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STABILITY AND DISSOLUTION BEHAVIOUR OF CYCLODEXTRIN MOLECULAR INCLUSION COMPLEXES OF ARTEMETHER

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ABSTRACT

This study aimed to assess the effect of inclusion complexation of artemether by 2-Hydroxypropyl-β-cyclodextrin on the dissolution and stabilization of the drug against degradation due to exposure to heat. Inclusion complexes of artemether with 2-Hydroxypropyl-β-cyclodextrin of molar ratios 1:1, 1:2 and 1:3 were prepared using the kneading method. Complexes were evaluated for dissolution rate via *in-vitro* dissolution studies using the Erweka dissolution apparatus at 37 ± 0.5^{o} C in simulated intestinal fluid without pancreatin and simulated gastric fluid without pepsin and compared with a commercial product. Stability tests were carried out on the complexes at room temperature for 10 weeks and at 50^{o} C for 72 h to accelerate the possible degradation of artemether in the presence of 2-Hydroxypropyl-β-cyclodextrin. The complexes exhibited higher and faster dissolution rate than the pure drug and a marketed product. The extent of dissolution enhancement varied with the host-guest molar ratios in the following order 1:3 M > 1:2 M > 1:1 M. However, the differences in dissolution between the pure drug and the inclusion complexes were found to be significant ($p < 0.05$). In simulated intestinal fluid without pancreatin, the prepared inclusion complexes yielded drug release profiles comparable and even slightly better than a commercial parenteral formulation. Stability data showed no significant differences in physical appearance and drug content over a reasonable period

indicating good stability of the formulation. We conclude that inclusion complexation of artemether with 2-Hydroxypropyl-β-cyclodextrin is a promising alternative to enhance the stability and dissolution rate of the drug.

KEYWORDS: Artemether, 2-hydroxypropyl-β-Cyclodextrin, inclusion complex, dissolution, stability.

INTRODUCTION

Cyclodextrins (CDs) can be considered as empty capsules of certain molecular size. They are cyclic oligosaccharides produced from the degradation of starch by cyclodextrin gluconotransferase (CGTase). The commercially successful CDs are α, β and γ-CDs which consist of 6,7 and 8 glucose molecules respectively.[1,2] CDs are able to form "inclusion complex" with a wide variety of hydrophobic guest molecules, this is crucial in their successful applications. The resulting complex hides most of the hydrophobic functionality in the interior cavity of the cyclodextrin while the hydrophilic hydroxyl groups on its external surface remain exposed to the environment.[3] One of the molecules referred to as the "host," includes, totally or partly, the hydrophobic guest molecule by physical forces.[2] Figure 1 gives a schematic illustration.

