



Effect of cyclodextrins on artemisinin stability and *in vitro* dissolution

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ABSTRACT

Artemisinin is extracted from the Asian plant *Artemisia annua L.* It has been proven to be, with its derivatives, the molecules that have shown so far the most powerful activity on malaria, even in its complicated forms and resistance cases. To resolve the problem of low aqueous solubility of this molecule, the use of cyclodextrins (CDs) was envisaged and gave good results in this topic. However, the impact on its stability and *in vitro* dissolution, important parameters for drug formulation and evaluation, was not evaluated. In this study, stability in accelerated degradation conditions and *in vitro* dissolution analysis of artemisinin were performed in comparison with complexes of artemisinin and cyclodextrins. The findings of this work allowed us to suggest that CDs improve the stability of artemisinin, especially when it's in aqueous solution. We also demonstrated the beneficial impact of cyclodextrins on the *in vitro* dissolution of artemisinin from capsules prepared with rough extracts of *A. annua*.

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Keywords: *Artemisia annua* capsules, malaria, lyoequivalence, dissolution apparatus, storage conditions.

INTRODUCTION

Malaria, a parasitic disease, is still a problem of public health in more than hundred countries. This represents about 2.4 billion people and 40% of the world population. The sub-Saharan African countries are particularly affected. Against this terrible infection,

artemisinin and its derivatives have been proved to be the most effective drugs, as well in the simple forms as in complicated ones and also effective in the chloroquino-resistant forms (Ferreira and Janick, 1995; White, 1999; Nosten et al., 2000). Thus they are recommended by WHO since 2001 for

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malaria treatment in Artemisinin Combined Therapy (ACT). However, the low aqueous solubility and high first-pass metabolism of artemisinin involve a poor and variable bioavailability (Titulaer et al., 1990). This may create problems of recrudescence and resistance at long-term. So, improving aqueous solubility of artemisinin may help to better bioavailability and cyclodextrins can be used for this purpose.

Cyclodextrins (CDs) are cyclic oligosaccharides made of six, seven, or eight (for α -, β - and γ -cyclodextrin, respectively) glucose units bound by 1,4-ether linkages containing a relatively hydrophobic central cavity and hydrophilic outer surface. By exchange with the water molecules present in their cavity in the crystalline state, cyclodextrins are able to include large organic molecules by noncovalent interactions forces (Frömring and Szejtli, 1994) leading to an increase of aqueous solubility and stability of these molecules (Loftsson and Brewster, 1996). However, this phenomenon is not always verified for all the substances; in fact the complexation ability depends on the geometry of the guest molecule inside the CD cavity and also its charge and polarity criteria (Frömring and Szejtli, 1994).

The interactions between artemisinin and different CDs was previously studied (Zime-Diawara et al., 2012) and it was concluded that there is no real inclusion. Artemisinin formed with the tested CDs non-inclusion complexes. In view of using these complexes in pharmaceutical preparations, one must know if this kind of complexes also influences stability of the molecule and its *in vitro* dissolution capacities.

In this study, we investigated the influence of artemisinin complexation with CDs on its stability and *in vitro* dissolution. The case of Crystmab, a new generation of

methylated β -cyclodextrin with a low degree of substitution (0.4 – 0.7), was particularly analysed.

MATERIALS AND METHODS

Materials

Standard artemisinin (ARTE, 98%) was purchased from Sigma-Aldrich (Darmstadt, Germany).

β -Cyclodextrin (β CD, 12.76% H₂O) was provided from Wacker-Chemie GmbH (Munich, Germany). Kleptose® Crystmab (Crystmab, D.S. , 3.99% H₂O) was obtained from Roquette Frères (Lestrem, France).

The seedlings of *Artemisia annua* used in this study were provided by the Laboratory of Applied Ecology of the Agronomic Faculty of Sciences of Abomey Calavi University in Benin. In fact, this Asian plant was acclimatized and set in culture in Benin. The seeds were obtained from Anamed (Winnenden, Germany).

