School of Pharmacy

Degradation of Artesunate in Aqueous Solution

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Abstract

Artesunate, ART, is an antimalarial drug which is the only soluble artemisinin available on the market. ART has a low stability in aqueous solution. The degradation rate of ART in aqueous solution in a range of pH values (2.00-10.50) and selected IV fluids at 37 °C was studied. The temperature dependence was also investigated. High-performance liquid chromatography, HPLC, with detection at 210 nm was employed. There were significant effects on the rate degradation of ART according to the pH value employed and temperature. Shelf-lives of ART were 2.5, 1.2 and 1.0 h when reconstituted at 0.6 mg mL^{-1} in Hartman's solution, 0.9% normal saline, and 5% glucose IV fluid at 37 °C respectively. The pH-rate profile demonstrated three general regions: decreased rate with pH fall to pH 7.50 followed by an increased rate. There was a combination of specific acid-base and carboxylate anion catalysis. Buffers may have a small effect on the degradation rates. The effect of ionic strength was slightly significant on the rat degradation of ART. A comparison in the rate degradation of ART with HPLC and LC-MS showed no significant difference in measured degradation rates**.**

The effect of inclusion complexes of ART with hydroxypropyl-ß-cyclodextrin (HP-ß-CD) at selected pH values on the phase solubility profile and stability of ART in aqueous solution was studied. The phase solubility profile of the complex was classified as AL- type, indicating the formation of a 1:1 stoichiometric inclusion complex. A complex of ART with HP-ß-CD (272 mg mL^{-1}) showed a 25-fold increase in solubility compared to ART at pH 3.00. Lineweaver-Burk plots were used to calculate the stability constants of the inclusion complexes (K_{st}) as well as the rate constants for degradation of the free and complexed drug. The stability

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constants K_{st} of the inclusion complexes were 83, 73 and 60 M^{-1} at pH 6.00, 7.00 and 8.00 respectively. The activation energies (Ea) were obtained from Arrhenius plots of degradation rate constants in the presence and absence of HP-ß-CD. The thermodynamic parameters of activation enthalpy and entropy were obtained from Eyring Equation. The activation energies in the absence and presence of HP-ß-CD were 93.4 and 95.8 $Kjmol^{-1}$ respectively. The shelf-life of ART in the presence of HP-ß-CD was increased five-fold. The shelf-life of ART at the pH minimum (pH 6.50) was 10.6 h. Then it was improved in the presence of 108 mg mL^{-1} HP-ß-CD to 46 h.

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I thankfully acknowledge my wife Ghaydaa and for her endless patience and encouragement.

Finally all thanks for my Lord Allah for keeping me healthy, giving me strength and insistence to finish my work.

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: .....

Date: 8.104.1.2011

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LIST OF ABBREVIATIONS

Artesunat	ART
High performance liquid chromatography	HPLC
Liquid chromatography mass spectroscopy	LC–MS
Hydroxypropyl-ß-cyclodextrin	HP-ß-CD
Stability constant of the inclusion complexes	K_{st}
Mole	M
activation energies	Ea
World Health Organization	WHO
Mole/Litter	mol L^{-1}
Temperature in Kelvin	T
Temperature in centigrade	t
minute	min
Dihydroartemisinin	DHA
Maximum blood concentration of drug	C_{max}
istandard free energy	ΔG
Enthalpy of activation	$\Delta H^{\ddagger}$
Entropy of activation.	$\Delta S^{\ddagger}$
Observed rate constant	k_{obs}
Rate constant at zero buffer concentration	$k^{'}$
The degradation rate of free drug	k_0
The degradation rate of complexed drug	k_c
Joule	J
Hour	h
Shelf-life	t_{90}
Atmospheric pressure chemical ionization	APCI
Selected ion monitoring	SIM
Intrinsic solubility	S_o

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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

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1 Introduction

1.1 Malaria

Malaria, the most prevalent parasitic disease in the world, is a life threatening disease caused by parasites that are transmitted to people through the bites of infected mosquitoes. The annual report of World Health Organization (WHO) estimated 881 000 malaria deaths in 2006, of which 91% were in Africa and 85% were children under five years of age.[1] Approximately half of the world's population is at risk of malaria.[1, 2] Most cases and deaths are in the tropical areas such as sub-Saharan Africa. However, Asia, Latin America, the Middle East and parts of Europe are also affected. The death toll of malaria is higher in rural areas where patients do not have access to health facilities. Therefore the mortality level could be reduced if the diagnosis and treatment of malaria occurred earlier.

Most cases of malaria are uncomplicated and patients can be cured effectively with appropriate oral antimalarial drugs. However, delay in the treatment of malaria, caused by plasmodium *Falciparum*, can rapidly lead to an inadequate immune response with serious consequences.[3, 4] Cerebral malaria, the major manifestation of severe malaria, has a mortality rate of 15–20% which can be increased to 30% with multiple vital organ dysfunction.[4] The WHO coordinated the production of guidelines for the management of severe and complicated malaria in 1990 and again in 2000. WHO determined the specific definitions of severe malaria based on the manifestations of malaria as shown in Table 1.[5] In 2007, WHO published evidence-based guidelines for the treatment of malaria. These guidelines included extensive

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advice on the management of severe malaria as well as a clinically useful distillation of the WHO severe malaria definitions.

Table 1: WHO criteria for severe malaria[5]

One or more of the following clinical or laboratory features	
1. Clinical manifestations	**2. Laboratory test**
✓ Prostration	✓ Severe anemia
✓ Impaired consciousness	✓ Hypoglycemia
✓ Respiratory distress (acidotic breathing)	✓ Acidosis
✓ Multiple convulsions	✓ Renal impairment
✓ Circulatory collapse	✓ Hyperlactatemia
✓ Pulmonary edema (radiological)	✓ Hyperparasitemia
✓ Abnormal bleeding	
✓ Jaundice	
✓ Hemoglobinuria	

The cinchona alkaloid quinine and especially chloroquine have for decades been used as chemotherapeutic drugs for the treatment of malaria. However plasmodium *Falciparum*, the most lethal human parasite, shows well-known resistance to commonly available anti-malarial drugs. This has called for reviews of guidelines and deployment of new anti-malarial drug policies.[6, 7] Chloroquine resistance was reported initially in Southeast Asia and South America and then spread in the vast majority of endemic countries.[7]

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1.2 Treatment of malaria

Two classes of medicines are available for the treatment of severe malaria: the cinchona alkaloids (quinine and quinidine) and the artemisinin derivatives (artesunate, artemether and artemotil). Parenteral or rectal dosage forms are essential for efficient and effective antimalarial treatment in severe malaria. However parenteral chloroquine is no longer recommended for the treatment of severe malaria, because of widespread resistance. Intramuscular sulfadoxine / pyrimethamine is also not recommended because of the rapid resistance to sulfadoxine / pyrimethamine in many areas where it has been deployed.[8] Furthermore, parenteral quinine as an intravenous infusion should be carried out over four hours to avoid its cardiotoxicity[9], therefore, it is often not a practically suitable treatment. It requires three times daily administration and has a number of adverse effects including hypoglycaemia, vomiting, headache and tinnitus.[10] Artemisinin and its derivatives, dihydroartemisinin (DHA), artesunate (ART) and artemether represent a new class of antimalarial drugs with potent activity against plasmodium *Falciparum*. Artemisinin is a sesquiterpene lactone isolated from the plant Artemesia annua L and its derivatives were originally developed in China.[11] They are the most potent antimalarial drugs available today, being safe and well tolerated.[11] They have a very short half-life and thus a multiple dose regimen is required to avoid recrudescence and achieve an acceptable cure rate. Artemisinin-based combination therapies are the preferred treatment for malaria today. They also enhance the efficacy of other antimalarial drugs to ensure the effective case management, lower malaria incidence and lower the rate at which resistance emerges and spreads.[12] Artemisinin derivatives have become an essential component of multi-drug treatment of resistant *Falciparum* malaria. Artesunate (ART) is the most researched derivative of this class and is

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available as oral, parenteral and rectal dosage forms.[13] According to the outcomes of the Seaquamat study, the WHO recommended ART as the drug of choice for the treatment of severe malaria in low transmission areas and in the second and third trimesters of pregnancy. [14]

1.3 Physical and chemical properties of ART

ART is $C_{19}H_{28}O_8$ (3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin-10-ol, hemisuccinate) as shown in Figure 1 (A). It is a fine, white crystalline powder, slightly soluble in water and soluble in sodium bicarbonate solution and most organic solvents. It has a molcular weight of 384.43 g/mol and a melting point of 142-144 ˚C.

ART is hydrolytically unstable under both acidic and neutral conditions. At pH 1.2, the conversion into DHA, Figure 1 (B), is rapid with $t_{1/2}$ 26 min and at pH 7.4 and 23 ˚C the $t_{1/2}$ was reported as 10 hours.[15] In the preparation of artesunate α-artesunate is exclusively formed. As the axial hydroxyl in the β-epimer experiences steric hindrance raises the energy of the transition state for acylation. This gives rise to exclusive acylation of the α-eprimer.[14]

The solubility of ART is 565 mg L^{-1} in aqueous solution at pH 7.0 which demonstrates a higher aqueous solubility than artemether (114 mg L^{-1}). It has pK_a of 4.6 indicating that > 99% of ART will be ionized at pH 7.40.[16] The predominant formation of the ionized species is reflected in the low octanol-water partition coefficient (Log P) of 1.59 at pH 7.50 while at pH 2 Log P is 2.77 which means that the unionized ART is a polar artemisinin derivative.[16] As only unionized ART will pass through a membrane by passive diffusion, at pH 7.20, ART uptake from intestinal tract will not be facile. Hence artemisinin, which is a low solubility drug, easily crosses the intestinal monolayers by passive diffusion while

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permeability was found to be much lower for sodium artesunate.[17] The oral absorption of ART is the result of interplay between many factors, such as drug solubility, dissolution rate, regional pH differences, and membrane permeability. Many drugs are weak acids and stomach pH is 1.00-2.00.

The peroxide moity within the artemisinin molcule and its derivative structure was found to be responsible for antimalarial activity.[18-20] However, the peroxide bridge is both a chemically reactive and heat sensitive group which renders artemisinin derivatives more difficult to handle in formulation development.[21] An ideal artemisinin related drug candidate should possess an external C-C bond at position 12 of the structure for increased chemical stability, and hence providing a longer half-life in the body. While possessing the carboxylate group mostly induced water solubility. It appeared that any derivative at carbon- 12 of the type -OCH_2CH_2R or -$O(C=O)CH_2R$ such as artemether and ART, respectively may not have sufficient stability in aqueous solution but they can be used as parenteral dosage forms.[22] Artesunic acid is the hemisuccinate of the α-anomer of DHA. ART is administered as the sodium salt of artesunic acid, but it hydrolyses to DHA in *vitro* and in *vivo* rapidly.[23, 24] All artemisinin derivatives are lipophilic, with the exception of ART and artelinic acid, which are formulated as their sodium salts.[25]

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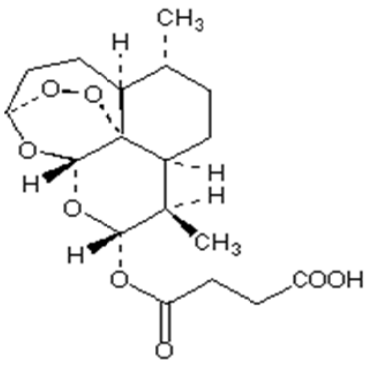
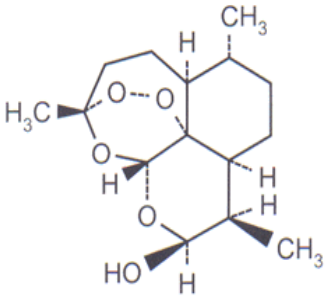
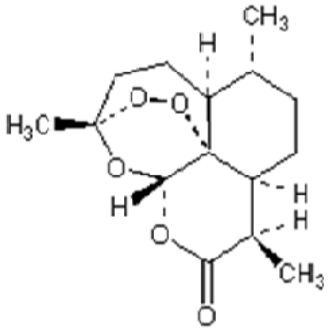


Artesunate



Dihydroartemisinin (DHA)



Artemisin

Figure 1. Chemical structures of Artesunate, DHA and Artemisinin

1.4 Pharmacokinetics of ART

Parenteral dosing is the preferred route for the treatment of patients with severe malaria. Intravenous administration is essential to achieve high bioavailability. However, the intramuscular route is often more convenient than the intravenous route, particularly when venous access is difficult to be established, as frequently in the case of severely ill young children. ART acts like a pro-drug with fast transformation into DHA and has an elimination half-life of less than half an hour. Absorption of intramuscular ART is rapid with a maximum concentration of DHA in serum being achieved in less than one hour in most children. The T-max of ART following oral administration in healthy adult volunteers varies between an average of 15 min and 39.6 min[23], and 1.7 h in children with *Falciparum*

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hyperparasitaemia.[26] In another study, the T-max was longer in convalescent than acute phase patients (30–60 min), following the administration of 200 and 100 mg of ART respectively.[27] The bioavailability of the pro-drug ART was low (15%) but the relative bioavailability of DHA was high (82%). ART is extensively hydrolysed to DHA in the gastro-intestinal lumen before first-pass metabolism in the gut wall and liver takes place. The profiles of ART and its metabolite are dictated by the pH-dependent rates of hydroxylation with esterases. In vitro data have indicated that the $t_{1/2}$ for hydroxylation of ART in the stomach at pH 1.20 is 10 min.[28]

The degree of binding for sodium artesunate to human serum or plasma proteins was found to be 59%.[29] As expected with a water soluble drug, the volume of distribution of ART was in the order of 0.15 L/kg or approximately 7-10 L in 50-70 kg patients. Given the plasma volume is approximately 3 L, extracellular fluid volume is 16 L and total body water is 40 L[30] , it is reasonable to assume that ART distribution into tissue is minimal.

The dose regimens of oral and intravenous ART in severe and uncomplicated malaria have been modelled according to the dose response. Although there was considerable inter-individual variation, the lowest oral dose was 2 mg/kg to give the maximum effect.[31] The currently recommended oral dose is 4 mg/kg/day of ART in combination therapy corresponding to an intravenous dose of 2.4 mg/kg.[32] The median ART C_{max} occurred within 20 min of injection with an elimination $t_{1/2}$ of 30 min.[32] Monotherapy ART treatment should be continued for at least 5 to 7 days to prevent recrudescence while combination therapy with mefloquine allows ART to be administered over 3 days or less, with a satisfactory clinical outcome maintained.

The pharmacokinetic data of ART and its metabolite DHA in healthy volunteers receiving a single daily oral administration of 200 mg ART for five consecutive days

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were measured using a liquid chromatography-mass spectrometry method.[33] There were no differences in pharmacokinetic parameters for either ART or DHA between the Day 1 and 5 date. The C_{max} and AUC_{∞} for DHA were 10 times and 20 times higher than those for ART respectively. With a short half-life of one hour and a once dose regimen, DHA reached maximum steady state concentration at two hours ($t_{ss\text{-}max}$) following the dosage, while the mean $C_{ss\text{-}max}$ value was 703 ± 94 ng mL^{-1} over five days.[33]

1.5 Synthesis of ART

ART was first prepared by Chinese scientists at the end of 1979.[34] ART was synthesized by the base catalyzed esterification of DHA with succinic anhydride according to Figure 2. ART is similar to DHA of owning two isomers, α and β, but only the α-isomer is easily sysnthesised and used in therapy. The semiacetal OH-group in the β-isomer of DHA is strictly hindered and therefore it is quite impossible for succinic anhydride to attack there, while the OH-group of the α-isomer is equatorial and interacts less with neighbouring groups, therefore the succinic anhydride can easily attack.[35]

Another consideration for the occurrence of the α-isomer is higher stability and thermodynamically favoured conformation compared to the β-isomer.[35]

This is the reason why only α-ART is produced by esterification of DHA with succinic anhydride. DHA suspended in ethyl acetate was cooled in an ice bath. Afterwards triethylamine was added and the mixture was stirred vigorously. Succinic anhydride was added to the cooled suspension step by step in small amounts over a period of 30 min. After further 10 min the ice bath was removed and the solution was stirred for two to three hours at room temperature. Cold water was added to the reaction mixture and then neutralised (pH 5.00) with 2 M H_2SO_4. The two phases

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were separated in a separating funnel and the aqueous phase was extracted three times with ethyl acetate. The combined ethyl acetate extracts were washed once with water. Afterwards, the extract was dried with Na_2SO_4, filtered and evaporated to dryness under reduced pressure.[36]

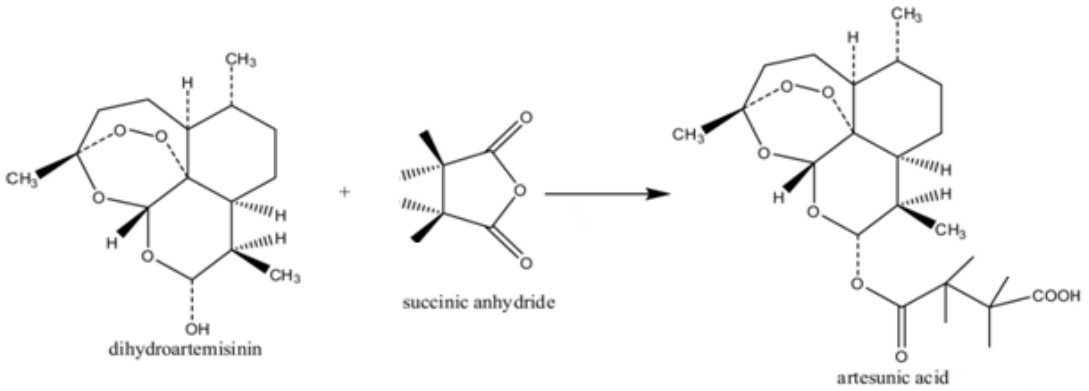

Figure 2. Synthesis of Artesunate

1.6 Mechanism of Action

Although it has been agreed that the endoperoxide linkage of artemisinin and heme iron play a major role in the mechanism of action of artemisinin, the detailed mechanism of action is still not fully understood. Artemisinins are relatively lipophilic in nature therefore readily cross both the erythrocyte and parasite membranes and concentrate in the digestive vacuoles and mitochondria of the parasites.[37, 38] It can be inactivated in the presence of iron. The malaria parasite is rich in heme-iron which is derived from breaking down host cell haemoglobin. The inhibition of hemozoin formation (haemoglobin catabolism) by artemisinin derivatives has been proposed, but is still controversial.[39] It has been proposed that the intraparasitic heme liberated during haemoglobin digestion might play an

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important role in the selective toxicity of artemisinin towards the parasite[40], and the reductive activation of artemisinin or its derivatives by Fe heme is probably a key point in the mechanism of action of these drugs. In the presence of synthetic heme models such as iron or manganese complexes of meso-tetraphenylporphyrin, it was recently found that artemisinin and many synthetic endoperoxides were able to generate alkylating intermediates via formation of either C-centered radicals [41] or other reactive species. For these compounds, the same mechanism of reductive activation by the synthetic metalloporphyrin or by heme itself gives rise to different drug-derived reactive species which can behave as alkylating agents with respect to heme or parasitic proteins. Such modifications of essential molcules of the parasite should be responsible for the antimalarial effect.

In summary, uptake of the preformed complex by the parasite has not yet been very clear. Based on the information available thus far, the proposed mechanism for the mode of action of artemisinin and related endoperoxide antimalarials is shown in Figure 3.[42]

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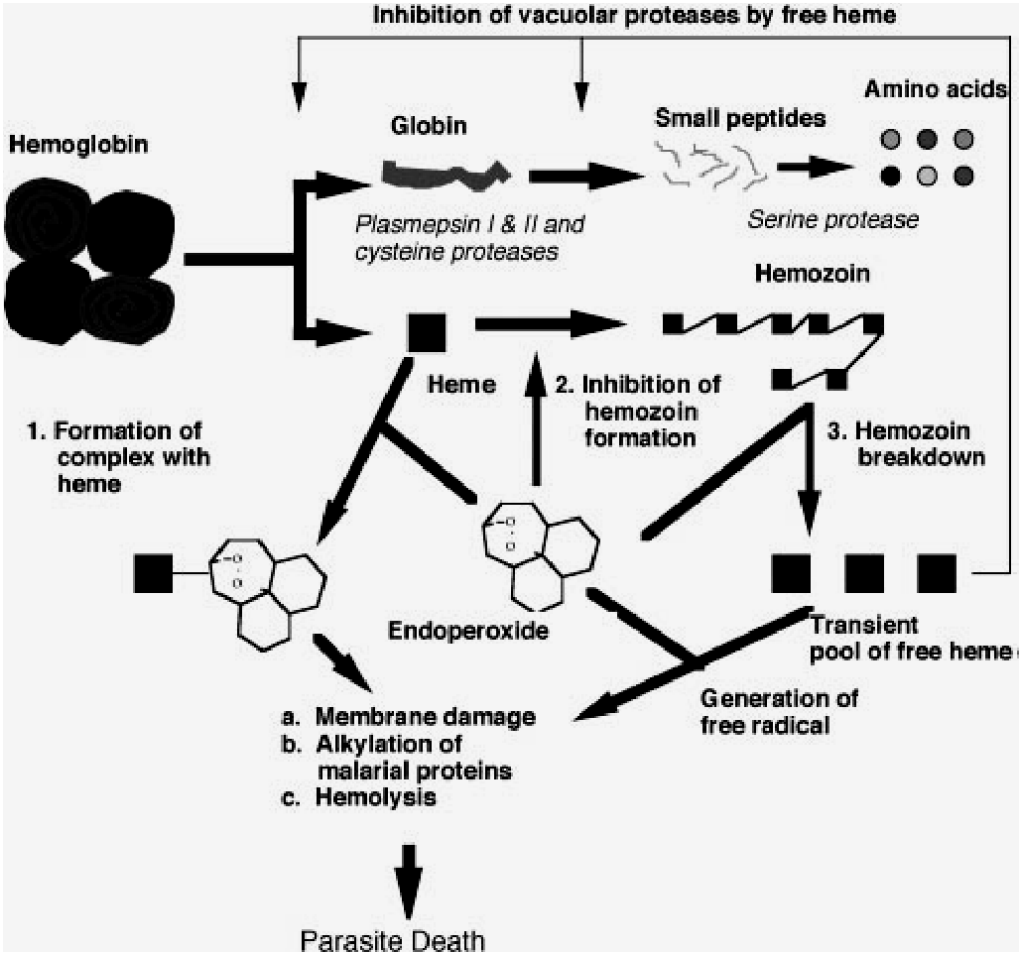

Figure 3. The proposed mode of action of artemisinin and related endoperoxide antimalarials.[42]

1.7 Combination therapy with ART

Owing the endoperoxide moiety, artemisinins have several advantages over existing antimalarial drugs. Firstly, there is no cross resistance with other antimalarial drugs. Secondly, the endoperoxides clear the peripheral blood of parasites more rapidly than any other available drug.[42] Finally, although widespread clinical use and the treatment of millions of patients, resistance to the endoperoxides has not developed significantly. Thus for the rapid onset of biological action and short biological half-life of artemisinin might retard large-scale development of resistance and

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treatment failure is not necessarily related to resistance as it could be caused by poor quality, or fake drugs. However, on theoretical grounds there may be several disadvantages for the artemisinins. The endoperoxides have short half-lives and effective levels in plasma are sustained for only relatively brief periods. For this purpose, the so called artemisinin based combination therapies should be used. These treatments combine an artemisinin derivative with a standard antimalarial drug with much longer half-life drugs and therefore are better in preventing recrudescence.

Currently, the prevalence of chloroquine-resistant *Plasmodium Falciparum* strains is high; therefore, the necessity for combination therapy has been established. The therapeutic efficacy of ART plus amodiaquine or sulfadoxine-pyrimethamine, and chloroquine plus sulfadoxine-pyrimethamine have been evaluated to enable the authorities to make an evidence-based choice.[43] ART combinations reduce the parasite load more quickly and effectively than therapy without ART.[43] The combination of ART and mefloquine is one of the most effective treatments against multidrug-resistant *Falciparum* malaria. Malaria treatments using combination therapy of ART rectal capsules plus mefloquine for two groups were significantly more effective than ART monotherapy (96% versus 94% versus 76% respectively).[44] It can be concluded that the combination of ART rectal capsules with mefloquine is effective and safe.[44]

1.8 Therapeutic uses of ART

Artemisinin derivatives have now been evaluated in various therapeutic fields. They were discovered primarily as compounds active against malaria but in the course of their development they were identified as possessing various degrees of activity against certain viruses including hepatitis C and cytomegalovirus. [45] [46] Other

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parasites were also found vulnerable to these drugs and animal and clinical studies confirmed this for leishmania and schistosomiasis infections. Exploratory studies suggest that they might be useful for trypanosoma infections causing either sleeping sickness or the variety causing Chagas disease. Clinical tests demonstrating their usefulness in the extended parasitic world are ongoing and their activity in schistosomiasis and cutaneous leishmaniasis is already confirmed.[47] Non-specific activity in rheumatoid arthritis has been suggested. Probably the most interesting feature is the activity of this class of compounds is against cancer for which a wealth of evidence is becoming available. They exert this action at the level of membrane destruction, interference with the genomic control of the tumor, inhibition of angiogenesis of the cancer growth and a variety of actions leading to tumor cell apoptosis and necrosis.[48, 49] There is a lack evidence that ART has a neurotoxic effect with high doses. It is likely that in the future these drugs will be classified as useful adjuvant drugs for cancer therapy in man and animals.[50]

1.9 Solubility of ART

The solubility of a compound depends upon the physical and chemical properties of the solute and the solvent, as well as upon factors such as temperature, pressure, pH of the solution and pKa of the solute. The pH of solution is regarded as an essential factor to influence the solubility of a drug dependent on the pKa of the drug and hence the chemical structure of the drug molcule. Therefore a buffer solution may be employed to keep pH value constant to maintain solubility. Changing the temperature of the solution may also affect the solubility of any drug.

Artemisinin derivatives are diverse in that their solubility in water varies from very poorly soluble to soluble. This range of solubility limits their formulation options for

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intravenous delivery and leads to irregular absorption upon oral administration. Artemisinin solubility in water was observed to be 12.5 µg mL^{-1} which indicated its poor solubility.[51] DHA solubility in water is 130 µg mL^{-1} [2] and artemether is 161 µg mL^{-1} at 25 ˚C [52] . These levels of poor solubility of artemisinin derivatives prompted the researchers to develop new delivery models to increase their solubility and stability in formulations and in vivo.

To some extent, ART is the only soluble derivative in the market as sodium artesunate injection. Since ART has acidic characteristics owing to its carboxyl group, the influence of pH of the solution and buffers on the solubility rate can be expected.

Recently, artemisinin solubility was greatly enhanced through inclusion complexation with cyclodextrins (CDs).[53] The artemisinin complexes were shown to display a faster rate and greater extent of dissolution compared to the uncomplexed form of the drug. Also, the saturation solubility of artemisinin in the complexed form was found to increase approximately two-fold.[53] Independent of pH and the buffer of selected, the apparent solubility of DHA increased as a function of hydroxylpropyl-β-cyclodextrin (HP-β-CD) concentration increased.[2] DHA solubility increased up to 89-fold with increasing CD concentration. Artemether solubility increased linearly with increasing HP-β-CD concentration.[54]

Artemisinin solubility was increased after the addition of different types of CDs. Table 2 shows the effects of conventional α-, β-, and γ-CD on the solubility of artemisinin in water at 25 °C.[54]

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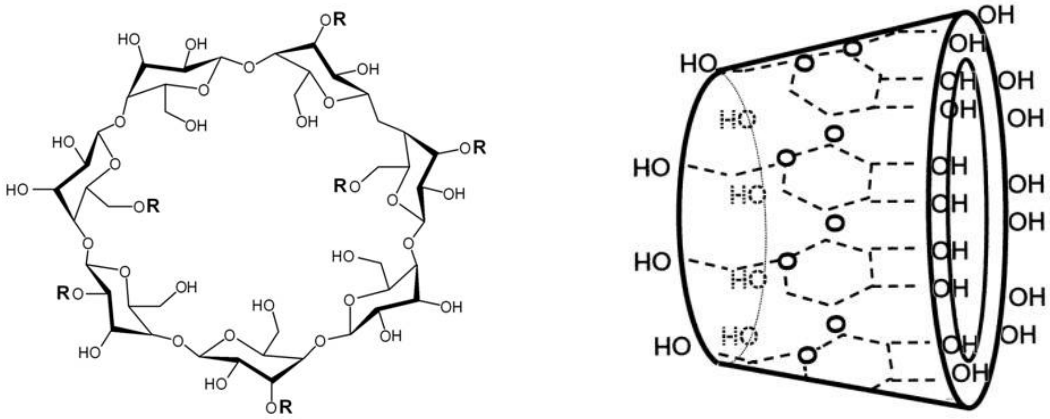
Table 2 Effects of α, β, and γ Cyclodextrins (10 mg mL^{-1}) on Solubility of Artemisinin in Water at 25 °C.[54]

	Solubility of artemisinin (mg mL^{-1})	Ratio (CD solution/water)
Without CD	0.084	—
α –CD	0.142	1.7
β –CD	0.517	6.2
γ –CD	0.427	5.1

1.9.1 Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides composed of a varying number of D-(+) glucopyranose units attached by α-(1, 4) glucosidal linkages. They are shaped like cones with secondary hydroxyl groups extended from the wider edge and the primary groups from the narrow edge. CDs have a lipophilic central cavity and hydrophilic outer surface structures are shown in Figure 4.



(A) **(B)**

Figure 4. The cone shape (A) and the chemical structure (B) of the α-cyclodextrin molcule.

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There are three natural CDs, α-, β-, and γ-CDs (with 6, 7, or 8 glucose units respectively), which differ in their ring size and solubility as shown in Table 3.

The large number of hydroxyl groups on the CDs imbues them with a high expectation of water solubility. Although β-CD contains a higher number of hydroxyl groups than the other CDs, β-CD solubility is the lowest compared to the α-CD or γ-CD. This is due to the formation of an internal hydrogen bond network between the secondary hydroxyl groups. Therefore disruption of the hydrogen bonding through molcular manipulation gives increased water solubility. For example, hydroxypropyl- β-CD (HP β CD) has an aqueous solubility more than 60% (w/w).[55]

Table 3. The 3 natural CD with their ring size and solubility.[56]

Type of CD	Cavity Diameter (Å)	Molcular Weight	Solubility (g/100 mL)
α-CD	4.7–5.3	972	14.5
β-CD	6.0–6.5	1135	1.85
γ-CD	7.5–8.3	1297	23.2

Cyclodextrins (CD) have complexation ability and some flexible characteristics which allow them to be used in a wide range of applications in different areas of drug delivery and pharmaceutical formulation. The most common pharmaceutical applications of CD's are to enhance the solubility, stability, safety and bioavailability of drug molcules.

In aqueous solutions, CDs are able to form inclusion complexes with many drugs by taking up the drug molcule or some lipophilic moiety of the molcule, into the central cavity. During complex formation , no covalent bonds are formed or broken, and the

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drug molcules in the complex are in rapid equilibrium with free molcules in the solution. The driving forces for the complex formation include release of enthalpy-rich water molcules from the cavity, hydrogen bonding, Van der Waals interaction, and charge transfer interaction. [57] The physicochemical properties of free cyclodextrin molcules differ from those in complexes. Evaluation of the complex stability and the numerical values of their stability constants can be determined by observing the changes in physicochemical properties such as solubility, chemical reactivity, UV/VIS absorbance (near-ultraviolet and visible light absorbance), chemical stability, effects on drug permeability through artificial membranes.[58]

Oral administration of α-CD is, in general, well tolerated and is not associated with significant adverse effects.[59, 60] β-CD can be found in numerous marketed oral dosage forms as well as in topical, buccal and rectal drug formulations. However β-CD has a relatively low aqueous solubility and can cause nephrotoxicity, therefore; β-CD can not be given parenterally.[61]

Toxicological studies indicate that HP-β-CD is safe, with no effect of dose levels and reversible histopathological and biochemical changes on the main target organs like kidney, liver, lungs and spleen.[62] The changes in the liver and kidney, such as renal cortical tubular vacuolation are consistent with those seen in several studies with the other α-, β- and γ-cyclodextrins.[63, 64]

1.9.2 Complex formation and phase solubility diagram

Inclusion complexation is accomplished by intermolcular interactions between CD and guest (drug) molcules. The most common type of inclusion complexation is the 1:1 drug/cyclodextrin complex (D/CD) which leads to the penetration of the guest

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molcule partly or completely into the cavity of the CD as shown in Figure 5. There is another type of inclusion complex which is 1:2 where one drug molcule (D) forms a complex with two CD molcules.

Phase–solubility evaluation is a traditional approach evaluating the effect of complexing agents on the compound being solubilized to determine the value of the stability constant and to give insight into the stoichiometry of the equilibrium.

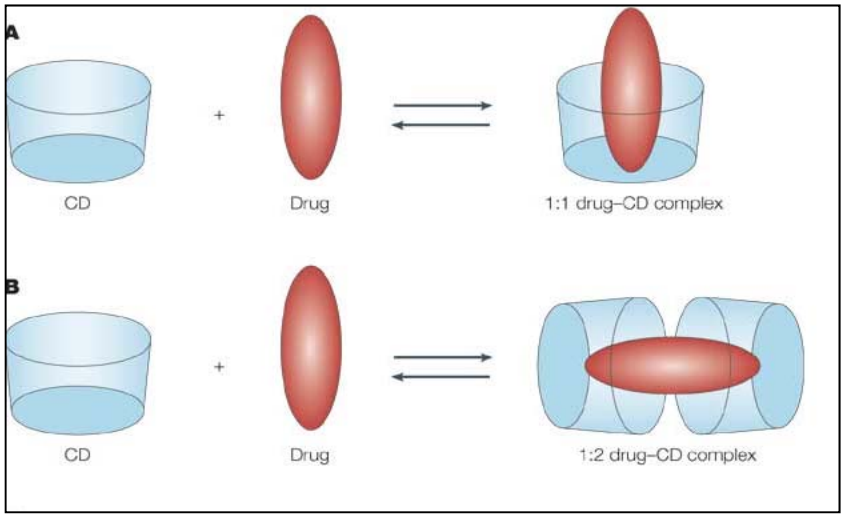

Figure 5. Types of cyclodextrin inclusion complex. **(A)** 1:1 drug–CD complex **(B)** 1:2 drug–CD complex.[65]

Phase–solubility analyses were developed by Higuchi and Connors.[66] Based on the shape of the generated phase–solubility relationship, several types of behaviours can be identified, however they fall into two major types: A and B as shown in Figure 6.[67]

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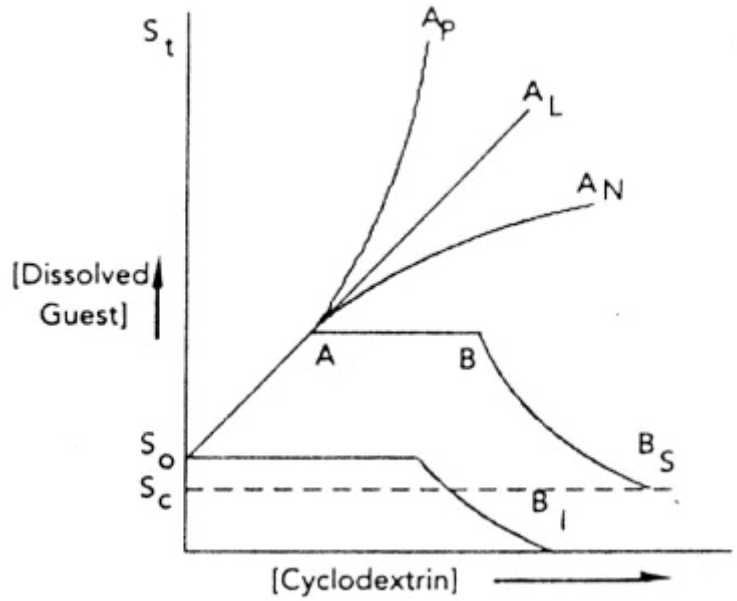

Figure 6. Phase solubility profile of drug with cyclodextrin.[68]

In A systems, the apparent solubility of the substrate increased as a function of CD concentration. Three subtypes have been defined: A_L profiles indicate a linear increase in solubility as a function of solubilizer concentration, A_P systems indicate an isotherm wherein the curve deviates in a positive direction from linearity (i.e. the solubilizer is proportionally more effective at higher concentrations) and A_N relationships indicate a negative deviation from linearity (i.e. the CD is proportionally less effective at higher concentrations). Taken as a whole, these isotherms indicate that water soluble complexes are being formed with solubilities higher than that of the uncomplexed substrate. A_L-type relationships indicate a 1:1 comlexation of drug and CD.

