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Kinetic Modeling and Release Behavior of PLGA-loaded Nanoparticle of Anti - Malarial Drug using Dialysis Membrane

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ABSTRACT

Malaria is one of the major critical issues in front of the world. It is a leading cause of sickness and death in the tropical and subtropical region of the world. Approximately 1-3 million people may die due to the cause of malaria. Malaria is caused by infection with a single-cell parasite, Plasmodium. Four Plasmodium spp. cause malaria in human. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. Artemisinin is of the newest and widely efficacious drugs, for the treatment of malaria, is also being compromised by the rise of Plasmodium falciparum strains with reduced clinical response to artemisinin-containing drug combinations. New anti malarial drugs must meet the requirements of rapid efficacy, minimal toxicity, and low cost. Due to its limited solubility in water and poor bioavailability nanotechnology play a vital role in this field. Targeting drugs specifically to their site of action have a great advantage in malaria since malaria parasites frequently develop drug resistance due to the administration of low drug concentrations in the presence of a high parasitic count. In the presented study PLGA used to prepare the nano-carriers with artemisinin by Nano-precipitation method, studied the entrapment of drug with PLGA, Morphology of nano-carriers and established the release kinetics with different models. It was found the nanoparticles were produced less than 200nm and release kinetics obeys Korsmeyer Peppas release mechanism.

INTRODUCTION

In the today scenario, Malaria is one of the major critical issues ahead of the world. It is a leading cause of sickness and death in the tropical and subtropical region of the world. Approximately 1-3 million people may die due to the cause of malaria every year. In the world, >40% of the population lives with some danger of constricting malaria [1-2]. The World Health Organization (WHO) reports that a child dies from malaria every 30 s, hence about 3000 children who are under the age of five die each day, The clinical manifestations of malaria are fever, chills, prostration, and anemia. Apart from that severe malarial infection, metabolic acidosis, cerebral malaria, multi-organ failure, coma, and death may additionally chase [3-5]. Malaria is caused by infection with a single-cell parasite, Plasmodium. Four Plasmodium spp. cause malaria in human beings: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. P. falciparum is the most important because it accounts for the majority of infections and causes the most severe symptoms. Malaria remains one of the leading

causes of morbidity and mortality in the tropics. According to the World Malaria Report (2011), there were 106 malaria endemic countries in 2010 [5-7].

Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world. Population movement has introduced resistant parasites to areas previously free of drug resistance. The economics of developing new pharmaceuticals for tropical diseases, including malaria, are such that there is a great disparity between the public health importance of the disease and the number of resources invested in developing new cures. The main drawbacks of conventional malaria [7-13].

Chemotherapy is the development of multiple drug resistance and the non-specific targeting to intracellular parasites, resulting in high dose requirements and subsequent intolerable toxicity. Nano sized carriers have been receiving special attention with the aim of minimizing the side effects of drug therapy, such as poor bioavailability and the selectivity of drugs [12-15]. Due to all these reasons recently, the last class of the newest and widely efficacious drugs, the artemisinins, is also being compromised by the rise of Plasmodium falciparum strains with reduced clinical response to artemisinin-containing drug combinations. New anti malarial drugs must meet the requirements of rapid efficacy, minimal toxicity, and low cost. So the nanotechnology plays a vital role in this field. Ideally, the nanoparticulate drug delivery system should selectively accumulate in the required organ or tissue and at the same time, penetrate target cells to deliver the bioactive agent [15-19]. These Nano carriers are known to improve the efficacy of currently available anti-malarial drugs and contribute to the formulation and delivery of new chemical entities. Targeting drugs specifically to their site of action have a great advantage in malaria since malaria parasites frequently develop drug resistance due to the administration of low drug concentrations in the presence of a high parasitic count. Furthermore, nanomedicine has the potential to restore the use of old and toxic drugs by modifying their bio-distribution, improve bioavailability and reducing toxicity [20-22]. PLGA use as a nano-carrier in the present study because it is a biodegradable polymer and it co-polymer including poly (esters), including poly (lactic acid), poly (glycolic acid) is nature biocompatibility and biodegradability provide a versatile range of polymer in pharmaceutical industry [22-24]. In present study author prepare the polymeric nanocarriers artemisinins with PLGA, characterize the polymeric nano carries also studied the release mechanism of the polymeric drug.