Ethanol (Ph Eur 96%) was acquired at VWR (Fontenay-sous-bois, France). Methanol was HPLC-Grade and was purchased, with KOH from VWR. All the other reagents (monopotassic and dipotassic phosphate and phosphoric acid 98%) were of analytical grade and acquired at Merck (Darmstadt, Germany) and Alfa Aesar (Karlsruhe, Germany), respectively.

Methods

Preparation of Cds-Arte Complexes

Excess amount of artemisinin were added to aqueous cyclodextrins solutions: β CD (10 mM) and Crystmab (50 mM). The flasks were then shaken continuously in a thermostatically controlled water bath set at 37 °C for 48 hours. This equilibrium time was determined experimentally by testing different times from 12 to 96 hours. All the conditions were realized in triplicate. After equilibrium

was attained, suspensions from the flasks were filtered through 0.45 μm PVDF membrane. After this step of inclusion, the mixtures were filtered and the resulting filtrates were freeze-dried to obtain ARTE- β CD and ARTE-Crysmeb complexes powders.

Characterization of Cds-Arte Complexes

This step was realized by Differential Scanning Calorimetry analysis (DSC) using a Mettler Toledo Star apparatus.

We examined different powders: artemisinin only, CDs only, complexes of artemisinin/CDs and physical mixtures of artemisinin with the CDs. All the samples were freeze-dried before the analysis. The temperature range went from 35 $^{\circ}\text{C}$ to 200 $^{\circ}\text{C}$ with a speed of 10 $^{\circ}\text{C}/\text{min}$.

The physical mixtures were prepared by mixing freeze dried artemisinin powder and freeze dried CDs powder in a mortar in the same proportions as in the complexes powders.

Stability studies

Stability studies were done at solid and liquid states on ARTE only, ARTE- β CD and ARTE-Crysmeb complexes. The conditions applied were a temperature of 40 ± 2 $^{\circ}\text{C}$ and relative humidity of 75 ± 5 during 2 months. These conditions correspond to subtropical area with possibilities of high humidity according to ICH classification.

For study in solid state, powder of the 3 compounds stored in the accelerated degradation conditions were weighted at each time of quantification and put in solution before prederivatization and analyze by HPLC.

For study in liquid state, solutions of the 3 compounds were realized at the beginning of the work (J1). These preparations were done with sterilized material under laminar flow cabinet and conditioned in several individual 1.5 mL –

vials to avoid microbial proliferation. These vials were stored at the conditions already specified.

Periodic collections were realized over 2 months (days 1, 3, 7, 14, 30, 45 and 60) for HPLC quantification.

In vitro dissolution studies

This study enters in a general topic of using *Artemisia annua* rough extracts to prepare antimalaric treatments. In fact, extracts of this plant can be obtained by non complicated operations and they contain other compounds which can be interesting in malaria treatment (with antipyretic properties, potentiation of artemisinin's antimalaric activity) (Elford et al., 1987).

So capsules were made with hydro alcoholic extracts of *Artemisia annua* (called capsules A). After that, we also made capsules containing this extract added to Arte- β CD and Arte-Crysmeb complexes respectively (called capsules B and C). The total artemisinin content was equal in these three kinds of capsules (approximately 8 mg/capsule).

The *in vitro* dissolution of the 3 kinds of capsules A, B and C was determined by using the paddle method (ERWEKA type DT6-K, Germany) in 500 mL of HCl (0.001 M), under sink conditions. The paddle rotation speed was set at 100 rpm. The temperature was maintained at 37 ± 0.5 $^{\circ}\text{C}$. Samples of 20 mL were collected at 15, 30, 45 and 60 minutes and were directly filtered. The filtrates were derivatized (as described below) and then quantified by HPLC.

Quantification of artemisinin

UV detection of artemisinin is not easy, due to the lack of chromophores. So, it must be pre- or post- derivatized. We used in this study prederivatization inspired from Zhao and Zeng (1985) method which has been modified and validated in presence of CDs (Zime-Diawara et al., 2011).