Type B phase–solubility profiles are indicative of the formation of complexes with limited water solubility and are traditionally observed with naturally occurring CDs, especially β-CD.[67]

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1.9.3 Stability of cyclodextrin inclusion complexed drugs

Free drug molcules are in equilibrium with these bound within the CD cavity. Measurements of stability constants or the dissociation constants of the D/CD complexes are important since this is an index of changes in physicochemical properties of a compound upon inclusion.

Although the fact that the forces which govern the complexation are not yet completely understood, it seems that it is the result of various effects:[69]

1. Replacement of polar–apolar interaction by apolar–apolar one, which means replacement of water included in the CD cavity by guest molcules which are less polar.
2. CD-ring strain released on complexation.
3. Van der Waals interactions and hydrogen bonding between CD and guest.

CDs are hydrophobic molcules, since their solubility may improve modestly when a small proportion of ethanol is added to water.[69] Water molcules in the CD cavity cannot satisfy their hydrogen bonding capacity as occurs with those in the bulk of the solvent. These water molcules have enhanced energy or enthalpy. The decrease in the energy of the system is caused by the reduction of the solvent-guest molcule and solvent-cavity interactions.

The thermodynamic parameters, i.e. the standard free energy change (ΔG), the standard enthalpy change ($\Delta H^{\ddagger}$), and the standard entropy change ($\Delta S^{\ddagger}$), can be obtained from the temperature dependence of the stability constant of the CD complex.[70] Thermodynamics for complexation of CDs with several series of drugs and other compounds have been determined and analysed.[71, 72] A number of forces have been examined for their role in driving complex formation including

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electrostatic interactions, van der Waals contributions, hydrogen bonding, release of conformational strain, exclusion of high energy water bound in the CD cavity and charge–transfer interactions.[73] The complex formation is almost always associated with a relatively large negative $\Delta H^{\ddagger}$ while the $\Delta S^{\ddagger}$ can be either positive or negative. This is because of liberating water molecules from the centre of CD cavity can lead to a large increase in entropy, more than sufficient to overcome the entropy associated with insertion of a drug molecule into the centre of CD cavity. Also, the complex formation is largely independent of the chemical properties of the guest (drug) molcules. The association or binding constants with substrate polarizability suggest that van der Waal's forces are important in the complex formation.[71] Classically, hydrophobic interactions are associated with a slightly positive ΔH and large positive ΔS indicating that they are entropy-driven in contrast to CD complexation, which is enthalpically driven. Based on the relatively nonpolar environment of the CD cavity, it may be expected that the water molcules situated therein do not have a full complement of hydrogen bonds and are at higher energy than those in the bulk media. Liberation of these “enthalpy-rich” molcules may represent a driving force in this context.[74]

1.10 Factors affecting on the stability of drugs

The stability of drugs is of continued importance for the drug industry. Temperature, relative humidity, pH of the solution, oxygen and light are among many factors affecting on the rate of degradation of a drug either chemically or physically. A formulated drug product must have a shelf-life sufficient to cover the time taken for transportation from the site of manufacture to the storage site until the drug administration process for clinical use is completed.

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1.10.1 Temperature effect

Artemisinins are used in countries within climatic zones either three (III) which is classified as hot and dry or zone four (IV) which is classified as hot and humid within an average temperature of 24 ˚C.[75] Any drug designed for use in climatic zones III and IV, long term thermal stress test by heating should be achieved at 30 ±2 ˚C and relative humidity of 65 ± 5% for 12 months and at 40 ±2 ˚C and relative humidity of 75 ± 5% , according to the WHO guidelines.[76] It also recommended that the threshold of unknown decomposition product based on a minimum daily dose of 100 mg should not exceed 0.2% and there should be < 1.5% decomposition of known degradation products.

Identification of thermal degradation products of solid artesunate (500 mg) which was heated neat under nitrogen at an oil bath temperature of 100 ºC for 39 h to give a mixture of ART and the products. The residue was (365 mg) whose components were verified by H NMR spectroscopic analysis. Beside the presence of β-artesunate (37 mg, 10%) and unchanged ART (175 mg, 48%) in the degradation product there were glycal (41 mg, 16 %), formate ester 10 (37 mg, 13%), β,β-artesunate dimer (27 mg, 4.4 %), α,β-artesunate dimer (25 mg 4.0 %), the α,α-artesunate dimer (10.5 mg, 1.7 %), the tricarbonyl compound 12 (2.5 mg, 1.1 %), and the peroxyhemiacetal (3.8 mg, 1.4%) to be present.

The degradation pathways of ART under such condition have different steps. For example, β-artesunate and the dimers are formed during thermolysis of artesunate. Protonation of the equatorial ester oxygen atom followed by loss of succinic acid generates oxonium ion. This undergoes axial addition, thermodynamically favoured by the anomeric effect with succinate to generate β-artesunate or with the free carboxyl group in artesunate to produce the α,β-dimer. The β,β dimer arises from

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equatorial–axial dimer by equilibration with artesunate via protonation at the equatorial ester followed by reaction of the incipient β-artesunate with the oxonium ion. The formation of the small proportions of α,α dimer may be due to the addition of α-artesunate to the oxonium ion. 9,10-anhydrodihydroartemisinin (glycal) arises via proton loss from the stabilised oxonium ion. The formate ester arises via axial addition of formate to the oxonium ion.

Treatment of artesunate with a 1:1 mixture of 5 mol aqueous hydrochloric acid ethanol at room temperature for 1.5 h gave DHA (12%), the peroxyhemiacetal (30%), the furanose acetal (1.2%), β-arteether (34%), and a-arteether (15%). No artesunate was recovered from this reaction. The arteether epimers were readily identified. Three more products glycal, 2-deoxyartemisinin, and the furanose acetal were obtained when the reaction was repeated in 1:1 2 mol aqueous hydrochloric acid–acetonitrile at room temperature for 17 h.

Identification and quantification the level of degradation of ART in rectal capsules by blending the capsule active ingredient with ART and then filled into sheets of wet gelatin film were fully analysed after drying them to a final water content of 5%.[21] Thermal stress testing of 100 mg ART capsules showed a percentage of decomposed ART against decomposed DHA according to the temperature, RH and period of storage as shown in Table 2. At 40 ˚C and 75% RH, solid ART had a decomposition rate lower than the formulated ART within the capsules. It showed 2% decomposition at four months and 5% at six months. Under the same conditions, solid DHA underwent 2% decomposition after one month and 2.9% after three months therefore solid ART considerably more stable than solid DHA.[21]

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Table 4. Stress testing results for 100-mg active artesunate powder in capsules

T [°C]	RH [%]	*t* [months]	3 [%] (ART) **a**	2 [%] (DHA)**b**
50	75	0.5	96.2	1.7
50	75	1	93.7	2.4
40	75	2	95.8	1.8
40	75	4	93.8	2.7
40	75	6	92.2	3.7
30	60	3	97.7	1.3
30	60	6	95.8	1.5
30	60	12	95.0	2.2
30	60	24	88.6	2.6
25	60	3	98.0	0.9
25	60	12	97.1	1.4

[a] Amount of ART remaining. [b] Amount of DHA formed.

Thuyle and his colleagues observed a spontaneous breakdown of stored ART to DHA in the plasma at 4 ˚C and at room temperature.[33] They found the changes in the measured concentrations of ART stored at 4 ˚C and room tepmerature for six months. At 4 ˚C, the quality control samples remained stable during the first month, and concentration deviation from the standards measured on day 1 were within 0.2%. Longer storage times resulted in decreased compound stability. The differences increased to 6-18%, 25-37% and 59-80% after 2, 3, and 6 months of storage, respectively. In addition the ratio of ART to its metabolite (DHA) was 11:1 on Day 1 and then decreased to 5:1, 3:1 and 1:1 on days 60, 90 and 180, respectively. These findings suggested that new standard solutions of ART must be prepared every two months.[33] The rate of transformation of ART to DHA increased considerably when the ART solutions were stored at room temperature. The ratio percents of ART to dihyroartemisinin were 7:1, 5:1, 4:1, 3:1, 2:1 and 0.2:1 on Days 1, 7, 15, 21, 30, 45 and 60, respectively.[33]

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Jansen and his colleagues evaluated the stability of ART tablets of different brands from Asia and Europe at 30 ˚C and 75% RH for 12 months.[77] they found that ART storage caused some degradation products and drug quality was marginally in line with International Pharmacopeia requirements as shown in Table 5. The typical degradation products are DHA, succinic acid, artemisinin and an impurity which elutes late was also found and characterized as a dehydrating product of DHA, therefore they concluded that ART should be retested after 9 months of storage.

Table 5. Stability Data of Artesunate at 30 ˚C and 75%RH

Time	**Water content**	**Related substances (%)**			
		α ART	DHA	Other impurities	Sum of impurities (%)
Initial	0.13	0.71	0.54	0.35	1.6
3 months	0.10	0.88	0.52	0.51	1.91
6 months	0.06	0.86	0.57	0.50	1.93
9 months	0.05	0.80	0.70	0.61	2.11
12 month	0.04	0.82	0.62	0.53	1.97

Data based on three different batches of artemisinin raw material.
Water contents were determined on the basis of Karl-Fisher test.

Data based on three different batches of artemisinin raw material and water contents were determined on the basis of Karl-Fisher test. When ART tablets were stored at 40 ˚C and 75% RH, significant degradation occurred, therefore; they recommended strict criteria must be set in order to store them for long period as shown in Table 6. This is not the case for artemether as either an oily solution or suspension is used which showed stability of storage at 30 and 40 ˚C for more than two and one years respectively, but artemether as a tablet or as a raw material decomposed significantly after 3 months of storage at 40 ˚C.[77]

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Table 6. Stability Data of Artesunate at 40 ˚C and 75% RH

Time	water content	Related subtances (%)			
		α AR	DHA	Other impurities	Sum impurities(%)
Initial	0.13	0.76	0.54	0.35	1.65
3 months	0.05	0.85	0.86	0.51	2.22
6 months	0.06	0.78	1.36	0.50	2.64

The stability of ART and DHA in the plasma was evaluated at two different concentrations and three temperatures.[78] ART and DHA were found to be stable in plasma for up to 5 h at room temperature (24 ˚C). Refrigerated storage of plasma samples was found to be acceptable, with stability limits of up to 6 days. Storage of ART and DHA at - 25 ˚C indicated no degradation result after 8 months.[78]

1.10.2 Effect of pH and general acid-base catalysis

A number of drugs undergo decomposition in solution upon the addition of acids or bases. Dependent on the pK_a most drugs are salts of either weak acids or bases in nature. Therefore in aqueous solution, drug molcules dissociate partially or completely. This dissociation usually has an effect on the drug efficacy or therapeutic effect. Obviously, there are often general acid-base catalysis (from buffer systems) or specific [H^+] and [OH^-] catalysis in aqueous solution. Although buffer salts are commonly used in pharmaceutical liquids to regulate the pH of the solution, some catalyse the degradation. For example penicillins, cephalosporins and carbapenems, due to the β-lactam ring in their structures, have demonstrated a general acid base catalysis and shown a significant increased rates of degradation when investigated with buffer systems.[79-82]

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Drug substances having succinate ester groups in their molcular structure, like ART, can be subjected to hydrolysis in the presence of water. The degradation process by hydrolysis is accelerated by the presence of increased concentrations of hydrogen or hydroxyl ions. It also can be accelerated by general acid base catalysis of buffer systems. Hydrolysis is one of the most common degradation pathways encountered with pharmaceuticals.

The hydrolysis of hydrocortisone 21-hernisuccinacinate above pH 8.00 has been studied by constant pH titrations in aqueous solution and appears to involve specific hydroxyl ion catalyzed hydrolysis as the only significant reaction pathway.[83] Below pH 8.00, certain chemical phenomena that may produce rates that exceed prediction of spontaneous hydrolysis or intra-molcular attack of the anion on the ester carbonyl carbon and a significant contribution of hydronium ion. Therefore this would involve a reaction accelerated by the attraction of opposite charges as explained in Equation 1.1.[83, 84]

$$H_3O^+ + R\text{-}OCOCH_2CH_2O^- \longrightarrow R\text{-}OH + HOOCCH_2CH_2COOH \text{ ..... Eq 1.1}$$

The stability of erythromycin, erythromycin ethylsuccinate , propionyl erythromycin and erythromycin estolate in methanol and acetonitrile was investigated.[85] Whilst erythromycin is stable in methanol, the esters are not, all of which showed extensive degradation in this organic solvent.[85] Furthermore, the degradation appeared to follow different pathways to those proposed for the aqueous acidic degradation of these compounds. For both the ethylsuccinate and propionyl esters, the reactions appeared to be simple solvolysis with base formation.

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The kinetics of hydrolysis of metronidazole monosuccinate in aqueous solution at pH 1.5–10 and 60 °C was investigated.[86] At any given pH the reactions displayed strict first-order kinetics and were subject to specific acid-base catalysis; no general acid-base catalytic effect by various buffer substances was observed. Maximal stability was observed at pH 3.00–6.00, where the degradation rate was independent of pH indicating that intra-molcular catalysis by the terminal carboxylate ion on the ester group was not involved in the hydrolysis reactions in this pH range.

Currently there is no study which has demonstrated the effect of aqueous buffer systems or pH on the degradation rate of ART. Liposomal suspensions of ART especially for parenteral administration, were evaluated for chemical and physical stability, including chemical degradation and crystallization of ART, and its release capacities.[87] Different concentrations of ART and lipids in three pH values (pH 5.00, 7.00 and 9.00) were investigated. The highest stability of ART was obtained with a phosphate buffer of pH 5.00 as ART was almost totally incorporated in the liposomes and solubility in the aqueous phase was low.

ART underwent a significant hydrolysis when diluted with glucose 5% w/v solutions which has a relatively low pH (approximately 5.00).[15]

1.10.3 Effect of ionic strength on the reaction rate

The ionic strength of a solution is a measure of the concentration of ions in that solution. The rate of degradation can be influenced by the ionic strength of the solution in accordance with Equation 1.2: [88]

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$\text{Log } k = \log k_0 + 1.02\ Z_A\ Z_B\ \sqrt{\mu}$ ..... Eq 1.2

Where as:

$Z_A + Z_B$ are the charges carried by the reacting species in a solution.

μ is the ionic strength.

k is the rate constant of degradation.

k_0 is the rate constant at infinite dilution.

Plotting the logarithm of the reaction rates versus the square root of the ionic strength, as shown in Figure 7 can allow us to determine whether an increase in ionic strength increases, decreases, or has no effect on the degradation rate.[88]

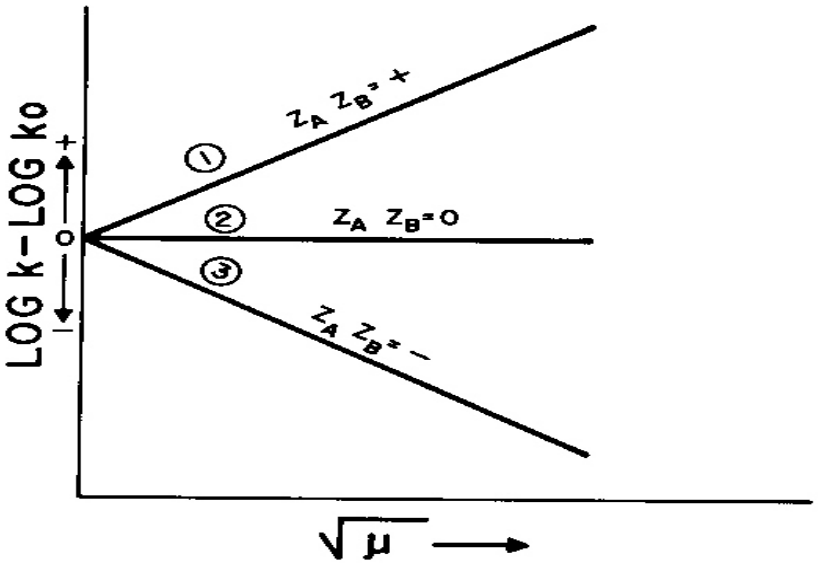

Figure 7. Dependence of reaction rates on ionic strength[89]

The ionized species of a buffer may have a catalytic effect on the rate of reaction whereas the corresponding non-ionized form may not. Increasing the ionic strength of the buffer by adding a neutral salt like sodium chloride or potassium chloride changes the concentration of ions present. Thus the rate of reaction can be influenced and this effect which is called the secondary salt effect. However when the ion contributes directly to the reaction rate, it is referred to as a primary salt effect.[89]

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1.11 Kinetic principles of drug degradation.

1.11.1 Influence of temperature on kinetic degradation

The temperature dependency of a reaction permits the prediction of the stability of a drug at ordinary shelf lives from data obtained under accelerated temperatures. The Arrhenius Equation (1.3) expresses of the influence of temperature on the reaction velocity constant

$k = A\ e^{-(Ea/RT)}$ ..... Eq 1.3

k = specific rate constant

A = frequency factor

T = temperature (Kelvin)

R = gas constant (8.314 joules $degree^{-1}$ mol)

Converting to $\log_{10}$

$\log k = \log A - (E_a / 2.303)\ (1/RT)$ ..... Eq 1.4

The activation energy can be evaluated by determining k at several temperatures and plotting log k versus (1/T). The activation energy represents the minimum energy required for the reactants to undergo the reaction. The higher the value of activation energy the more the reaction is temperature-dependent.

An alternative to Arrhenius equation is Eyring equation, which in contrary to the empirical Arrhenius equation, this model is theoretical and based on statistical thermodynamics.[90] This equation can be expressed as following:

$\ln k/T = (\ln k_B / h_P) + (\Delta S^{\ddagger}/R) - (\Delta H^{\ddagger}/RT)$ ..... Eq 1.5

Where $\Delta S^{\ddagger}$ = entropy of activation.

$\Delta H^{\ddagger}$ = enthalpy of activation.

h_P = Planck's constant.

k_B = Boltzmann's constant

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A plot of logarithm of k against the reciprocal of absolute temperature generally yields a linear relationship, therefore the frequency factor and the activation energy are regarded as independent of temperature and the activation energy is used as a measure of the temperature dependence of the rate constant. However, the Eyring equation suggests that both frequency factor and activation energy should be temperature dependent. E_a can be shown to be related to activation enthalpy ($\Delta H^{\ddagger}$) as indicated in the following equation:

$E_a = \Delta H^{\ddagger} - RT$ ..... Eq 1.6

Stability of ART in sodium chloride minibags at 9, 23 and 36.5 ˚C was investigated. The first order degradation profile of ART and t_{90} values were calculated as shown in Table 7.[15] However normal saline is not a buffer to keep the pH of the solution constant hence calculation of Ea and shelf-life may not be accurate.

Table 7. Stability data for ART (30 mg) in 50 mL minibags normal saline injection at 9, 23 and 36.5 ˚C[15]

Temperature (˚C)	t_{90} (h)	Degradation rate constant (h^{-1})
9	130	3.6×10^{-4}
23	10.6	4.3×10^{-3}
30	4	Not mentioned
36.5	1.6	2.8×10^{-2}

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1.11.2 Influence of pH on the kinetic degradation

Hydrogen ion catalysis is predominant at the lower pH's, whereas hydroxide ion hydrolysis operates at higher pH's. At the intermediate pH range, the rate can be independent pH or catalysed by either hydrogen or hydroxide ions. The pH stability profile can be determined by plotting of logarithm of the rate constant at zero buffer concentration versus pH.

The pH-rate profile has different shapes which are characteristic of reactions susceptible to specific acid-base catalysis. However the most common one is the U shaped one which represents three regions of acid, independent and base catalytic reactions.[91]

1.11.3 Influence of buffer species on kinetic degradation

The catalytic effect of buffer species in kinetic studies was determined at constant temperature, pH, ionic strength and drug concentration, the only variation being in total buffer concentration. This is repeated at several pH values within the effective range of the buffers employed. Several studies have demonstrated the catalytic effect of the buffer which increased linearly with the buffer concentration.[92, 93]

For example, Mendez et al. calculated the rate constants of the acetate buffer species at pH 4.20 to 5.50 dependent on Equation 1.5.[94]

$$k_{obs} = k' + k_{AcH}[HAc] + k_{Ac^-}[Ac^-] \quad \text{.....} \quad \text{Eq 1.7}$$

Where: k_{obs} is represent the observed degradation rate constant of the drug

[HAc] is the concentration of undissociated acid

$[Ac^-]$ is the concentration of dissociated acid ion.

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k' is the rate constant at zero buffer solution and the other k values are the catalytic rate constants due to the acetate buffer species.

From the value of the slopes at the two pH values k_{HAc} and k_{Ac}^- can be calculated using simultaneous equations.

1.11.4 Influence of cyclodextrin inclusion complex on the kinetic degradation

The most common type of cyclodextrin complexes is the 1:1 drug/cyclodextrin complex (D/CD) where one drug molcule (D) forms a complex with one cyclodextrin molcule (CD):

$$D + CD \overset{K_{1:1}}{\rightleftharpoons} D/CD$$

The value of the stability constant ($K_{1:1}$) is used to compare the affinity of drugs for different cyclodextrins. Under conditions for an A_L-type phase-solubility diagram, the stability constant ($K_{1:1}$) of the complex can be calculated from the slope and the intrinsic solubility (S_o) of the drug in aqueous complexation media (i.e, drug solubility when no CD is present and is given in Higuchi and Connors Equation (1.8)

$$K_{1:1} = \frac{\text{Slope}}{S_0\,(1 - \text{Slope})} \qquad \text{..... Eq 1.8}$$

The stability constant of the inclusion complex is usually governed by the behaviour of the guest molcule in the presence of cyclodextrin and the complex itself in the solution. The rate of degradation of free drug (k_0) and the complexed drug (k_c) have a role in determination of the stability of the drug when complexed with cyclodextrin as explained in the Scheme 1.

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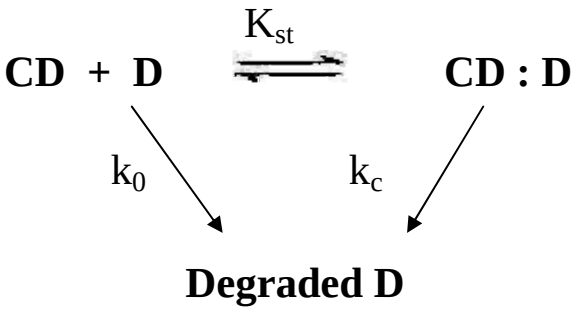

Scheme 1 a model representing the effect of complex stability constant on drug degradation.

The observed reaction rate for the degradation of the guest molcule (D) in the presence of CD is an average of the degradation rate of free and complexed D. In addition, K_{st}, the apparent stability constant of the complex can be determined by relating the observed rate constant with the concentration of the added CD. Actually, the measurable rate constant which on rearrangement gives the Lineweaver–Burke Equation (1.9).

$$\frac{1}{k_o - k_{obs}} = \frac{1}{K_{st}(k_o - k_c)} \frac{1}{[CD]} + \frac{1}{k_o - k_c} \quad \text{..... Eq 1.9}$$

The plot of $1/k_o - k_{obs}$ versus $1/[CD]$ gives $1/ K_{st} (k_o - k_c)$ as the slope and $1/k_o - k_c$ as the intercept.

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The enthalpies of reactions can likewise be determined from $K_{1:1}$ obtained at various temperatures using the van't Hoff equation 1.10. If two sets of data are available (i.e. Two K_c values determined at two different temperatures in K) then:

$$\frac{k_2}{k_1} = \frac{-\Delta H}{2.303\ R}\ \frac{T_2 - T_1}{T_1 T_2} \quad \text{..... Eq 1.10}$$

On the other hand, if a range of values are available, the ΔH values can be obtained from a plot of ln K versus 1/T using the equation 1.11.

$$\log K = -\frac{\Delta H}{2.303\ R}\ \frac{1}{T} + \text{constant} \quad \text{..... Eq 1.11}$$

Where the slope will provide the enthalpy data. The entropy for the complexation reaction can be calculated using equation 1.12.

$$\Delta G = \Delta H - T\Delta S \quad \text{..... Eq 1.12}$$

1.12 Qualitative and quantitative detection of ART in the samples

Sensitive methods for determination of ART and its degradation products or metabolites in biological fluids or solutions are needed when conducting therapeutic drug monitoring or aqueous drug stability. The development of sensitive and selective analytical methods for ART and its metabolites or degradation products has been challenging. Acid or base hydrolysis of ART producing an UV-chromophore prior to high performance liquid chromatography (HPLC) analysis has been employed,[95] but this approach lacks specificity.[96] A technique that met the best

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sensitivity and specificity of detection of ART and metabolite or its degradation products is liquid chromatography mass spectroscopy (LC-MS) or even the more sensitive one which is the LC-MS/MS, which now is being used for pharmacokinetic and metabolic studies. In spite of such systems being expensive and complex in the operation and maintenance, several research groups are able to use them routinely to assay ART and its metabolites.[97-99]

ART is relatively regarded the only soluble derivative of artemisinins in the market. However, currently there is no study demonstrates the stability data of ART in aqueous solution or evaluates the stability of ART in aqueous solution with different buffer solutions or different pH values. In addition there is no study which has evaluated the stability or solubility of ART with cyclodextrins, therefore it is valuable to investigate the extent of ART stability in aqueous solution with or without cyclodextrin.

1.13 Objectives

1- To determine the stability of ART in aqueous buffer solutions at selected temperatures

2- To evaluate the temperature dependence of ART in buffered solutions at selected pH values

3- Evaluate the influence of complexation of ART with HP-ß-CD at selected pH values and temperatures.

4- To investigate the stability of ART in commonly used intravenous fluids.

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Chapter 2

EXPERIMENTAL AND METHODOLOGY

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2 Experimental

2.1 Materials

2.1.1 Investigated product

The product in this study was artesunate powder. Batch number (27851A), Control number 24445. Apin Chemical Ltd, UK. This is α-artesunate.

2.1.2 Water

Water was obtained from a Milli-Q Ultrapure water system, Millipore, Australia (4-bowl ultrapure cartridge kit) with a conductivity of 0.05 µS.

2.1.3 Materials used for buffer solutions

1. Citric acid powder, MW 210.14, Batch number (53572), B.D.H. Chemicals, Australia.
2. Tri-sodium citrate, MW 294.10, Batch number (9682), B.D.H. Chemicals, Australia
3. Acetic acid Glacial, MW 60.05, Batch number (A8401), Lab-Scan Laboratory, Bangkok, Thailand
4. Sodium acetate anhydrous, MW 82.03, Batch number (912122), Ajax Chemicals, Australia.
5. Di-sodium hydrogen orthophosphate anhydrous, MW 141.96. Batch number (19322), Merck Pty, Australia.
6. Sodium di-hydrogen orthophosphate monohydrate, MW 137.90, Batch number (16256), Merck Pty, Australia.
7. Tris (hydroxymethyl) methylamine, Batch number 51068, Ajax chemicals Ltd.
8. Sodium hydrogen carbonate, MW 84.01, Batch number (001764), Ajax Chemicals, Australia.
9. Sodium carbonate anhydrous, MW 105.99, Batch number (32467), BioLab, Australia.
10. Ammonium acetate, Batch number, Merck Pty, Australia.

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2.1.4 Materials used for HPLC and LCMS mobile phases

1. Orthophosphoric acid 85%, MW 98, Batch number (10173), Analar Grade, Australia.
2. Acetonitrile, MW 41.05. Batch number 5110218, J.T.Baker in U.S.A.
3. Freshly prepared 5 mol/L sodium hydroxide solution.
4. Ammonium formate, Batch number 9429JC, Sigma-Aldrich Chemical, UK
5. Formic acid 90%, Batch number 284295F, VWR International Ltd, England

2.1.5 Materials used for IV fluids

1. Glucose monohydrate, Batch number 202634, Chem-supply Pty, Australia.
2. Sodium lactate, Batch number AF611419, Ajax fine chemical, Australia.
3. Calcium chloride, Batch number 240363, Biolab Ltd, Australia.
4. Potassium chloride, Batch number 243524, Biolab Ltd, Australia.
5. Sodium chloride, MW 58.44, Batch number (18858), Merck Pty, Australia.

2.1.6 Material used for inclusion complex.

1. HP-ß-C powder, MW 1309.23, Batch Number (Y1933), Cavitron Laboratory, Ceretar USA Inc.

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2.2 Equipment

2.3 Chromatographic equipment

2.3.1.1 HPLC

The high performance liquid chromatography instruments consisted of a high pressure pump (Waters 501, HPLC pump, Millipore USA), a Apollo C18 (5 micron) column of 150 mm length and ID of 4.6mm, a Rheodyne Model 7125 syringe loading sample injector with 20 µL sample loop, a ultraviolet detector (Waters 484, Tunable Absorbance Detector, Millipore, USA) and an integrator/printer (Hewlett Packard HP 3396A integrator).

2.3.1.2 LCMS

LC-MS analysis was performed with Shimadzu liquid chromatography system coupled to Mass detector (LC-MS 2020). System includes a binary pump 20AD, a auto injector SIL 20 AC HT, an column oven CTO 20A, a PDA detector SPD M 20A, an Mass analyser MS2020 and Communication Unit CBM 20A. Data were acquired, processed and analysed using a chromatographic software LABSOLUTIONS. An Eclipse XDB-C8 (150 mm × 2.1 mm, 3.5 µm) column was incubated in a thermostatted column compartment set at 30 °C.

2.3.2 Diode array spectrophotometer

Hewlett Packard diode array spectrophotometer, model 8452A.

2.3.3 Balance

The balanced used was Sartorius digital balance.

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2.3.4 pH meter

Hanna pH meter, model 8417 (Singapore). It was calibrated with standared buffer solutions: (A_2 49, Batch No. AF 402226, Labchem, Australia, pH 7.00 ± 0.02 at 20 ˚C) and (A_2 491, Batch No. AF 402121, Labchem, Australia, pH 7.00 ± 0.02 at 20 ˚C).

2.3.5 Water bath

Julabo circulating water bath, model p/1 (Germany). It has a temperature stability of ±0.02.

2.4 Assay methods

2.4.1 Assay of artesunate

ART was assayed by reverse phase HPLC. The mobile phase used was phosphate buffer (50 mmol L^{-1}) and acetonitrile (70:30). The pH of the mobile phase was adjusted with sodium hydroxide to 7.00. The mobile phase was filtered through a filter (Millipore, FH), 0.45 µm and degassed before use. The flow rate was 1.5 mL min^{-1}. The monitoring UV wavelength was 210 nm.

2.4.2 Assay validation

Samples of 200, 300, 400, 500, 600, and 700µg mL^{-1} ART in 40% acetonitrile solutions were freshly prepared and analysed by HPLC. The peak area (area under the curve), which quantitatively represents the concentration of ART in the samples, versus concentration of the samples was plotted and calibration curve was obtained. The within-day (repeatability) precision of 600 µg mL^{-1} ART in 40% acetonitrile was determined using seven replicate determinations. Similarly the intraday (or

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reproducibility) precision assay was determined by comparing the assay of 600 µg mL^{-1} concentration on two consecutive days. The results were expressed as the relative standard deviation (RSD).

2.4.3 Stability indicating HPLC methods

Samples of 600 µg mL^{-1} ART were prepared in 2% ethanol solution and subjected to 0.1 M hydrochloric solution (pH 1.00) at 25 ˚C, 0.1 M sodium hydroxide solution (pH 13.00) at 25 ˚C and heat at temperature 50 ˚C with purified water separately to determine whether the degradation products of ART interfered with the peak of ART.

An aliquot was drawn at specified time intervals, filtered with a syringe equipped with a 0.45 µm filter and analysed by HPLC. The degradation process was evaluated either by identifying the required time for the HPLC drug peak to completely disappear, reduce or not change.

2.4.4 Method of ART stability study

Accurate amount of ART powder (60 mg) was dissolved in 2 mL of absolute ethanol, added to 98 mL of buffer solutions and sonicated for one minute to achieve a concentration of 600 µg mL^{-1}. Buffer solutions (phosphate at pH 2.00, 2.50, 5.50, 6.50, and 7.50, citrate at pH 3.50, acetate at pH 4.00 and 5.00, Tris at 8.50 and carbonate at pH 9.50 and 10.50) were employed to evaluate the degradation of ART. These buffer solutions of varying concentrations were prepared to evaluate the rate of degradation of ART at a constant pH, temperature (37 ºC), and ionic strength of 0.5 $mol.L^{-1}$. An aliquot was withdrawn at intervals and the concentration of residual drug was determined by HPLC. A plot of log percent drug concentration versus time

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was plotted to determine the rate of degradation and to find the rate constant of the reaction.

2.4.4.1 Preparation of buffer solutions

According to the Henderson-Hasselbalch Equation 2.1, the amount of acid and conjugate base was determined.

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{acid}]} \quad \text{..... Eq 2.1}$$

Where the pKa is the dissociation constant of the weak acid. The concentration of conjugated acid and conjugated base was calculated depending on the specific total concentration of the buffer solution at the given pH.