MATERIAL AND METHODS

The Drug Artimisnins obtained from IPCA Labs Ltd. Ratlam as a gift sample. PLGA (Meark), Hydrochloric acid (SD fine chemical), and potassium dihydrogen phosphate (SD fine chemical), PVA Parched from the market. All chemicals are used as an analytical grade.

Preparation of PLGA loaded Nano-particles

The PLGA nanoparticles with Artimisnins were prepared by an emulsion–solvent evaporation method. Typically, 100 mg of PLGA in 10 ml of Methylene Chloride (DCM) with Artimisnin (10% w/w), was mixed with 16 ml of 5% PVA aqueous solution. This mixture was sonicated for 5min to produce the oil-in-water emulsion. The nanoparticles were formed from nano droplets by evaporating a highly volatile organic solvent at room temperature using rotatory vacuum evaporator. The nanoparticles were recovered by centrifugation at 15,000 rpm for 25 min. The nanoparticles were rinsed twice with double distilled water in order to remove absorbed PVA on the surface of nanoparticles. Purified nanoparticles were lyophilized. The final product was stored in a vacuum desiccators prepare the nanoparticle in the different ratio of Drug: Polymer: stabilizer that is 1:1:1, 1:5:5 and 1:10:10 accordingly (Figure 1).

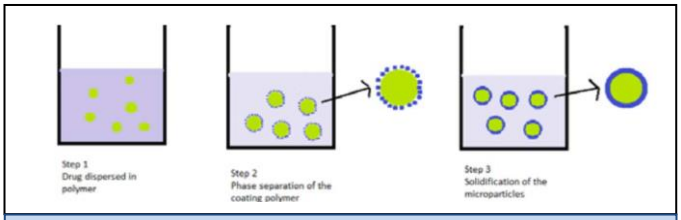

Figure 1: Diagrammatic preparation of Nano-particle by Emulsion solvent evaporation method.

Determination of particle size and size distribution

The nanoparticles size and size distribution were determined in double distilled water at 27^{0}C by Dynamic Light Scattering using a Zeta sizer Malvern Instruments, measurements,100µl of the nanoparticles suspension were dispersed in 1 ml of distilled water and sonicated during 1 min. The analyses were

performed at a scattering angle of 90^0 and at a temperature of 25^0C. For each sample, the mean diameter and the standard deviation of ten determinations were calculated using multimodal analysis. Zeta potential was indicated the surface charge of the loaded sample it indicates the particle stability of in dispersion zeta potential was calculable on the premise of action quality of particle underneath the electrical field. Phase Analysis.

Morphological study of Nanoparticle

The morphology of N-Arti determines by the transmission electron microscope CM-10 Philips-Netherlands. Before the analysis sample was treated by 0.5% (w/v) phosphor tungstic acid and fixed on a copper grid for observation

The size and the morphology are scanning through scanning electron microscopy. In the SEM setup the nanoparticulate sample In N-Arti coated with platinum as a conductive is scanned in a high vacuum chamber with a focused electron beam.

Entrapment study of Artimisnins Drug with PLGA

Accurately weighed, 40 mg of Arti -loaded PLGA were taken in a 100ml of phosphate buffer (pH 7.4), and Further dilute 10ml to 100ml obtained the concentration 40µg/ml. Also, prepare a standard solution of 40µg/ml using Artimisnins working standard having potency 98.1%w/w. The solution was filtered through Whatman® filter paper (no. 20) and drug content in the filtrate was determined using a visible spectrophotometer (Lambda-1700). Drug Entrapment Efficiency (EE) of Arti was calculated using.

$$IE = \frac{AT}{AS} \times \frac{Sc}{Tc} \times \frac{PE}{100}$$

AT= Absorbance of test solution.

AS= Absorbance of standard solution.

Sc= Concentration of standard solution in µg/ml

St= Concentration of sample solution in µg/ml

IE% total am out of Arti to quantify in preparing nanoparticle

***In vitro* release study of N-Arti**

Several methods have been reported for the *in-vitro* release study of nanoparticulate drugs but the dialysis method (DM) is the most versatile and popular.

The dissolution medium consisted of 250 mL of freshly prepared phosphate buffer (pH 7.4)Maintained at 37.2 ^{0}C. The polycarbonate dialysis membrane used was pre-treated by soaking it in the dissolution medium for 24 h prior to the commencement of each release (Figure 2).