For the prederivatization, one ml of sample (extract solution or standard solution) was transferred into a 10 ml measuring flask. Four ml of 0.2% (m/v) NaOH solution was added in the flask, and then left to react at 50 °C for 30 min. After cooling for 10 min, 1 ml of ethanol was added. Finally the flask was filled with acetic acid 0.2 N.

All solutions were filtered on a PTFE 0.45 µm membrane before HPLC analysis. The HPLC apparatus used for artemisinin quantification was a Hitachi Alliance from

VWR with LaChrom Elite software for data acquisition.

Chromatographic separation was performed with a reversed phase RP-18 LiChroCART column (250 mm × 4 mm I.D.; particle size: 5 µm). Mobile phase consisted in a mixture of methanol and phosphate buffer (0,005 M; pH: 6.3) (45/55, v/v). A flow rate of 1 ml/min and detection at 260 nm were used. The column was maintained at 35 °C and the injection volume was of 20 µl.

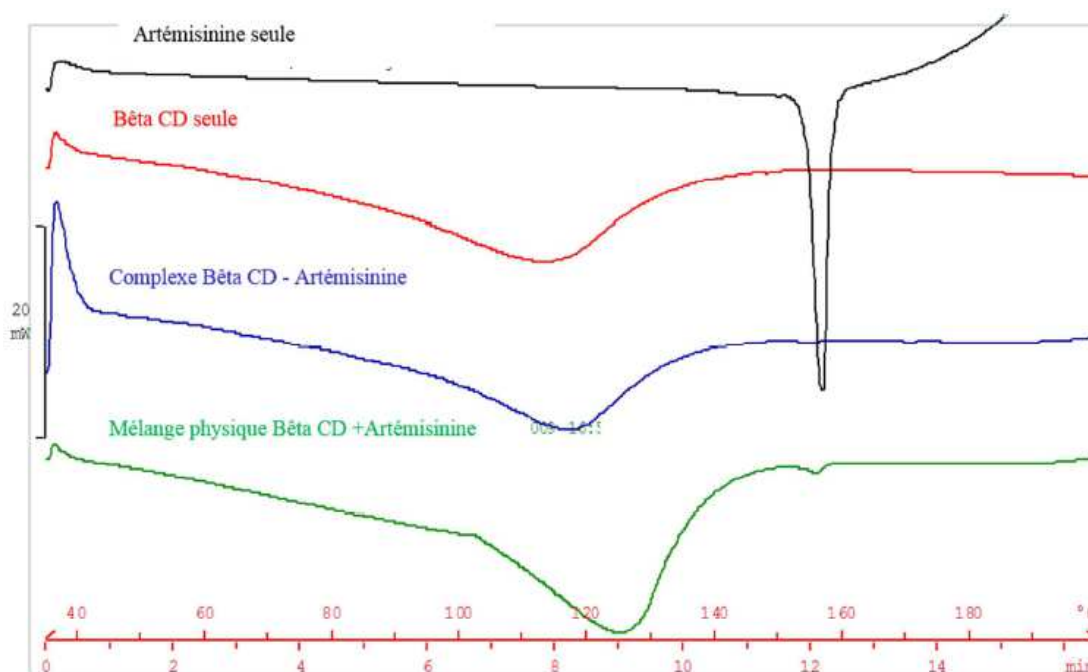


Figure 1: Thermograms of artemisinin only (in black), β -CD only (in red), complex of artemisinin with β -CD (in blue) and physical mixture of artemisinin and β -CD (in green); up to down.