Each buffer solution was prepared in three different concentrations to investigate any effect of the buffer species on the degradation rate of drugs. This was performed at a specific pH, 37 ˚C and ionic strength of 0.5mol L^{-1}. The buffer solutions, used in this study, were:

1- Citrate buffer solution which was prepared from citric acid and tri-sodium citrate.
2- Acetate buffer solution which was prepared from acetic acid and sodium acetate.
3- Phosphate buffer solution which was prepared from sodium di-hydrogen orthophosphate monohydrate salt and di-sodium hydrogen orthophosphate anhydrous salt.
4- Tris buffer solution which prepared from dissolving the required amount of Tris base in the water.

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5- Carbonate buffer solution which was prepared from sodium carbonate salt and sodium hydrogen carbonate salt.
6- Ammonium formate buffer solution which was prepared from ammonium format and formic acid.

Ionic strength was adjusted at 0.5mol L^{-1} for all buffer solutions by adding sodium chloride salt. The sodium chloride required was calculated according to following equation:

$$\mu = \frac{1}{2} \sum c_i z_i^2 \quad \text{..... Eq 2.2}$$

where $\sum$ is summation of the product of cz^2 terms for all the ionic species in the solution.

c_i is concentration in mols per litre of any of the ions.

z_i is valance of the species.

2.4.4.2 Preparation of samples

60mg of ART was dissolved with 2 mL of absolute alcohol in a small flask and sonicated for one minute until complete solution occurred. A required amount of buffer species was dissolved in 98 mL of purified water in a second flask. The two solutions were mixed to produce ART concentration of 600 µg mL^{-1} and placed in a thermostated water bath at 37˚C (± 0.2 ˚C) for 5 min to achieve equilibration. The pH of the solution was adjusted if necessary to a required pH by adding dropwise of sodium hydroxide or hydrochloric acid. Immediately an aliquot of the mixed solutions were withdrawn from the flask and analysed by HPLC. The time, when the first sample was withdrawn, was regarded as a zero time. Subsequently samples were

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withdrawn at intervals for analysis until at least three half-lives of the reaction was completed.

2.4.5 Method of ART inclusion complex

2.4.5.1 Phase solubility study

Samples of HP-ß-CD powder were mixed with 50 mL of 0.2 M phosphate buffer solutions of pH values of 3.00, 4.00, 5.00, and 6.00 to produce concentrations of 0, 68, 136 and 272 mg mL^{-1}. One gram of ART added and mixed with each sample. All mixed samples were kept at 100 rpm and 25 °C ± 0.2 in an orbital shaker. Aliquots were withdrawn from each mixed sample at intervals, filtered with a syringe equipped with a 0.45 µm filter, diluted with water and analyzed by HPLC until the equilibrium was achieved. A plot of solubility of ART versus the concentration of HP-ß-CD was plotted.

2.4.5.2 ART inclusion complex stability study

Samples of 2 mg mL^{-1} of ART in phosphate buffer solution of pH values of 6.00, 7.00 and 8.00 were prepared. Graded amounts of HP-ß-CD were added to the samples to make concentrations of 13.6, 27.2, 54.4 and 108.8 mg mL^{-1}. All the samples were kept in a thermostated water bath at 37 ˚C (± 0.2 ˚C). Aliquots were withdrawn at intervals, filtered with a syringe equipped with a 0.45 µm filter, diluted with water to make concentration of 500 µg mL^{-1} ART and analyzed by HPLC. A plot of log residual percent of ART versus time was plotted.

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2.4.6 Method of ART stability study with LCMS

ART powder was added to three different concentrations of buffer solution to make ART nominal concentration of 600 µg mL^{-1}. Ammonium acetate buffer solution at pH 3.00, 6.50 and 8.50 was employed. All the samples were kept in thermostated water bath at 37 ˚C. An aliquot was withdrawn at intervals, filtered with a syringe equipped with a 0.45 µm filter, diluted with water to make concentration of 10 µg mL^{-1} and analyzed by LCMS. A plot of log residual percent of ART versus time was plotted.

The LCMS system consisted of a binary pump using mobile phase of ammonium formate 20 mmol L^{-1} (pH 5.40) as a phase A (35%) and acetonitrile as phase B (65%). The acquisition data were atmospheric pressure chemical ionization (APCI), positive ionization, heat block 400 ˚C, dry gas was 10 L min^{-1}, nebulizer gas was 1.5 L min^{-1}, flow rate was 0.2 mL $min^{-1}$and m/z was 402.

2.5 Treatment of kinetic runs

Each experimental run was performed by mixing the drug with the buffer solution to produce ART concentration of 600 µg mL^{-1}. A sample was taken immediately following admixture as a zero time. After analysing by HPLC, a plot of log remaining drug concentration versus time was plotted. From this plot the observed rate constant was obtained for each condition. A plot of buffer concentration versus the rate constant of each condition was plotted. Rate constant at zero buffer concentration at each pH value were obtained from the intercept of each linear relationship with the y axis.

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The condition selected follow second order kinetic. However under condition where $[H^+]$ or $[OH^-]$ remains essentially constant pseudo first-order kinetic are observed. Hence kinetically first-order reaction can be expressed as in Equations 2.3, 2.4 and 2.5.

$$- dc/dt = kc \quad \text{..... Eq 2.3}$$

$$\text{In } c - \text{In } c_0 = - k t \quad \text{……..……..... Eq 2.4}$$

$$\log c = \log c_0 - (kt/2.303) \quad \text{..... Eq 2.5}$$

The linear expression in Equation (2.5) indicated that the plot of log concentration against time is linear with the slop of ($- k_{obs} / 2.303$) from which the value of k_{obs} is obtained. Hence when k_{obs} was plotted versus total buffer concentration the slope can be represented by Equation (2.4).

$$\text{Slope} = k_A [AH] + k_B [B] \quad \text{……..…….…..... Eq 2.6}$$

Hence the rate constant values for general catalysis of each buffer species (k_A and k_B) can be obtained from Equation (2.4) by using two slopes at different pH values. The observed rate constant indicated by Equation 2.7.

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$k_{obs} = k' + k_A [AH] + k_B [B]$ ……..……..... Eq 2.7

k_{obs} observed rate constant

k´ rate constant at zero buffer concentration

k_A catalytic rate constant of the buffer acid

[AH] concentration of the acid

k_B catalytic rate constant of conjugate base

[B] concentration of conjugate base

2.6 Temperature dependence studies

ART samples of 600 µg mL^{-1} in hydrochloric acid (pH 1.20), water (pH 6.50) and sodium hydroxide pH (10.50) were prepared. All samples were incubated in 22, 30 and 37 ˚C separately. Analysis of first sample by HPLC was regarded as zero time and subsequently more samples were withdrawn at selected intervals. The pH of the solution was recorded in the beginning and the end of the experiment.

A log percent remaining drug as a function of time was plotted for each experiment. The log rate constant of each reaction at specific temperature versus reciprocal temperature in Kelvin was also plotted. Activation energy can be calculated by substituting the slope of log rate constant versus reciprocal temperature in Equation (1.3) to result Equation 2.8

E_a = - slope (2.303. R)..... Eq 2.8

Samples of 108 mg mL^{-1} HP-ß-CD were prepared in 0.2 mol L^{-1} phosphate buffer solution at pH 7.00. An accurate amount of ART was mixed with HP-ß-CD solution to make a concentration of 600 µg mL^{-1} ART. All samples were incubated at 37 ˚C.

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A plot of log percent remaining drug as a function of time was plotted for each experiment. Shelf-life can be kinetically calculated according to the Equation 2.9 and activation energy can be also calculated according to the Equation 2.8.

$t_{90} = 0.105/ k_{obs}$ ……. Eq 2.7

2.7 Errors

Linear relationships were fitted by the method of least were fitted by the method of least squares. Errors in the slope and intercept values are expressed at the 95% confidence interval. Where runs were repeated three times, the rate constant values were normally within ± 2%.

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Chapter 3

EFFECT OF TEMPERATURE AND BUFFER SOLUTIONS ON THE STABILITY OF ARTESUNATE

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3 Results and discussion

3.1 Analytical methods

3.1.1 Assay validation

A reverse phase HPLC method was validated by determining the linearity of the detector response for a range of ART concentrations. The assay was found to give a linear relationship between the peak area and concentration of 200, 300, 400, 500, 600, and 700µg mL^{-1} ART in 40% acetonitrile solutions with a regression coefficient (R^2) of 0.9995 as shown in Figure (8)

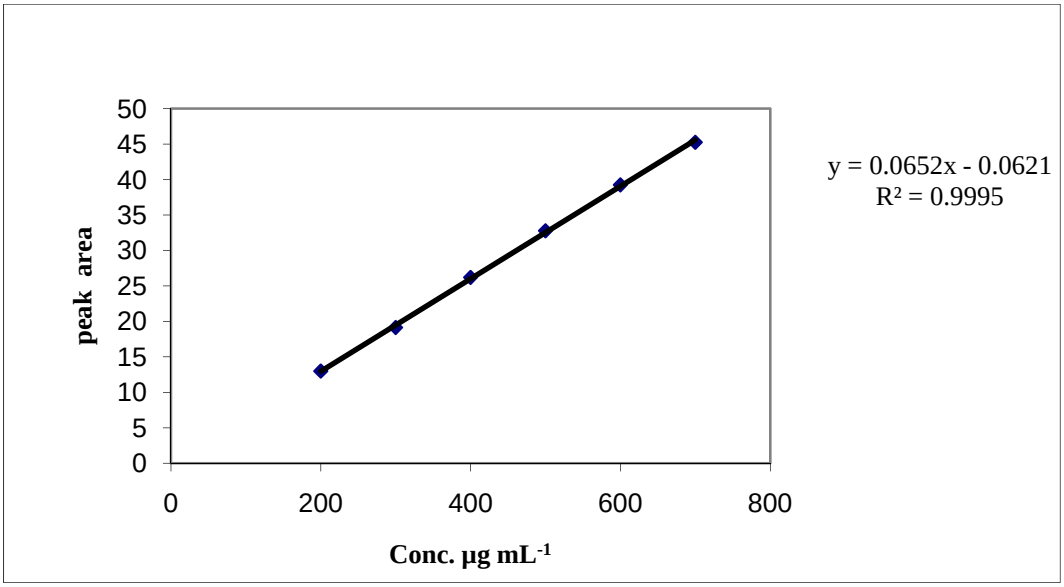

Figure 8. Calibration curve of artesunate.

The repeatability (precision) of the method was found by calculating the relative standard deviation (RSD) for the area counts of seven repeated injections. The value of the RSD for ART was 0.40%. The intraday precision of the method was determined by comparing the area counts of ART samples of 600 µg mL^{-1}

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concentration on two consecutive days. The RSD value for intraday precision for ART was 0.53%.

3.1.2 Stability indicating HPLC method

The stability indicating nature of the assay method was determined by inducing forced degradation of the drug in alkali (pH 13.00), acid (pH 1.00), and in purified water at 50 ˚C. The stability indicating method was also established to ascertain peak purity.

3.1.2.1 Acid degradation

A sample of 600 µg mL^{-1} ART was prepared in 2% ethanol solution and subjected to 0.1 M hydrochloric acid solution (~ pH 1.00) at 25 ˚C and ionic strength of 0.5 mol. L^{-1}. The degradation progress of ART was investigated by HPLC over 4 h. Figure 9 showed the detectable ART peaks at zero and at 4 h. There was no interference between the peaks at the retention time of ART with the others which were related to the degraded products. This showed an 83% less than the zero time concentration after 5 h exposure.

3.1.2.2 Alkali degradation

A sample of 600 µg mL^{-1} in 0.1 M sodium hydroxide was prepared and was incubated at 25 ˚C. The degradation process was investigated over 25 min until the smallest detectable ART peak was seen with retention time of 6.8 min as shown in Figure 10. There was no significant interference between the peaks at the retention time of ART with the others which were related to the degraded products. The slight change of retention time is common which is possibly related to the change of theoretical plates, column temperature or slight pH changes of the mobile phase.

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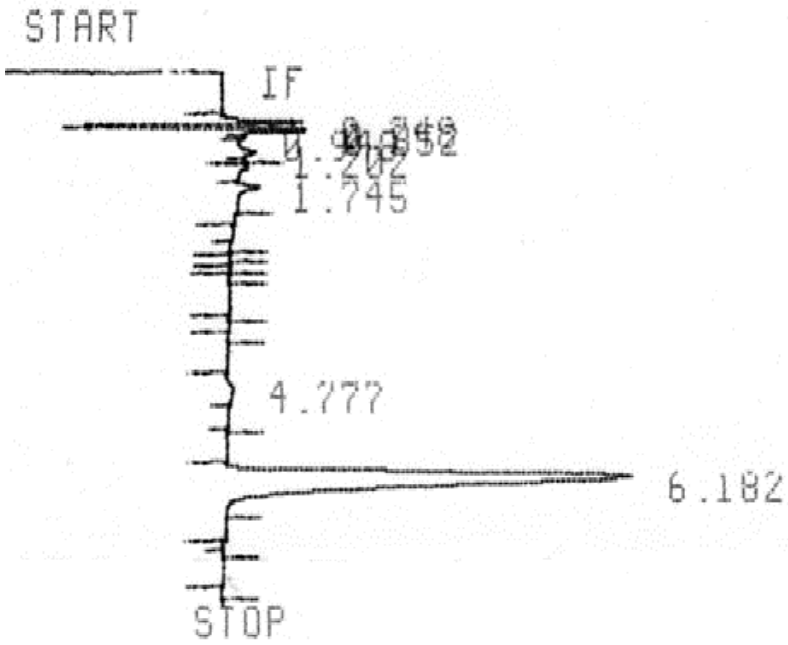
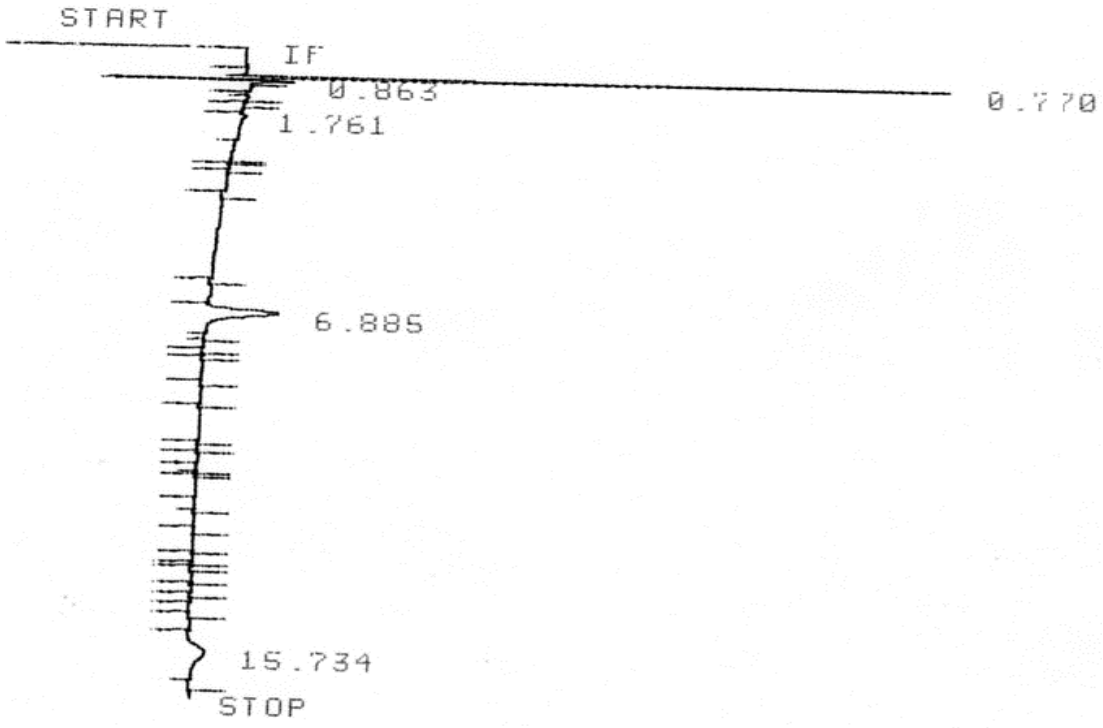


(A) At zero time



(B) After 4 h

Figure 9. Representative chromatogram of degradation of ART in 0.1 M HCL (~ pH 1.00) at 25 ˚C and μ of 0.5 mol L^{-1} . **(A)** at zero time and **(B)** after 4 h.

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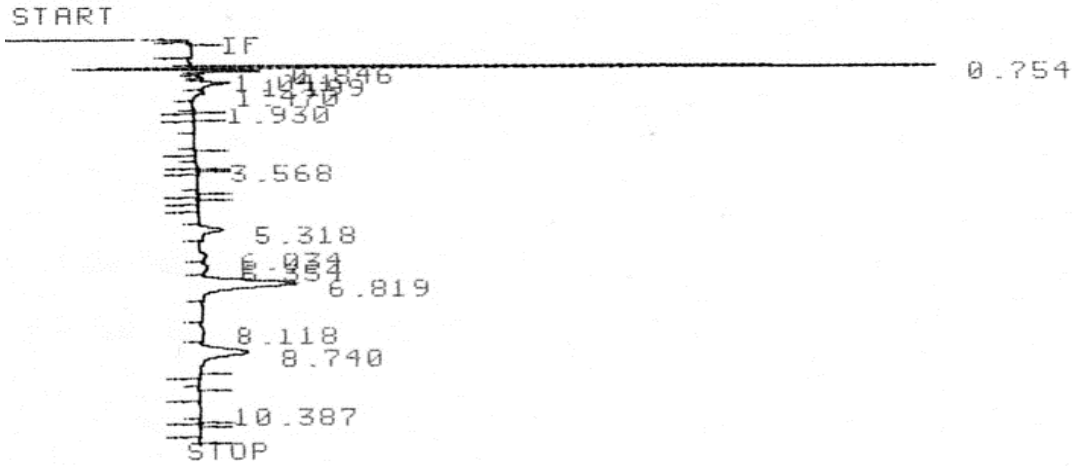
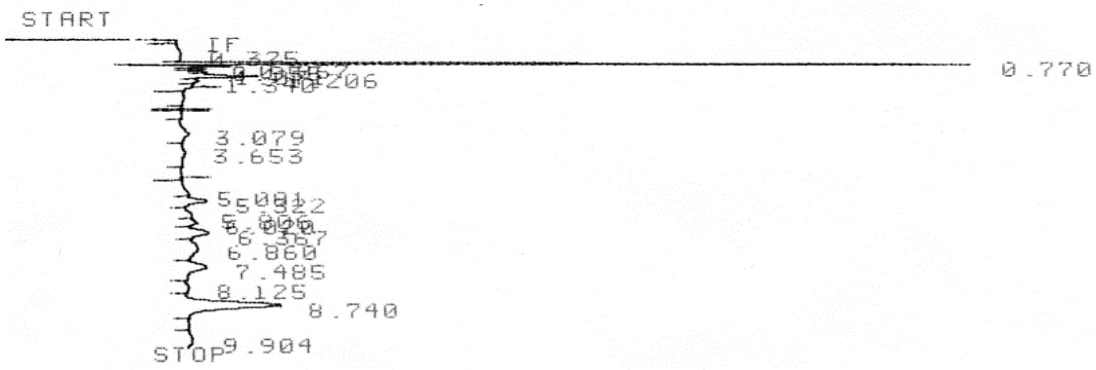


(A) At zero time



(B) After 25 min

Figure 10. Representative chromatogram of degradation of ART in 0.1 M NaOH (~ pH 13.00) at 25 ˚C and μ of 0.5 mol L^{-1} . **(A)** at zero time and **(B)** after 25 min.

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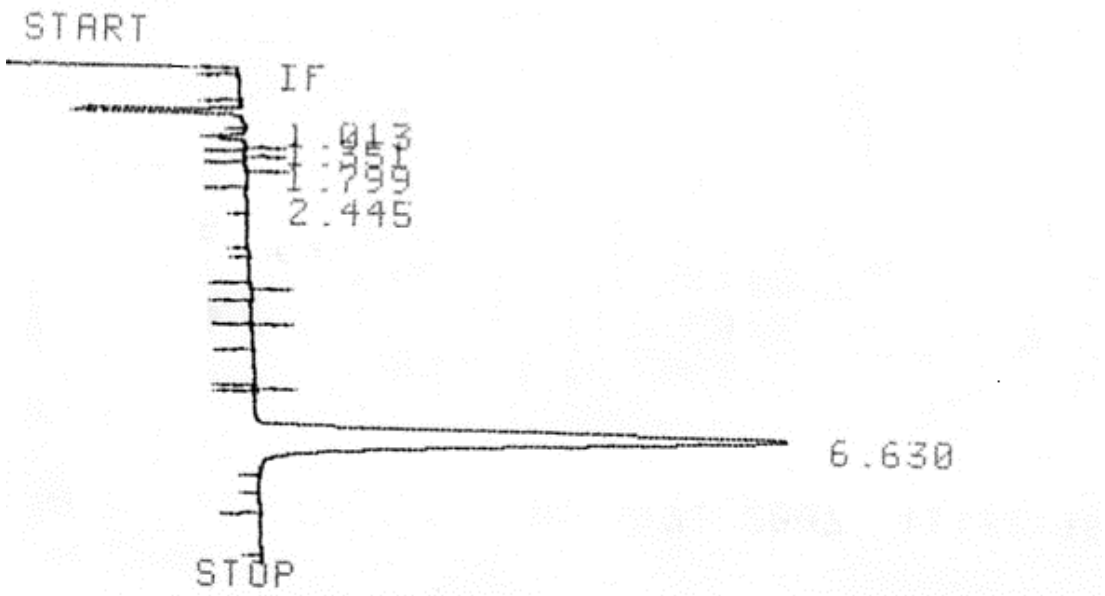
3.1.2.3 Forced degradation of ART in purified water.

A sample of 600 µg mL^{-1} ART in purified water was stored at 50 ˚C in a water bath. The pH of the solution was adjusted at 7.00 by adding dropwise sodium hydroxide. The degradation process was investigated for 5 h and there was no interference between the peak retention times of ART with the degraded products as shown in Figure 11. This showed a 54% lower concentration after 5 h and no evidence of interfering peaks. There is no evidence of any β-epimer and the degradation product is DHA.



(A) At zero time

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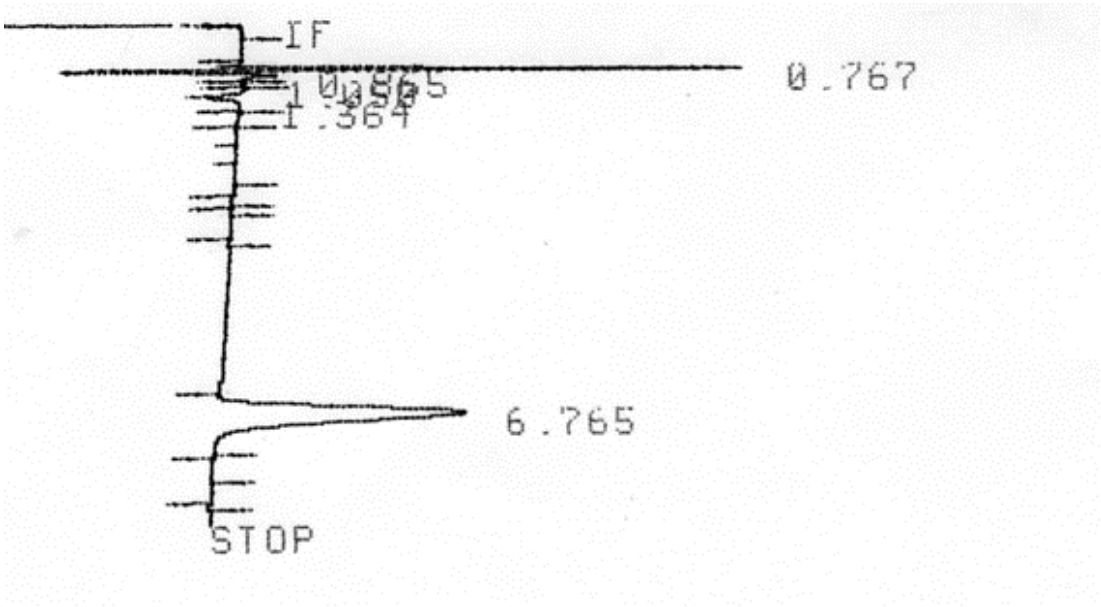

(B) After 5 h

Figure 11. Representative chromatogram of degradation of ART in purified water (pH 7.00) at 50 ˚C. **(A)** at zero time and **(B)** after 5 h.

3.2 Stability studies for ART

The stability of ART in aqueous solutions was investigated at different temperatures (22, 30 and 37 ˚C) and 0.1, 0.15 and 0.2 mol L^{-1} buffer solutions (phosphate at pH 2.00, 2.50, 5.50, 6.50, and 7.50, citrate at pH 3.50, acetate at pH 4.00 and 5.00, Tris at 8.50 and carbonate at pH 9.50 and 10.50) and ionic strength of 0.5 mol L^{-1}. Preparation of all ART samples in aqueous solutions at pH less than 6.50 required the addition of 2 mL of ethanol per 100 mL of final solution to ensure ART solubility in the solution.

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3.2.1 Effect of temperature on degradation of ART

The temperature dependence of ART was studied at three temperatures (22 and 30 and 37 ˚C) separately. Degradation of ART at each temperature was investigated by employing three pH values (1.20, 6.50 and 10.50). The rate constants were determined from plotting the log remaining percent concentration versus time. As expected, the rate of degradation of ART increased with increased temperature. Table 8 shows the rate constant values of ART with the three employed temperatures. There was obviously an increase in the observe rate constant values with increase of temperature.

Table 8. The observed rates constants values of ART and standard errors at 22, 30 and 37 ˚C

Temp. ˚C	k_{obs} h^{-1}(pH 1.20)	k_{obs} h^{-1} (pH 6.50)	k_{obs} h^{-1} (pH 10.50)
22	$02.57 \pm 0.03 \times 10^{-1}$	$0.99 \pm 0.011 \times 10^{-2}$	$3.4 \pm 0.02 \times 10^{-1}$
30	$7.22 \pm 0.012 \times 10^{-1}$	$2.87 \pm 0.024 \times 10^{-2}$	$10.7 \pm 0.01 \times 10^{-1}$
37	$16.8 \pm 0.026 \times 10^{-1}$	$7.43 \pm 0.044 \times 10^{-2}$	$22.0 \pm 0.47 \times 10^{-1}$

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Linear relationships (R^2 > 0.99) were obtained for all reactions at the three temperatures. Hence at the selected temperatures the degradation process of ART can be described by apparent first-order kinetics.

The Arrhenius plot was obtained by the normal procedure as shown in Figure (12).

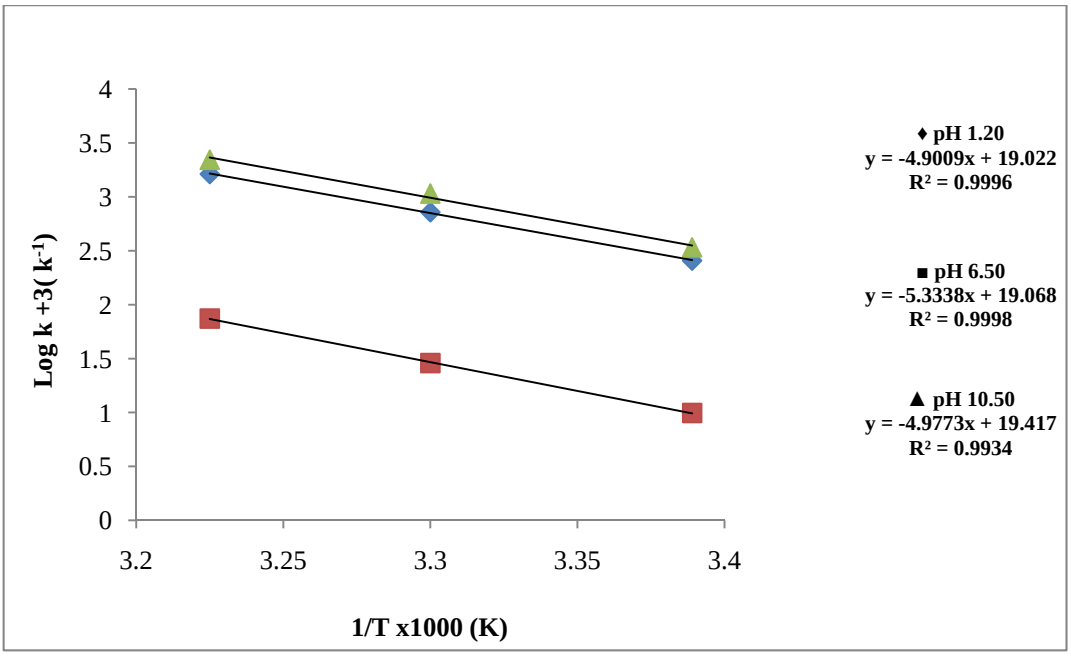

Figure 12. Temperature dependence of ART at 22, 30 and 37 ˚C.

From the slopes of all reactions, shelf-lives of ART can be calculated according to Equation 2.7. Table 9 shows shelf lives values of ART at the selected three temperatures.

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Table 9. Shelf lives values of ART at 22, 30 and 37 ˚C

Temp. (ºC)	(pH 1.20) t_{90} h	(pH 6.50) t_{90} h	(pH 10.50) t_{90} h
22	0.41	10.60	0.31
30	0.15	3.6	0.08
37	0.06	1.40	0.05

The activation energy (Ea) values were calculated according to the equation (2.8) and the values are listed in Table 10.

Arrhenius plots generally yields a straight line relationship. The frequency factor A in Equation 1.3 and activation energy are regarded as independent of temperature, and the activation energy is used as measure of temperature dependence of the rate constant. However, the Eyring equation suggests that both A and Ea should be temperature dependent. Activation energy can be shown to be related to change in the enthalpy ($\Delta H^{\ddagger}$) as indicated in Equation 1.6.

In order to better investigate the extent of temperature sensitivity for the ART rate constants, an analysis for the thermodynamic parameters of enthalpy and entropy change was carried out. The linear form of the Eyring equation represents a plot of logarithm of (k/T) versus 1/T with slope of $-\Delta H^{\ddagger}/R$ from which the enthalpy of activation ($\Delta H^{\ddagger}$) can be derived and with intercept ($\ln k/h + \Delta S^{\ddagger}/R$) from which the entropy ($\Delta S^{\ddagger}$) of activation is derived. Where *k* is the Boltzmann's constant and h is Planck's constant. A linear relationship (correlation coefficient r > 0.99) was produced from the plots of (ln k/T) versus 1/T as shown in Figure 13. According to

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those conditions, which were employed in this investigation, Ea, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are listed in Table 10.

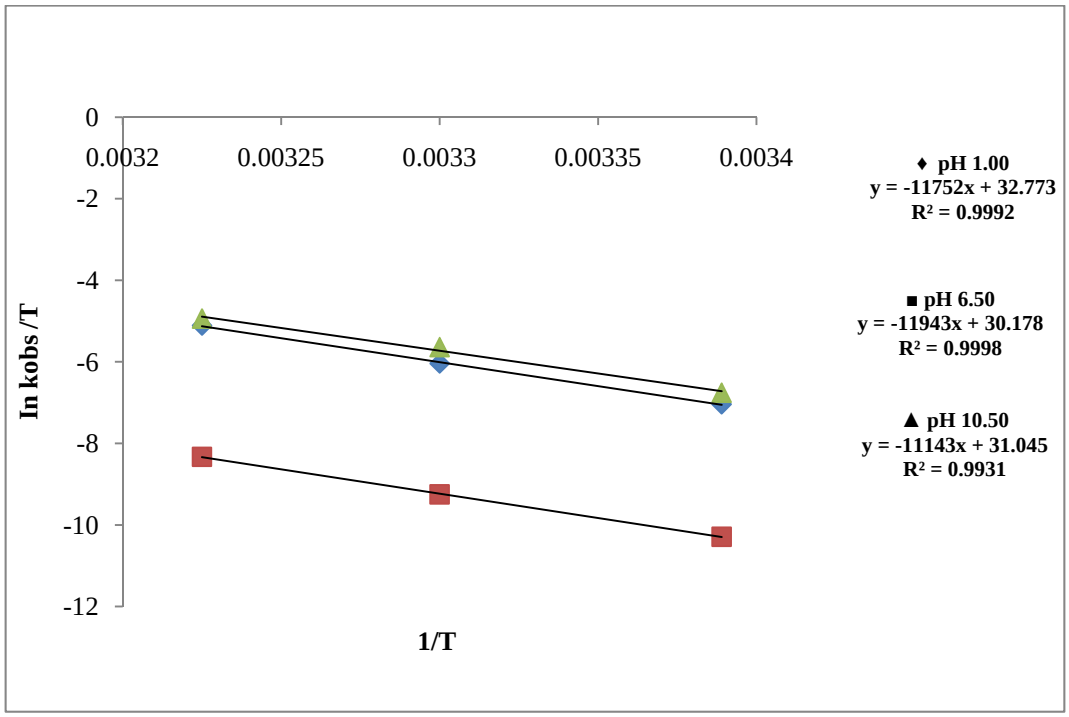

Figure 13. Enthalpy plot of degradation of ART at 22, 30 and 37 ˚C and in pH 1.20, 6.50 and 10.50.

Table 10. Activation energy, enthalpy and entropy of activation changes with standard errors values for ART at 22, 30 and 37 ˚C.

pH	Ea ($kJmol^{-1}$)	$\Delta H^{\ddagger}$ ($kJmol^{-1}$)	$\Delta S^{\ddagger}$ ($J\ K^{-1}mol^{-1}$)
1.2	93.8 ± 0.044	97.7 ± 0.044	74.9 ± 0.032
6.5	101.2 ± 0.005	99.3 ± 0.005	53.3 ± 0.003
10.5	95.3 ± 0.320	92.6 ± 0.320	60.5 ± 0.210

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The results in Table 10 show relatively high values of Ea and $\Delta H^{\ddagger}$ which indicates a significant temperature dependence for ART degradation. Hence storage at ambient temperature in warmer climates will have a marked negative effect on the shelf-life of ART in the solution. The smaller value for $\Delta S^{\ddagger}$ change indicates that much of the free energy change is embodied in $\Delta H^{\ddagger}$**.** These data together with shelf-life data in Table 9 indicate a very short shelf-life of reconstituted ART solutions.

3.2.2 Stability studies of ART in buffer solutions using HPLC

Degradation of ART was studied in phosphate, citrate, acetate, Tris and carbonate buffer solutions at a range of pH values (2.00 – 10.50).

Mixing ART with the buffer solution caused a decrease in pH due to the acidic nature of the drug. Therefore the pH was adjusted by the dropwise addition of sodium hydroxide solution to the required pH value. The pH of the admixture was measured at the beginning and end of each analysed run. There was a difference of not greater than ± 0.02 in the pH of the admixture over the course of run, which was not considered significant. The catalytic effects of all buffer solutions, which were used in this study, were investigated at a concentration of 600 µg mL^{-1} of ART at 37 ˚C, ionic strength of 0.5 mol L^{-1} and at a given pH; the only variation was in the overall concentration of the buffer. Three different buffer concentrations (0.1, 0.15 and 0.2 M) were employed in all investigations.