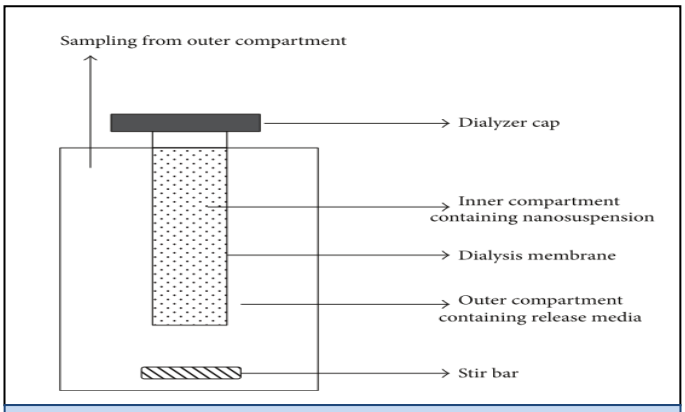

Figure 2: Dissolution through the dialysis membrane.

Experiment: In each case, 0.05 g of the formulated NPs was placed in the dialysis membrane containing 5 mL of the dissolution medium, securely tied with a thermo-resistant thread and then immersed in the dissolution medium under agitation provided by the paddle at 100 rpm. At predetermined time intervals, 5 mL portions of the dissolution medium were withdrawn, filtered and analyzed spectrophotometrically (lamda-1700) at 254 nm. For each sample withdrawn, an equivalent volume (5 mL) of phosphate buffer maintained at the same temperature was added to the contents of the dissolution medium to maintain sink conditions throughout the release period. The amount of drug released at each time interval was determined with reference to the standard in the same concentration and calculate the % amount of release drug using the following formula

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Std}} \text{ x } \frac{\text{Std dilution}}{\text{Test dilution}} \text{ x } \frac{\text{potency}}{100} \text{ x } \frac{100}{\text{Lable claim}}$$

Determination of Hemolytic Activity

Hemolytic assay with uninfected RBC, determine according to their hemolytic potentials of Artemisinin placebo, PLGA nanoparticles, and Artemisinin PLGA Human RBC. Incubate the human RBC after phosphate-buffered saline (PBS) according to their molecular weight. Heparinized fresh blood was rinsed with PBS and centrifuges the RBCs. After that, it is resuspended in PBS at 4% hematocrit. Increasing concentration of nanoparticle preparations was added to uninfected erythrocyte (2% hematocrit) in a 96-well plate for 42 h at 37

^{0}C. PBS alone and 0.4% Triton X-100 (for 100% hemolysis) were added to attain negative and positive control respectively After incubation at 37 ^{0}C with stirring, the samples were centrifuged and the hemolytic activity was determined by measuring the absorbance at 405 nm. Hemolytic of infected red blood cells: To assess the hemolytic of infected cells, cultures were exposed to increasing concentrations of the Artemisinins, placebo PLGA nanoparticles of different molecular weight of various molecular weight for 42 h. The optical density in the supernatant was determined after centrifugation, and the percent lysis compared to the amount of full lysis of the cells present in the culture was calculated. Hemolytic activity data were obtained from at least two different sets of experiments.

RESULTS AND DISCUSSION

Particle size and encapsulation study

The mean particle size and PI of the Arti were less than 200 nm and 0.45, respectively, except for Arti: PLGA :PVA at 1:1:1 and 1:5:5 (522.9 nm and 0.82±0.01, 465.21 nm and 0.71±0.01) and The ratio of the Co-polymer and stabilizer also play a very important role. Results indicated in table-1 that the small mean size and PI value can depend on the proportion of PLGA. The possible explanation was that a high proportion of PLGA could provide sufficient stabilization to the nanoparticles system, and reduces their particle size and sized Distribution. In contrast, the Arti: PLGA: PVA at 1:1:1& 1;5:5 was insufficient to stabilize the nanoparticles system and would lead to mutual coalescence between particles forming a larger size [25].

Table 1: Mean particle size, PI, Encapsulation efficiency of the drug.