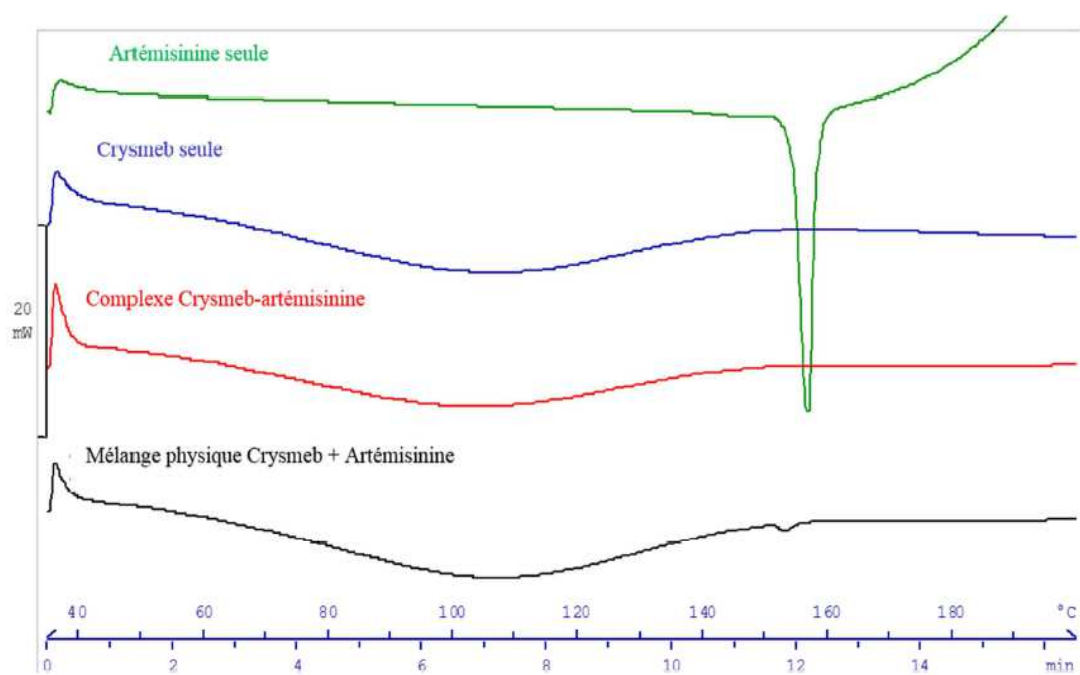


Figure 2: Thermograms of artemisinin only (in green), Crysmeb only (in blue), complex of artemisinin with Crysmeb (in red) and physical mixture of artemisinin and Crysmeb (in black); up to down.

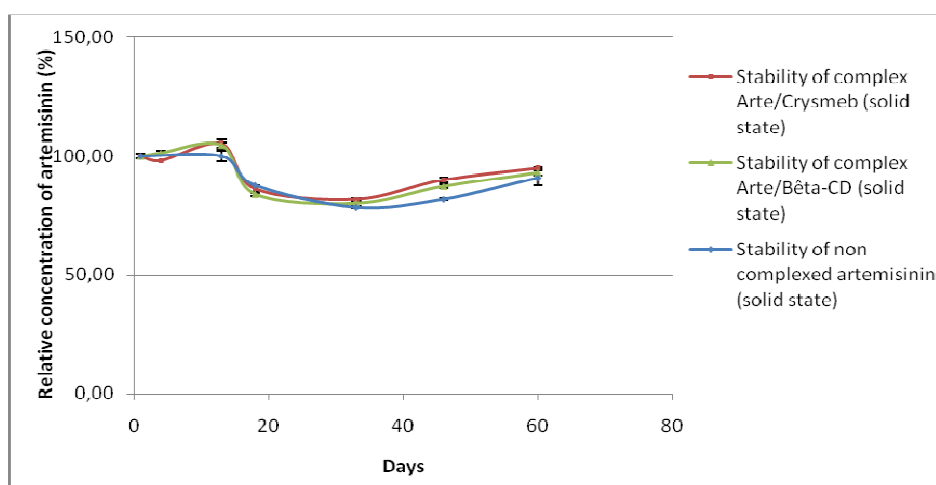


Figure 3: Compared stability in solid state of non complexed artemisinin (down), complexed with β -CD (middle) and Crysmeb (up).

