

## Original Research Article

# Preparation and characterization of artemether inclusion complexes with hydroxypropyl- $\beta$ -cyclodextrin

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Sent for review: 8 April 2017

Revised accepted: 18 September 2017

## Abstract

**Purpose:** To investigate experimentally the inclusion of artemether into the cavity of hydroxypropyl- $\beta$ -cyclodextrin and examine its effect on the solubility and dissolution rate of the drug.

**Methods:** Inclusion complexes of artemether with hydroxypropyl- $\beta$ -cyclodextrin of molar ratios 1:1, 1:2 and 1:3 were prepared using the kneading method. Phase solubility analysis and *in vitro* dissolution studies were utilized in evaluating the influence of inclusion complex formation on the solubility and dissolution rate of the drug. The complexes were characterized using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR). The inclusion complex containing equimolar concentrations of artemether and hydroxypropyl- $\beta$ -cyclodextrin was then formulated into tablets via direct compression and evaluated for various pharmaceutical characteristics including hardness, friability, absolute drug content and comparative *in vitro* dissolution profiles with some commercially available brands of artemether.

**Results:** The phase solubility diagram for the formed complexes in water at 37 °C indicated a linear curve soluble complex system (referred to as the  $A_L$  system), and a stability constant ( $K_c$ ) value of 143  $M^{-1}$ . Evidence consistent with inclusion complex formation was obtained using FT-IR and DSC. The formulated inclusion complex tablets exhibited a higher rate of dissolution than the pure drug and commercial brands, showing 3.9-, 1.8- and 1.6-fold increases, respectively, over a period of 15 min.

**Conclusion:** Inclusion complexation of artemether with hydroxypropyl- $\beta$ -cyclodextrin is a promising approach to enhance the solubility and dissolution rate of the drug.

**Keywords:** Artemether, 2-Hydroxypropyl- $\beta$ -cyclodextrin, Dissolution, Solubility enhancement, Inclusion complex

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## INTRODUCTION

All drugs, regardless of their route of administration, should exhibit at least limited aqueous solubility for therapeutic efficiency. Thus relatively insoluble compounds can exhibit erratic or incomplete absorption, and it might be appropriate to use more soluble salts or chemical

derivatives. Complexation, solid dispersion or micronizing techniques might alternatively be employed [1]. Molecular complexation is the interaction between "guest" molecules and cyclodextrins (CD) to form reversible inclusion complex which has been shown to be an effective means of increasing the aqueous solubility of hydrophobic compounds [2].

Cyclodextrins are torus shaped molecules of D-glucopyranose obtained by the enzymatic degradation of starch [2,3]. There are three types of natural cyclodextrins – alpha ( $\alpha$ ), beta ( $\beta$ ), and gamma ( $\gamma$ ) with six, seven and eight glucopyranose units, respectively. The cyclodextrin cavity dimension, which is important for guest-host interaction, ranges from 0.174 to 0.427 nm for alpha and gamma cyclodextrins respectively [2,3].

Inclusion complex formation is mediated majorly by van der Waals attraction and hydrophobic interaction. The “host,” includes, totally or partly, the hydrophobic molecule [2,3].

Artemether is a potent and rapidly acting antimalarial agent available for the treatment of severe multi-resistant malaria and is included in World Health Organization (W.H.O) list of essential medicines [4]. It is active against *P. vivax* as well as chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*; it is also indicated for the treatment of cerebral malaria [4]. Unfortunately, this potent blood schizonticidal belonging to Class II of Biopharmaceutical Classification System shows poor water solubility and low bioavailability following oral administration [5]. The aim of this study is to utilize 2-hydroxypropyl- $\beta$ -cyclodextrin for improving the solubility and dissolution rate of this poorly aqueous soluble drug via inclusion complex formation.

## EXPERIMENTAL

### Materials

2-Hydroxypropyl- $\beta$ -cyclodextrin, Molecular weight (Mw) = 1540 (Sigma-Aldrich, U.S.A), CAS No. 128446-35-5. A pure sample of artemether (Mw = 298.4, Batch No. ATM 121003) was a kind donation from Afrab Chem. Nigeria Ltd, Lagos. Other reagents and chemicals were of analytical reagent grade.