The decomposition rate of ART in each buffer solution was found from plotting of logarithm of concentration of drug remaining versus time. Methods of calculation of rate constants are discussed in detail by Martin et al.[70] A straight line obtained from

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these plots over 2-3 half-lives of reaction indicated that the reaction was apparent first order in nature.

3.2.2.1 Degradation of ART in phosphate buffer solutions at pH 2.00 and 2.50

Decomposition of 600 µg mL^{-1} ART in 0.1, 0.15 and 0.2 mol L^{-1} phosphate buffer solution (pH 2.00 and 2.50) was studied at 37 ˚C for three halves lives of reaction. All reactions showed linear relationships (R^2 > 0.99). These are shown in Figures 14 and 15.

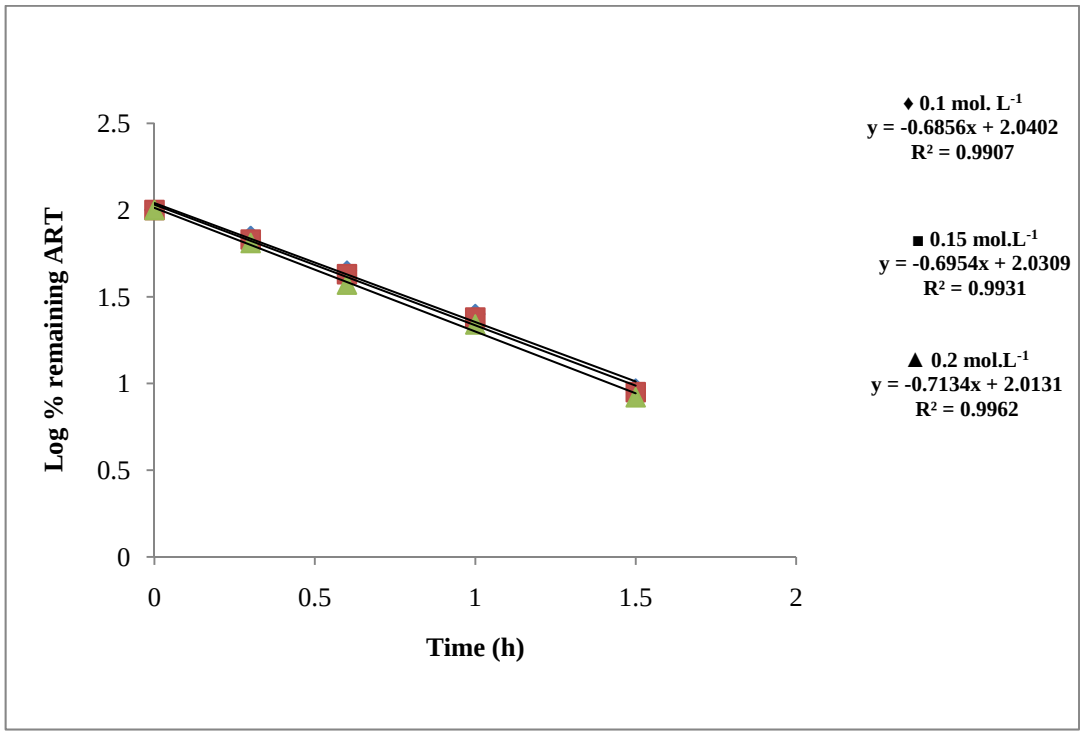

Figure 14. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} phosphate buffer solution (pH 2.00) at 37 ˚C and µ of 0.5 mol L^{-1}.

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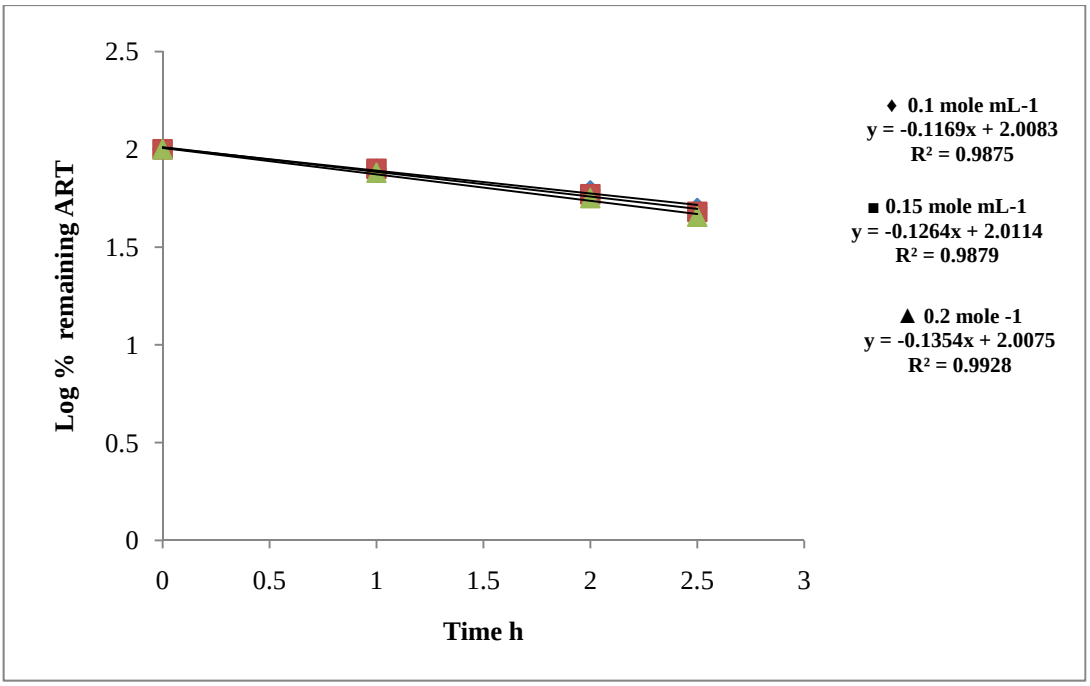

Figure 15. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} phosphate buffer solution (pH 2.50) at 37 ˚C and µ of 0.5 mol l^{-1}.

The catalytic effect of the selected buffer system was evaluated and examined at 600 µg mL^{-1} ART, 37 ˚C and ionic strength of 0.5 mol L^{-1} at a given pH. The overall constant (k_{obs}) for the degradation of ART in buffer can be calculated as in Equation 2.3. The observed rates constant values of ART at pH 2.00 were 1.57, 1.60 and 1.64 h^{-1} and pH 2.50 were 0.269, 0.291 and 0.311 h^{-1}. Plots of observed rates constant values of ART at pH 2.00 and 2.50 versus phosphate buffer concentrations were plotted as in Figure 18. There was no significant effect on the rate of degradation of ART with increased concentration of buffer solution at pH 2.00 or 2.50. For example at pH 2.00 the rate constant increased from1.51 h^{-1} at 0.1 M buffer to 1.64 h^{-1} at 0.2 M buffer concentration.

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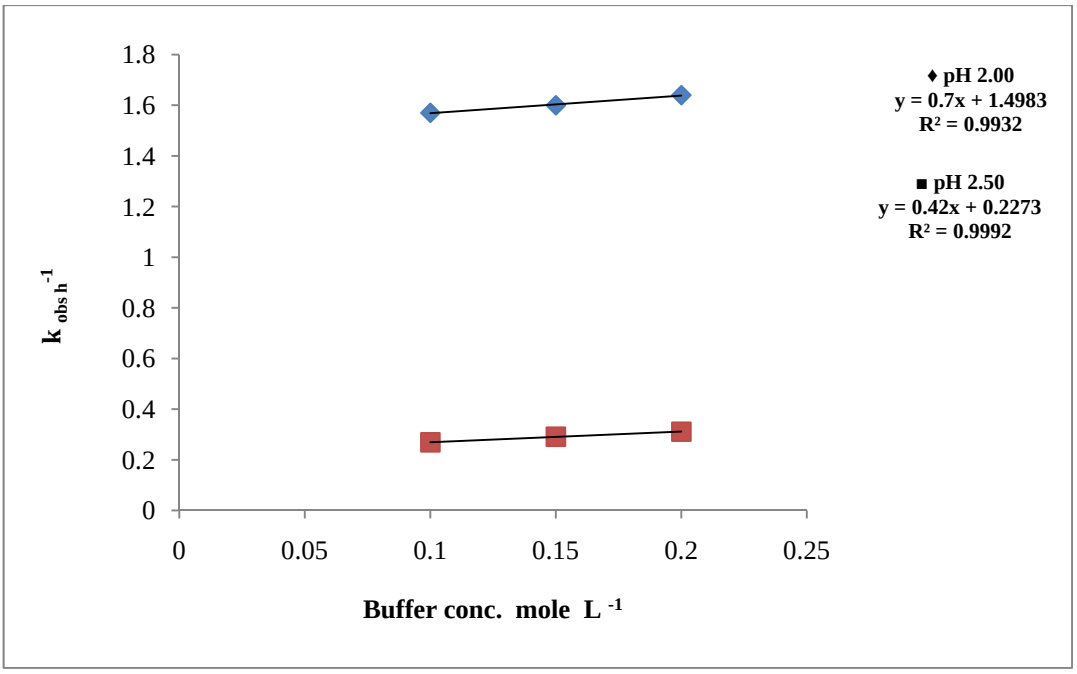

Figure 16. Catalytic effect of phosphate buffer solution on rate degradation of ART at pH values of 2.00 and 2.50 and 37 ˚C.

3.2.2.2 Degradation of ART in the ammonium acetate buffer solution at pH 3.00.

Decomposition of ART at 600 µg mL^{-1} in ammonium acetate buffer solution of different concentrations was evaluated at 37 ˚C and ionic strength of 0.5 mol L^{-1}. All reactions showed linear relationships (R^2 > 0.99) which indicated that all reactions were first-order as shown in Figure 17. Ammonium acetate was selected as a buffer solution at pH 3.00 for evaluation of ART degradation within HPLC and LC-MS. Ammonium acetate or formate buffer solution can be used within LC-MS because they are volatile buffer solutions in the LC-MS system.

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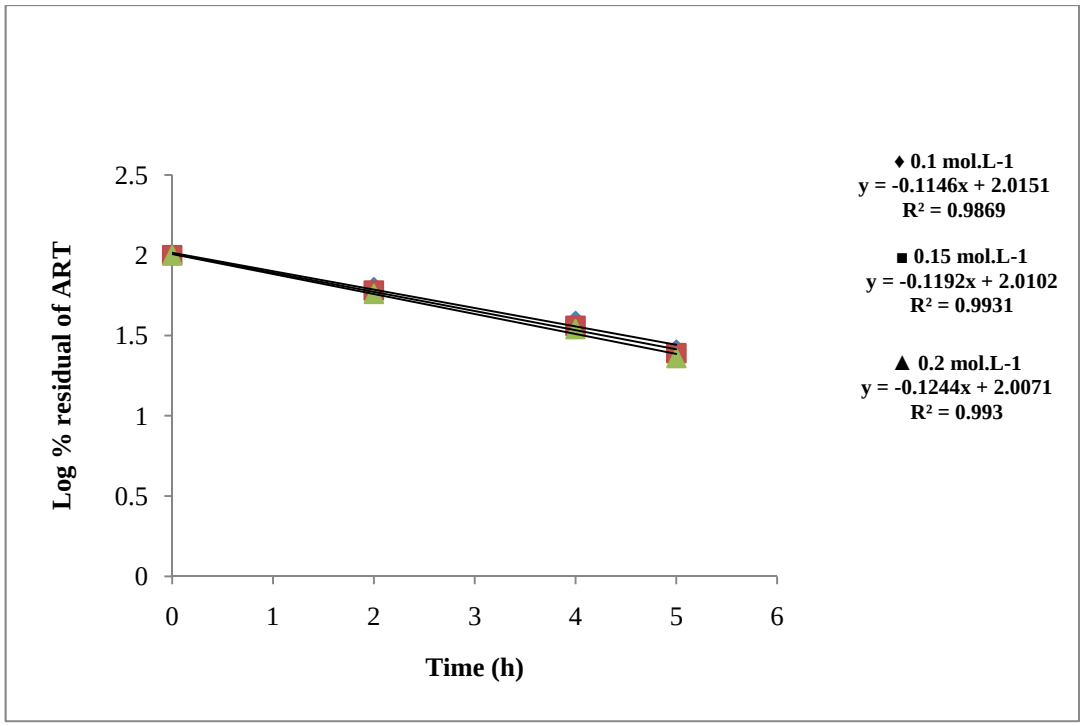

Figure 17. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} ammonium acetate buffer solutions (pH 3.00) at 37 ˚C and μ of 0.5 mol L^{-1}.

3.2.2.3 Degradation of ART in citrate buffer solution at pH 3.5

Decomposition of ART at 600 μg mL^{-1} in citrate buffer solution of different concentrations was evaluated at 37 ˚C and ionic strength of 0.5 mol L^{-1}. All reactions showed linear relationships (R^2 > 0.99) which indicated that all reactions were first-order as shown in Figure 18.

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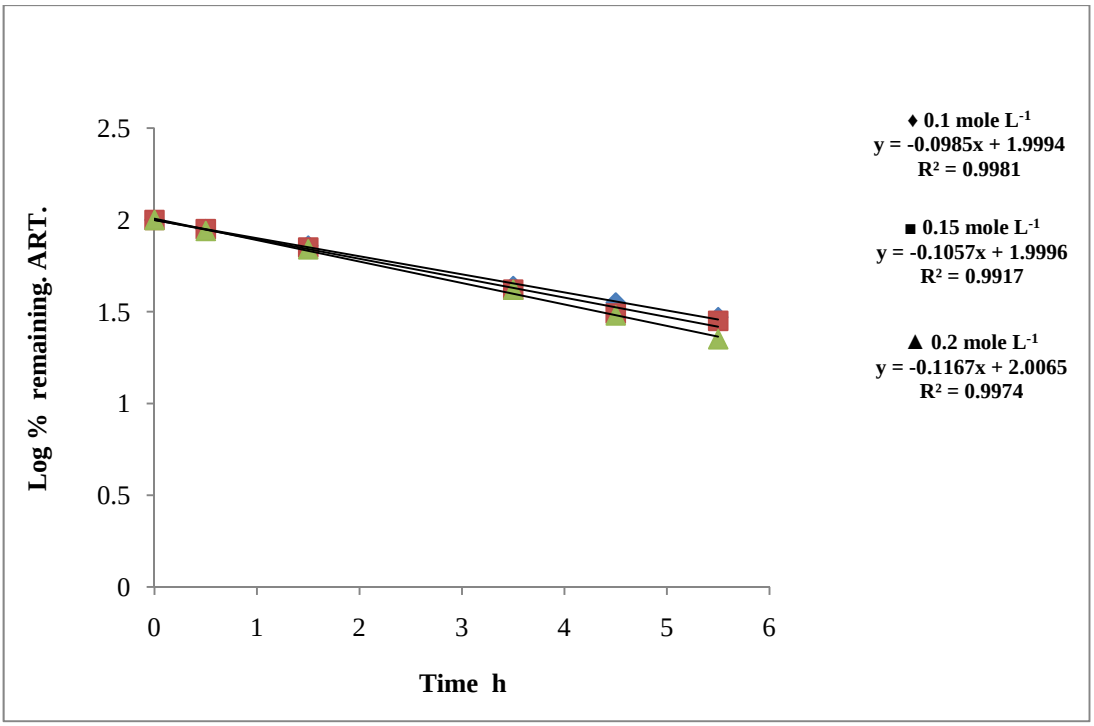

Figure 18. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} citrate buffer (pH 3.50) at 37 ˚C and μ of 0.5 mol L^{-1}.

3.2.2.4 Degradation of ART in acetate buffer solution at pH 4.00 and 5.00

Degradation of ART in acetate buffer solutions (pH 4.00 and 5.00) of different concentrations was investigated at 37 ˚C and ionic strength of 0.5 mol L^{-1}. The degradation process of ART was investigated over three consecutive half lives. Figures 19 and 20 demonstrated linear relationships ($R^2 > 0.99$) which indicated that such reactions obeyed first-order reaction kinetics.

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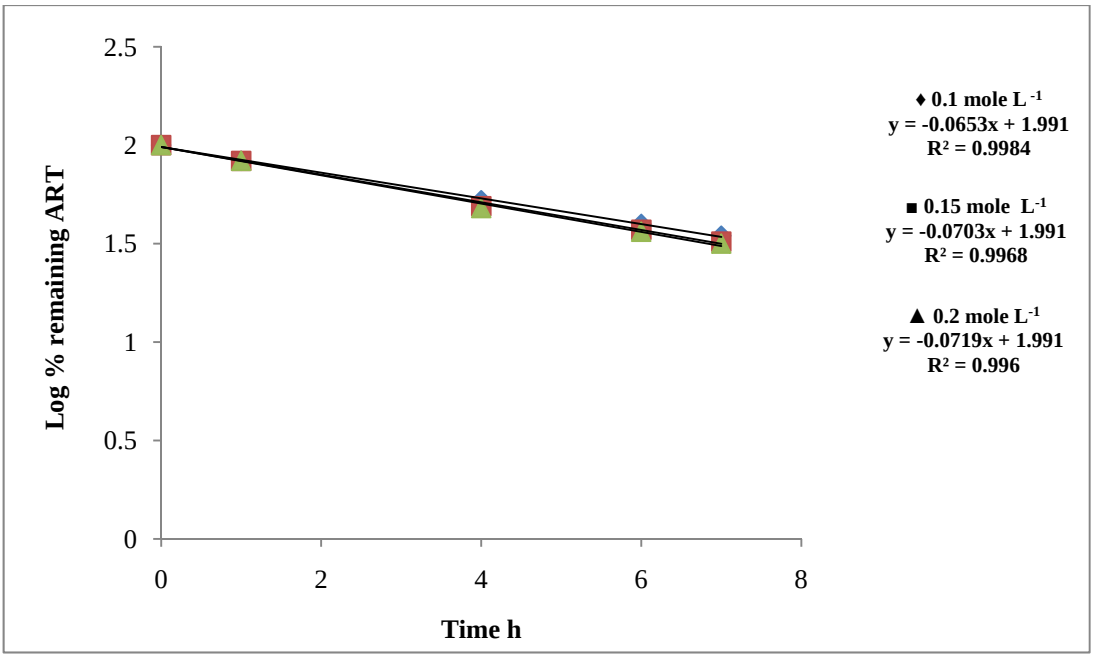

Figure 19. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} acetate buffer solutions (pH 4.00) at 37 ˚C and μ of 0.5 mol L^{-1}.

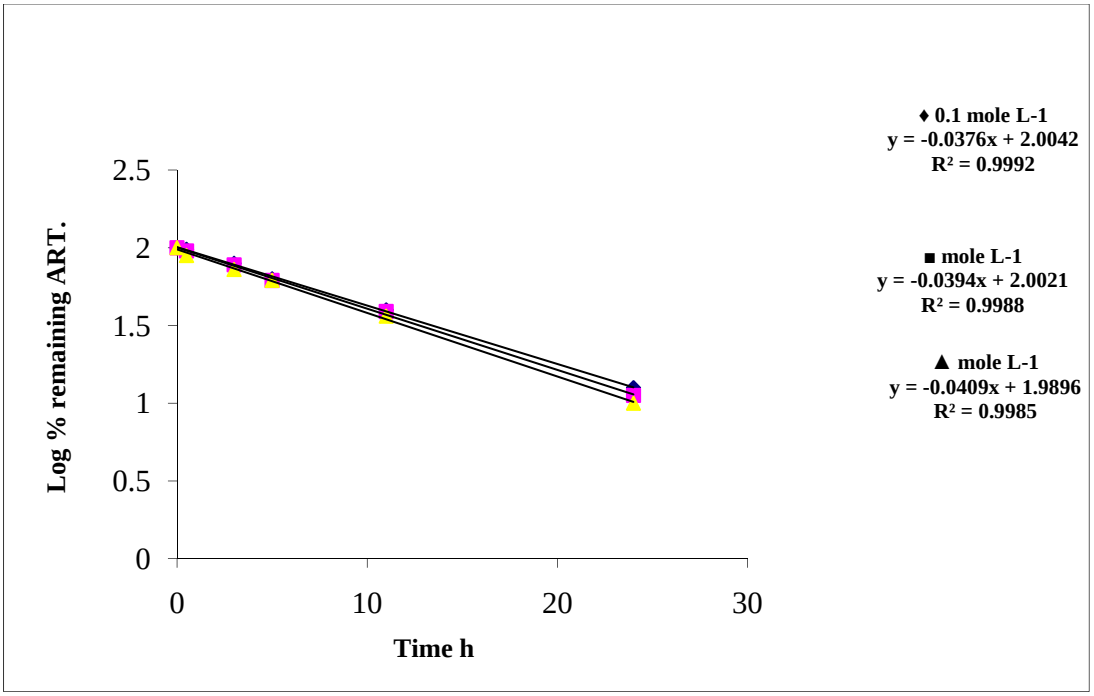

Figure 20. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} acetate buffer solutions (pH 5.00) at 37 ˚C and μ of 0.5 mol L^{-1}.

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The observed rate constants of ART at pH 4.00 and 5.00 versus acetate buffer concentrations were plotted as in Figure 21. There was a small effect on the rate of degradation of ART with increased concentration of buffer components at pH 4.00 or 5.00. The lower slope value at pH 5.00 may indicate that the dissociated form of ART or acetate ion is less catalytic.

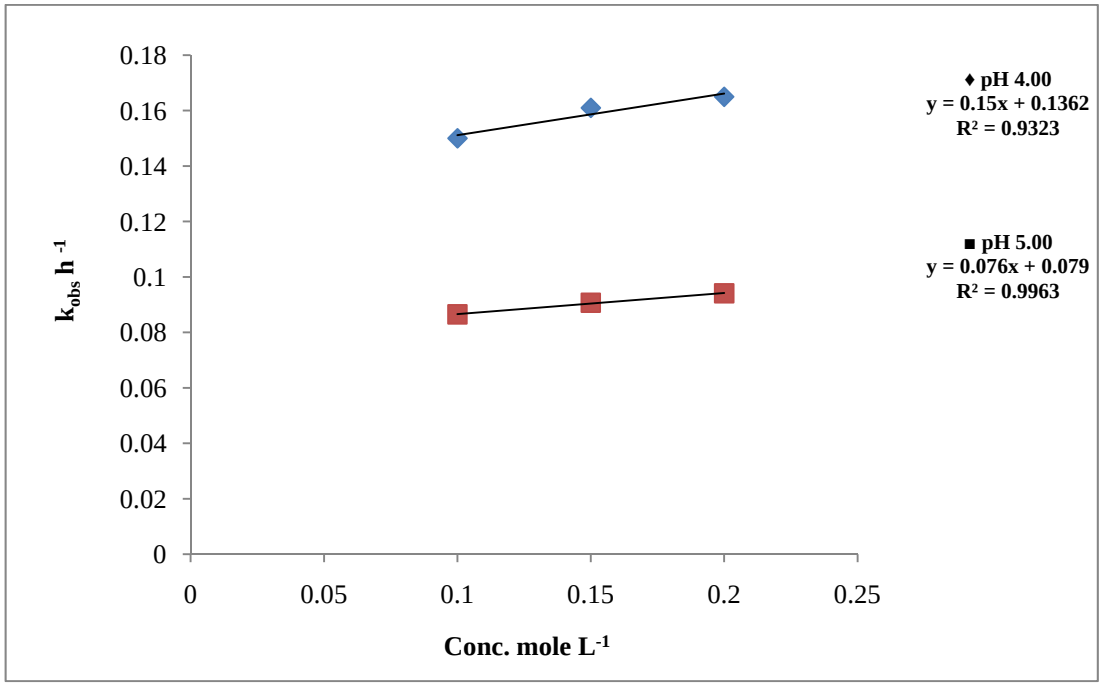

Figure 21. Catalytic effect of acetate buffer solution on rate degradation of ART at pH 4.00 and 5.00 and 37 ˚C.

3.2.2.5 Degradation of ART in phosphate buffer solutions at pH 5.50, 6.50 and 7.50.

Degradation of ART in phosphate buffer at pH 5.50, 6.50 and 7.50 was investigated and evaluated at 37 ˚C and ionic strength of 0.5 mol L^{-1} over three half lives of reaction. Linear relationships ($R^2 > 0.99$) were produced indicating a first-order reaction. Figures 22, 23 and 24 show the rates degradation of ART at pH 5.50, 6.50 and 7.50 respectively.

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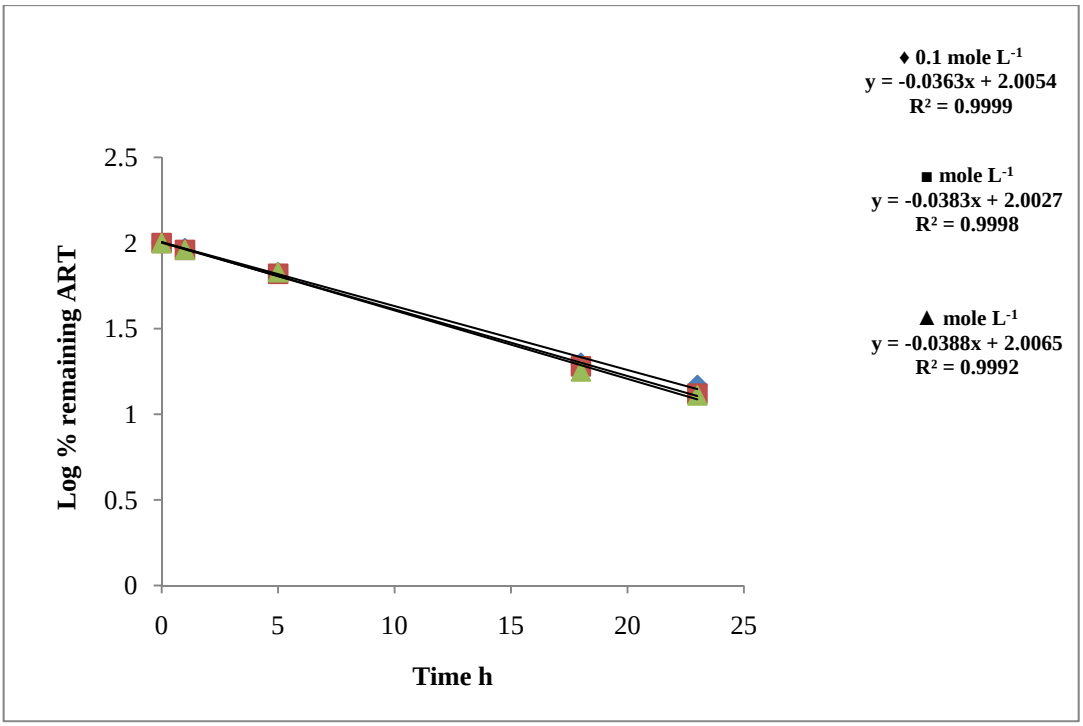

Figure 22. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} phosphate buffer solution (pH 5.50) at 37 ˚C and μ of 0.5 mol L^{-1}.

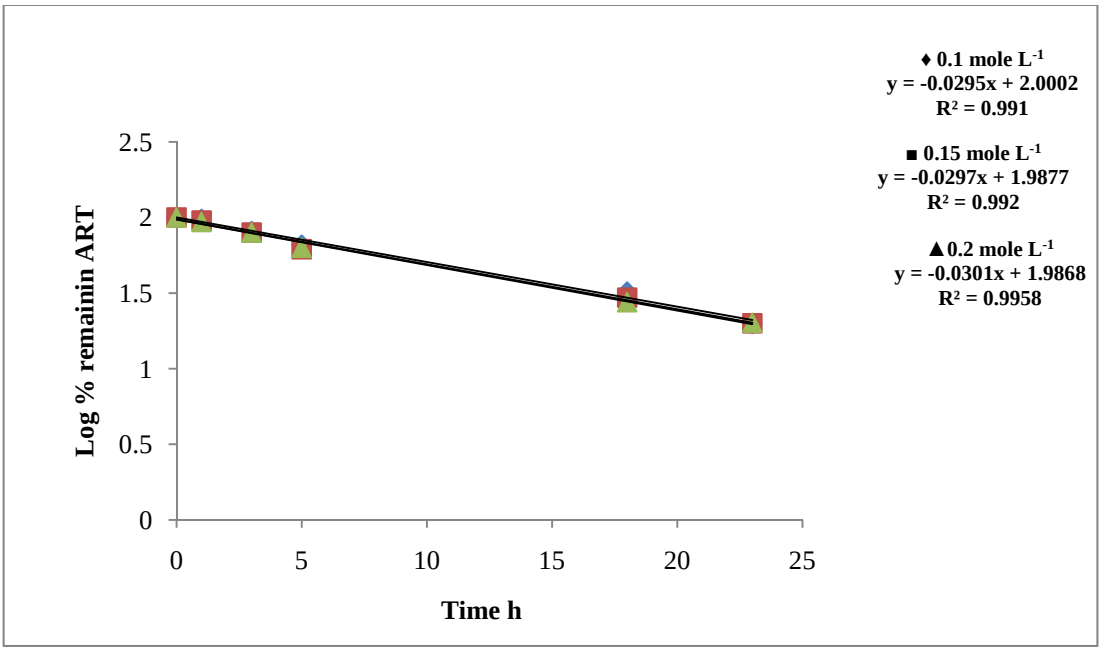

Figure 23. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} phosphate buffer solution (pH 6.50) at 37 ˚C and μ of 0.5 mol L^{-1}.

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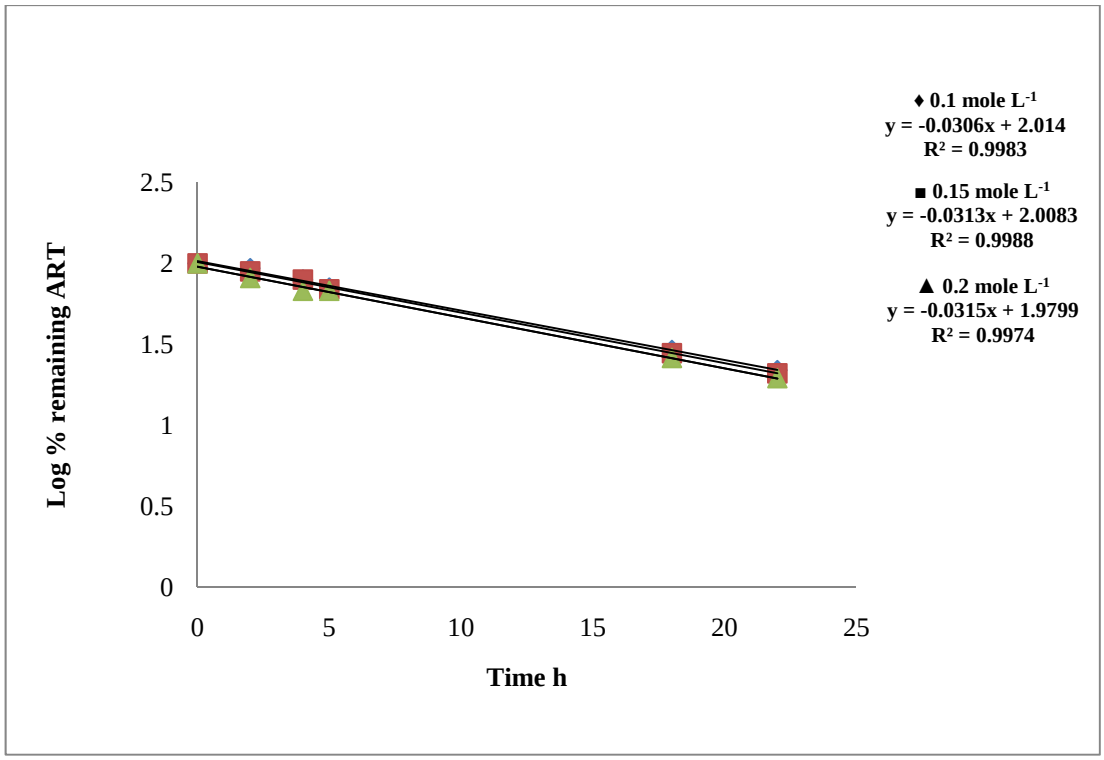

Figure 24. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} phosphate buffer solution (pH 7.50) at 37 ˚C and µ of 0.5 mol L^{-1}.

The observed rates constant of ART at pH 5.50, 6.50 and 7.50 were obtained and plotted against phosphate buffer concentrations as shown in Figure 25. There was no marked effect on the rate degradation of ART with increased concentration of buffer solution. For example at pH 6.50 the catalytic rate constant at 0.1 M buffer was 0.0680 h^{-1} and at 0.2 M was 0.0693 h^{-1} which indicates no marked catalytic effect. Same comparison can be demonstrated at pH 7.50 where the catalytic rate constant at 0.1 M buffer was 0.070 and at 0.2 M buffer was 0.072 h^{-1}.

The lower slope values at pH 6.50 and 7.50 in respect to the pH 5.50 may indicate that dissociated form of ART or phosphate ion is less catalytic.

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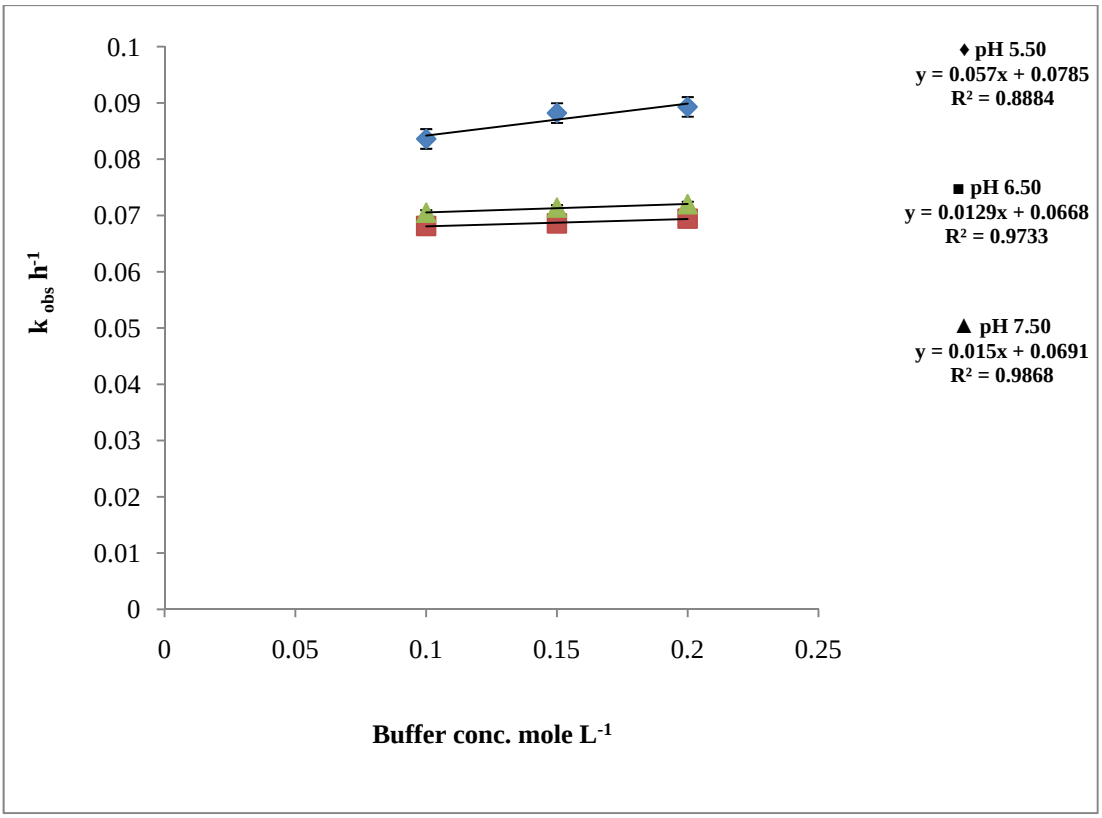

Figure 25. Catalytic effect of phosphate buffer solution on rate degradation of ART at pH 5.50, 6.50 and 7.50.