Formulation	Drug-polymer and Stabilizer Ration	Mean particle size (nm)	Polydispersity	Encapsulation efficiency
Artemisinin	-	4567		
ARTI-1	1:1:1	522.9	0.81	73.5%
ARTI-2	1:5:5	465.21	0.7	89.9%
ARTI-3	1:10:10	195	0.50	97.8%

The ratio of Arti:PLGA:PVA 1:10:10 give good enough reduces particle size, a possible reason PLGA is a hydrophilic block copolymer due to their hydrophilic nature the block copolymer has the self-ability arrange in a queous solutions forming micelles and micelle aggregates, which have proven to be an excellent candidate for increasing the surface area and reduce the particle size [26]. The encapsulation efficiency of ARTI was obtained between 73.5.8 to 98.4 as the content of PLGA increased, and encapsulation efficiency of the drug was enhanced. The most likely reason was that the lipophilic character of Arti and PVA can be miscible in the internal organic phase, leading to a stronger affinity between them. Consequently, a higher ratio of PVA with ARTI was preferentially dispersed in the internal organic phase, and a slight amount of drug was lost in the aqueous phase during the process of Nanoparticles preparation [22-23] Furthermore, the encapsulation efficiencies of the drug could also be influenced by adjusting the proportion of PVA and also adjusting the proportion of PLGA. (Table 1) showed that encapsulation efficiencies of ARTI increased with enhancing the content of PLGA. The possible reason was that the hydrophobic portion of PVA interpenetrated into the PLGA chains during nanoprecipitation and remained trapped to the polymeric matrix of the nanoparticles. Accordingly, the addition of PVA easily formed an interconnected network with PVA–ARTI and thus elevated the encapsulation efficiencies of the Drug [27,28].

A second possible mechanism to improve encapsulation can be understood by the ionic strength of copolymer can influence significantly the solubilization of a drug in micelle solutions, especially in the case of biodegradable copolymers. The addition of small amounts of stabilizer decreases the repulsion between the similarly charged ionic polymer head groups, thereby decreasing the CMC and increasing the aggregation number and volume of the micelles. The increase in aggregation number favors the encapsulation of hydrophobic drugs in the inner core of the micelle. On the other hand, the decrease in mutual repulsion of the ionic head groups causes closer packing of the copolymer molecules in the palisade layer decreasing the volume available for encapsulation. of polar drugs. The addition of a drug to solutions of PLGA block copolymer may also increase the extent of encapsulation of hydrophobic drugs because of the increase in aggregation number (Figure 3) [29-31].

Morphology of nano-particle

The nanoparticles formed with the PLGA Derivatives encapsulating Artimisnins were analyzed by SEM (Figure 4A, B,C). Due to the chemical interaction with drug and polymer encapsulated resulted in nanoparticles have a certain size and shape. The Shapes of the particle was spherical with mean long axes/diameters, respectively, ranging from 100 nm.

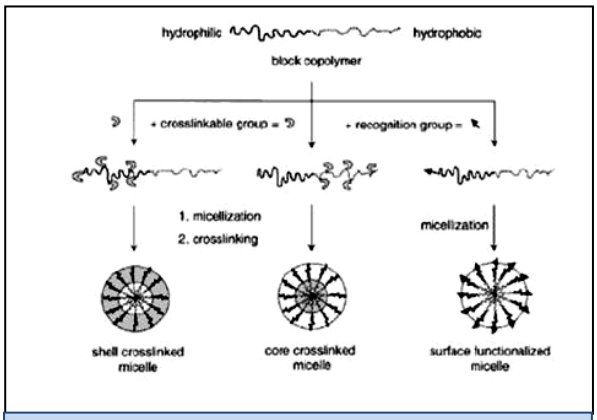

Figure 3: Scheme for Drug encapsulation with micelles.

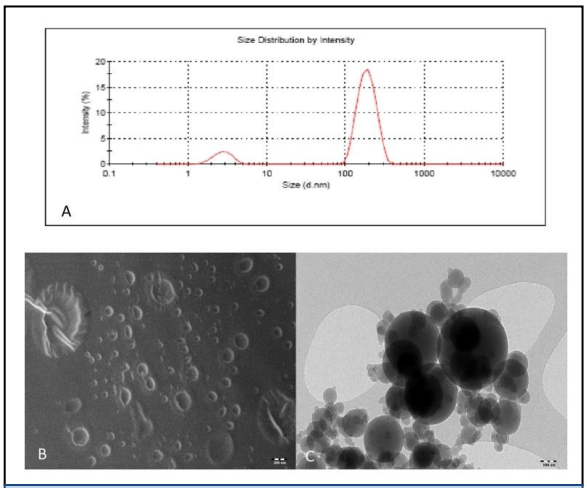

Figure 4: A- Size Distribution study, B.-SEM Image, C. TEM Image of Nanoparticles.