Brand A (Coartem<sup>®</sup> tablets); Batch No. X1571, Manufac. date Jan. 2012, Expiry date Dec. 2013, NAFDAC Reg. No. 04 -275. Manufactured by Beijing Novartis Pharma Ltd, Beijing China.

Brand B (Lumartem<sup>®</sup> tablets); Batch No. FD2011, Manufac. date Jan. 2012, Expiry date Dec. 2013, NAFDAC Reg. No. A4 -4845. Manufactured by CIPLA Ltd, India.

### Spectrophotometric characterization

The spectrophotometric analysis of artemether in samples was carried out after derivatization.

Derivatization was done by treating samples with 1 M ethanolic hydrochloride and heated at 60 °C for 3 h, this enabled the formation of acid decomposition product described as  $\alpha$ ,  $\beta$ -unsaturated decalone [6-8] which absorbs at 254 nm.

### Phase solubility studies and determination of apparent stability constants ( $K_c$ )

Phase solubility behavior was studied according to the method proposed by Higuchi and Connors [9]. An excess of the drug (200 mg) was added to 20 mL portions of distilled water in six conical flasks, each containing variable amounts of 2-HP- $\beta$ -CD (0, 3, 6, 9, 12, and 15  $\times 10^{-3}$  moles/Liter). The solutions were shaken on a flask shaker (Grant OLS 200, Cambridge – England) for 24 h at  $37 \pm 2$  °C, filtered and 100 mL of 1 M ethanolic HCl added to 2 mL of each filtrate and shaken for 5 s. This was heated for 3 h at 60 °C and allowed to cool to room temperature. Their absorbance was taken at 254 nm wavelength.

The solubility of the artemether in every 2-HP- $\beta$ -CD solution was calculated and phase solubility diagram was drawn between the solubility of artemether and different concentrations of 2-HP- $\beta$ -CD.

The stability constant ( $K_c$ ) of artemether:2-HP- $\beta$ -CD complex was calculated using Higuchi and Connor's equation [10].

$$K_c = \frac{\text{slope}}{\text{Intercept} (1 - \text{slope})} \dots\dots\dots (1)$$

### Preparation of inclusion complexes in solid state

This was prepared using artemether and 2-hydroxypropyl- $\beta$ -cyclodextrin in different molar ratios (1:1, 1:2 and 1:3 M). First, a small quantity of 50 % ethanol was added into cyclodextrin in a glass mortar and triturated until a paste was obtained. Then, the drug was added into the paste and kneaded for 1 h. The product was then air dried at room temperature (28 °C) for 24 h, pulverized, passed through a sieve of size 90  $\mu$ m and stored in a desiccator over fused calcium chloride.

### Differential scanning calorimetry

Thermograms of pure artemether, 2-hydroxypropyl- $\beta$ -cyclodextrin and inclusion complex were recorded by heating the samples

at a temperature range of 30 to 300 °C at a rate of 10 °C/min.

### Fourier transform-infrared spectroscopy (FT-IR)

The FT-IR spectra of artemether, its complexes, and 2-hydroxypropyl- $\beta$ -cyclodextrin were obtained by KBr pellet method using FTIR spectrophotometer (SHIMADZU FTIR 8400S) over the range 500 - 4,500  $\text{cm}^{-1}$ .

### Formulation of artemether-cyclodextrin inclusion complex tablets

Inclusion complex of artemether-cyclodextrin of molar ratio 1:1 was used for the preparation of tablets. The complex of artemether: 2-HP- $\beta$ -CD containing the equivalent of 20 mg artemether was compressed into tablets by direct compression method. The complex, maize starch and microcrystalline cellulose were mixed together after which talc and magnesium stearate were added and further mixed for 5 min. The mixture was then compressed into tablets (using a compression force of 10.5 metric ton and a punch size of 12.5 mm). The tablets were evaluated for thickness, diameter, weight uniformity, crushing strength, friability, disintegration and dissolution time.

### In vitro dissolution studies for artemether-CD inclusion complex tablets

*In vitro* dissolution of artemether inclusion complex tablets containing 20 mg of artemether was studied in Erweka dissolution apparatus (Erweka DT, Germany). Five hundred millilitres of simulated gastric fluid was used as dissolution medium. The basket was rotated at 50 rpm and the temperature of dissolution medium maintained at  $37.0 \pm 0.5$  °C. Five millilitre aliquots were withdrawn at predetermined intervals (5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min) and filtered using Whatman filter paper no. 41. At each interval, withdrawn volume was replaced with a fresh quantity of dissolution medium. After suitable dilution, the withdrawn samples were derivatized by treating with 1 M ethanolic hydrochloride and heated at 60 °C for 3 h, then analyzed spectrophotometrically at  $\lambda_{\text{max}}$  of 254 nm. The percentage amount of artemether released was calculated and plotted against time. This was compared with both the dissolution profile of the pure artemether and the commercial products.