3.2.2.6 Degradation of ART in Tris buffer solution at pH 8.5.

Decomposition of ART in Tris buffer solution of different concentrations was evaluated at 37 ˚C and ionic strength of 0.5 mol L^{-1}. All reactions showed linear relationships (R^2 > 0.99) which indicated that all reactions were first-order as shown in Figure 26.

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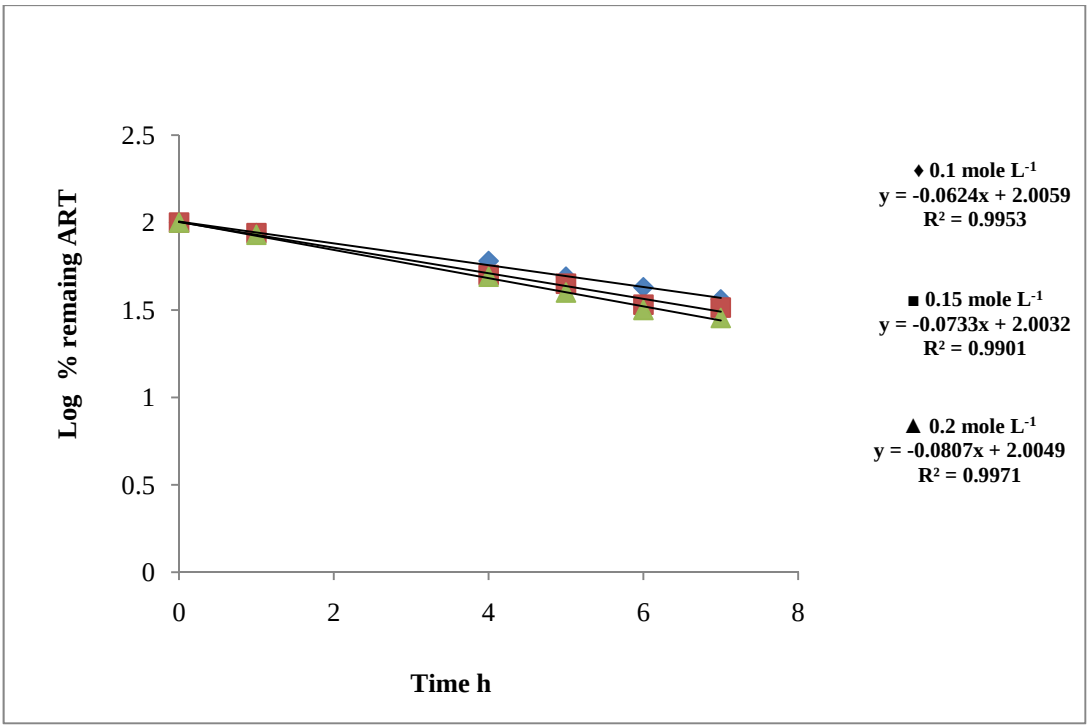

Figure 26. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} Tris buffer solution (pH 8.50) at 37 ˚C and μ of 0.5 mol L^{-1}.

3.2.2.7 Degradation of ART in carbonate buffer solution at pH 9.50 and 10.50.

Degradation rates of ART in carbonate buffer solution (pH 9.50 and 10.50) at 37 ˚C and ionic strength of 0.5 mol L^{-1} was investigated. All reactions showed linear relationships ($R^2 > 0.99$) which indicated that all reactions were first-order as shown in Figures 27 and 28. The observed rate constants were plotted against buffer concentrations as shown in Figure 29. There was no marked effect of buffer concentration on the rate of degradation of ART, especially at pH 9.50.

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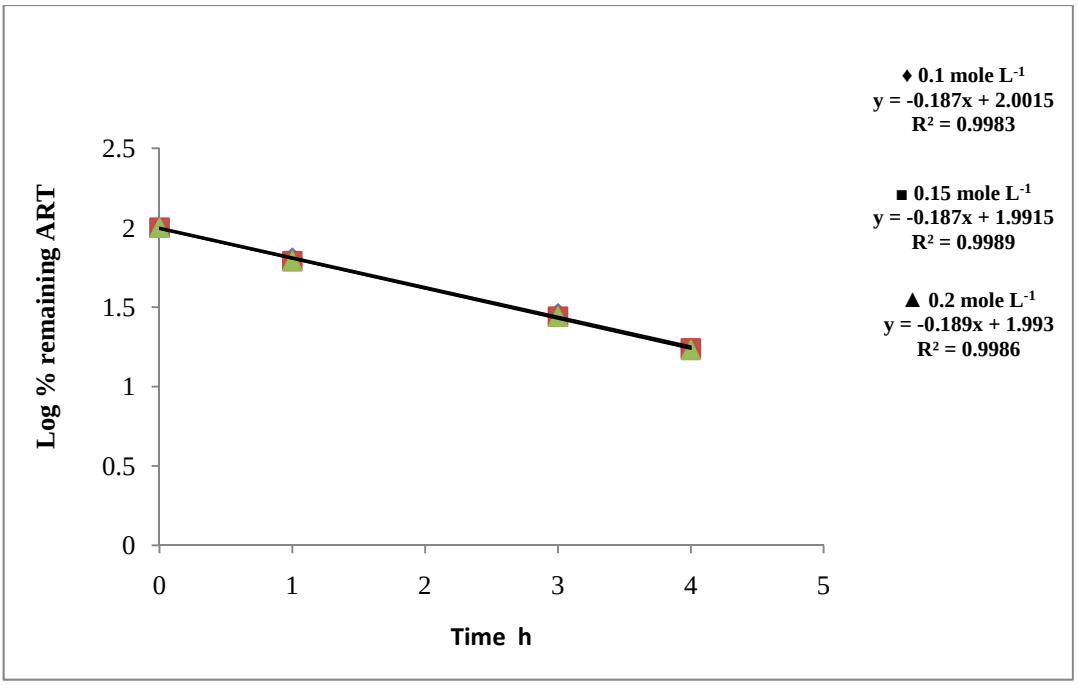

Figure 27. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} carbonate buffer solution (pH 9.50) at 37 ˚C and μ of 0.5 mol L^{-1}.

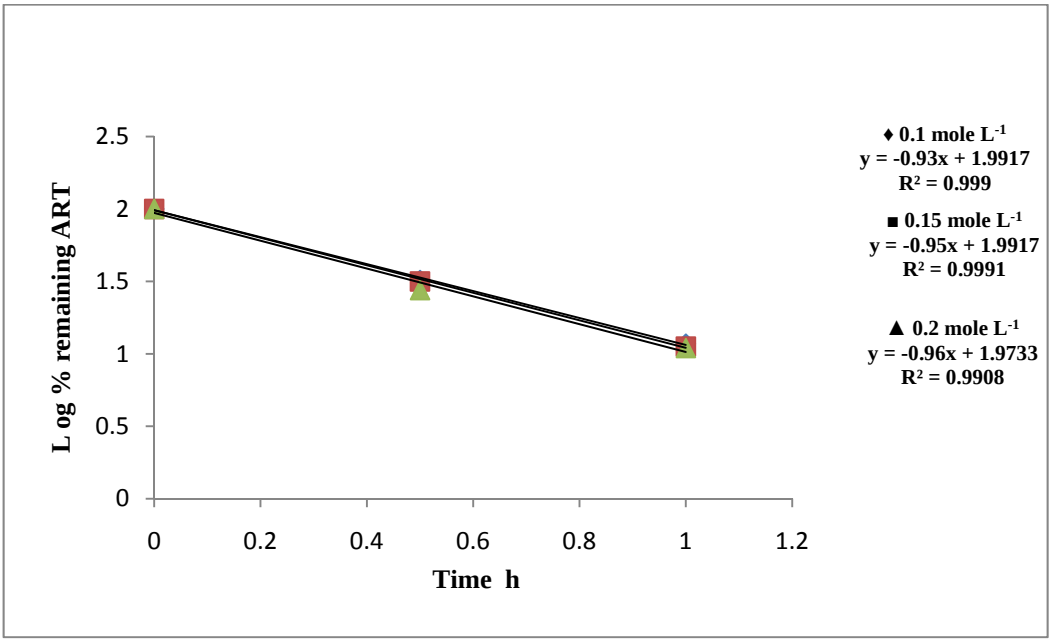

Figure 28. Degradation of ART in 0.1, 0.15 and 0.2 mol L^{-1} carbonate buffer solution (pH 10.50) at 37 ˚C and μ of 0.5 mol L^{-1}

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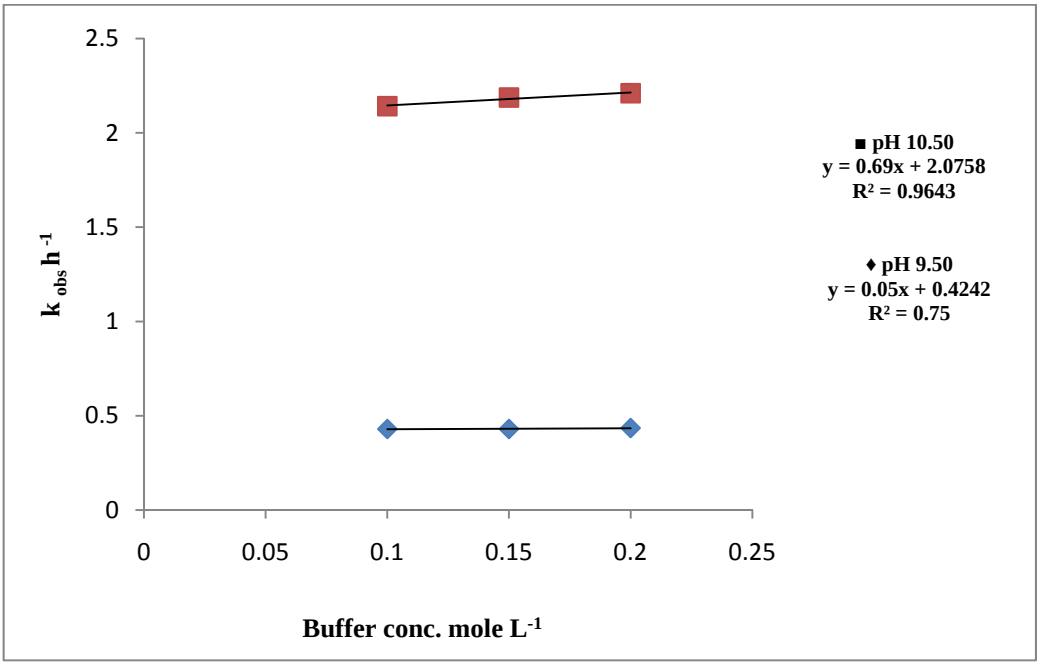

Figure 29. Catalytic effect of carbonate buffer solution on rate degradation of ART at pH 9.50 and 10.50.

Table 11 shows all of the observed rates constants of ART at pH range of 2.00-10.50. Data in Table 11 indicated no marked increased of rates constants with increased buffer concentration and it shows the lowest observe rate constant was at pH 6.50. This would indicate that formulation in various buffers is not likely to have a major effect on the shelf-life. For example formulation at pH 6.50 at zero buffer concentration has a rate constant of 0.067 h^{-1} and shelf-life of 1.57 h. At 0.2 M phosphate buffer this has increased to 0.069 h^{-1} and the shelf-life of 1.52 h

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Table 11. List of observe rate constants of ART and standard error (SE) at pH 2.00-10.50 and 37 ºC

Buffer Conc.	**0.1 mol L^{-1}**	**0.15 mol L^{-1}**	**0.2 mol L^{-1}**
pH	**k_{obs} h^{-1} ± SE**	**k_{obs} h^{-1} ± SE**	**k_{obs} h^{-1} ± SE**
2.00	1.57 ± 0.02	1.60 ± 0.015	1.64 ± 0.013
2.50	0.27 ± 0.005	0.29 ± 0.004	0.31 ± 0.007
3.00	0.26 ± 0.003	0.27 ± 0.003	0.29 ± 0.004
3.50	0.226 ± 0.003	0.24 ± 0.003	0.27 ± 0.002
4.00	0.15 ± 0.001	0.161 ± 0.001	0.165 ± 0.002
5.00	0.086 ± 0.0003	0.090 ± 0.0003	0.094 ± 0.0003
5.50	0.083 ± 0.0004	0.088 ± 0.0004	0.089 ± 0.0004
6.50	0.068 ± 0.0006	0.0684 ± 0.0004	0.069 ±0.0003
7.50	0.070 ± 0.0004	0.072 ± 0.0004	0.0725 ± 0.0004
8.50	0.143 ± 0.0013	0.169 ± 0.0015	0.186 ± 0.0016
9.50	0.430 ± 0.0037	0.430 ±0.002	0.435 ± 0036
10.50	2.14 ± 0.043	2.19 ±0.041	2.21 ± 0.049

`

3.2.3 Influence of buffer species

In order to evaluate the effect of the buffer species on the rate of degradation of ART, a series of buffer concentrations with the same [B]/[AH] ratio, where [AH] and [B] are the concentrations of the weak acid and the conjugate base respectively, using at least two pH values and constant ionic strength. If the observed rate constant increased with increasing buffer concentration**,** one or both of the buffer species is exerting a catalytic effect. The observed rate constant of ART in buffer solutions can be expressed by Equation 2.5

$k_{obs} = k' + k_A [AH] + k_B [B]$ ……..……..... Eq 2.7

Where k_{obs} = the total observed rate constant of ART in buffer solution.

The total buffer concentration (C_T) can be written as in Equation 3.1.

$C_T = [AH] + [B]$ ……..... Eq 3.1

The slopes of plots in Figures 16, 21, 25 and 29 represent the summation values of k_A [AH], k_B [B] and the pH effect. The molar ratio of acid and its conjugate base can be calculated by the Henderson-Haselbach Equation at any pH value after correction for the pKa of the employed acid in the buffer solutions. For the accurate pKa determination of an acid-base pair, correction should be made for pKa due to the effect of ionic strength. For example the pKa of phosphate is 7.1 while the corrected pKa (pKa′) was 6.94 which is calculated by using Davies' Equation.[100] Table 12

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illustrates the total observed rates constant values of ART in the phosphate and carbonate buffer solutions.

In addition the pH was measured for this study which is a measure of a_{H^+} (activity of hydrogen ion). This value is dependent on ionic strength.[101] For example at 37 ˚C and ionic strength 0.5 mol L^{-1}

$$C_{H^+} \cdot C_{OH^-} = 5.01 \times 10^{-14} \quad \text{…….....} \quad \text{Eq 3.2}$$

And $a_{H^+} \cdot a_{OH^-} = 2.3 \times 10^{-14}$ ……..…... Eq 3.3

Combining Equation 3.2 and 3.3 will give

$$a_{H+} = C_{H^+} + y_{H^+}$$

And $a_{OH^-} = C_{OH^-} + y_{OH^-}$

Gives $y = 0.57$

Hence $\log C_{H^+} = 0.24 - pH$

And $\log C_{OH^-} = pH - 13.54$

`

Table 12. Effect of phosphate and carbonate buffer concentration on the rate constant values of ART and standard error at 37 ˚C, μ of 0.5 mol L^{-1}.

pH	k_{obs} (h^{-1}) ± SE		
	0.1 (mol L^{-1})	**0.15 (mol L^{-1})**	**0.2 (mol L^{-1})**
5.50	9.20 ± 0.00045	10.10 ± 0.00043	10.96 ± 0.00041
6.50	7.96 ± 0.00069	8.61 ± 0.00048	9.25 ± 0.00028
7.50	8.40 ± 0.00047	9.15 ± 0.00046	9.90 ± 0.00042
9.50	27.00 ± 0.00038	74.00 ± 0.0019	86.00 ± 0.00036
10.50	181.00 ± 0.00043	311.00 ± 0.00041	345.00 ± 0.00049

3.2.4 Comparison between HPLC and LC-MS for measurement of rate degradation of ART in buffer solution.

Evaluation of the rates degradation of ART in buffer solutions at three selected pH values (3.00, 6.50 and 8.50) was performed by using LC-MS and HPLC separately. Samples of 0.6 mg mL^{-1} of ART in 0.1, 0.15 and 0.2 mol L^{-1} acetate buffer solution were prepared and incubated at 37 ˚C. As was expected all plots of remaining ART concentrations versus time demonstrated straight lines which indicated a first-order reaction. The retention time was 5.8 min as it shown in the chromatogram Figure 30. The observed rate constants of ART at different pH values are illustrated in Table 13. There was no marked difference in the observed rate constants investigated in HPLC or LC-MS.

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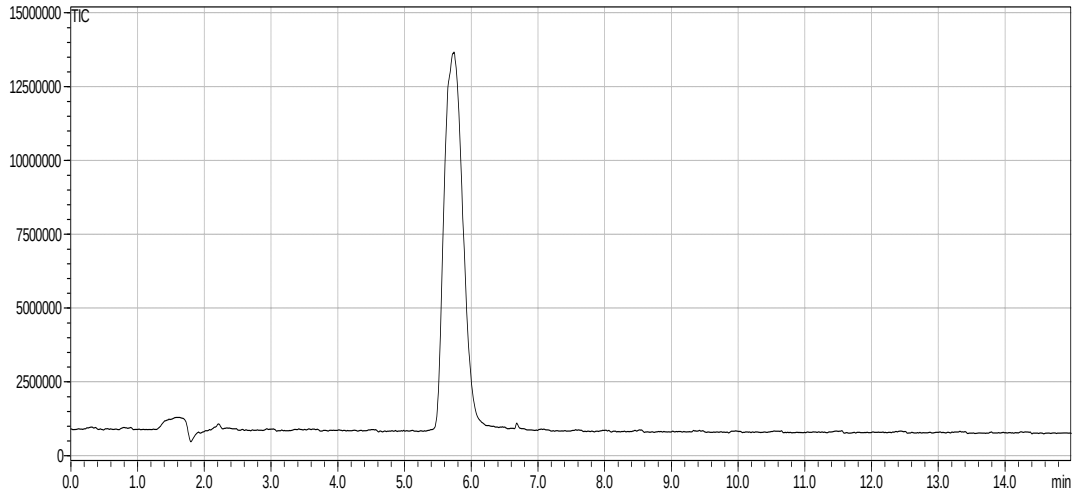

Figure 30. Chromatogram for ART within LC-MS.

Table 13 Observed rate constants of 0.6 mg mL^{-1}ART and standard error in buffer solution at pH values of 3.00, 6.50 and 8.50 using HPLC and LC-MS.

	k_{obs} (h^{-1}) ± SE		
Buffer conc.	**0.1 (mol L^{-1})**	**0.15 (mol L^{-1})**	**0.2 (mol L^{-1})**
HPLC pH 3.00	26.4 ± 0.013 × 10^{-2}	27.4 ± 0.016 × 10^{-2}	28.6 ± 0.017× 10^{-2}
pH 6.50	7.0 ± 0.065× 10^{-2}	7.3 ± 0.042× 10^{-2}	7.4 ± 0.062 × 10^{-2}
pH 8.50	15.0 ± 0.13× 10^{-2}	18.0 ± 0.16×10^{-2}	20.0 ± 0.166× 10^{-2}
LC-MS pH 3.00	23.2 ± 0.24× 10^{-2}	24.3 ± 0.24× 10^{-2}	25.5 ± 0.43× 10^{-2}
pH 6.50	7.2 ± 0.06 × 10^{-2}	7.25 ± 0.03 × 10^{-2}	7.3 ± 0.085 × 10^{-2}
pH 8.50	14.0 ± 0.095× 10^{-2}	17.8 ± 0.0357 × 10^{-2}	19.9 ± 0.029× 10^{-2}

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3.2.5 pH rate profile of ART

The rate constant values at zero buffer concentration, k´(Equation 2.5), were obtained from the plots of first-order rate constants versus the total buffer concentration. At zero buffer concentration only specific acid-base catalysis (H^+ / OH^-) or a spontaneous water reaction occurs under normal conditions. The rate constant values, which represent the intercept of the plots on the Y axis are listed in Table 14.

Table 14 First-order rate constants (k´) for the degradation of ART at zero buffer concentration at 37 ˚C.

pH	k´ h^{-1}	Log k´ h^{-1}	log k´ +2 h^{-1}
2.00	1.0900	0.04	2.037
2.50	0.2630	-0.58	1.420
3.00	0.2322	-0.63	1.366
3.50	0.1835	-0.74	1.264
4.00	0.1367	-0.86	1.136
5.00	0.0791	-1.10	0.898
5.50	0.0785	-1.11	0.895
6.50	0.0660	-1.18	0.820
7.50	0.0690	-1.16	0.839
8.50	0.0910	-1.04	0.959
9.50	0.3765	-0.42	1.576
10.50	2.0759	$0.32	2.317

`

From the data in Table 14 the pH for the maximum stability of ART can be identified. The lowest rate of degradation of ART at zero buffer concentration is in the region of pH 6.50 and is the optimum pH value for stability of ART in solution at 37 ˚C and μ of 0.5 mol L^{-1}. Below and above this pH region the degradation rate is increased.

The logarithms of these rate constants are plotted versus pH in the pH range of 2.00 – 10.50. The pH-rate profile Figure 31 has a U-shape somewhat characteristic of pH-rate profile, but the slope of (-1) and (+1) associated with specific acid-base catalysis is only evident and very low and high pH values. This would indicate that other factors could be influencing the degradation profile of ART.

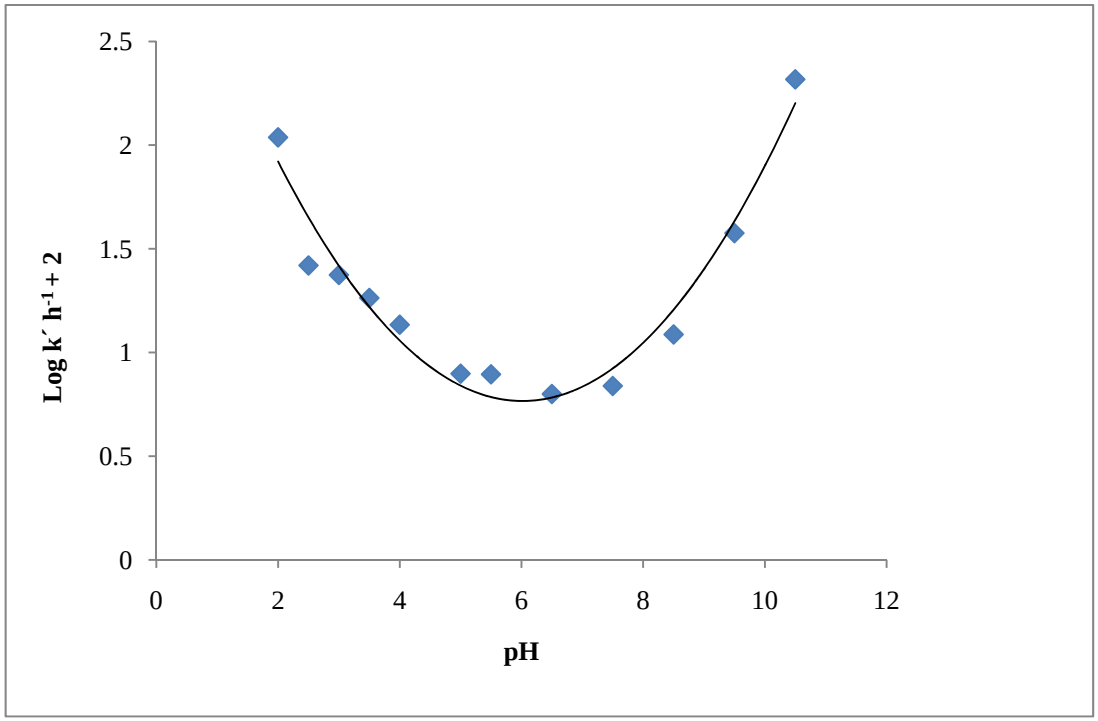

Figure 31. pH-rate profile of ART at 37 ˚C and μ of 0.5 mol L^{-1}.

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3.2.6 Effect of ionic strength on the rate degradation of ART

Another aspect of ART stability is the influence of ionic strength on the rate degradation. Investigation of effect of ionic strength on rate degradation of ART was evaluated by varying sodium chloride concentrations. Dependent on Equation 3.4 three amounts of salt were calculated and added to three samples of 600 µg mL^{-1} ART in 0.2 mol L^{-1} phosphate buffer solution.

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \quad \text{.....3.4}$$

Where I is ionic strength, c_i is the molar concentration of ion i (mol L^{-1}), z_i is the charge number of that ion, and the sum is taken over all ions in the solution.

All ART samples were incubated at 37 ˚C and aliquots were drawn at intervals for three half lives of reaction. A plot of log percent remaining concentration of ART versus time is shown in Figure 32.

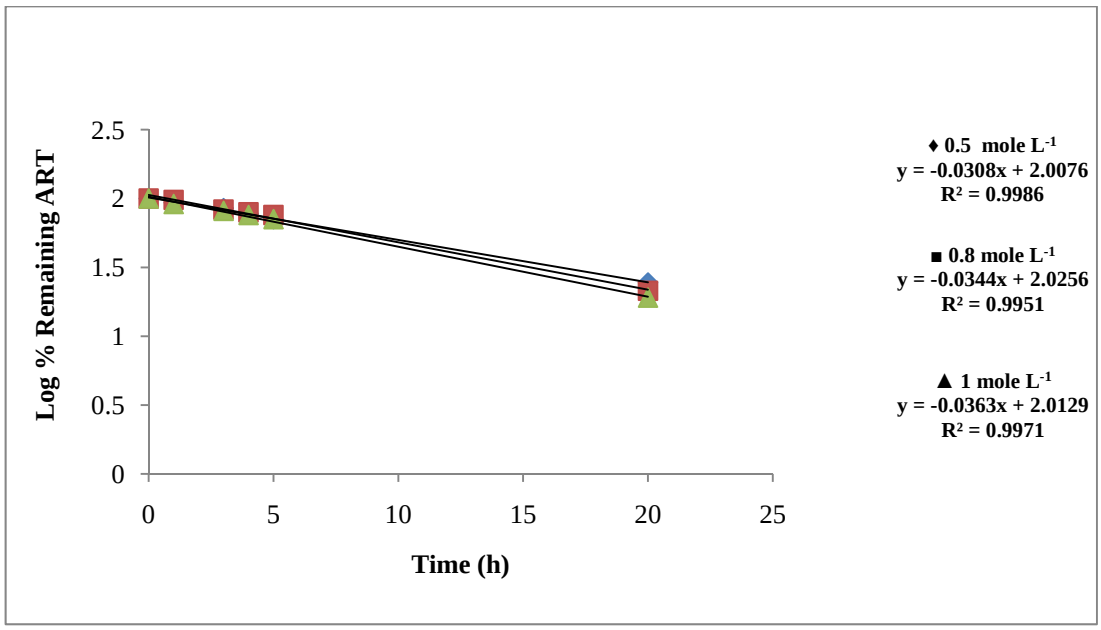

Figure 32. Degradation of ART in 0.2 mol L^{-1} phosphate buffer solution of µ 0.5, 0.8 and 1 mol L^{-1}.

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Plotting of logarithm of observes rate constant of ART versus the square root of the ionic strength demonstrated that increased ionic strength produced a small increased rate of degradation of ART as shown in Figure 33. It is noted that integer slopes as predicted by the Debye-Huckel theory were not evident. This would tend to indicate secondary salt effects. Over the ionic strength range of 0.5-1.0 mol L^{-1} a rate increase of approximately 20% occurred. This would indicate that minimising the ionic strengths in formulations would be advantageous.

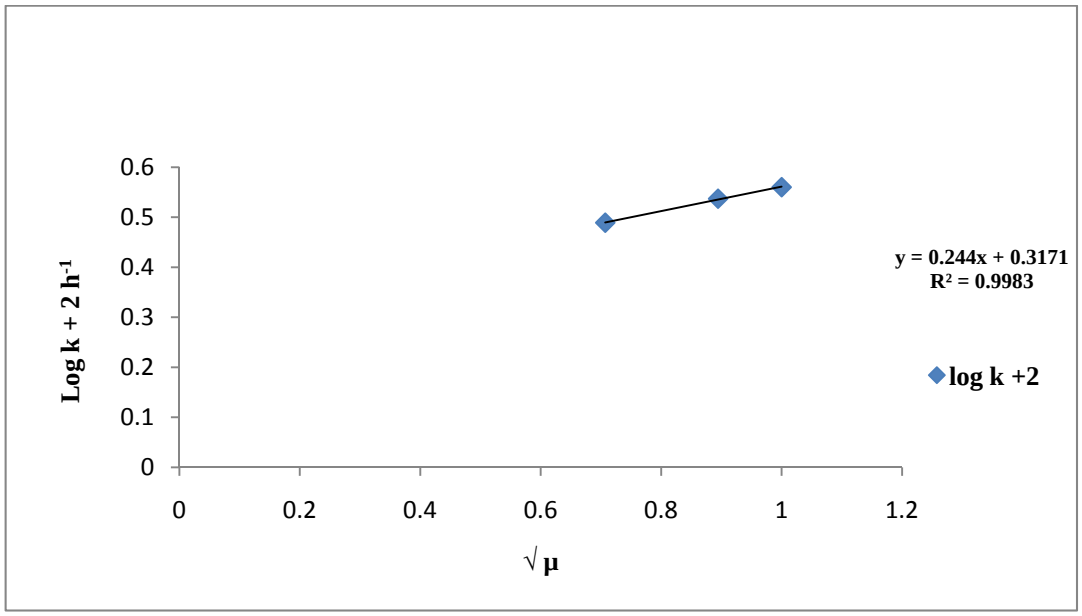

Figure 33. Effect of ionic strength on the rate degradation of ART at 37 ºC.

3.2.7 Evaluation of shelf-lives of ART in buffer solution

During storage of ART in the buffer solutions, the degradation rate constant would be expected to be influenced by changes in pH. The shelf life of the drug is the therapeutic indicator of stability. The shelf life can be calculated by substituting the rate constant at zero buffer solution of each pH in Equation (2.7) will give the t_{90} of ART at 37 ˚C. Shelf lives of ART in different pH are shown in Table 15. Under these

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conditions, it indicated that ART more stable at pH 6.50. This would indicate the meaningful measurement of stability of ART in the formulation.

Table 15. List of shelf-lives of 600 µg mL^{-1} ART in zero buffer concentration (pH 2.00 -10.50) at 37 ˚C and µ of 0.5 mol L^{-1}.

pH	k´ h^{-1}	t_{90} (h)
2.00	1.0900	0.10
2.50	0.2630	0.40
3.00	0.2322	0.45
3.50	0.1835	0.60
4.00	0.1367	0.75
5.00	0.0791	1.30
5.50	0.0785	1.35
6.50	0.0660	1.60
7.50	0.0690	1.50
8.50	0.0910	1.15
9.50	0.3765	0.30
10.50	2.0759	0.05

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3.3 Evaluation of ART stability in commonly used intravenous fluids

The stability of ART in selected intravenous solutions was studied. Admixtures of 600 μg mL^{-1} of ART were prepared in 5% dextrose, 0.9% sodium chloride and Hartman's solution. The pH value of each IV solution was measured in the beginning of experiment. pH values of 5% dextrose, 0.9% sodium chloride and Hartman's solution were 5.80, 6.60 and 7.10 before mixing with ART and 3.70, 3.77 and 5.38 after mixing respectively. The decrease in pH values related to the acidic nature of ART. At the end of experiment, pH values of 5% dextrose, 0.9% sodium chloride and Hartman's solution were 3.55, 3.51 and 5.18 respectively. The decrease in the pH value in comparison with beginning may relate to the acidic nature of the degradation product which is the succinic acid hemiester. All samples were incubated in a water bath at 37 ˚C and aliquots were drawn at intervals for approximately three half lives of reaction. All reactions showed linear relationships ($R^2 > 0.99$). Figure 34 which indicated that all reactions were first-order. Shelf-lives of ART in 5% dextrose, 0.9% sodium chloride and Hartman's solution were 1.0, 1.2 and 2.5 h respectively. This result indicated that stability of ART in Hartman's solution was double that in 5% glucose solution. It is noted that these solutions are not buffered but there was insufficient change in pH to markedly influence the nature of the kinetics. Some of this may arise that the pH-rate profile was dampened by other effects in this pH range.

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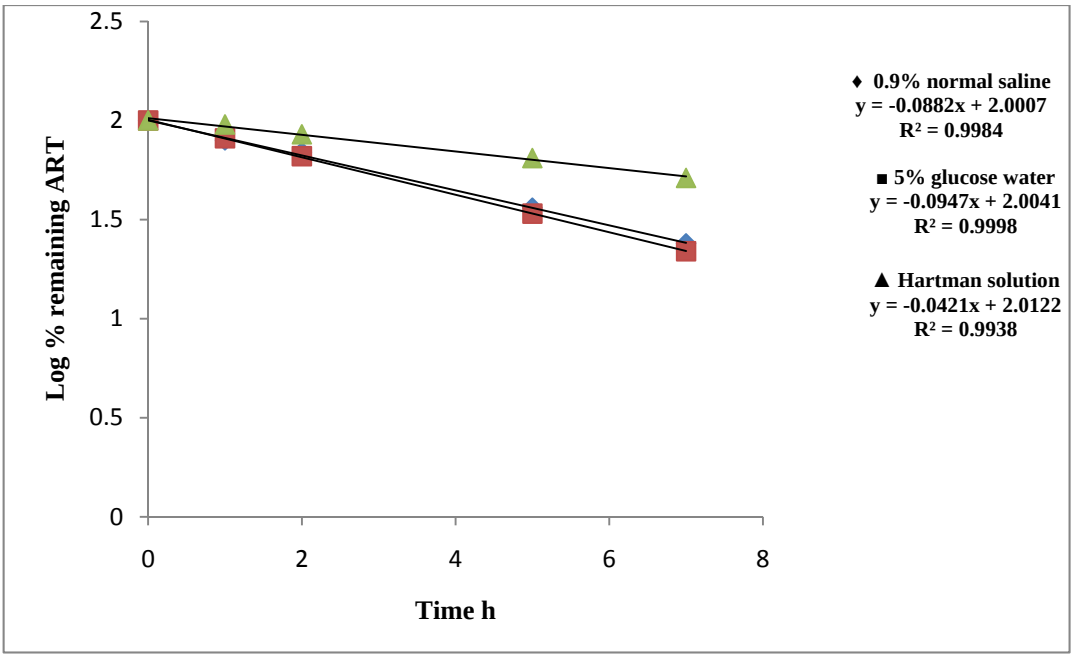

Figure 34. Stability of 600 µg mL^{-1} ART in 5% glucose water, 0.9% normal saline and Hartman's solution at 37 ˚C.