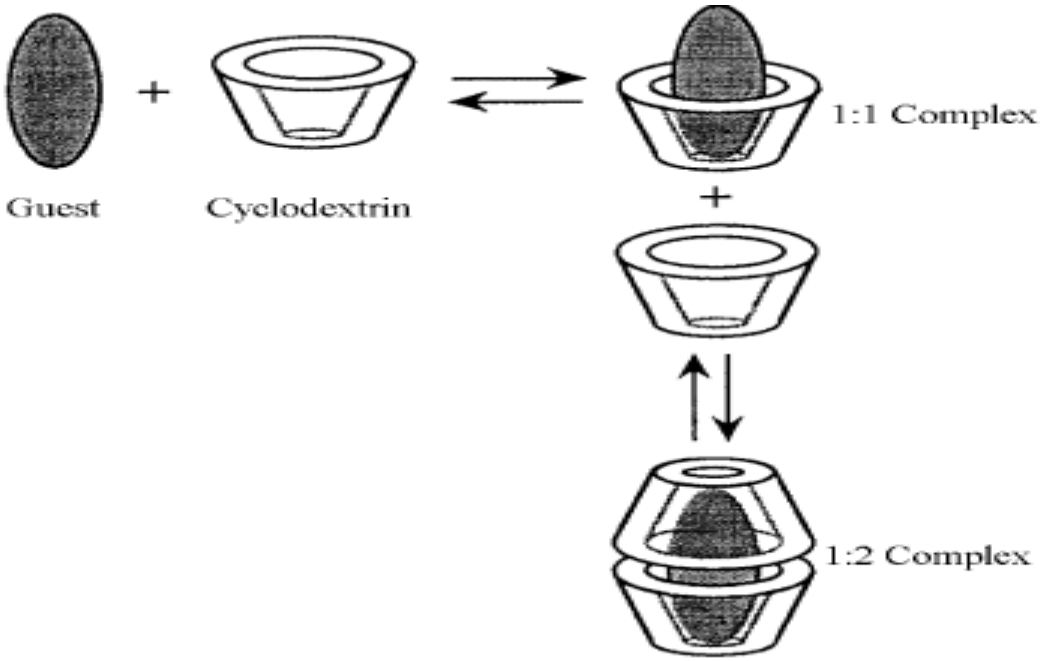

Figure 1: Scheme illustrating equilibrium binding of gust and CD to form a 1:1 complex and a 1:1 complex with a second molecule of CD to form a 1:2 complex.[2]

Inclusion complexation is a molecular phenomenon where a molecule of guest and a molecule of CD combined to form a complex.[2,4] Inclusion complexation with CD has been used to increased solubility of the guest; stabilization of the guest to prevent volatilization, oxidation and degradation due to exposure to light and heat; elimination or reduction of undesired taste or odors.[5,6,7] Complexation is mediated primarily by the following.[8, 9]

- Substitution of energetically unfavored polar-polar interactions (between the included water and the CD cavity one hand and between water and the guest on the other) by the more favored apolar-apolar interaction (between the guest and the cavity) and the polar-polar interaction (between bulk water and the released cavity-water molecules).
- CD-ring strain release on complexation.
- Van der Waals interactions and hydrogen bonds between host and guest.

The bond between the guest and the host is non-covalent. A variety of non-covalent forces like van der Waals forces, hydrogen bonding, dipole-dipole interaction, London dispersion forces and other hydrophobic interactions are responsible for the formation of a stable complex.[3] Artemether is a derivative of artemisinin which is highly potent and schizonticidal. It is classified as a class II drug in the Biopharmaceutical Classification System (BCS); poor solubility in water and low bioavailability when administered orally. This considerably hampers its therapeutic potential and limits its efficacy.[10,11] To overcome these problems, various formulation strategies are reported in the literature including the use of micro emulsions.[11] Inclusion complex formation with methyl-β-cyclodextrin.[12] and co-administration with fatty meals.[13] The drug is also sensitive to light and heat, hence the need for it to be kept in a tightly closed container and protected from light and heat.[14]

In this work, we studied the effect of inclusion complexation of artemether by 2-Hydroxyl propyl-β-CD (2-HP-β-CD) on the dissolution and stabilization of the drug against degradation due to exposure to heat. The preparation and characterization of the inclusion complexes used in this study have earlier been evaluated.[15]

MATERIALS AND METHOD

2-Hydroxypropyl-β-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. Sodium Chloride (BDH Chemicals Ltd Poole-England), Monobasic potassium phosphate (Sigma chemical Co.,

USA), Sodium hydroxide pellets (Avondale Laboratory Ltd Banbury, England), Hydrochloric acid (May and Baker, Lagos- Nigeria), DRUTEMAL® injection; Batch no. HJ110801, Manufac. date Aug. 2013, Expiry date Aug. 2016, NAFDAC Reg. No. A4-3232, Drugfield Pharmaceutical Ltd, Nigeria. All other reagents (chemicals and solvents) were of analytical reagent grade and used without further purification.

Preparation of artemether inclusion complexes

The inclusion complexes of artemether with 2-hydroxypropyl-β-cyclodextrin were prepared at stoichiometric molar ratios of 1:1, 1:2 and 1:3 using the kneading method as earlier reported.[15]

Physical appearance assessment and drug content estimation

A quantity of the complex equivalent to 50 mg of artemether was accurately weighed and dissolved in sufficient quantity of dehydrated ethanol to produce 100 mL. Two milliliters of this solution was derivatized by treating with 1 M ethanolic hydrochloride and heated at 60^{o} C for 3 h, then assayed spectrophotometrically at λmax of 254 nm. The color and texture of the formed complexes were also examined.