### Statistical analysis

The data generated from the various determina-

tions were analyzed using SPSS 20.0 software (SPSS, Chicago, IL, USA) and are presented as mean  $\pm$  standard deviation (SD). The differences between the data sets were determined using one-way analysis of variance (ANOVA) and  $p < 0.05$  was considered statistically significant.

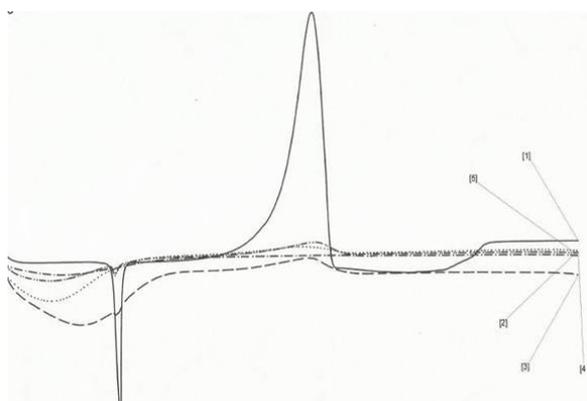
## RESULTS

### Phase solubility

The phase solubility diagram gave a straight line with  $R^2$  value of 0.9963 and  $y = 0.0014x + 7E-06$ . The phase solubility diagram for the formed complexes in water at 37 °C indicated a linear curve soluble complexes system (referred to as the  $A_L$  system) following the Higuchi and Connors classification [11]. A stability constant ( $K_c$ ) value of  $143 \text{ M}^{-1}$  was obtained.

### Thermal characteristics

The DSC thermograms of the pure drug, the 2-hydroxypropyl- $\beta$ -cyclodextrin and the prepared inclusion complexes are given in Figure 1.



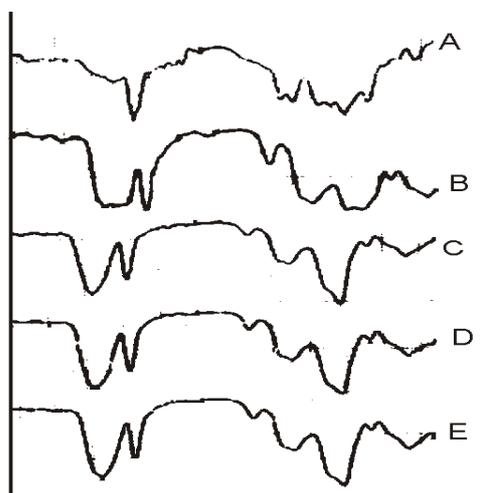
**Figure 1:** DSC thermograms of pure artemether sample (1), 2-hydroxypropyl- $\beta$ -cyclodextrin (5), artemether-2-HP- $\beta$ -CD (1:1) complex (4), artemether-2-HP- $\beta$ -CD (1:2) complex (2), artemether-2-HP- $\beta$ -CD (1:3) complex (3)

The DSC thermogram of pure artemether exhibits a sharp endothermic peak at 89.6 °C and an exothermic peak at 180 °C, which is consistent with the findings of Yang *et al* [12]. The 2-hydroxypropyl- $\beta$ -cyclodextrin exhibited a typical broad endothermic peak between 50 - 100 °C, which attained a maximum at 87.2 °C, consistent with the findings of Liu and Zhu [13]. The DSC thermograms of the inclusion complexes exhibited mark decrease in intensity to even complete disappearance of the artemether endothermic and exothermic peaks. Changes in the exothermic peak (corresponding to its decomposition temperature) ranging from a

marked reduction in intensity to a complete disappearance suggest that the 2-HP- $\beta$ -CD-artemether complex is more thermally stable than the artemether alone and this further supports the fact that inclusion complex formation enhanced drug stability [14].