***In-vitro* Release Kinetics**

The graphical presentation of cumulative drug release show the release profile of Artimisnins showed a significant initial burst by releasing 20% of the drug in the first 10 min due to the immediate release profile. But the release profile of the pure drug shows a significant difference as compared with PLGA loaded Artimisnins (ARTI-3). PLGA loaded drug show the 95.5% release in 45 min but cumulative drug release of drug show 74.2% in 60 min probably is due to its low solubility

To investigate the drug release kinetics, data were fitted to various kinetic models such as Zero-order, First-order, Higuchi model and Korsmeyer Peppas model. Based on the regression study of ARTI, having consistency having good drug release show in Korsmeyer Peppas model. The regression analysis was done for both samples. The Korsmeyer-Peppas model shown higher correlation coefficient (R2) = 0.9970, the value of 'n' and the obtained rate constant is given under the (Table 2). A more comprehensive, but still very simple semi-empirical equation is the so-called power law equation given by Korsmeyer and peppas [32-34].

Table 2: R^2 and Rate constant of PLGA Loaded Drug.

Parameters	Korsmeyer Peppas (Min^{-1})	First Order (Min^{-1})	Zero order ($mol^{-1}L^{-1}min^{-1}$)	Higuchi (Min^{-1})
R^2	0.9970	0.9251	0.9650	0.9712
Rate Constant	$6x10^{-3}$	$1.3x10^{-2}$	$2.4x10^{-3}$	$1x10^{-1}$

$$\frac{Mt}{M\infty} = kt^n \quad \ldots\ldots\ldots\ldots\ldots 1$$

Where M∞ and Mt is the amount of drug released at infinite and "t" time, "k" is a constant and "n" is an exponent, characterizing release process. The above equation has to be used with great care for the classification of release nature on the basis of "n" values. For the analysis, data in the range 5-60% of drug released were used.

Indeed, it is well known that, despite fickian diffusion in a matrix, boundary conditions such as the presence of a stagnant layer give origin to release kinetics characterized by 'n' greater than 0.5 or nearer to 1. Thus, Eq. (1) has two distinct physical realistic meanings in the two special cases of n=0.5 (indicating diffusion-controlled drug release) and n=1.0 (indicating swelling-controlled drug release). Values of 'n' between 0.5 and 1.0 can be regarded as an indicator for the superposition of both phenomena (anomalous transport). It has to be kept in mind that the two extreme values for the exponent 'n' 0.5 and 1.0 are only valid for slab geometry. My experiments the results of 'n' = 0.40 - 0.41 clearly indicated t hat diffusion is the dominant mechanism of drug release from these formulations. The drug is released by Fickian diffusion

[7,35]. The drug release rate versus time curve was calculated by the Incremental slope of the curves of Fig. Using a two-point slope Formula

The results showed biphasic profile describe the rapid initial rate decrease, followed by a more steady release rate. This behavior can be attributed to the 'burst' release, typical of hydrophilic matrices, occurring before the formation of the controlling gel layer on the matrix surface. The diffusion mechanism of a drug shown in (Figure 5) [36-40].

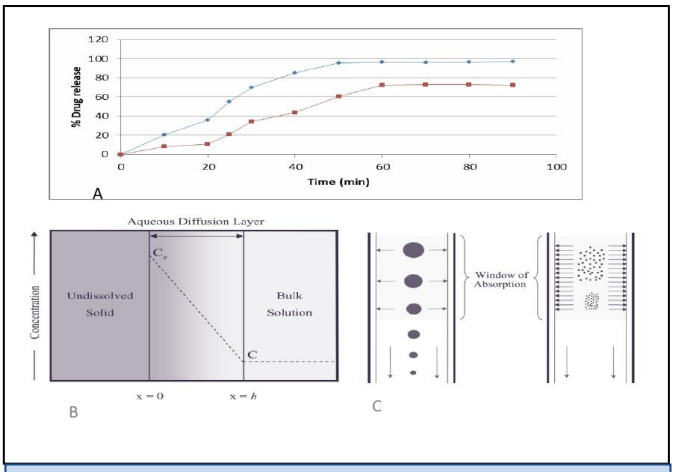

Figure 5: Drug Release behavior through the dialysis membrane.