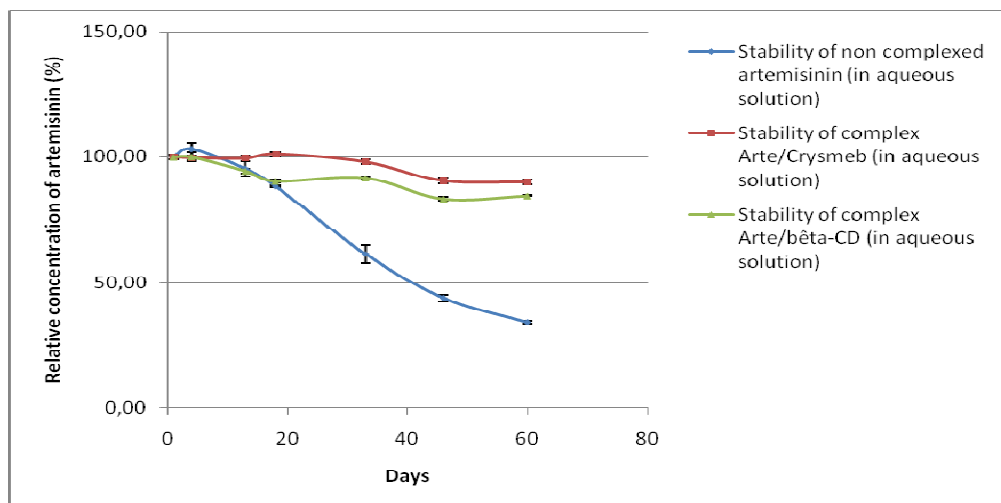


Figure 4: Compared stability in aqueous solution of non complexed artemisinin (down), complexed with β -CD (middle) and Crysmeb (up).

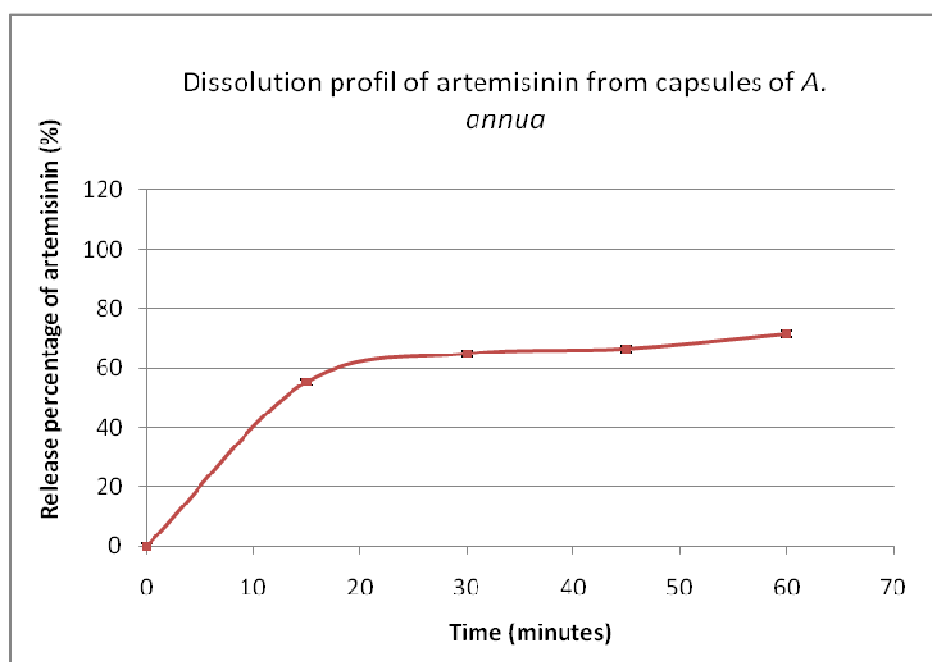


Figure 5: Dissolution profil of artemisinin from capsules A (*A.annua* only). N=6.