### Infrared spectra

The FT-IR spectra of the pure artemether and its inclusion complexes over the frequency range of 500 – 4500  $\text{cm}^{-1}$  are given in Figure 2. The characteristic FT-IR peaks of the pure artemether occurred at 3198.08  $\text{cm}^{-1}$  due to O-H stretching vibration, C-H stretching at 2932.86  $\text{cm}^{-1}$ , C-H bending at 1020.38  $\text{cm}^{-1}$ , C-O bending at 1020.38  $\text{cm}^{-1}$ , C-O-O-C bending vibration at 1195.95  $\text{cm}^{-1}$ , O-O-C stretching at 857  $\text{cm}^{-1}$  and O-O stretching at 746  $\text{cm}^{-1}$  respectively. These values are comparable to those earlier reported by Fule *et al* [15].



**Figure 2:** FT-IR spectrum of pure artemether sample (A), 2-hydroxypropyl- $\beta$ -cyclodextrin (B), artemether-2-HP- $\beta$ -CD (1:1) complex (C), artemether-2-HP- $\beta$ -CD (1:2) complex (D) and artemether-2-HP- $\beta$ -CD (1:3) complex (E)

In the FT-IR spectra of 1:1 M inclusion complex, the bands of the pure drug due to O-H bending

and C-O bending vibration were shifted to higher frequencies of 3396 and 1037.74  $\text{cm}^{-1}$  respectively while those due to C-H stretching and C-H bending were shifted to lower frequencies 2930.93 and 1373.36  $\text{cm}^{-1}$  with marked decrease in intensity. In 1:2 M molar inclusion spectra the decrease in peak intensity was more compared to what was observed in the 1:1 M inclusion complex. In addition, peaks at 3198.08 and 1020.38  $\text{cm}^{-1}$  were shifted to higher frequencies and appeared at 3424 and 1043.32  $\text{cm}^{-1}$  respectively. Those at 2932 and 1449.55  $\text{cm}^{-1}$  were shifted to lower frequencies and appeared at 2928.04 and 1368.54  $\text{cm}^{-1}$  respectively. In addition, there was a disappearance of bands at 4594.59, 4241.61, 4066.08 and 3862.58  $\text{cm}^{-1}$ .

For the 1:3 M inclusion complex spectra, peaks at 3198.08 and 1020.38  $\text{cm}^{-1}$  were equally shifted to higher frequencies while those at 2932.86 and 1449.55  $\text{cm}^{-1}$  were shifted to a lower frequency.

### Tablet properties

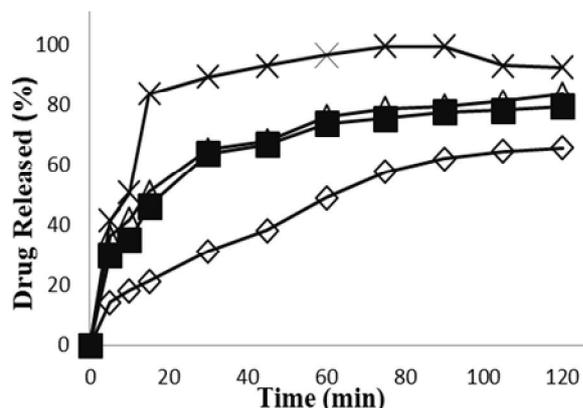
The properties for the commercial and prepared tablets of cyclodextrin-artemether inclusion complex are given in Table 1.

For the prepared inclusion complex tablet; the crushing strength was in the range of 10.0 – 10.5 kg/F, the percentage friability was 0.048 which is less than 1 % (fulfilling the official requirements), the drug content was 95.5 % and the disintegration time was 9.28 min which is within Pharmacopoeia limits (15 min for uncoated tablets), [16]. The mean weight of 402.85 mg was within the Pharmacopoeia limits, which specifies that for tablets weight > 250 mg,  $\pm 5$  % weight variation is allowed [16]. The inclusion complex tablets prepared, therefore, fall within the limit considering the 400 mg theoretical weight.