3.4 General discussion

3.4.1 Assessment of experimental design

Mobile phase admixtures and various solvents, pH values and concentration of the buffer were studied and modified until an apparent separation of ART with a suitable retention time occurred within HPLC. Using a non-nucleophilic mobile phase (phosphate buffer acetonitrile) was important to avoid nucleophilic attack during the analysis. Several pH values were investigated until a relatively sharp peak of ART on the chromatogram was observed at pH 7.00 and an acceptable retention time which was around 6.50 min. The monitoring UV wavelength was 210 nm which based on diode-array UV spectrophotometer scan of ART as show in Figure 35. Several previous studies have also selected approximately the same wavelength which was an end-effect of the ART.[87, 102, 103]

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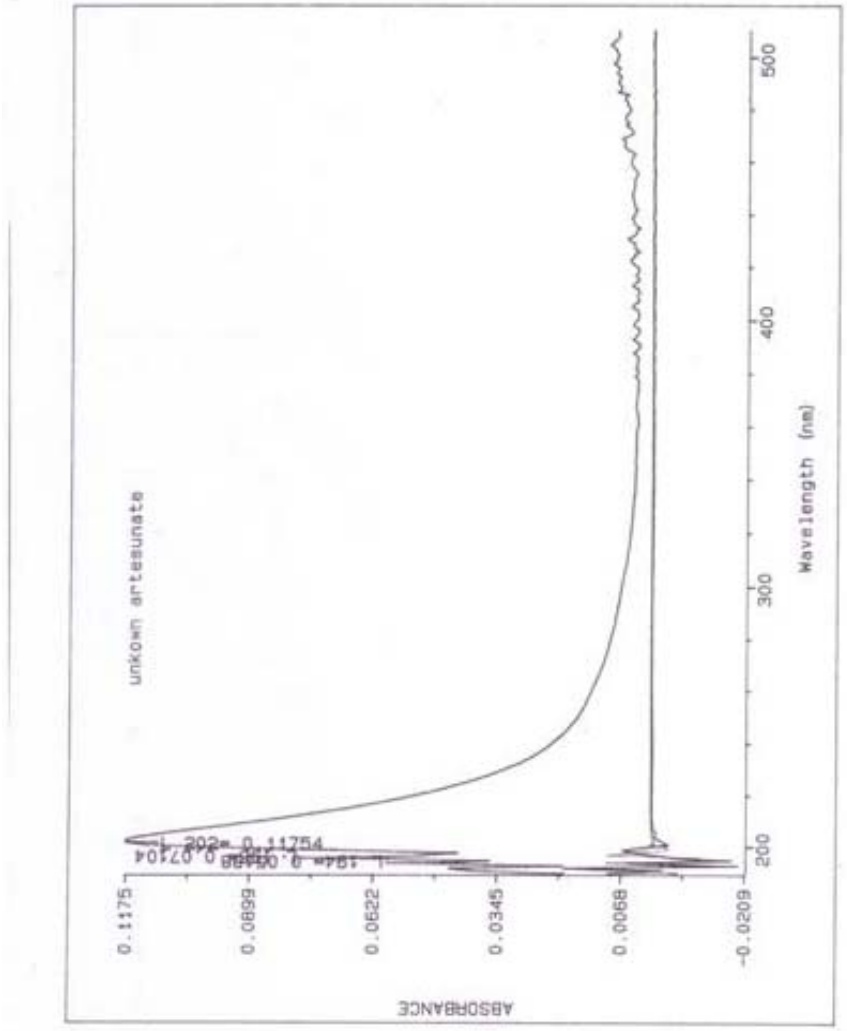

Figure 35. Wavelength scan of ART from 200 to 500 nm and ART concentration of 0.6 mg mL^{-1}.

This has occurred because of the lack of a UV chromophone in the ART molcule. However it is reliable in that ART concentrations could be adequately evaluated over the concentration range employed in this study.

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The mobile phase employed to detect and separate ART for LC-MS was ammonium formate: acetonitrile 65% at pH 5.80. Although negative ion mode electrospray ionization can be a choice for ART analysis because of its carboxylic acid functionality, but positive ion mode produces higher sensitivity, and ensures detection of other compounds that might be present in the sample. Quantification was undertaken using SIM (selected ion monitoring) in APCI (atmospheric pressure chemical ionization) mode. ART ammonium adduct ion $[M + NH4]^+$ (m/z 402) was monitored for quantification purpose as shown in Figure 36. A clear ART peak was observed within retention time of 5.8 min.

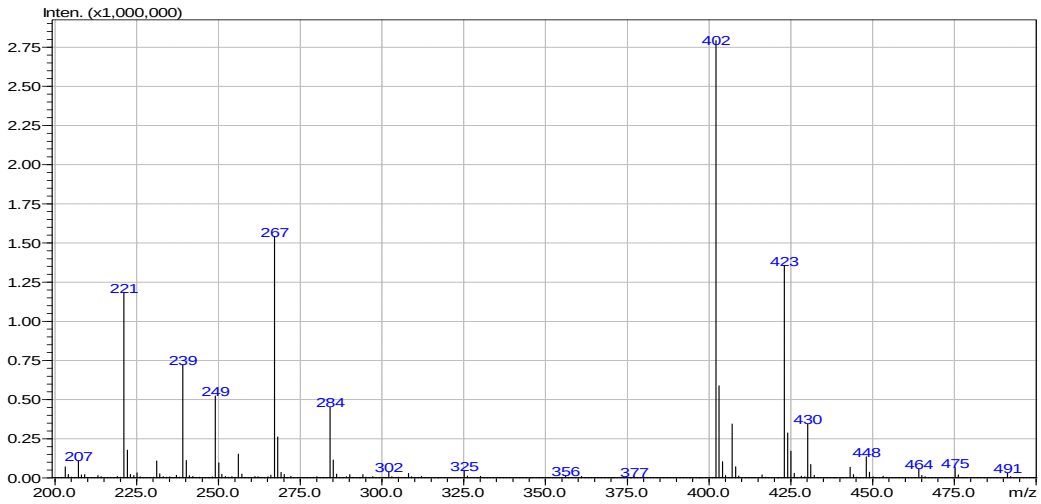

Figure 36. Full mass spectra of ART.

The validity of the method was determined from linear regression analysis of a representative calibration curve resulted in R^2 of 0.9995 for ART. In addition no detectable peak interference was produced from accelerated degradation of ART in other parameters according to ICH like acid, or alkali by heat. A range of (± 0.02) differences in the pH values was observed between the beginning and the end measurement of experimental reads which was not significant. The reverse phase

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HPLC method was therefore validated at 210 nm. Although several studies was mentioned this wave length for ART, this will provide an additional confirmation of the methodology employed in this study.

3.4.2 Assessment of ART stability studies

There are no published studies on the kinetics of this antimalarial drug in aqueous solution over a wide pH range. The present research deals with the kinetic aspects of the degradation of ART in buffer solutions of various pH values and temperature dependence of ART in aqueous solution.

Solutions of ART prepared at pH 1.20, 6.50 and 10.50 exhibited significant temperature dependence (22, 30 and 37 ˚C). All ART reactions in such solutions behaved as first-order kinetics. This result is supported by Batty[15] who found that the rate degradation constant of ART injection, which is sodium artesunate in buffer powder, in normal saline increased with increasing temperature. He found that the degradation rate constant of ART in the normal saline at 23 ˚C was 4.3×10^{-3} h^{-1} much the same result was obtained in this study at 22 ˚C (4.29×10^{-3} h^{-1}). Even at temperature 36.5 ˚C, Batty reported a rate constant of 2.8×10^{-2} h^{-1} which was similar to this study (2.23×10^{-2} h^{-1}).

Shelf-lives of ART were decreased with increased temperature as expected and were similar to as reported by Batty as shown in Table 16.

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Table 16. comparison shelf-lives of ART in this study and Batty study.[15]

Batty study		**This study**	
Temp. (˚C)	**t_{90} (h)**	**Temp. (˚C)**	**t_{90} (h)**
23.0	10.6	22	10.60
30.0	4.0	30	3.60
36.5	1.6	37	1.40

There was slightly increase of Ea value of ART at pH 6.50 in comparison with pH 1.20 and 10.50.

ART is a hemiester of succinic acid with has a free carboxylic acid end group. Thus, it is potentially susceptible to both hydrolysis by the water and to transesterification and other reactions as shown in Figure 37.

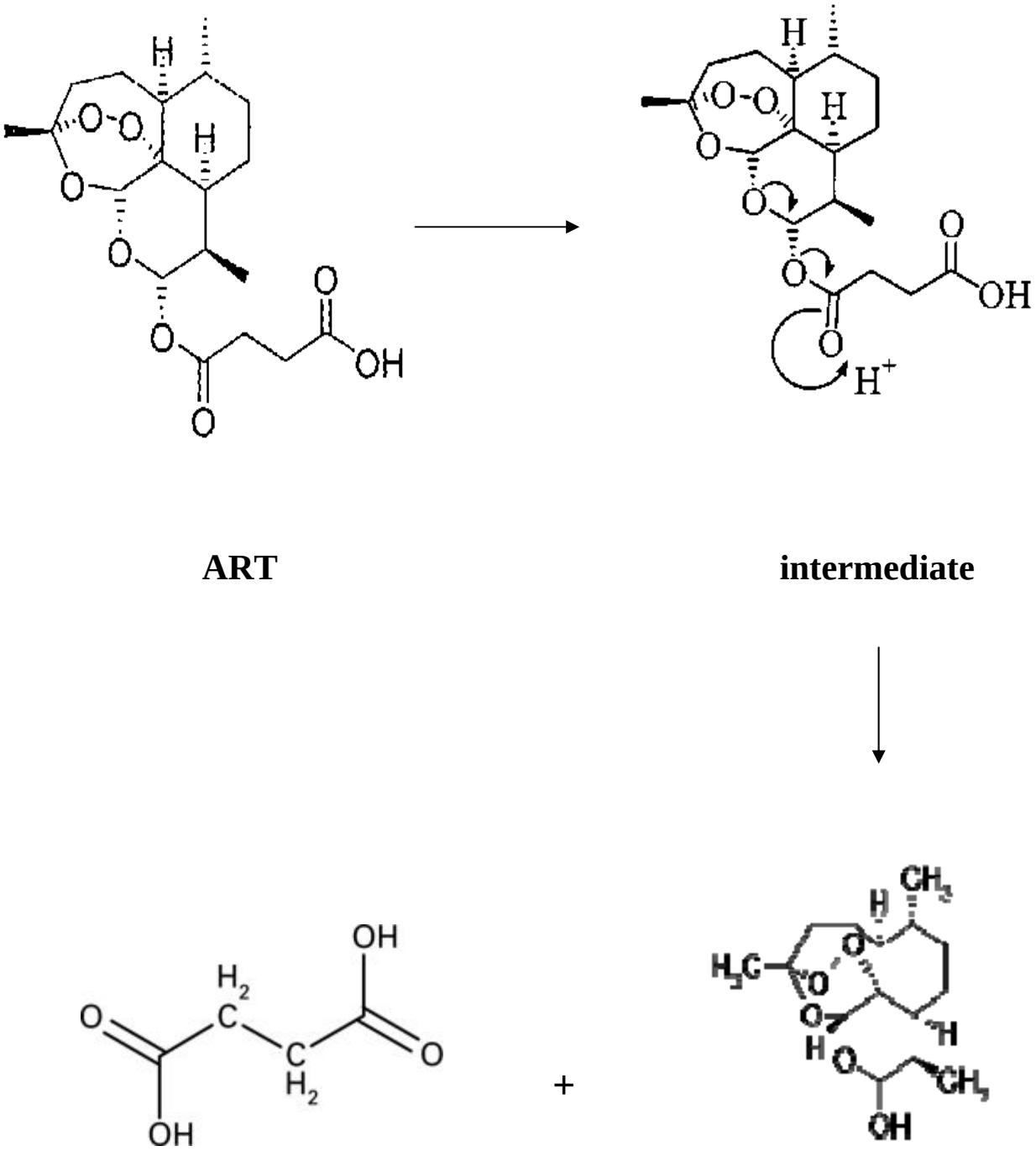

Figure 37. Hydrolysis pathway of ART in the presence of water.

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The free carboxylic acid group will also deprotonate to give the salt form which on pH first from sodium hydroxide addition forms sodium artesunate as shown in Figure 38. As the pKa is 4.6 ART will be essentially fully ionized at pH value above 6.60.

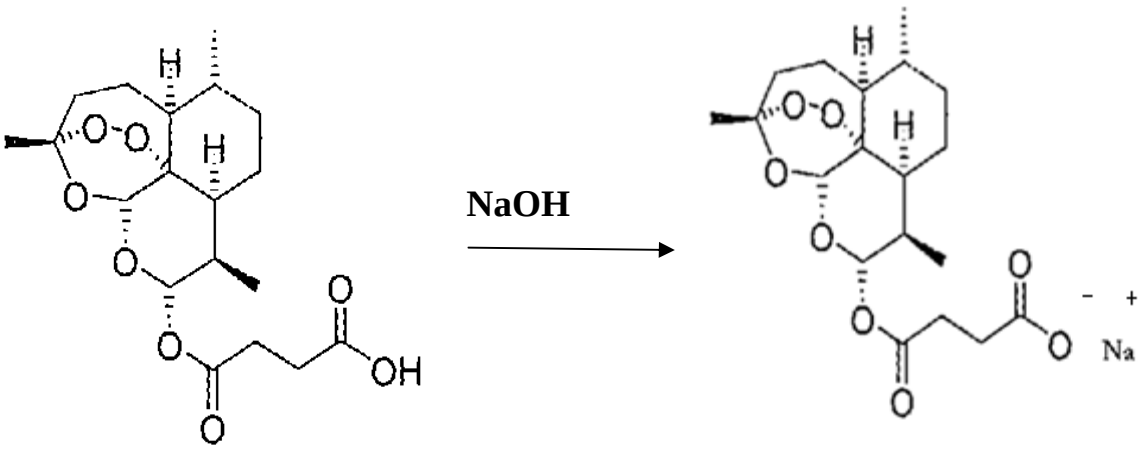

Figure 38. Conversion of ART in the presence of NaOH to sodium ART.

Since artesunic acid is almost completely ionized at a pH of 7.40 (99.8%) therefore it is available on the market as sodium artesunate tablet or injection.

The overall rates constants (k_{obs}) for the degradation of ART in phosphate buffers at pH 2.00, 2.50, 5.50, 6.50 and 7.50 or in acetate at pH 3.00, 4.00 and 5.00 were not increased linearly with increase of buffer concentration at a constant pH and temperature.

Similarly the observed rat constants of ART in carbonate buffer solution at pH 9.50 and 10.50 were not increased linearly with increased buffer concentration at constant pH and temperature.

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Data in Table 12 of buffer species did not show marked differences in general catalysis in respect to the buffers used over the pH range. Both species of phosphate and carbonate buffer solution had some catalytic effect towards the degradation of ART but there was no predominance of any species.

The pH-rate profile of ART Figure 31 can be demonstrated kinetically as follows: there were three important pH regions. One where the acid catalysed reaction took place, which was evaluated at low pH. At higher pH values the logarithm of the rate constant did not indicate +1 or -1 slopes with pH change. The apparent rates of ART hydrolysis are greater than the extrapolation of the specific hydrogen or hydroxyl ion catalysis rate expressions would predict. The effect of the succinate on the rate degradation of ART can be demonstrated and explained as the following: three points in the acid catalysis region which represent straight line on the pH-rate profile of ART have been selected. On the other hand, three points in the base catalysis region which represents a straight line have been also selected and plotted as shown in Figure 39. The equation produced from straight line plots in the acid region can be employed to calculate the expected second order rate constant for the pH values below pH 2.00. Figure 40 represent the experimental pH-rate profile of ART. The small change in log k with pH over the pH range is postulated k arise from succinate catalysis of the reaction species.

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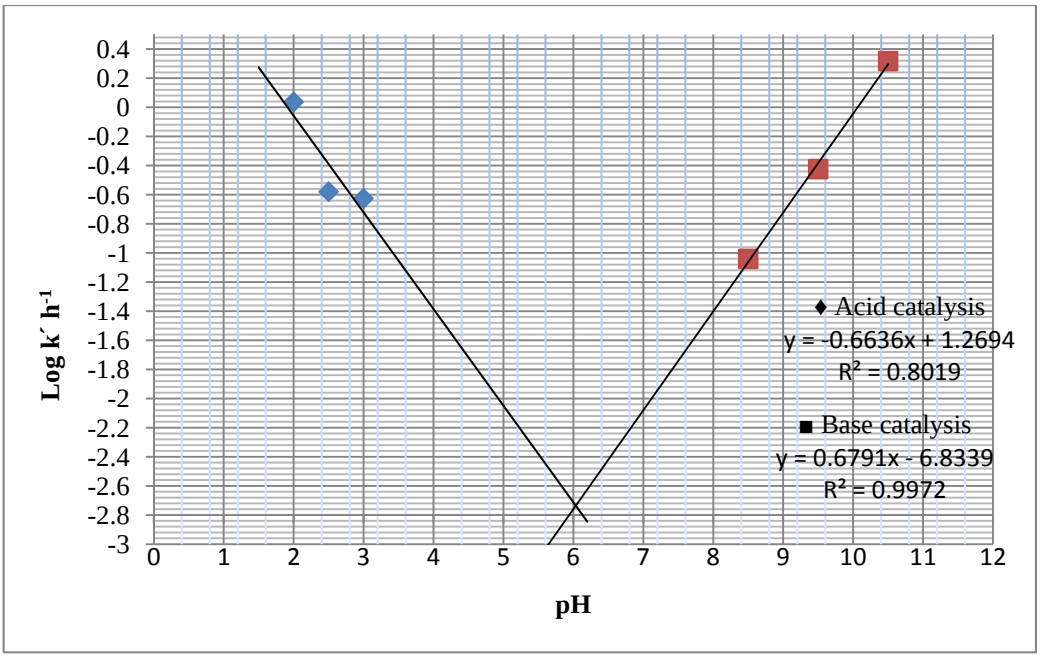

Figure 39. The pH-rate profile of ART of three points selected in both acidic and alkali catalysis regions.

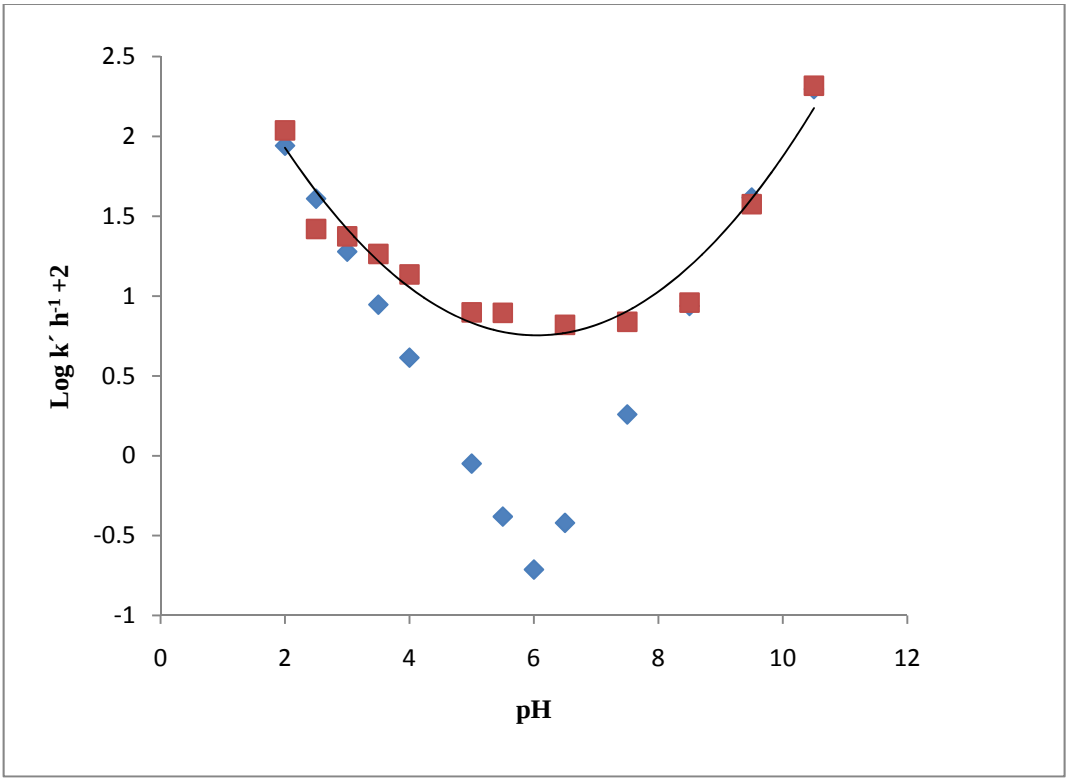

Figure 40. Experimental and expected pH-rate profile of ART.

(■ Experimental plot, ♦ Expected plot)

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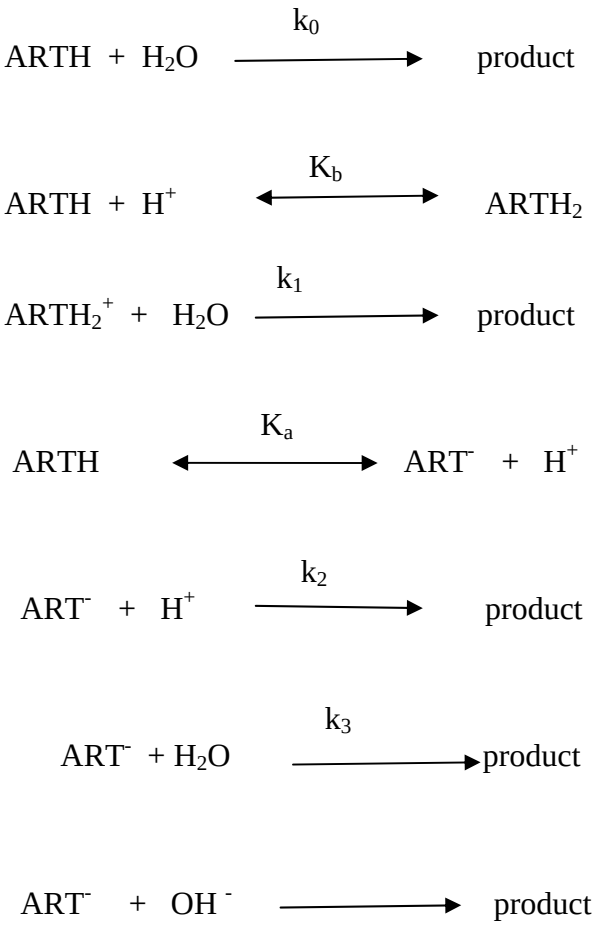
The overall expected mechanism for ART hydrolysis would be as follows:



Where ARTH is unionized ART and $ARTH_2^+$ is the protonated ester and ART^- the deprotonated form. The pKa for ART is 4.6. This would indicate that above pH 2.60 succinate anion will be formed by deprotonation of the hemiester. It can be seen that the slope from pH 2.00 to 2.50 as approximately -1 indicating specific acid catalysis of $ARTH^+$. In addition there is some protonation of the artemisinin structure at points 10 to 12 at low pH that may influence the kinetics. At high pH values [$COOH^-$] will be constant hence increased rate is likely to be largely from increased concentration of [OH^-]. The slope in this region pH 9.50-10.50 is close to +1.

The region from pH 3.00 to 8.50 shows a marked increased rate over that predicted from specific acid base catalysis. It is not possible to identify the acid catalysis

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component of deprotonated ARTas it is masked by succinate catalysis. This therefore does not allow the magnitude of the succinate catalysis to be quantified.

Garrett [83] found succinate catalysis of hydrocortisone hemisuccinate and identified a method for its quatitation.

That is not possible in this case as access to the absence of its affect is not available over the pH range 3.00 and above. This is complicated by any effect of the deprotonation of the ester has only on $[H^+]$ and $[OH^-]$ and the water reaction.

Hence the reaction is complicated by the impact of carboxylate catalysis on the following species.

$ARTH_2^+ + COO^- \longrightarrow$ Product

$ART^- + COO^- \longrightarrow$ Product

This indicates that a succinate ester is not a good choice from a stability perspective for ART and a range of esters should be investigated for new products.

On the basis of the evaluation of ART stability in respect to the ionic strength of a buffer solution, there was a slightly increased rate degradation of ART with increase of ionic strength. This is related to the interaction between ions of the buffer species and solvent resulting in nonideal behaviour (i.e. the effective concentration of the solute species is often quite different from its real, known concentration. The effective concentration is called the activity of the species and may be less than, equal to, or greater than the molar or formal concentration of the species.[89]

The Debye-Hückel equation simply states that the activity coefficient of any ion depends on the ionic strength of the solution. This equation also predicts that Ka'

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(apparent dissociation constant) will also depend on ionic strength. Hence the pKa' will also depend on ionic strength. It is common practice to use pKa values when calculating pH and buffer problems instead of pKa' values, appropriate to the buffer concentration. The pKa' values are known, as a function of buffer concentration, for only a few common buffers, but it is possible to calculate approximate pKa' from pKa values for a given ionic strength. Use of pKa' allows more accurate prediction of buffer pH.

For example: what is pKa' for acetic acid at 37 ˚C in buffer solution, 0.1 mol L^{-1} ionic strength? The pka at 37 ˚C is 4.77. At 37 ˚C and μ of 0.1 mol and for z = 0 the correction factor, (pKa' – pka) is 0.11. Hence pKa' = pka - (pKa' – pka) = 4.77 – 0.11= 4.66.

Evaluation of ART stability in 5% dextrose, 0.9% sodium chloride and Hartman's solution demonstrated greater stability with Hartman's solution rather compared to dextrose or sodium chloride solution. This result is related to lack of buffer capacity of each of these solutions. Hence although the pH of normal saline is near the pH minimum addition of the hemiester markedly lowers the pH and the low initial pH value of glucose 5% in water which is around pH 5.00 gives a lower final pH after addition of ART.

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Chapter 4

EFFECT OF HP-ß-CD ON THE SOLUBILITY AND STABILITY OF ARTESUNATE

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4 Solubility and stability studies

4.1 Solubility of ART

Investigation of the solubility of ART was studied on samples of 20 mg mL^{-1} ART in 0.2 mol L^{-1} phosphate buffer solution of pH 3.00, 4.00, 5.00 and 6.00 at 25 ˚C. Graded quantities of HP-ß-CD were added to produce concentrations of 0, 68, 136 and 272 mg mL^{-1}. The equilibrium was achieved within 3 h. Aliquots were withdrawn, filtered and diluted with distilled water then assayed by HPLC for ART concentration. Solubilities of ART at different pH values with different concentrations of HP-ß-CD are listed in Table 17. Plots of ART concentrations versus HP-ß-CD concentrations at each pH value are shown in Figure (41). All plots in Figure 41 show linear relationships ($R^2 > 0.99$). The linear relationship indicates the formation of a 1:1 complex. In this case, the apparent stability constant of the complex, K_{st}, can be calculated from Eq. (1.8) which was deduced by Higuchi and Connors (Higuchi and Connors, 1965). The reaction is:

$$\text{ART} + \text{HP-ß-CD} \xleftrightarrow{K_{st}} \text{ART- HP-ß-CD}$$

The slopes and intercepts of the curves (S_0) were found from the linear fits of the curves in Figure 41. The apparent stability constants of the formulated complex at each pH values are listed in Table 17.

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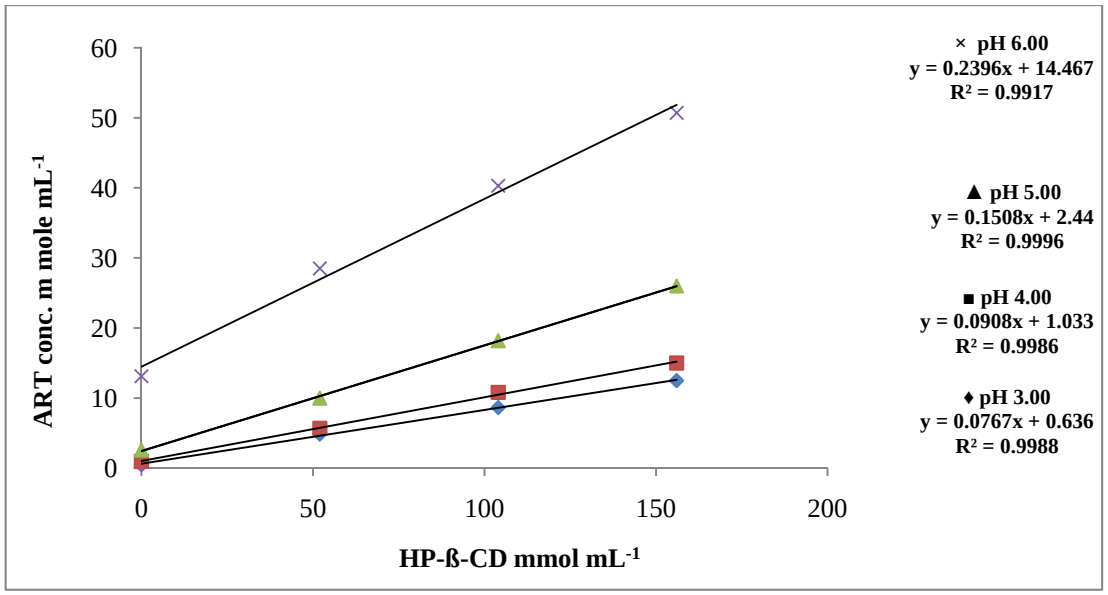

Figure 41. Phase solubility diagram of ART-HP-ß-CD complexes in aqueous solution at pH 3.0, 4.0, 5.0 and 6.0 and 25 °C.

Solubility diagram shows as described by Higuchi and Connors indicating a linear relationship classified as AL- type of overall concentration of ART in the presence of HP-ß-CD.

Table 17. Rate solubilities, standard errors and K_{st} values of ART in phosphate buffer at pH 3.00, 4.00, 5.00 and 6.00 and 25 °C with different concentrations of HP-ß-CD.

HP-ß-CD (mg mL^{-1})	**0**	**68**	**136**	**272**	**K_{st} (M^{-1})**
pH	**Solubility mg mL^{-1}**				
3.00	0.2 ± 0.002	1.9 ± 0.05	3.3 ± 0.08	5.0 ± 0.10	130
4.00	0.4 ± 0.005	2.2 ± 0.06	4.2 ± 0.09	10.9 ± 0.20	96
5.00	1.0 ± 0.03	4.7± 0.11	7.8 ± 0.15	15.4 ± 0.25	72
6.00	5.0 ± 0.12	12.1± 0.29	17.1 ± 0.3	19.4 ± 0.40	21

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Data in Table 17 shows that ART solubility is markedly increased in the presence of HP-ß-CD especially at lower pH values. The complex interaction is an equilibrium governed by the constant K_{st} therefore ART showed high complex interaction at pH 3.00 as well as a 25 fold increase in the solubility with HP-ß-CD.

Plotting the apparent stability constant versus pH gave a sigmoid relationship between K_{st} and pH as shown in Figure 42. These points on the sigmoid curve reflect the affinity of ART to the HP-ß-CD in correlation to the pH values.

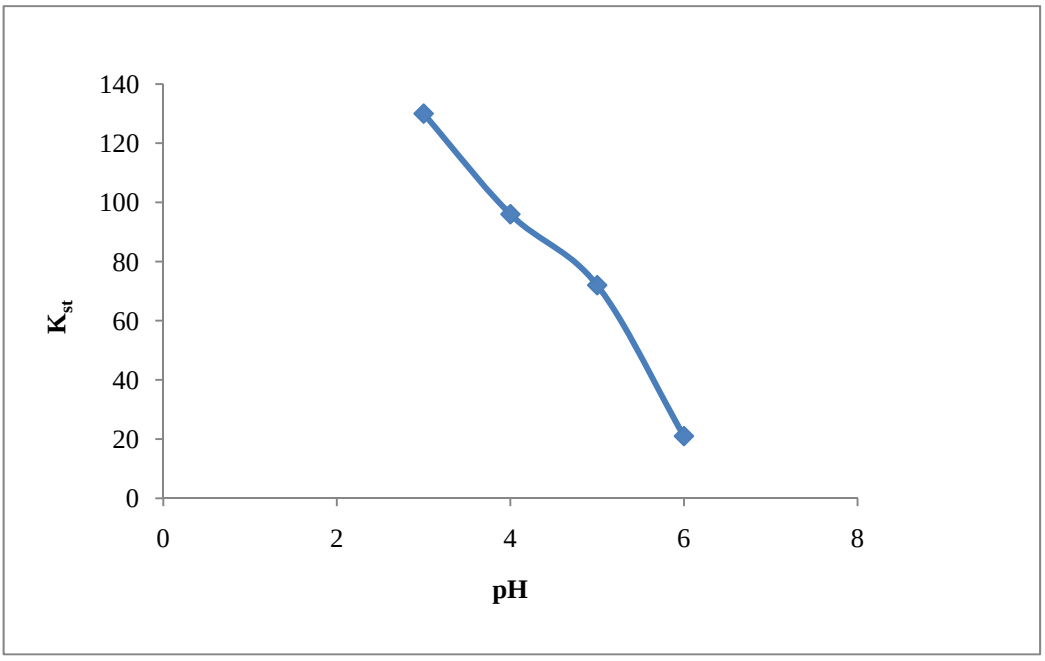

Figure 42. Correlation between stability constant K_{st} and pH.

Since ART has a pKa of 4.6 most is in the molcular form at pH 3.00. This explains the lower solubility value and higher K_{st} value. At pH 6.00 the majority is in the anion form giving higher solubility and a lower K_{st} value. Although the influence of HP-ß-CD is lower at pH 6.00 it still improves the solubility almost four-fold to give the highest solubility value.