Drug release is also affected by particle size. Smaller particles have a larger surface area-to-volume ratio; therefore, most of the drug associated with small particles would be at or near the particle surface, leading to faster drug release. In contrast, larger particles have large cores, which allow more drugs to be encapsulated per particle and give slower release. Thus, control of particle size provides a means of tuning drug release rates. The Possible molecular mechanism of Artimisnins release through nano-carries given in (Figure 6). If a polymeric carrier is to be used, the next step is to design a type of polymeric structure that will permit obtaining the desire release condition. in order to have an idea about the nature of the interaction between the release of drug and the polymer the release kinetics well concluded as earlier. In the first step it was found that drug molecule bounded by polymer by covalent bonding through two lone pair of electron of the oxygen atom. They form a compact structure of the transient state. This state is in fast step and breaks into encapsulated drug and unused polymer as well as the drug.

Hemolytic assay

The Hemolysis assay was performed to check when polymer loaded drug enters in systematic blood circulation could cause to damage the erythrocytes membrane which is the main target in malaria activity. The results of hemolytic assay show there have no hemolytic activity show by the Artimisnins placebo and the sample loaded with PLGA show very little hemolytic activity But the mechanism anti-malarial activity of PLGA loaded Artimisnins is little controversial (Figure 6) there have several mechanisms has been reported [41,42] Author proposed PLGA work as a carrier till the outer membrane of erythrocytes cell. Fe (III) that present in red blood cell work as oxidizing agent and it reduces the Artemisinin In addition, it has also been suggested that non-peroxide oxygen play a role in facilitating the ring opening and to stabilized the positive charge high the activation energy of reducing molecule is high and it converts into a peroxide free radical and finally it converts to an open ring hypo peroxide artemisinin its show the anti malarial activity [43-44] (Figure 7).

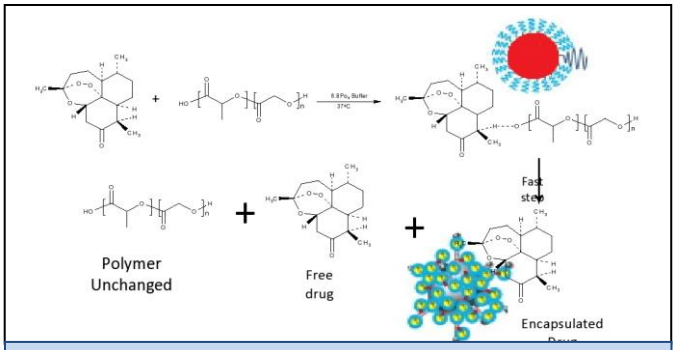

Figure 6: Drug Encapsulation mechanism.

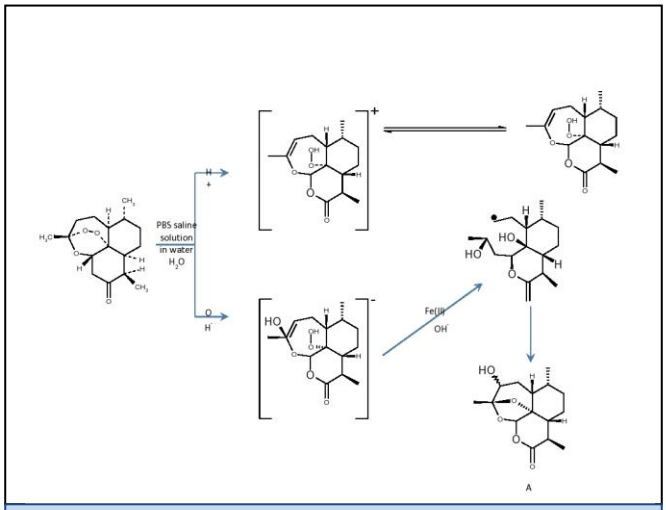

Figure 7: Release of Artemisinin in red blood cells.

CONCLUSION

The present study investigated the targeted drug delivery of anti-malarial drug using the PLGA as a nano-carrier. 1:10:10 ratio of Drug: PLGA: PVA is good enough to produce the less than 200nm nano-particle studied the release kinetics and n-Arti follow the Korsmeyer Peppas model independent release followed by the fickian diffusion method. Arti-3 found the best choice of the combination for archive the high encapsulation of drug as well as the better dissolution results. The experimental result demonstrates PLGA is more bio-relevant to increase the bioavailability of anti-malarial drug and open the new door to improve the formulations of anti-malarial drugs.

AUTHORS' CONTRIBUTIONS

RJ and SS carried out all labs experiments under the guidance and super vision of AP. All authors participated in manuscript preparation and involved in the result discussions. All authors read and approved the final manuscript.

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