**Table 1:** Tablet characteristics of commercial and formulated artemether tablets (mean  $\pm$  SEM)

Tablet property	Brand A	Brand B	Artemether-2-HP- $\beta$ -CD complex (1:1) tablet	P-value
Thickness (mm)	3.22 $\pm$ 0.01	3.52 $\pm$ 0.01	3.54 $\pm$ 0.02	0.922
Uniformity of weight (mg)	240.65 $\pm$ 0.33	344.95 $\pm$ 5.17	402.85 $\pm$ 1.39	0.774
Diameter (mm)	8.88 $\pm$ 0.02	10.09 $\pm$ 0.03	12.07 $\pm$ 0.02	0.612
Crushing strength (kg/F)	11.7 $\pm$ 0.12	7.40 $\pm$ 0.40	10.90 $\pm$ 0.20	0.193
Drug content (%)	98.5 $\pm$ 0.00	97.00 $\pm$ 0.03	95.50 $\pm$ 0.07	0.917
Disintegration time (Min)	3.37 $\pm$ 0.03	3.30 $\pm$ 0.25	9.28 $\pm$ 0.04	0.003**
Friability (%)	0.048 $\pm$ 0.000	0.083 $\pm$ 0.001	0.058 $\pm$ 0.001	0.074

\*\* indicates significance at 5% level ( $P < 0.05$ )



**Figure 3:** Dissolution profiles of pure artemether (◇), brand A (Δ), brand B (■) and inclusion complex (1:1) tablet (X) in SGF, pH 1.2

### Dissolution

The dissolution profile of the inclusion complex (1:1 M) tablet, marketed tablets (brands A and B) and the pure drug sample in simulated gastric fluid, pH 1.2 are shown in Figure 3.

## DISCUSSION

The phase solubility diagram showed that the solubility of artemether increased as the concentration of 2-hydroxypropyl-β-cyclodextrins increased. It further shows that each complex contains only one molecule of the complexing agent. Because the linear host-guest correlation obtained had a slope less than one (1) it suggest that the stoichiometry of the complexes is 1:1 (mol/mol) artemether-2-HP-β-CD [17].

The value of the stability constant ( $K_c$ ) falls within the range of 50-2000  $M^{-1}$  suggesting a favorable interaction that may contribute to the improved dissolution of hydrophobic drugs occurred [17,18]. It has been reported that when a guest molecule is incorporated in CD cavity, its physicochemical properties are usually modified [3,19,20].

Earlier reports have shown that inclusion of drugs into cyclodextrin temporarily locked or caged the drug within the host cavity, giving rise to beneficial modifications like solubility enhancement, stabilization of labile guest molecule against oxidation, visible or UV light, heat and so forth [21]. These observations, therefore, clearly indicate strong evidence of inclusion of the drug into the cyclodextrin cavity. All inclusion complexes (1:1, 1:2 and 1:3 M) exhibited pronounced enhancement with comparable values of dissolution rate; therefore, inclusion complex of molar ratio 1:1 was used for the preparation of tablets since it contains lesser

amount of cyclodextrin than complexes of molar ratios 1:2 and 1:3. The prepared tablets were of good properties and did not exhibit chipping, capping or sticking. The tested commercial tablets equally conformed to the compendial specifications for uniformity of weight, percentage friability and disintegration time.

The dissolution rates of the inclusion complex tablets (from inclusion complex of 1:1 M ratio) were found to be higher than that of the commercial brands. Tablets formulated from the inclusion complexes exhibited 3.9-fold, 1.8-fold, and 1.6-fold increases, respectively over the pure sample and the commercial brands A and B in the amount of drug released in 15 min. The amount of artemether dissolved after 45 min ( $t_{45}$ ) from inclusion complex tablets was 93.1 %, while corresponding values of 68 and 67 %, respectively, were dissolved from A and B. The increased dissolution rate of artemether - cyclodextrin inclusion complexes may be attributable to improvement in drug wettability, decrease in drug crystallinity, increase in hydrophilicity or a reduction in the interfacial tension between the drug and the dissolution medium due to the surfactant-like properties of CDs [22].

## CONCLUSION

Molecular inclusion complex formation between 2-HP-β-CD and artemether has been confirmed with the inclusion complex displaying enhanced solubility of artemether in distilled water. Tablets formulated from 1:1 M ratio of the artemether-2-HP-β-CD complex have higher dissolution rate than the selected commercial artemether tablets. Thus, the developed artemether/2-HP-β-CD complex can be further explored to develop artemisinin-combination therapies with enhanced bioavailability for more effective treatment of malaria.

## DECLARATIONS

### Acknowledgement

The authors are thankful to the management of Afrab Chem Nigeria Ltd for the kind donation of pure samples of artemether.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by

the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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