***In vitro* dissolution studies for artemether-HP-β-CD complexes**

Dissolution rates in simulated gastric fluid (SGF) without pepsin (pH 1.2) and simulated intestinal fluid (SIF) without pancreatin (pH 7.4) were evaluated using the Erweka dissolution apparatus in 500 mL of medium at 37 ± 0.5°C. In each case, inclusion-complex or pure artemether equivalent to 40 mg of artemether was transferred into a dialyzing membrane, securely tied at both ends and placed in the dry basket with the apparatus being set to a rotational speed of 50 rpm. Five-milliliter 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. The volume withdrawn at each time interval was replaced with a fresh quantity of dissolution medium. Two milliliters of the filtrate was derivatized by treating with 1 M ethanolic hydrochloride and heated at 60^{o} C for 3 h, then analyzed spectrophotometrically at λmax of 254 nm. The amount of artemether released (percent) was calculated and plotted against time.

The dissolution rate of the complexes in SIF without pancreatin (pH 7.4) was compared with that of a commercial parenteral product; an 80 mg/mL archis oil intramuscular preparation of artemether.

Stability Studies

Stability studies of the artemether complexes were carried out at different temperature conditions such as room temperature for 10 weeks and at 50^{o} C to accelerate the possible degradation of artemether in the presence of 2-hydroxypropyl-β-cyclodextrin for 72 h.

The inclusion complex samples of the artemether complexes were stored at room temperature for 10 weeks. Formulation equivalent to 50 mg artemether were withdrawn at the end (after 10 weeks), weighed accurately and dissolved in sufficient dehydrated ethanol to produce 100 mL. Two milliliters of the filtrate was derivatized by treating with 1 M ethanolic hydrochloride and heated at 60^{o} C for 3 h, then analyzed spectrophotometrically at λmax of 254 nm. The same treatment was given to samples stored at 50^{o} C for 72 h.

Statistical Analysis

The results generated from the various determinations were expressed as mean ± standard error of mean. The differences between the data sets were determined using one-way analysis of variance (ANOVA) and ρ value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Physical appearance assessment and drug content estimation

The results of physical appearance, texture and drug content of the prepared inclusion complexes are given in Table 1.

Table 1: Color, Texture and Drug content of formed Complexes.

Drug to carrier ratio	Color	Texture	Drug content (%)
1:1 M complex	White	Fine and non-sticky	87.5
1:2 M complex	White	Fine and non-sticky	87
1:3 M complex	White	Fine and non-sticky	86

All the prepared inclusion complexes were white, without any stickiness and were free-flowing. The drug content of all inclusion complexes was in the range of 86% - 87.5%. This indicates that the drug was present in the inclusion complexes in substantial amounts and further lends credence to the effectiveness of kneading method in inclusion complex formation.[16]

Dissolution studies

The dissolution profiles for the systems under study are presented in Figures 2 and 3, while the drug release parameters at some specific time intervals are given in Tables 2 and 3.

