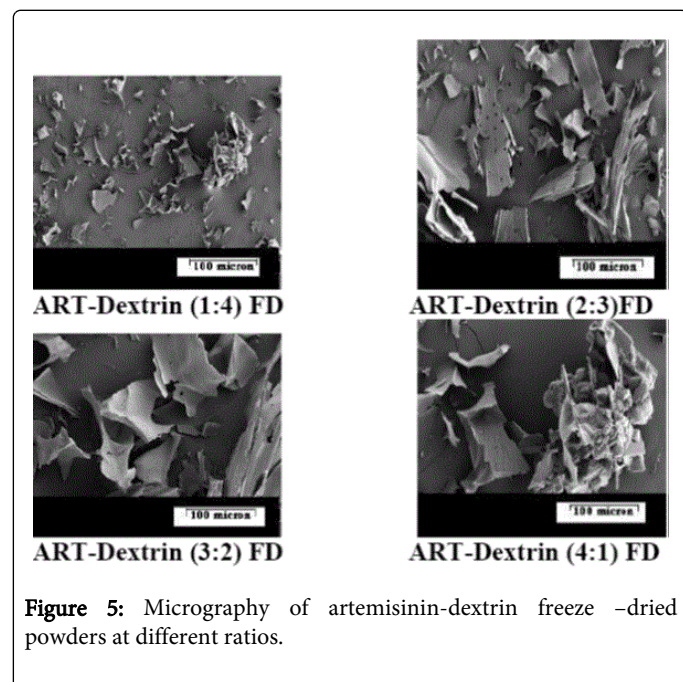
### Scanning electron microscopy (SEM) studies

Figure 4 illustrated the SEM micrographs of artemisinin, PVP K-25, dextrin, their physical mixture and freeze-dried product of artemisinin-PVP K-25 / dextrin. From the micrographs it can be noted that 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 these crystals 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 was not visible at the ratio 1:4 and 2:3. But when the ratio of artemisinin to dextrin was increased to (3:2) and (4:1), the characteristic crystals of artemisinin were clearly visible, as shown in Figure 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.





**Figure 4:** Micrographs of artemisinin, carriers, physical mixture (PM) of artemisinin-different carriers and freeze-dried (FD) product prepared of artemisinin-PVP K-25.



**Figure 5:** Micrograph of artemisinin-dextrin freeze-dried powders at different ratios.

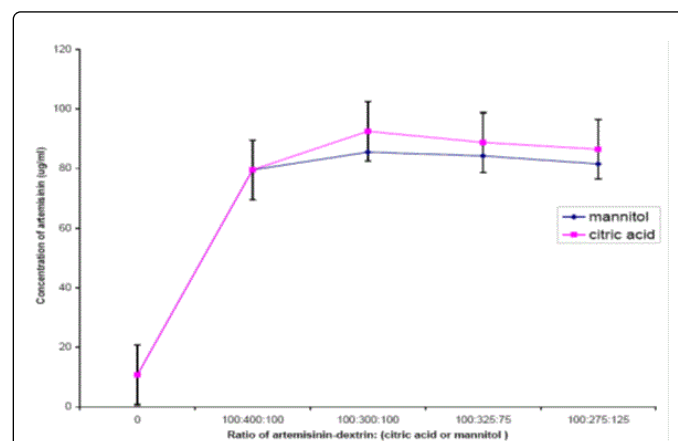
### Study of effect of additional cryoprotectants

Molpeceres et al. [17] and Ozaki and Hayashi [18] reported that for freeze-dried products, addition of cryoprotectant was essential for the maintenance of the initial formulation characteristics. Therefore, in the present study, to enhance the solubility of the freeze-dried artemisinin-dextrin powder, cryoprotectants were added.

Chacón et al. [19] used mannitol and other cryoprotectants to enhance the stability of the freeze-dried products of cyclosporine-loaded poly (D, L lactide glycolide). Table 1 shows the formula used for the preparation of the freeze-dried product of artemisinin-dextrin

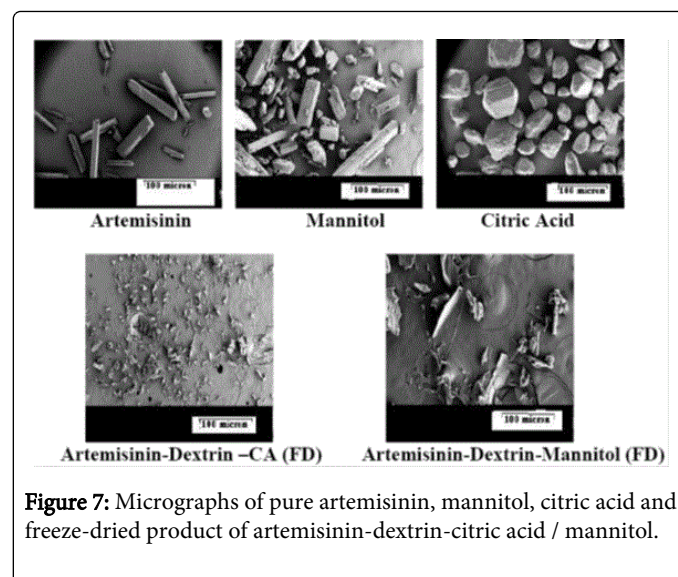
using different ratios of two cryoprotectants, mannitol or citric acid [20].