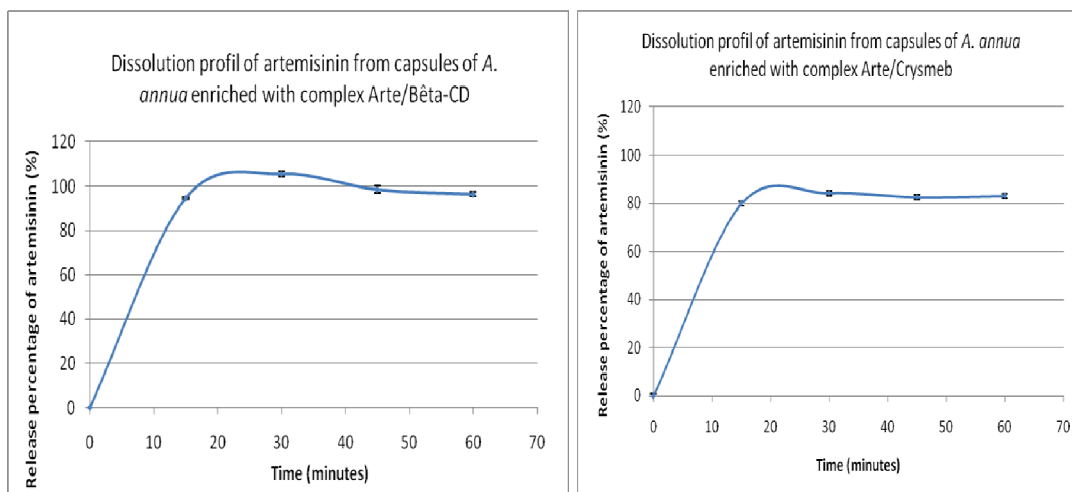


Figure 6: Dissolution profil of artemisinin from capsules B (*A. annua* enriched with β CD/Arte complex on left) and from capsules C (*A. annua* enriched with Crysmeb/Arte complex on right). N=6 in each case.

RESULTS AND DISCUSSION

DSC analyses

Figures 1 and 2 show the thermograms obtained with β -CD and Crysmeb respectively. Each figure represents four thermograms: artemisinin only, CD only, physical mixture of artemisinin and the CD and complex of artemisinin with the CD.

Artemisinin showed a melting point at 153 °C. This value is close to that reported by Jinadasa (1996) for the orthorhombic polymorphic form of the drug.

We can observe the disappearance of the peak of artemisinin on the thermogram of the complexes, which is the proof of the transformation of crystalline form in amorphous form. So, we have effective formation of complexes in this powder. At the difference, in the physical mixtures, the presence of the two forms (amorphous and crystalline) is observed.

The powders of complexes prepared are thus homogeneous and don't contain any more non-complexed artemisinin.

As it has been proved that there is no real inclusion of artemisinin in CDs (Zime-Diawara et al., 2012), this analysis comes to confirm that the DSC analysis can't be use to conclude on an inclusion or not. It is just an element to confirm or invalidate complexation of CDs with a molecule.

Effect of cyclodextrins on artemisinin stability

In solid state (Figure 3), complexation with CDs seemed to have no significant effect on stability of artemisinin after a sixty days observation with storage in conditions of accelerate degradation previously described. In fact, it remains 90% of non complexed artemisinin compared to 93% and 95% when artemisinin is complexed with β -CD and Crysmeb respectively.

However, in aqueous solution (Figure 4), we can note an important degradation (66%) for non complexed artemisinin while β CD/Arte and Crysmeb/Arte complexes showed moderate degradation (15% and 10% for β -CD and Crysmeb respectively) after

sixty days storage stress. This suggested an improvement of artemisinin stability in solution when complexed with CDs.

Ansari et al. (2009) have obtained the same results with dihydroartemisinin/HP β -CD complexes. The complexes showed a 40% increase in thermal stability (50 °C) and a 29-fold decrease in hydrolysis rates compared with dihydroartemisinin.