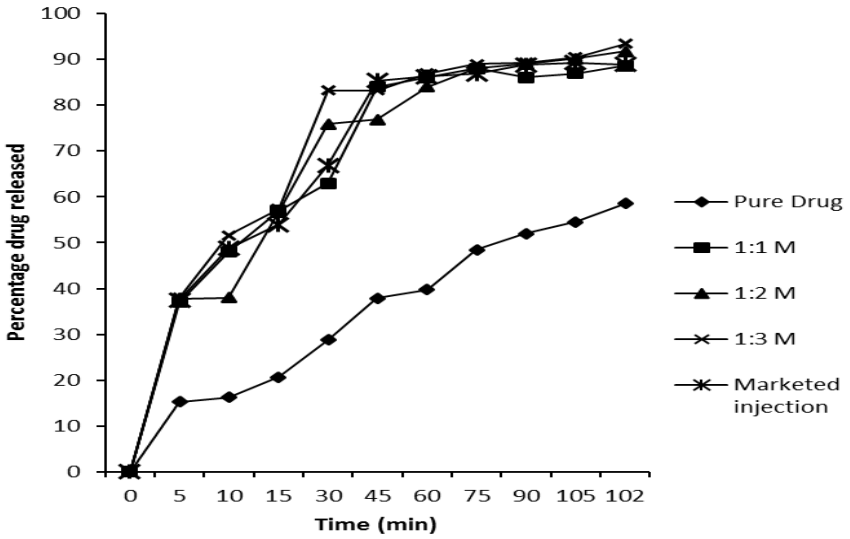

Figure 2: Dissolution profile of pure artemether, artemether-2-hydroxypropyl-β-CD complexes and a commercial parenteral product in simulated intestinal fluid without pancreatin (pH 7.4).

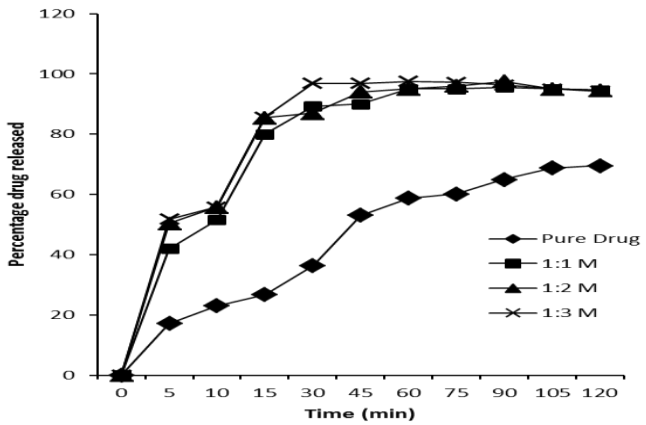

Figure 3: Dissolution profile of pure artemether and artemether-2-hydroxypropyl-β-CD complexes in simulated gastric fluid without pepsin (pH 1.2).

Table 2: Parameters for the dissolution profile of pure artemether, 2-hydroxypropyl-β-cyclodextrin inclusion complexes of artemether and a marketed parenteral product in simulated intestinal fluid without pancreatin (pH 7.4).

Parameter	Pure drug	1:1 M complex	1:2 M complex	1:3 M complex	Marketed injection
D.R_{15}min (%)	16.3	57.0	56.7	57.4	54
D.R_{45}min (%)	37.9	84	76.9	83.2	85.4
$t_{50\%}$ (min)	80	11.3	12.5	10	11.3
$t_{90\%}$ (min)	>120	>120	104	97.5	>120

D.R: Drug release

Table 3: Parameters for the dissolution profile of pure artemether, 2-hydroxypropyl-β-cyclodextrin inclusion complexes of artemether of (molar ratio 1:1 M, 1:2 M and 1:3 M) in simulated gastric fluid without pepsin (pH 1.2).

Parameter	Pure drug	1:1 M complex	1:2 M complex	1:3 M complex
D.R_{15} min (%)	26.8	80	85.4	87.5
D.R_{45} min (%)	53.1	90	94.0	96.9
$t_{50\%}$ (min)	39.5	8.5	4.8	4.0
$t_{90\%}$ (min)	>120	45	36.8	21

D.R: Drug release

Dissolution of the drug from oral solid dosage forms is a necessary criterion for drug bioavailability (that is, the drug must be solubilized in the aqueous environment of the gastrointestinal tract to be absorbed). For this reason, dissolution testing of drug products has emerged as one of the most important control tests.[17] The dissolution studies of a marketed parenteral product in comparison with all the batches of the inclusion complexes was carried out in SIF without pancreatin pH 7.4 to simulate the tissue fluid and blood.