In Figure 6 it can be noticed that both citric acid and mannitol at different ratios increased the solubility of artemisinin-dextrin freeze-dried products. Each carrier was incorporated into the mixture of artemisinin-dextrin in water and then mixtures were freeze-dried. The freeze-dried powder containing citric acid was able to significantly increase the solubility of artemisinin compared to the one with mannitol.

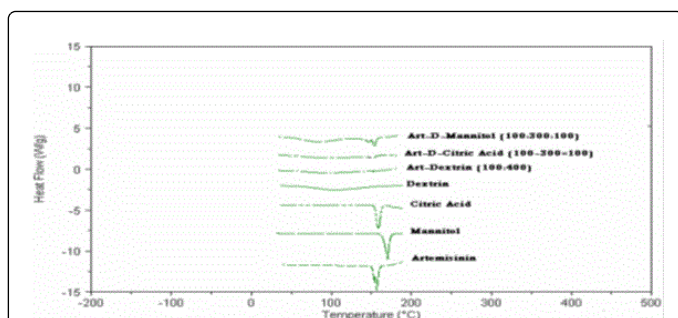


**Figure 6:** Artemisinin solubility in ART:dextrin: citric acid/ mannitol freeze-dried powder.

The increase in solubility was well supported by SEM and DSC studies, as shown in Figures 7 and 8, respectively. In Figure 5 it can be noticed that in the artemisinin freeze-dried powders containing either citric acid (CA) or mannitol, these ingredients were present as irregular particles in which the original morphology of all components disappeared in the case of citric acid, where tiny aggregates of amorphous small pieces of irregular size were present, compared to mannitol. Therefore reduced particle size resulted in an increase in surface area responsible for enhancing the solubility of the drug [20].



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



**Figure 8:** Thermograms of artemisinin and artemisinin-dextrin (D)-mannitol / citric acid at a ratio of 1:3:1.

Figure 5 presents the DSC curves of pure artemisinin, dextrin, citric acid, mannitol and artemisinin-dextrin freeze-dried powders containing citric acid / mannitol at a ratio of 1: 3: 1. 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 [15], whereas 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 was still in its crystalline form.

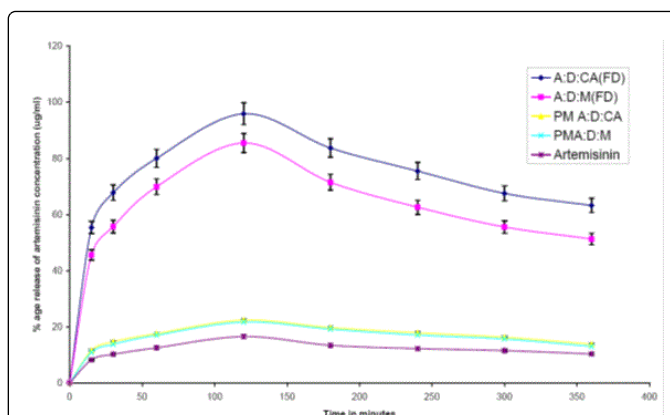
## Dissolution

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

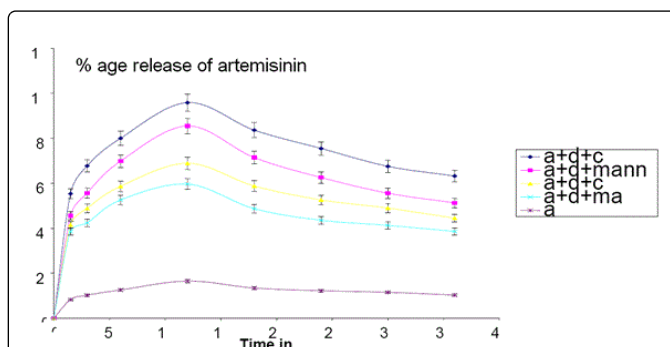
The high rate and the extent of dissolution from freeze-dried powder of artemisinin-dextrin may be attributed to the hydrophilic effect of dextrin, which can reduce both the hydrophobicity of artemisinin as well as interfacial tension between artemisinin and the dissolution medium, so the particle size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

## Conclusion

From the above-mentioned results it can be concluded that the freeze-drying technique can be employed to prepare artemisinin-dextrin freeze-dried products capable of increasing the solubility and dissolution rate of artemisinin. Moreover, incorporation of citric acid into the mixture of artemisinin-dextrin leads to a further enhancement in the dissolution rate.



**Figure 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.



**Figure 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.

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