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4.2 Stability study of ART in the presence of HP-ß-CD

Stability of ART in the presence of HP-ß-CD was evaluated at different pH values (6.00, 7.00 and 8.00). This range of pH values showed the best ART stability in the mid-pH region among the other pH values which were employed in the evaluation of effect of buffer solutions on the stability of ART (Chapter 3). Different concentrations of HP-ß-CD in phosphate buffer solution were mixed with ART and the rate degradation of ART was investigated. Temperature dependence of ART in the presence of HP-ß-CD was also investigated in 23, 30 and 37 ˚C.

4.2.1 Stability of ART in different pH values the presence of HP-ß-CD

Samples of 0, 13.6, 27.2, 54.4 and 108.8 mg mL^{-1} HP-ß-CD were prepared in 0.2 mol L^{-1} phosphate buffer solution at pH 6.00, 7.00 and 8.00. Accurate amounts of ART were added to produce a concentration of 2 mg mL^{-1}. All the samples were sonicated and incubated in a water bath at 37 ˚C. An aliquot was withdrawn at intervals for three half lives of reaction diluted and assayed by HPLC. Plots of log remaining ART versus time were plotted as shown in Figures 43, 44 and 45.

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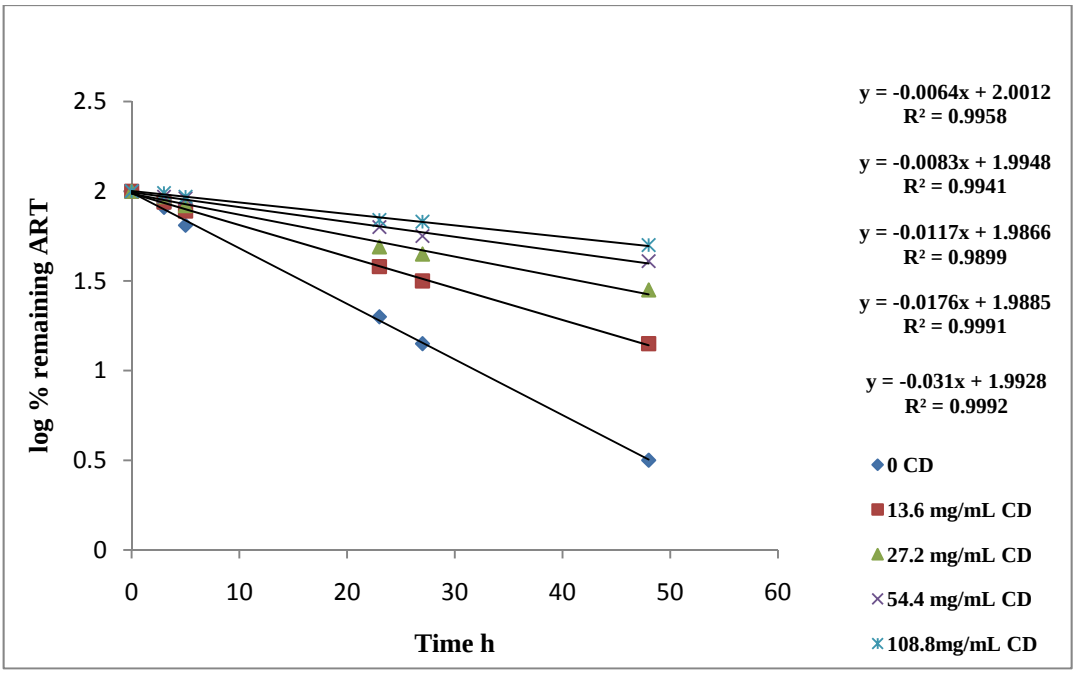

Figure 43. Degradation of ART in the presence of HP-ß-CD in 0.2 mol L^{-1} phosphate buffer solution (pH 6.00) at 37 ˚C.

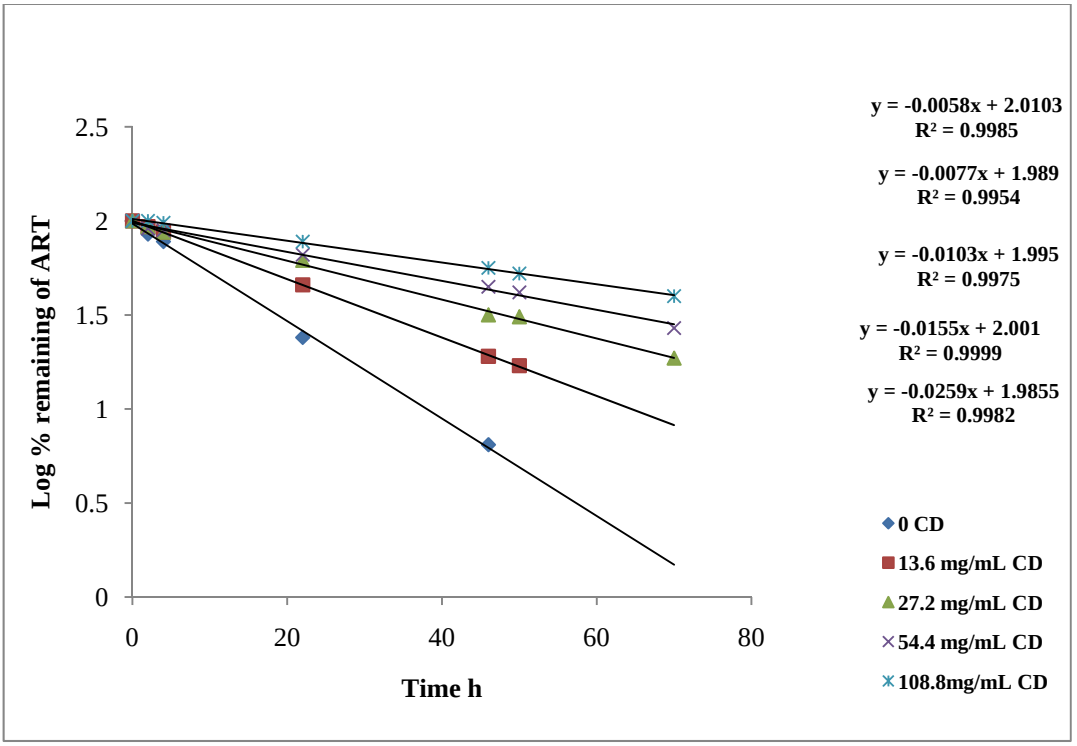

Figure 44. Degradation of ART in the presence of HP-ß-CD in 0.2 mol L^{-1} phosphate buffer solution (pH 7.00) at 37 ˚C.

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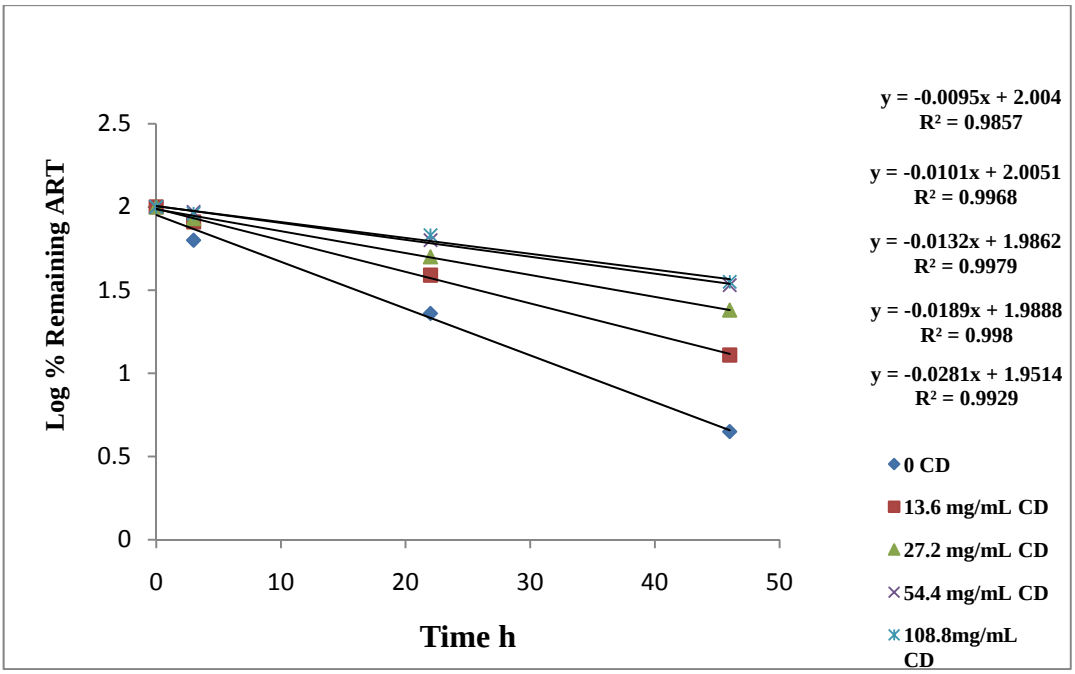

Figure 45. Degradation of ART in the presence of HP-ß-CD in 0.2 mol L^{-1} phosphate buffer solution (pH 8.00) at 37 ˚C.

Figures 43, 44 and 45 demonstrated linear relationships (R^2 > 0.98) which indicated that such reactions obeyed first-order reaction. The observed rate constant was decreased with increased HP-ß-CD concentration. This indicated that the stability of ART was increased with the increase HP-ß-CD concentration. The observed rate constants in regarding graded HP-ß-CD concentration are listed in Table 18.

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Table 18. List of observed rate constants of ART in the presence of HP-ß-CD with standard error at pH 6.00, 7.00 and 8.00 and 37 ºC.

HP-ß-CD (mg mL^{-1})	**k_{obs} (h^{-1}) ± SE**		
	pH 6.00	**pH 7.00**	**pH 8.00**
0	71.3 ± 0.44 × 10^{-3}	59.6 ± 0.38 × 10^{-3}	64.7 ± 0.13 × 10^{-3}
13.6	40.5 ± 0.50 × 10^{-3}	35.7 ± 0.40 × 10^{-3}	43.50 ± 0.49 × 10^{-3}
27.2	26.9 ± 0.50 × 10^{-3}	23.7 ± 0.33× 10^{-3}	30.4 ± 0.67 × 10^{-3}
54.4	19.1 ± 0.30 × 10^{-3}	17.7 ± 0.12 × 10^{-3}	23.2 ± 0.12 × 10^{-3}
108.8	14.7 ± 0.51× 10^{-3}	13.3 ± 0.53 × 10^{-3}	21.8 ± 0.16× 10^{-3}

To explain kinetically how the complex increased ART stability, the apparent stability constant K_{st} can be calculated from the Lineweaver Burke equation. This linear model was used to illustrate the kinetic behaviour of the guest molcule in the presence of HP-ß-CD. The stability constant was determined by the following model:

$$-d(ART)/dt = k_0(ART) + k_c(ART - CD)$$

Where k_0 and k_c represent the rate constants for degradation of free and complexed ART respectively. The observed reaction rate for the degradation of ART in the presence of HP-β-CD is an average of the degradation rate of free and complexed ART as defined by K_{st}. A plot of $1/(k_o - k_{obs})$ versus 1/[CD] will give a linear relationship assuming a 1:1 complex is formed with a y-intercept of $1/(k_c - k_o)$ and a slope of $1/K_{st}(k_c - k_o)$ from which values of k_c and K_{st} can be derived. Table 19 lists

`

the values of 1/[CD], k_0 and $1/ k_0-k_{obs}$ which were obtained from Lineweaver Burke equation (1.9)

$1/k_0 – k_{obs} = 1/ K_{st} (k_0 – k_c) \times 1/(CD) + 1/k_0 – k_c$ ..... Eq 1.9

Table 19. List of values of 1/[CD], k_0 and $1/ k_0-k_{obs}$.

	pH 6.00	**pH 7.00**	**pH 8.00**
k_0 (h^{-1})	0.0714	0.0596	0.0647
1/CD (M^{-1})		**$1/ k_0-k_{obs}$**	
96	32.5	41.8	47.1
48	22.5	27.8	29.1
24	19.1	23.8	24.0
12	17.6	21.6	23.3

Plotting of $1/ k_0-k_{obs}$ versus 1/[CD] as shown in Figure 46 gives $1/K_{st}(k_0 – k_c)$ as the slope and $1/ k_0 - k_c$ as the intercept.

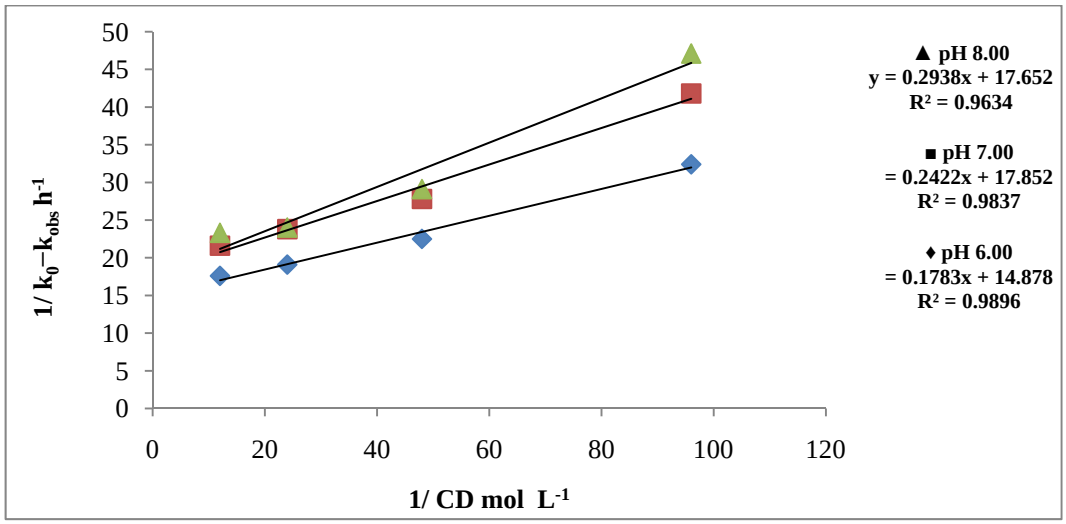

Figure 46. Graph of the rate data according to the Lineweaver–Burke equation at pH 6.00, 7.00 and 8.00.

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Table 20 shows the values of $k_{0,}$ K_{st} and k_c of ART at pH 6.00, 7.00 and 8.00. ART is most stable within the complex at pH 7.00 according to the equilibrium between the apparent stability constant of the complxed ART with the degradation rates of the free and the total complxed ART. This reflects the same order of stability as for free ART, however here is an approximate improvement in stability of approximately five-fold.

Table 20. The rate and equilibrium values of ART in HP-ß-CD at 37 °C.

pH	k_0 h^{-1}	K_{st} M^{-1}	k_c h^{-1}
6.00	7.14×10^{-2}	83	4.18×10^{-3}
7.00	5.96×10^{-2}	73	3.58×10^{-3}
8.00	6.47×10^{-2}	61	8.04×10^{-3}

4.2.2 Temperature dependence of ART in the presence of HP-ß-CD

The temperature dependence of ART in the presence HP-ß-CD was studied at three temperatures 23, 30 and 37 ˚C separately. Degradation of ART at each temperature was investigated at pH 7.00. The observed rates constants were determined from plotting the log remaining percent concentration versus time. As expected, the degradation of ART increased with increased temperature. Table 21 shows the rate degradation values of ART at the three employed temperatures. There was obviously an increase in the observed rate constant values with increased temperature.

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Table 21. list of observe rate constants of ART with standard errors at the 95% confident interval in the presence of HP-ß-CD at 23, 30 and 37 ˚C.

HP-ß-CD	**k_{obs} h^{-1} ± SE**		
mg L^{-1}	**23 ˚C**	**30 ˚C**	**37 ˚C**
0	$16.0 \pm 0.37 \times 10^{-3}$	$49.5 \pm 0.52 \times 10^{-3}$	$59.6 \pm 0.61 \times 10^{-3}$
108	$2.3 \pm 0.03 \times 10^{-3}$	$6.4 \pm 0.04 \times 10^{-3}$	$13.3 \pm 0.07 \times 10^{-3}$

Linear relationships (R^2 > 0.99) were obtained for all reactions at the three temperatures. Hence at the selected temperatures the degradation process of ART can be described by first-order kinetics.

The Arrhenius plot was obtained by the normal procedure of plotting log observed rates constants of ART versus reciprocal temperature in Kelvin as shown in Figure 47.

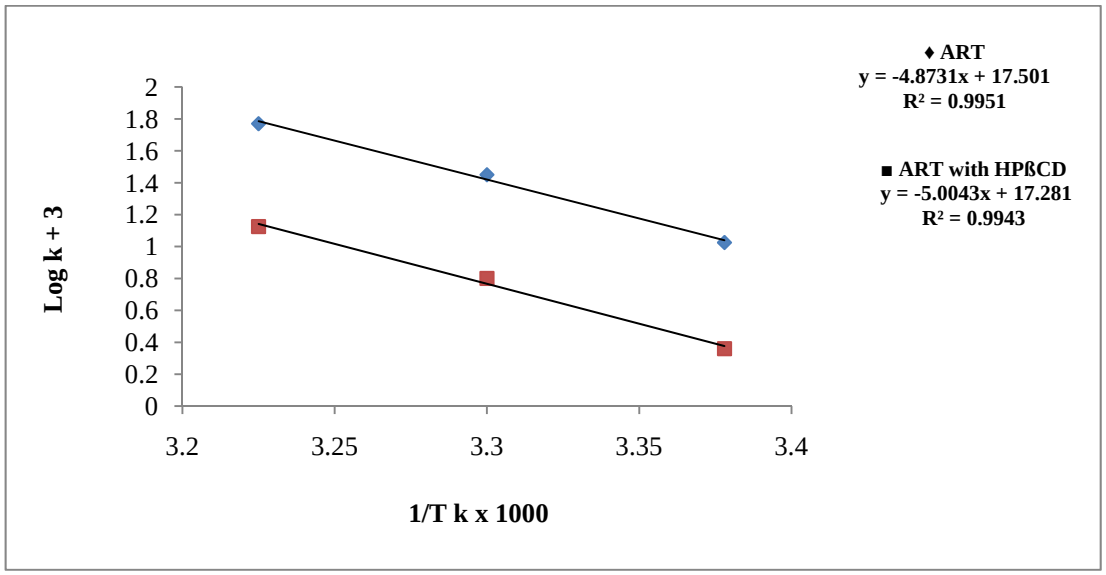

Figure 47. Temperature dependence of ART in the presence or absence of HP-ß-CD at 23, 30 and 37 ˚C and pH 7.00.

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According to the Equations 2.7 and 2.8 activation energy and shelf-lives of ART at three temperature degrees were calculated and listed in Table 23.

There were significantly increased shelf-lives of ART in the presence of HP-ß-CD. At 23 ˚C, which is almost the ambient storage temperature, there was approximately a 5 fold increase in the shelf-life.

As it has been previously mentioned that the linear form of the Eyring equation represents a plot of logarithm of (k/T) versus 1/T with slope of $-\Delta H^{\ddagger}/R$ from which the enthalpy of activation can be derived and with intercept $\ln k/h + \Delta S^{\ddagger}/R$ from which the entropy of activation is derived. A linear relationship produced from the plots of (k/T) versus 1/T as shown in Figure 48. Ea, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are listed in Table 20.

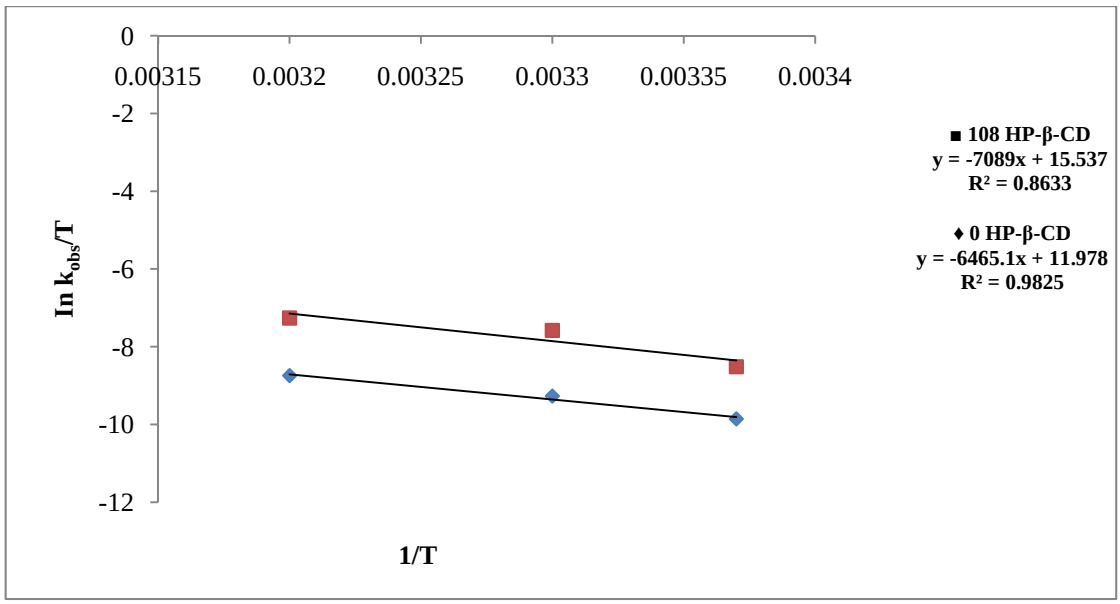

Figure 48. Eyring plot of degradation of ART in the presence of HP-ß-CD at 23, 30 and 37 ˚C and in pH 7.00.

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Table 22. List values of Ea, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of ART in the presence HP-ß-CD at pH 7.00.

HP-ß-CD (mg mL^{-1})	Ea ($kJmol^{-1}$)	$\Delta H^{\ddagger}$ ($kJmol^{-1}$)	$\Delta S^{\ddagger}$ ($J\ k^{-1}mol^{-1}$)
0	93.40 ± 0.09	94.25 ± 0.09	22.96 ± 0.04
108	95.80 ± 0.67	92.14 ± 0.67	24.69 ± 0.20

The result shows similar Ea and $\Delta H^{\ddagger}$ values in the presence of HP-ß-CD. The smaller value for $\Delta S^{\ddagger}$ change indicates that much of the free energy change was embodied in $\Delta H^{\ddagger}$**.** These results show little evidence for the improved stability in the presence of HP-ß-CD.

Table 23. List of shelf-lives of ART in the presence and absence of HP-ß-CD.

Temperature	ART Shelf-lives (h)	
	0 mg mL^{-1} HP-ß-CD	108 mg mL^{-1} HP-ß-CD
23 °C	9.9	46.0
30 °C	3.0	16.3
37 °C	1.7	7.9

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Chapter 5

GENERAL DISCUSSION

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5 General discussion

5.1 Assessment of solubility study

Although ART is the only soluble artemisinin formulation available in the market, investigation of ART solubility in aqueous solution at a range of pH values is important. ART is slightly soluble at low pH values because it has pKa of 4.6. The solubility rates have a wide range between pH 3.00 which was 0.2 mg mL^{-1} to 5 mg mL^{-1} at pH 6.00 Table 17. This means there was 25 fold increase of solubility at pH 6.00 than at 3.00. This result was expected due to the pKa of ART, where at pH values more than 4.60, ART ionises and is more readily soluble.

Inclusion complexation is a common method to enhance the solubility of most drugs. HP-ß-CD has been selected among several types of cyclodextrin due to being effective, safe and relatively cheap compared to other derivatives.[104] Selection of cyclodextrin type for new formulation of ART depends on the strength or magnitude of complexation of ART within the cyclodextrin. The complexation capability of cyclodextrins with artemisinin has been demonstrated in the order of α- < γ- < β-cyclodextrin.[53] This result reveals the structural compatibility between the molcular size of artemisinin and the diameter of the cyclodextrin cavities. Several studies on the formulation of drug inclusion complexes with β-cyclodextrin especially HP-β-CD showed relatively higher solubility and stability among the other cyclodextrins.[53, 55, 105] Therefore selection of HP-β-CD inclusion complex with ART as a formulation was appropriate rather than the other cyclodextrins. Solubility of ART increasingly improved and showed a 25-fold increase in solubility with highest HP-ß-CD concentration employed at pH 3.00. This is due to high affinity of

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hydrophobic nature of ART which favours inclusion into the cavity of the cyclodextrin molcule.

Ansari found that DHA solubility increased up to 89-fold (32.62 m mol) in water containing 206.4 m mol HP-ß-CD.[2] In phosphate buffer solution (pH 7.40), DHA solubility in the presence of HP-ß-CD was 17.1 m mol. Since the main degradation product of ART is DHA which usually precipitates in the solution therefore a further study needs to investigate the solubility of the candidate drug and its degraded compound in the solution up to the shelf-life of ART.

The phase solubility diagram shows that the solubility increased in linearly manner as a function of the HP-β-CD concentration and revealed a 1:1 drug/cyclodextrin complex which was typical A_L-type. This result can be supported by most derivatives of artemisinin which showed similar phase solubility profiles.[2, 53, 106 105] This result also reflects the extent of hydrophobicity of this antimalarial group which can be supportive in the formulation.

The apparent stability, $K_{st,}$ of the ART/ CD complex correlated with the employed pH values as shown in Table 19 revealed that the affinity of ART to be complexed with HP-ß-CD was decreased with increased of pH values. This phenomenon is expected since the inclusion complex formation is dependent on the hydrophobic interactions between the drug and the nonpolar cavity of HP-ß-CD. Thus, this interaction is favoured when the molcular species of ART is present in the medium.

5.1.1 Assessment of stability study

Stability of ART in the presence of HP-ß-CD was performed at three pH values 6.00, 7.00 and 8.00. Linear relationships ($r > 0.99$) were produced indicating a first-order

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reaction. Low observed rates constants were found at pH 7.00. ART has a pK_a of 4.6 indicating that > 99% of ART will be ionized at pH 6.60 and above. Ionized ART is more polar which can not easily transfer to the nonpolar cavity of cyclodextrin. Hence more of the free ART will be subjected to degradation in the aqueous medium as indicated by K_{st} values at pH 6.00, 7.00 and 8.00 were 83, 73 and 61 M^{-1} respectively.

To evaluate the temperature dependent parameters, the solubilities of ART in HPβCD were studied at 23, 30 and 37°C. Various thermodynamic parameters such as Ea, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ were determined Table 21. There was a significant increase of shelf-lives in correlation to the different HP-β-CD concentrations. Shelf-life of ART increased 5-fold in the presence of HP-β-CD which gives a significant promising approach in drug formulation. The Ea of ART in the absence and presence of HP-β-CD were 93.4 and 95.8 kJ/mol respectively, Table 22. This indicated there was essentially no difference in the activation energies in the presence or absence of HP-β-CD. It is also reliable that the high Ea values indicate a marked effect of temperature on reaction rate. This is a major factor for the storage of reconstituted ART in tropical climates. The addition of HP-β-CD to the reconstitution fluid will markedly improve the shelf-life. The observation of positive ΔH change and small positive ΔS in ART in aqueous and HP-β-CD are indicative of classical hydrophobic and endothermic interactions.

Batty found that ART in glucose 5% injection was stable for approximately 12 h at ambient temperature in an air-conditioned environment.[78] He also mentioned that ART in glucose 5% in syringes was stable for approximately 7 h at 30 ºC, a temperature that is consistent with ambient conditions in tropical countries.

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The recommended diluents for ART, when prepared for intravenous injection, are sodium chloride 0.9% or glucose 5% injection.[107] Glucose 5% solution may have a low pH value and this could lead to accelerated degradation. However the data in this study showed the stability of ART was greater in Hartman's solution therefore it can be recommended the reconstitution of ART in Harman's solution to increase the shelf-life.

Whilst 'stability' represents the time for the concentration to fall to 90% of original concentration, assuming no toxic degradation products are formed.[108] A precipitate was observed in the samples at different times. At low and high pH values the precipitation happened at 5h while in the pH range of 4.00 – 8.50 the precipitation started to be observed at the 9 h. Batty also observed a precipitate in the syringes stored at 36.5 ºC at the 9 h and also at 24 h at ambient temperature.[78]

Unidentified peaks appeared in the chromatogram as the ART degraded but not before 12 h in the pH range of 5.50-7.50 and 37 ºC.

The apparent stability constant, $K_{st,}$ of ART inclusion complex with HP-β-CD appeared stronger at low pH value (at pH 3.00 K_{st} value was 130 M^{-1}). This is due to the affinity of ART molcule as unionized form at low pH value to favour the HP-β-CD cavity.

Gabriels investigated the stability and solubility of ART in liposomes at different concentrations of ART and lipids in three buffers (pH 5.00, 7.00 and 9.00). [87] ART seemed to be stable at pH 5.00 rather than 7.00 and 9.00 and almost totally incorporated in the liposomes and solubility in aqueous phase was nil. This can be related to the predominant unionized form of ART molcule (pKa 4.6) at pH 5.00

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which tended to incorporate in the liposome (lipid compounds) and leave the aqueous phase.

The pH rate profile of ART demonstrated at region from pH 3.00 to 8.50 with a marked increased rate over that predicted from specific acid base catalysis. This was assumed to be a catalytic effect of succinic acid on the ART. The deviations of rate constants in specific acid and specific hydroxyl ion catalyzed hemiester hydrocortisone hydrolysis in the intermediate pH regions greatly exceeded the rate constant values predictable for these pH values from the extrapolations of the right and left arms of the curve.[109]

Not only hydrogen and hydroxyl ions and solvent alone catalyze the decomposition of drugs in solution, but charged species contributed from excipients or buffers may do so as well. Another example is the intra-molcular catalysis in the independent pH region for the hydrolysis of the aspirin anion rather than general acid-base catalysis by water since variations in acetic acid-acetate buffer concentrations had negligible effect on the hydrolysis.[110]

A further investigation is required for ART to develop a new ester rather than succinate ester due to the intra-molcular catalysis.

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6 Conclusions

The degradation rate of ART in buffered systems obeyed pseudo first-order kinetics and with general acid-base catalysis, however; it was not a major factor in ART degradation. Maximum stability of ART in aqueous solution was between pH 5.50 and 7.50. Stability was markedly temperature dependent. The small change between the predicted and experimental log k with pH over the studied pH range was postulated to arise from succinate catalysis of the reaction species. Over the ionic strength range of 0.5 - 1.0 mol L^{-1} the rate increased by approximately 20%. There was no marked difference in the observed rate constants investigated in HPLC or LC-MS. Shelf-life of ART at pH 6.50 and 30 ˚C, which consistent with tropical countries, was approximately 3.5 h whereas it was double in Hartman's solution compared with normal saline or glucose 5% solution. Solubility of ART in aqueous solution was pH-dependent and increased linearly with increased HP-ß-CD concentration showing a 1:1 complex. Solubility increased 25-fold in the presence of the highest concentration of HP-β-CD examined at pH 3.0. Stability of ART increased significantly in the presence of HP-ß-CD and shelf-life values increased almost 5 fold in the presence of HP-ß-CD.