The release of artemether from both the pure drug and inclusion complexes was found to be higher in SGF without pepsin, pH 1.2 than in SIF without pancreatin, pH 7.4. This is in agreement with literature report that release of artemether from products is higher in simulated gastric fluid and food modified simulated gastric fluid than in simulated intestinal fluid and food modified simulated intestinal fluid.[18] All systems with cyclodextrin exhibited better dissolution properties than the pure drug alone. The calculated dissolution parameters revealed that pure artemether exhibited a slow initial dissolution rate with 69.5% of the drug being released after 120 min. There was a marked enhancement of dissolution of artemether from the artemether-2-HP-β-CD complex when compared with the dissolution of artemether from the pure sample. The extent of this dissolution enhancement varied with the host-guest

molar ratios. Although the ranking of dissolution rate was in the order 1:3 M > 1:2 M > 1:1 M, all dissolution rate values obtained were comparable. However, the differences in dissolution between the pure drug and the inclusion complexes were found to be significant ($p < 0.05$).

It is equally discernible from Fig. 2 that the prepared inclusion complexes yielded drug release profiles comparable and even slightly better than a commercial parenteral formulation. While the pure artemether gave a percentage drug release of 58.6% in SIF without pancreatin after 120 min, inclusion complexes released 88.6%, 91.8% and 93.4% respectively for molar ratios of 1:1, 1:2 and 1:3 while the commercial parenteral formulation released of 88.9% of artemether after 120 min.

The increased dissolution rate of artemether - cyclodextrin inclusion complexes has been attributed to improvement in drug wettability, a decrease of drug crystallinity, formation of readily soluble complexes in dissolution medium, 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.[2,9,19]

Stability studies

There were no significant differences ($\rho = 0.999$) for the accelerated stability testing and $\rho = 0.960$ (for the short-term stability testing) observed in the color, texture and drug content of the complexes after the stability testing as shown in Tables 4 and 5.

Table 4: Results of short-term stability studies of the inclusion complexes and the formulated tablet at room temperature (28-30^{O}C).

Sample	Color		Percent drug content at room temperature (28-30)^{O} C	
	Day one (1)	After 10th week	Day one (1)	After 10th week
1:1 M complex	White	white	87.50	87.30
1:2 M complex	White	white	87.00	86.96
1:3 M complex	White	white	86.00	86.00
t-test df = 6 T-calculated = 0.053 T- tabulated =1.872 ρ-value = 0.960				

Table 5: Results of accelerated stability studies of the inclusion complexes and the formulated tablet of artemether at 50^{O} C.

Sample	Color				Percentage drug content at 50^{O} C upon storage			
	HOURS				HOURS			
	0	24	48	72	0	24	48	72
1:1 M complex	white	White	white	White	87.5	87.5	87.3	87.2
1:2 M complex	white	White	white	White	87.0	86.8	86.8	86.7
1:3 M complex	white	White	white	White	86.0	86.0	85.8	85.6
ANOVA: df = (3,12)		F-Calculated = 0.005			F-tabulated = 3.89		ρ = 0.999	

This result suggests that inclusion of artemether into 2-HP-β-CD cavity conferred a high level of stability not only on the complexed drug but on the complex as a whole. These imply that the formulations are stable. Since the guest is surrounded by cyclodextrin after inclusion complex formation, there is no crystalline guest structure to absorb energy leading to stabilization of the guest to degradation due to exposure to light and heat.[2,20] This is consistent with our finding as earlier reported that using differential scanning calorimetry, no energy absorption was observed at the melting temperature of artemether after inclusion complex formation with 2-HP-β-CD.[15] It further corroborates other early reports.[2,5]

CONCLUSION

The stability and dissolution behavior of inclusion complexes of artemether with 2-Hydroxypropyl-β-cyclodextrin were investigated. Results showed that 2-Hydroxypropyl-β-cyclodextrin could enhance the dissolution rate and stability of artemether. Inclusion complexes of artemether with 2-Hydroxypropyl-β-cyclodextrin could, therefore, prove useful in the design of novel medicinal artemether formulations to confront the scourge of malaria.

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