Moreover, similar results of increasing stability from complexation and/or inclusion affinity with CDs were obtained with astaxanthin, warfarin, camptothecin, and taxol (Chen et al., 2007; Dordunoo and Burt, 1996; Kang et al., 2002; Zingone and Rubessa, 2005).

A comparison of the two type of complexes (Figure 4) shows that the degradation is a little more marked with β CD/Arte complex than Crystmeb/Arte one. This could be explained by interactions more strong in Crystmeb/Arte complex than in β CD/Arte. The NMR results in the study of interactions of artemisinin with CDs (Zime-Diawara et al., 2012) corroborate this hypothesis.

In vitro dissolution studies

The solubility of artemisinin in water is reported as 10.6 mg/L (Mueller et al., 2000) and 8.6 mg/L (Usuda et al., 2000).

However, this solubility is improved when it is in *A. annua* extracts. In fact, Mueller et al. (2000) obtained 10.6 mg/L with 250 mg artemisinin in 1L water; while with 5g of *A. annua* containing 0.5% artemisinin (only 25 mg artemisinin), they found 12.0 mg/L. This increase in solubility (ten fold increasing) was attributed to other constituents in the plant (flavonoid, glucosides or saponins) which may help to dissolve the lipophilic plant compounds in an aqueous medium.

It has been proved that CDs increase the aqueous solubility of pure artemisinin (Usuda et al., 2000; Wong and Yuen, 2003;

Zime-Diawara et al., 2012). We can thus expect that the *in vitro* dissolution of artemisinin will be improved by these molecules. However, a verification of this phenomenon is necessary, especially in the present situation where artemisinin is in form of plant extracts.

Figures 5 and 6 show the dissolution profiles of artemisinin from the capsules A, B and C. We notice that the dissolution is better when artemisinin is complexed with a cyclodextrin. In fact, for the dissolution of artemisinin from capsules A, after 60 min, we reached 71% of solubilization (Figure 5) whereas with capsules B and C, we have 100 and 85% respectively (Figure 6).

The dissolution rate obtained with capsules of *A. annua* only (71%) is higher than that found by Hoa et al. (1996) for *in vitro* dissolution of pure artemisinin (60%). This confirm that the *in vitro* dissolution of artemisinin from preparations with extracts of *A. annua* is better than when it is in commercial preparations with pure artemisinin. The presence of the other constituents of the plant (flavonoids and other sesquiterpens) is thus important.

β -CD seems to have more impact than Crystmeb on artemisinin *in vitro* dissolution from *Artemisia annua* capsules. With β -CD, the dissolution is completed after 30 minutes. While with Crystmeb, the total dissolution is not reached but stabilization is obtained at about 80-85%. Thus, cyclodextrins increase the *in vitro* dissolution of artemisinin from *Artemisia* capsules and β -CD has a higher impact than Crystmeb.

Similar results were obtained for tablets of pure artemisinin (Wong and Yuen, 2003). In fact, they concluded that the tested complexes (α -CD/Arte, β -CD/Arte and γ -CD/Arte) showed an increased dissolution rate as compared with the commercial preparation of pure artemisinin.

So, the presence of the other components of the extracts doesn't hind the action of the CDs.

This improvement of the *in vitro* dissolution suggested a possible beneficial impact on the bioavailability of these preparations and also a better efficiency of the treatment.

Conclusion

This study shows that the complexation of artemisinin with cyclodextrins improves *in vitro* dissolution and makes it possible to obtain a better stability of artemisinin in aqueous medium. Crysmeb seems to have a light advantage compared to β -CD, in fact, the stability of capsules containing *A. annua* extract enriched with Crysmeb complex is better than those enriched with β -CD one . This is interesting for a development and distribution of galenic formulations in African countries. Even if its dissolution is lower than that of β -CD, it remains in conformity to the pharmacopeias recommendations.

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