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8 Appendices

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} phosphate buffer solution (pH 2.00) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.30	73.98	70.79	67.56	70.48	67.61	64.75	67.91	64.57	63.30
0.60	46.41	44.67	42.90	43.81	41.49	42.66	37.15	37.15	37.15
1.00	25.19	25.12	24.44	23.99	23.99	23.99	22.11	21.74	21.81
1.50	9.40	9.34	9.33	8.91	9.69	9.20	8.36	8.38	8.39

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
0.30	1.87	1.85	1.83	1.85	1.83	1.81	1.83	1.81	1.80
0.60	1.67	1.65	1.63	1.64	1.62	1.63	1.57	1.57	1.57
1.00	1.40	1.40	1.39	1.38	1.38	1.38	1.34	1.34	1.34
1.50	0.97	0.97	0.97	0.95	0.99	0.96	0.92	0.92	0.92

	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L-1	0.2 mol. L^{-1}	0.1 mol. L-1	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
0.30	1.85	1.83	1.82	0.02	0.02	0.02	1.07	1.01	0.87
0.60	1.65	1.63	1.57	0.02	0.01	0.00	1.03	0.72	0.00
1.00	1.40	1.38	1.34	0.01	0.00	0.00	0.52	0.00	0.29
1.50	0.97	0.97	0.92	0.00	0.02	0.00	0.16	1.90	0.08

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} phosphate buffer solution (pH 2.50) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	77.45	81.50	77.93	79.49	76.71	81.59	75.44	73.99	73.85
2.00	62.09	63.68	60.29	58.73	57.35	58.81	57.10	57.14	56.09
2.50	55.82	45.36	49.06	48.53	47.73	48.12	40.59	50.36	44.75

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
1.0	1.89	1.91	1.89	1.90	1.88	1.91	1.88	1.87	1.87
2.0	1.79	1.80	1.78	1.77	1.76	1.77	1.76	1.76	1.75
2.5	1.75	1.66	1.69	1.69	1.68	1.68	1.61	1.70	1.65

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. $^{L-1}$	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. $^{L-1}$	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. $^{L-1}$
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
1.0	1.90	1.90	1.89	0.0121	0.0134	0.0051	0.013	0.014	0.005
2.0	1.79	1.77	1.75	0.0119	0.0061	0.0046	0.013	0.007	0.005
2.5	1.698	1.68	1.65	0.0455	0.0036	0.0469	0.054	0.004	0.057

Degradation of 0.6 mg L^{-1}ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 3.00) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.00	73.82	77.85	69.93	74.65	77.17	71.79	68.17	65.21	65.93
4.00	56.33	54.70	51.20	60.83	62.62	63.92	56.42	54.80	58.22
5.00	49.81	51.33	48.36	48.74	48.10	49.04	44.34	44.19	44.36

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.0	1.87	1.89	1.84	1.87	1.89	1.86	1.83	1.81	1.82
4.0	1.75	1.74	1.71	1.78	1.80	1.81	1.75	1.74	1.77
5.0	1.70	1.71	1.68	1.69	1.68	1.69	1.65	1.65	1.65

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0	1.87	1.87	1.83	0.02	0.02	0.01	1.25	0.84	0.55
4.0	1.73	1.80	1.75	0.02	0.01	0.01	1.23	0.60	0.75
5.0	1.697	1.69	1.65	0.01	0.00	0.00	0.76	0.25	0.05

Degradation of 0.6 mg L^{-1}ART in 0.1, 0.15 and 0.2 mol. L^{-1} citrate buffer solution (pH 3.50) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.5	83.5	92.5	88.7	89.8	90.3	87.8	85.7	86.0	88.9
1.5	72.7	74.2	72.0	69.8	74.1	67.8	69.2	66.3	72.0
3.5	46.4	44.1	40.9	41.1	41.0	42.0	41.7	42.1	41.2
4.5	38.3	34.8	33.1	30.5	30.9	30.9	30.2	30.7	30.1
5.5	29.5	32.7	26.4	30.7	27.9	26.9	22.6	22.4	21.6

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
0.5	1.92	1.97	1.95	1.95	1.96	1.94	1.93	1.93	1.95
1.5	1.86	1.87	1.86	1.84	1.87	1.83	1.84	1.82	1.86
3.5	1.67	1.64	1.61	1.61	1.61	1.62	1.62	1.62	1.61
4.5	1.58	1.54	1.52	1.48	1.49	1.49	1.48	1.49	1.48
5.5	1.47	1.51	1.42	1.49	1.44	1.43	1.35	1.35	1.33
Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.000	0.000	0.000	0.000	0.000	0.000
0.5	1.95	1.95	1.94	0.022	0.007	0.009	0.023	0.007	0.009
1.5	1.86	1.85	1.84	0.007	0.020	0.018	0.007	0.021	0.019
3.5	1.64	1.62	1.62	0.028	0.006	0.005	0.034	0.007	0.006
4.5	1.55	1.49	1.48	0.032	0.004	0.005	0.042	0.005	0.007
5.5	1.47	1.45	1.35	0.046	0.030	0.010	0.063	0.041	0.015

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 4.00) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	83.18	86.15	80.34	80.28	83.18	86.05	83.18	83.18	80.25
4.00	52.48	55.26	49.65	48.98	47.52	50.99	47.86	47.86	46.18
6.00	39.81	41.48	39.53	37.15	37.15	37.15	36.31	36.31	35.03
7.00	34.67	34.67	34.67	32.36	32.36	32.36	31.62	31.76	30.51

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
1.00	1.92	1.94	1.90	1.90	1.92	1.93	1.92	1.92	1.90
4.00	1.72	1.74	1.70	1.69	1.68	1.71	1.68	1.68	1.66
6.00	1.60	1.62	1.60	1.57	1.57	1.57	1.56	1.56	1.54
7.00	1.54	1.54	1.54	1.51	1.51	1.51	1.50	1.50	1.48

	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L-1	0.2 mol. L^{-1}	0.1 mol. L-1	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.92	1.92	1.92	0.02	0.02	0.01	0.79	0.79	0.47
4.00	1.72	1.69	1.67	0.02	0.02	0.01	1.35	0.91	0.54
6.00	1.60	1.57	1.55	0.01	0.00	0.01	0.70	0.00	0.58
7.00	1.54	1.51	1.50	0.00	0.00	0.01	0.00	0.00	0.64

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 5.00) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	% Conc. 0.1 mol. L^{-1}			% Conc. 0.15 mol. L^{-1}			% Conc. 0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.50	99.20	98.06	96.94	99.14	97.57	98.23	92.68	90.38	87.14
3.00	81.57	80.72	80.41	79.38	79.71	79.95	74.07	73.85	73.70
5.00	64.40	63.58	63.44	63.82	64.04	63.89	61.84	62.26	61.47
11.00	40.55	41.94	39.44	39.32	40.22	40.98	36.46	36.66	36.32
24.00	12.84	12.62	12.45	11.86	11.21	11.11	9.80	10.20	10.22

Log % of ART Residual

Time h	Log % Conc 0.1 mol. L^{-1}			Log % Conc 0.15 mol. L^{-}			Log % Conc 0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
0.5	2.00	1.99	1.99	2.00	1.99	1.99	1.97	1.96	1.94
3.0	1.91	1.91	1.91	1.90	1.90	1.90	1.87	1.87	1.87
5.0	1.81	1.80	1.80	1.80	1.81	1.81	1.79	1.79	1.79
11.0	1.61	1.62	1.60	1.59	1.60	1.61	1.56	1.56	1.56
24.0	1.11	1.10	1.10	1.07	1.05	1.05	0.99	1.01	1.01
	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
0.5	1.99	1.99	1.95	0.0050	0.0035	0.0135	0.005	0.003	0.014
3.0	1.91	1.90	1.87	0.0032	0.0015	0.0011	0.003	0.002	0.001
5.0	1.80	1.81	1.79	0.0035	0.0008	0.0028	0.004	0.001	0.003
11.0	1.61	1.60	1.56	0.0134	0.0090	0.0020	0.017	0.011	0.003
24.0	1.10	1.06	1.003	0.0067	0.0153	0.0103	0.012	0.029	0.021

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} phosphate buffer solution (pH 5.50) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	97.75	93.83	92.85	95.09	90.79	90.04	90.56	89.23	92.26
4.00	73.31	71.29	70.00	74.98	72.33	69.94	68.68	72.55	73.31
5.00	68.66	71.09	64.87	67.53	68.04	65.83	67.29	70.72	62.72
18.00	22.36	22.31	22.40	19.05	18.96	18.90	17.62	17.69	17.57
23.00	15.03	15.02	15.03	13.73	13.67	13.54	13.64	14.42	14.23

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
1.00	1.99	1.97	1.97	1.98	1.96	1.95	1.96	1.95	1.96
4.00	1.87	1.85	1.85	1.87	1.86	1.84	1.84	1.86	1.87
5.00	1.84	1.85	1.81	1.83	1.83	1.82	1.83	1.85	1.80
18.00	1.35	1.35	1.35	1.28	1.28	1.28	1.25	1.25	1.24
23.00	1.18	1.18	1.18	1.14	1.14	1.13	1.13	1.16	1.15

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L-1	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L-1	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.98	1.96	1.95	0.01	0.01	0.01	0.60	0.65	0.37
4.00	1.85	1.86	1.85	0.01	0.02	0.02	0.54	0.81	0.82
5.00	1.83	1.83	1.82	0.02	0.01	0.03	1.09	0.41	1.44
18.00	1.35	1.28	1.25	0.00	0.00	0.00	0.07	0.14	0.13
23.00	1.18	1.15	1.15	0.00	0.00	0.01	0.02	0.26	1.09

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 phosphate buffer solution (pH 6.50) at 37 C and 0.5 µ

				% of ART Residual					
Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	94.89	94.30	94.54	95.62	96.03	96.05	93.91	93.93	93.94
3.00	79.50	81.30	78.01	81.98	83.44	80.55	78.54	76.95	79.71
5.00	65.47	66.49	64.03	64.99	64.54	65.26	66.01	67.19	65.39
18.00	32.36	32.49	31.86	29.51	29.02	30.15	27.54	28.05	26.46
23.00	19.16	20.05	19.66	19.96	19.86	19.02	19.83	19.61	19.93

				Log % of ART Residual					
Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
0.50	1.98	1.97	1.98	1.98	1.98	1.98	1.97	1.97	1.97
3.00	1.90	1.91	1.89	1.91	1.92	1.91	1.90	1.89	1.90
5.00	1.82	1.82	1.81	1.81	1.81	1.81	1.82	1.83	1.82
18.00	1.51	1.51	1.50	1.47	1.46	1.48	1.44	1.45	1.42
23.00	1.28	1.30	1.29	1.30	1.30	1.28	1.30	1.29	1.30
	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L-	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L-	0.2 mol. L^{-1}
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
0.50	1.98	1.98	1.98	0.00	0.00	0.00	0.07	0.06	0.00
3.00	1.90	1.91	1.89	0.01	0.01	0.01	0.47	0.40	0.00
5.00	1.82	1.81	1.82	0.01	0.00	0.01	0.45	0.13	0.00
18.00	1.51	1.47	1.44	0.00	0.01	0.01	0.30	0.57	0.90
23.00	1.29	1.30	1.30	0.01	0.01	0.00	0.76	0.88	0.28

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 phosphate buffer solution (pH 7.50) at 37 C and 0.5 µ

				% of ART Residual					
Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.00	95.05	95.02	94.78	90.61	93.67	91.17	82.47	81.48	85.16
4.00	82.71	76.23	81.28	81.95	78.50	84.95	69.23	68.04	70.35
5.00	68.42	72.37	73.09	70.11	71.88	69.06	71.60	68.35	65.99
18.00	29.17	28.78	29.54	27.86	28.61	27.52	27.38	25.67	25.27
22.00	21.96	21.65	22.17	20.17	20.41	20.05	19.35	19.13	19.50

				Log % of ART Residual					
Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.00	1.98	1.98	1.98	1.96	1.97	1.96	1.92	1.91	1.93
4.00	1.92	1.88	1.91	1.91	1.89	1.93	1.84	1.83	1.85
5.00	1.84	1.86	1.86	1.85	1.86	1.84	1.85	1.83	1.82
18.00	1.46	1.46	1.47	1.44	1.46	1.44	1.44	1.41	1.40
22.00	1.34	1.34	1.35	1.30	1.31	1.30	1.29	1.28	1.29

	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L-	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L-	0.2 mol. L^{-1}
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
2.00	1.98	1.96	1.93	0.00	0.01	0.01	0.03	0.39	0.51
4.00	1.90	1.91	1.84	0.02	0.02	0.01	0.98	0.90	0.39
5.00	1.85	1.85	1.84	0.02	0.01	0.02	0.83	0.48	0.97
18.00	1.46	1.45	1.42	0.01	0.01	0.02	0.39	0.60	1.31
22.00	1.34	1.32	1.29	0.01	0.00	0.00	0.39	0.30	0.33

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} Tris buffer solution (pH 8.50) at 37 C (HPLC)

				% of ART Residual					
Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	83.18	86.15	80.34	83.18	80.25	86.05	83.18	83.18	80.25
4.00	53.32	55.26	51.35	48.11	44.59	45.91	47.59	48.14	46.99
6.00	40.65	41.48	40.38	34.26	34.23	34.28	27.97	30.72	29.60
7.00	36.36	36.06	36.66	29.78	30.02	29.51	26.62	26.45	25.36
				Log % of ART Residual					
Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
1.00	1.92	1.94	1.90	1.92	1.90	1.93	1.92	1.92	1.90
4.00	1.73	1.74	1.71	1.68	1.65	1.66	1.68	1.68	1.67
6.00	1.61	1.62	1.61	1.53	1.53	1.54	1.45	1.49	1.47
7.00	1.56	1.56	1.56	1.47	1.48	1.47	1.43	1.42	1.40
	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L-1	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.92	1.92	1.92	0.02	0.02	0.01	0.79	0.79	0.47
4.00	1.73	1.66	1.68	0.02	0.02	0.01	0.92	1.00	0.31
6.00	1.61	1.53	1.47	0.01	0.00	0.02	0.38	0.02	1.39
7.00	1.56	1.47	1.42	0.00	0.00	0.01	0.23	0.25	0.81

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} carbonate buffer solution (pH 9.50) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	64.94	67.26	62.57	63.25	64.60	61.86	62.74	63.56	61.83
3.00	29.31	30.04	30.00	28.18	27.91	28.52	29.00	28.68	29.34
4.00	17.43	17.60	17.27	17.93	17.77	18.13	17.00	16.81	17.15

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
1.0	1.81	1.83	1.80	1.80	1.81	1.79	1.80	1.80	1.79
3.0	1.47	1.48	1.48	1.45	1.45	1.46	1.46	1.46	1.47
4.0	1.24	1.25	1.24	1.25	1.25	1.26	1.23	1.23	1.23

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
1.0	1.81	1.80	1.80	0.0157	0.0094	0.0060	0.867	0.523	0.334
3.0	1.47	1.45	1.46	0.0060	0.0047	0.0049	0.409	0.325	0.336
4.0	1.241	1.25	1.23	0.0041	0.0044	0.0043	0.333	0.348	0.349

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} carbonate buffer solution (pH 10.50) at 37 C and 0.5 µ

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
0.50	32.55	35.92	29.01	32.46	32.03	32.77	28.55	27.50	29.57
1.00	12.04	12.23	11.81	10.76	10.56	10.91	11.88	11.68	12.10

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
0.5	1.51	1.56	1.46	1.51	1.51	1.52	1.46	1.44	1.47
3.0	1.08	1.09	1.07	1.03	1.02	1.04	1.07	1.07	1.08

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
0.5	1.51	1.51	1.47	0.0464	0.0050	0.0158	3.074	0.332	1.073
3.0	1.08	1.03	1.08	0.0076	0.0071	0.0076	0.699	0.687	0.711

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 3.0) at 37 C (LC/MS)

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.00	74.02	74.60	74.32	76.46	76.60	77.02	74.02	83.66	73.02
4.00	49.01	49.74	50.25	54.52	56.65	52.36	52.81	53.58	53.01

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.0	1.87	1.87	1.87	1.88	1.88	1.89	1.87	1.92	1.86
4.0	1.69	1.70	1.70	1.74	1.75	1.72	1.72	1.73	1.72

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
2.0	1.87	1.88	1.89	0.0017	0.0017	0.0325	0.091	0.088	1.718
4.0	1.70	1.74	1.73	0.0055	0.0171	0.0033	0.321	0.985	0.190

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 6.50) at 37 C (LC/MS)

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.00	95.72	96.02	95.42	83.45	83.90	81.84	84.30	84.93	75.79
16.00	31.33	31.81	30.84	30.97	30.94	30.01	32.24	33.15	28.34
20.00	24.10	23.73	24.43	22.77	22.92	22.45	22.86	23.69	21.36

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.0	1.98	1.98	1.98	1.92	1.92	1.91	1.93	1.93	1.88
16.0	1.50	1.50	1.49	1.49	1.49	1.48	1.51	1.52	1.45
20.0	1.38	1.38	1.39	1.36	1.36	1.35	1.36	1.37	1.33

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
2.0	1.98	1.92	1.92	0.0014	0.0057	0.0277	0.069	0.295	1.439
16.0	1.50	1.49	1.49	0.0068	0.0078	0.0364	0.453	0.523	2.434
20.0	1.382	1.36	1.35	0.0064	0.0046	0.0229	0.460	0.339	1.692

Degradation of 0.6 mg L^{-1} ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 8.50) at 37 °C (LCMS)

				% of ART Residual					
Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.00	91.14	97.29	85.57	88.42	89.93	87.04	86.63	86.68	89.66
19.00	20.14	19.41	20.79	21.80	21.52	22.08	21.82	21.78	22.24
				Log % of ART Residual					
Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.0	1.96	1.99	1.93	1.95	1.95	1.94	1.94	1.94	1.95
19.0	1.30	1.29	1.32	1.34	1.33	1.34	1.34	1.34	1.35
Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol.L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.0000	0.0000	0.0000	0.000	0.000	0.000
2.0	1.96	1.95	1.94	0.0279	0.0071	0.0085	1.423	0.365	0.441
19.0	1.30	1.34	1.34	0.0150	0.0056	0.0050	1.152	0.417	0.375

Degradation of 0.6 mg L^{-1}ART in 0.1, 0.15 and 0.2 mol. L^{-1} acetate buffer solution (pH 8.50) at 37 C (HPLC)

% of ART Residual

Buffer Con	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.00	82.74	85.19	80.84	89.91	89.65	90.22	81.56	83.12	79.64
16.00	30.06	30.47	29.91	30.20	29.34	30.92	31.06	31.46	30.64
20.00	25.27	25.63	25.27	23.70	23.45	23.98	21.30	21.48	21.09

Log % of ART Residual

Time h	0.1 mol. L^{-1}			0.15 mol. L^{-1}			0.2 mol. L^{-1}		
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.0	1.92	1.93	1.91	1.95	1.95	1.96	1.91	1.92	1.90
16.0	1.48	1.48	1.48	1.48	1.47	1.49	1.49	1.50	1.49
20.0	1.40	1.41	1.40	1.37	1.37	1.38	1.33	1.33	1.32

Buffer Con	Avarage of Log % ART			STDV			STD Error		
Time h	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}	0.1 mol. L^{-1}	0.15 mol. L^{-1}	0.2 mol. L^{-1}
0.0	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
2.0	1.92	1.95	1.93	0.01	0.00	0.01	0.60	0.07	0.48
16.0	1.48	1.48	1.49	0.00	0.01	0.01	0.28	0.77	0.38
20.0	1.405	1.37	1.33	0.00	0.00	0.00	0.25	0.35	0.30

Degradation of 0.6 mg L^{-1} ART in 5%glucose water, 0.9% normal saline and Hartman's solution at 37 °C

% of ART Residual

IV FLUID	5% GLUCOSE WATER			0.9% NORMAL SALINE			HARTMAN'S SOLUTION		
Time h	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1.00	79.99	75.08	78.95	81.41	79.95	84.36	96.28	97.86	93.22
2.00	66.59	62.97	64.87	66.63	65.94	68.53	81.04	85.08	82.19
5.00	36.68	32.49	35.49	34.52	33.78	36.15	65.14	66.19	68.53
7.00	21.88	21.09	20.09	24.45	24.55	25.04	49.76	49.45	48.59

Log % of ART Residual

Time h	5% GLUCOSE WATER			0.9% NORMAL SALINE			HARTMAN'S SOLUTION		
0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
1.00	1.90	1.88	1.90	1.91	1.90	1.93	1.98	1.99	1.97
2.00	1.82	1.80	1.81	1.82	1.82	1.84	1.91	1.93	1.91
5.00	1.56	1.51	1.55	1.54	1.53	1.56	1.81	1.82	1.84
7.00	1.34	1.32	1.30	1.39	1.39	1.40	1.70	1.69	1.69
	Avarage log % ART residual			STDV			STD Error		
Time h	G.W	N.S	H	G.W	N.S	H	G.W	N.S	H
0.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.89	1.91	1.97	0.01	0.01	0.01	0.77	0.62	0.55
2.00	1.81	1.83	1.92	0.01	0.01	0.01	0.67	0.47	0.57
5.00	1.54	1.54	1.82	0.03	0.02	0.01	1.77	0.98	0.62
7.00	1.32	1.39	1.69	0.02	0.01	0.01	1.40	0.40	0.32

Degradation of 0.6 mg L^{-1} ART in 0.2 mole L^{-1} phosphate buffer solution (pH 7.50) at 37 °C and 0.5 µ

	% of ART Residual			Log % of ART Residual				
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV
0.0	100.0	100.0	100.0	2.0	2.0	2.0	2.0	0.0
1.0	98.5	98.7	98.6	2.0	2.0	2.0	2.0	0.0
3.0	96.5	96.9	96.8	1.9	1.9	1.9	1.9	0.0
4.0	94.5	94.6	94.6	1.9	1.9	1.9	1.9	0.0
5.0	92.5	92.7	94.8	1.9	1.9	1.9	1.9	0.0
20.0	69.5	69.0	69.7	1.4	1.4	1.4	1.4	0.0

Degradation of 0.6 mg L^{-1} ART in 0.2 mole L^{-1} phosphate buffer solution (pH 7.50) at 37 °C and 0.8 µ

	% of ART Residual			Log % of ART Residual				
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV
0.0	100.0	100.0	100.0	2.0	2.0	2.0	2.0	0.0
1.0	99.5	99.9	99.7	2.0	2.0	2.0	2.0	0.0
3.0	96.0	96.4	96.3	1.9	1.9	1.9	1.9	0.0
4.0	95.0	95.4	99.0	1.9	1.9	2.0	1.9	0.0
5.0	94.0	94.3	94.5	1.9	1.9	1.9	1.9	0.0
20.0	66.5	66.9	66.8	1.3	1.3	1.3	1.3	0.0

Degradation of 0.6 mg L^{-1} ART in 0.2 mole L^{-1} phosphate buffer solution (pH 7.50) at 37 °C and 1 µ

	% of ART Residual			Log % of ART Residual				
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV
0.0	100.0	100.0	100.0	2.0	2.0	2.0	2.0	0.0
1.0	98.0	98.4	98.3	2.0	2.0	2.0	2.0	0.0
3.0	95.5	95.8	95.8	1.9	1.9	1.9	1.9	0.0
4.0	94.0	94.2	94.2	1.9	1.9	1.9	1.9	0.0
5.0	92.5	92.8	92.6	1.9	1.9	1.9	1.9	0.0
20.0	64.0	64.4	64.3	1.3	1.3	1.3	1.3	0.0

Degradation of 0.6 mg mL^{-1} ART at pH 10.50 and 22 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
0.25	94.22	93.89	94.13	1.97	1.97	1.97	1.97	0.0008	0.040
0.75	79.56	81.27	77.79	1.90	1.91	1.89	1.90	0.0095	0.500
1.75	56.63	56.45	56.69	1.75	1.75	1.75	1.75	0.0010	0.056
2.75	40.24	40.85	37.03	1.60	1.61	1.57	1.59	0.0229	1.438
3.75	28.42	28.74	28.36	1.45	1.46	1.45	1.45	0.0031	0.214

Degradation of 0.6 mg mL^{-1} ART at pH 10.50 and 30 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
0.25	73.61	70.86	76.17	1.87	1.85	1.88	1.87	0.0157	0.840
0.50	54.81	52.98	56.58	1.74	1.72	1.75	1.74	0.0143	0.821
0.75	44.17	42.76	45.56	1.65	1.63	1.66	1.64	0.0137	0.836
1.00	34.21	34.42	34.33	1.53	1.54	1.54	1.54	0.0014	0.088
1.25	25.25	24.88	25.85	1.40	1.40	1.41	1.40	0.0084	0.602

Degradation of 0.6 mg mL^{-1} ART at pH 10.50 and 37 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
0.50	32.46	31.73	24.39	1.51	1.50	1.39	1.47	0.0690	4.705
1.00	11.89	12.07	9.27	1.08	1.08	0.97	1.04	0.0645	6.195

Degradation of 0.6 mg mL^{-1} ART at pH 1.20 and 22 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
0.15	93.25	90.97	89.60	1.97	1.96	1.95	1.96	0.0088	0.447
0.50	84.99	85.78	91.17	1.93	1.93	1.96	1.94	0.0166	0.854
1.50	69.11	62.98	65.35	1.84	1.80	1.82	1.82	0.0203	1.117
3.50	39.65	38.40	44.02	1.60	1.58	1.64	1.61	0.0310	1.928
4.00	37.22	32.42	34.16	1.57	1.51	1.53	1.54	0.0303	1.971

Degradation of 0.6 mg mL^{-1} ART at pH 1.20 and 30 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
0.25	91.27	92.24	93.53	1.96	1.96	1.97	1.97	0.0053	0.271
0.75	63.25	61.54	67.01	1.80	1.79	1.83	1.81	0.0189	1.046
1.00	48.54	49.66	56.18	1.69	1.70	1.75	1.71	0.0342	1.998
1.50	34.72	34.20	35.75	1.54	1.53	1.55	1.54	0.0098	0.635

Degradation of 0.6 mg mL^{-1} ART at pH 1.20 and 37 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	101.22	100.00	100.00	2.01	2.00	2.00	2.00	0.0030	0.152
0.50	39.62	39.62	38.38	1.60	1.60	1.58	1.59	0.0080	0.500
1.00	16.88	19.57	19.93	1.23	1.29	1.30	1.27	0.0395	3.104

Degradation of 0.6 mg mL^{-1} ART at pH 6.50 and 22 °C									
	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
4.00	96.95	94.07	100.81	1.99	1.97	2.00	1.99	0.0151	0.758
27.00	80.55	77.96	83.39	1.91	1.89	1.92	1.91	0.0146	0.766
51.00	58.71	56.70	60.62	1.77	1.75	1.78	1.77	0.0145	0.822
118.00	30.75	32.81	30.77	1.49	1.52	1.49	1.50	0.0162	1.081
Degradation of 0.6 mg mL^{-1} ART at pH 6.50 and 30 °C									
	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
2.00	96.18	98.21	91.24	1.98	1.99	1.96	1.98	0.0165	0.832
4.00	89.92	91.59	88.15	1.95	1.96	1.95	1.95	0.0083	0.425
24.00	51.61	52.51	47.65	1.71	1.72	1.68	1.70	0.0225	1.323
26.00	46.11	47.46	48.89	1.66	1.68	1.69	1.68	0.0127	0.756
28.00	46.61	43.58	45.94	1.67	1.64	1.66	1.66	0.0154	0.930
Degradation of 0.6 mg mL^{-1} ART at pH 6.50 and 37 °C									
	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.0	100.00	100.00	100.00	2.00	2.00	2.00	2.00	0.0000	0.000
1.0	94.16	96.18	91.66	1.97	1.98	1.96	1.97	0.0105	0.531
3.0	78.54	76.95	79.71	1.90	1.89	1.90	1.89	0.0077	0.406
5.0	69.13	65.61	63.96	1.840	1.817	1.806	1.821	0.017	0.945
18.0	27.54	28.05	26.46	1.440	1.448	1.423	1.437	0.013	0.902
23.0	17.13	16.94	17.22	1.234	1.229	1.236	1.233	0.004	0.294

Degradation of 0.6 mg mL^{-1} ART in the presence of HPβCD at pH 7.0 and 22 °C									
	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.0	100.0	100.0	100.0	2.00	2.00	2.00	2.00	0.00	0.00
2.0	99.1	99.1	98.8	2.00	2.00	1.99	2.00	0.00	0.03
24.0	98.8	98.7	98.6	1.99	1.99	1.99	1.99	0.00	0.02
48.0	93.3	93.3	93.2	1.97	1.97	1.97	1.97	0.00	0.02
72.0	89.0	88.9	88.8	1.95	1.95	1.95	1.95	0.00	0.02
96.0	82.7	82.6	82.6	1.92	1.92	1.92	1.92	0.00	0.02
121.0	76.4	76.4	76.3	1.88	1.88	1.88	1.88	0.00	0.02
145.0	74.1	74.0	74.0	1.87	1.87	1.87	1.87	0.00	0.02
170.0	69.0	68.9	68.8	1.84	1.84	1.84	1.84	0.00	0.02

Degradation of 0.6 mg mL^{-1} ART at pH 7.0 and 22 °C									
	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.0	100.0	100.0	100.0	2.00	2.00	2.00	2.00	0.00	0.00
2.0	98.8	98.8	98.8	1.99	1.99	1.99	1.99	0.00	0.00
24.0	77.2	77.4	77.1	1.89	1.89	1.89	1.89	0.00	0.04
48.0	62.0	60.3	64.6	1.79	1.78	1.81	1.79	0.02	0.84
72.0	21.4	22.1	21.4	1.33	1.34	1.33	1.34	0.01	0.57
96.0	37.3	37.9	36.9	1.57	1.58	1.57	1.57	0.01	0.36
121.0	29.3	29.9	28.7	1.47	1.48	1.46	1.47	0.01	0.58
145.0	21.4	22.1	21.4	1.33	1.34	1.33	1.34	0.01	0.57
170.0	16.4	16.5	16.6	1.22	1.22	1.22	1.22	0.00	0.16

Degradation of 0.6 mg mL^{-1} ART in the presence of HPβCD at pH 7.0 and 30 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.0	100.0	100.0	2.00	2.00	2.00	2.00	0.00	0.00
20.00	93.8	91.0	96.0	1.97	1.96	1.98	1.97	0.01	0.58
24.00	93.1	92.3	93.9	1.97	1.96	1.97	1.97	0.00	0.19
46.00	79.3	79.6	78.8	1.90	1.90	1.90	1.90	0.00	0.12
70.00	68.9	65.8	72.2	1.84	1.82	1.86	1.84	0.02	1.09
94.00	61.0	58.0	64.1	1.79	1.76	1.81	1.79	0.02	1.21
120.00	53.9	52.6	55.2	1.73	1.72	1.74	1.73	0.01	0.62
145.00	46.5	44.8	48.5	1.67	1.65	1.69	1.67	0.02	1.04
170.00	39.4	37.7	41.1	1.60	1.58	1.61	1.60	0.02	1.16
194.00	33.31	32.33	34.33	1.52	1.51	1.54	1.52	0.01	0.86

Degradation of 0.6 mg mL^{-1} ART at pH 7.0 and 30 °C

	% of ART Residual			Log % of ART Residual					
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV	STDE
0.00	100.0	100.0	100.0	2.00	2.00	2.00	2.00	0.00	0.000
20.00	65.5	66.8	64.0	1.82	1.82	1.81	1.82	0.01	0.501
24.00	54.7	56.1	53.0	1.74	1.75	1.72	1.74	0.01	0.710
46.00	27.5	25.4	29.6	1.44	1.40	1.47	1.44	0.03	2.327
70.00	17.3	17.9	16.8	1.24	1.25	1.22	1.24	0.01	1.129
94.00	8.3	8.3	8.4	0.92	0.92	0.92	0.92	0.00	0.366

Degradation of 0.6 mg mL^{-1} ART in the presence of HPβCD at pH 7.0 and 37 ºC

	% of ART Residual			Log % of ART Residual				
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV
0.0	100.0	100.0	100.0	2.0	2.0	2.0	2.0	0.0
2.0	100.0	100.0	99.8	2.0	2.0	2.0	2.0	0.0
4.0	99.5	99.7	99.6	2.0	2.0	2.0	2.0	0.0
22.0	94.5	94.9	94.6	1.9	1.9	1.9	1.9	0.0
46.0	87.5	87.7	87.7	1.8	1.8	1.8	1.8	0.0
50.0	86.0	86.5	86.3	1.7	1.7	1.7	1.7	0.0
70.0	80.0	81.0	80.9	1.6	1.6	1.6	1.6	0.0

Degradation of 0.6 mg mL^{-1} ART in the presence of HPβCD at pH 7.0 and 37 ºC

	% of ART Residual			Log % of ART Residual				
Time h	1st	2nd	3rd	1st	2nd	3rd	Avarage	STDV
0.0	100.0	100.0	100.0	2.0	2.0	2.0	2.0	0.0
2.0	96.5	96.8	96.6	1.9	1.9	1.9	1.9	0.0
4.0	94.5	94.9	94.8	1.9	1.9	1.9	1.9	0.0
22.0	69.0	69.4	69.2	1.4	1.4	1.4	1.4	0.0
46.0	40.5	40.0	40.8	0.8	0.8	0.8	0.8	0.0

Degradation of ART in presence of graded concentration of HPβCD at pH 6.00 and 37 ºC

% of ART Residual

CD(m mol)	0		5.2		10.4		20.8		41.6	
Time	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0	100	100	100	100	100	100	100	100	100	100
3	82.3	81.8	88.5	88.5	94.3	95.0	95.0	95.3	98.6	98.6
5	65.3	65.5	79.2	796.2	83.7	84.3	90.9	91.2	94.6	94.8
23	20.6	20.7	37.9	38.1	49.1	49.4	63.8	63.8	70.0	70.2
27	14.5	14.5	32.1	32.3	44.9	45.3	57.5	57.6	67.8	68.0
48	3.4	3.4	13.9	14.0	26.2	26.4	41.0	41.1	51.2	51.3

Log % of ART Residual

Time	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
3	1.92	1.91	1.95	1.95	1.97	1.98	1.98	1.98	1.99	1.99
5	1.81	1.82	1.90	2.90	1.92	1.93	1.96	1.96	1.98	1.98
23	1.31	1.32	1.58	1.58	1.69	1.69	1.81	1.80	1.85	1.85
27	1.16	1.16	1.51	1.51	1.65	1.66	1.76	1.76	1.83	1.83
48	0.53	0.53	1.14	1.14	1.42	1.42	1.61	1.61	1.71	1.71

Time	Average (0) CD	Average(5.2) CD	Average(10.4) CD	Average(20.8) CD	Average(41.6) CD
0	2.00	2.00	2.00	2.00	2.00
3	1.91	1.95	1.98	1.98	1.99
5	1.82	2.64	1.92	1.96	1.98
23	1.32	1.58	1.69	1.80	1.85
27	1.16	1.51	1.65	1.76	1.83
48	0.53	1.14	1.42	1.61	1.71

Degradation of ART in presence of graded concentration of HPβCD at pH 7.00 and 37 °C

% of ART Residual

CD(m mol)	0		5.2		10.4		20.8		41.6	
Time	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	100	100	100	100	100	100	100	100	100	100
2.0	85.7	85.8	93.8	93.7	94.1	940.0	91.5	91.4	100.1	10.0
4.0	78.1	77.9	90.8	90.7	87.0	86.9	89.5	89.4	98.2	9.8
22.0	24.5	24.3	46.9	4.7	62.2	62.1	66.8	66.8	78.8	7.9
26.0	2.0	2.0	40.7	40.6	50.3	50.2	62.0	62.0	72.3	7.2
46.0	0.0	0.0	17.7	17.7	29.0	29.0	42.5	41.6	53.2	5.3
50.0	0.0	0.0	0.0	0.0	29.0	29.0	42.5	41.6	53.2	5.3
70.0	0.0	0.0	0.0	0.0	20.3	2.0	30.1	30.1	39.8	39.8

Log % of ART Residual

Time	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
2.0	1.93	1.93	1.97	1.97	1.97	2.97	1.96	1.96	2.00	1.00
4.0	1.89	1.89	1.96	1.96	1.94	1.94	1.95	1.95	1.99	0.99
22.0	1.39	1.39	1.67	0.67	1.79	1.79	1.82	1.82	1.90	0.90
26.0	0.31	0.31	1.61	1.61	1.70	1.70	1.79	1.79	1.86	0.86
46.0	0.00	0.00	1.25	1.25	1.46	1.46	1.63	1.62	1.73	0.73
50.0	0.00	0.00	0.00	0.00	1.46	1.46	1.63	1.62	1.73	0.73
70.0	0	0	0	0	1.31	0.31	1.48	1.48	1.60	1.60

Time	Average (0) CD	Average(5.2) CD	Average(10.4) CD	Average(20.8) CD	Average(41.6) CD
0.0	2.00	2.00	2.00	2.00	2.00
2.0	1.93	1.95	1.97	1.97	2.47
4.0	1.89	1.92	1.96	1.95	1.94
22.0	1.39	1.53	1.17	1.23	1.79
26.0	0.31	0.96	1.61	1.66	1.70
46.0	0.00	0.62	1.25	1.36	1.46
50.0	0.00	0.00	0.00	0.73	1.46
70.0	0.00	0.00	0.00	0.65	0.81

Degradation of ART in presence of graded concentration of HPβCD at pH 8.00 and 37 ºC

% of ART Residual

CD(m mol)	0		5.2		10.4		20.8		41.6	
Time	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	100	100	100	100	100	100	100	100	100	100
3.0	68.4	47.0	81.9	81.9	86.7	86.7	63.8	63.8	67.9	68.0
22.0	23.8	23.8	39.3	39.3	51.7	51.7	63.9	63.9	67.9	68.0
27.0	17.2	17.2	32.5	32.5	48.7	48.7	51.1	51.1	56.5	56.5
46.0	4.5	4.6	13.4	13.4	24.5	24.5	37.7	37.7	32.4	32.4

Log % of ART Residual

Time	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
3.0	1.8	1.7	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8
22.0	1.4	1.4	1.6	1.6	1.7	1.7	1.8	1.8	1.8	1.8
27.0	1.2	1.2	1.5	1.5	1.7	1.7	1.7	1.7	1.8	1.8
46.0	0.7	0.7	1.1	1.1	1.4	1.4	1.6	1.6	1.5	1.5

Time	Average (0) CD	Average(5.2) CD	Average(10.4) CD	Average(20.8) CD	Average(41.6) CD
0.0	2.0	2.0	2.0	2.0	2.00
3.0	1.8	1.9	1.9	1.8	1.83
22.0	1.4	1.6	1.7	1.8	1.83
27.0	1.2	1.5	1.7	1.7	1.75
46.0	0.7	1.1	1.4	1.6	1.51