

## RESEARCH ARTICLE

## OPEN ACCESS

## Comparative Evaluation of Amodiaquine and Quinine Sulphate Microspheres in The Treatment of Leishmaniasis

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### Abstract

Leishmaniasis is an infectious protozoan disease caused by the Leishmania parasite and transmitted by the female phlebotomine sand fly. Pentavalent antimicrobials have been the backbones for treatment however certain antiprotozoal such as quinine sulphate and amodiaquine among others have been proven to be effective in this regard. The numerous side effects, cost and low availability of current medication has necessitated the need for alternate delivery of this drug hence the emergence of the formulation of drugs into microparticles. Microparticles are known to possess unique physicochemical properties that make them suitable for the management of this debilitating and disfiguring disease. The aim of this research was to specifically assess an alternate formulation for the treatment of leishmaniasis by making microparticulate forms of amodiaquine and quinine sulphate for use in the treatment of leishmaniasis. Using bovine serum albumin as the polymer matrix, amodiaquine and quinine sulfate microparticles were formulated using the spray drying method by crosslinking in solution. The microparticles formed were characterized then the particle size was determined using a Scanning Electron Microscope and the zeta potential determined. The drug content and encapsulation were then determined by preparing standard solutions of each drug. With the aid of the UV/Vis spectrophotometer and CytoFluor well plate reader, the absorbance and intensity were measured respectively and the parameters calculated. An *in vitro* release studies was then performed. The results showed that both amodiaquine and quinine sulphate microparticles had a percentage release of 100% and 2.6% respectively over 24 hours and encapsulation efficiency of 94.5% and 111% respectively. This study served as a significant breakthrough as amodiaquine, and quinine sulphate microparticles were successfully formed using the spray dryer method by solution crosslinking paving the way for use in leishmaniasis.

**KEYWORDS:** amodiaquine, quinine sulphate, encapsulation, pharmacokinetics, microparticle

### INTRODUCTION

Leishmaniasis is a spectrum of vector-borne disease that threatens about 350 million men, women and children around the world in 88 countries around the world. The prevalence and incidence rates currently stands at 12 million and 1-2 million per year. It is endemic in tropical and subtropical countries particularly in rainforests and deserts [1]. With the exception of Australia and Antarctica, it is present in all continents. Essentially, transmission and maintenance of the life cycle of Leishmaniasis is either anthroponotic or zoonotic. Typically, the protozoan parasite is transmitted by a bite from an infected female sand-fly of genera Phlebotomus (Old World) and Lutzomyia (New World) [2,3]. A large number of medications have been approved for use in leishmaniasis infections in addition to other therapies due to the evidence of shortcomings in the recent treatment [4]. Chemotherapy of leishmaniasis has evolved from pentavalent antimonials; sodium stibogluconate and meglumine antimoniate,

amphotericin B and its lipid formulation AmBisome® and pentamidine [5-10]. Treatment of this disease continues to evolve as specific antineoplastic agents like miltefosine has been found to be successful in its cure [11-12]. Efficacy against leishmaniasis has also been proven for a range of antifungal imidazole, including itraconazole, ketoconazole, and fluconazole [13-16]. As well as the aminoglycoside antibiotic, paromomycin [17-20].

Protein-based microparticles among the potential drug carrier systems have greater stability during storage and in-vivo, is non-toxic and non-antigenic and can easily be scaled up during manufacturing hence it is the most of the time chosen over the other systems, the most widely used among them is albumin [21,22].

Microparticles made from albumin are biodegradable, easy to prepare and reproducible. Due to the high protein capacity of most drugs, the matrix of the albumin nanoparticles easily

incorporates them [23] The well-defined albumin primary structure and high content of charged amino acids allow the electrostatic adsorption of positively or negatively charged molecules without the requirement of other compounds [24, 25] These particles show smaller particle sizes compared to microparticles and better-controlled release traits than liposomes which may improve patient compliance and acceptance.

Albumin is derived from egg white (ovalbumin), bovine serum (BSA) and human serum (HAS). It is also available from soya beans, milk and grains [26].

Bovine Serum Albumin of molecular weight 69,323D and an isoelectric point of 4.7 in water (at 25°C) is widely used for drug delivery systems because of its medical importance, abundance, low cost, ease of purification, unusual ligand binding properties and its wide acceptance in the pharmaceutical industry [27,28]. BSA could be substituted by HAS in order to a possible immunologic response in vivo.

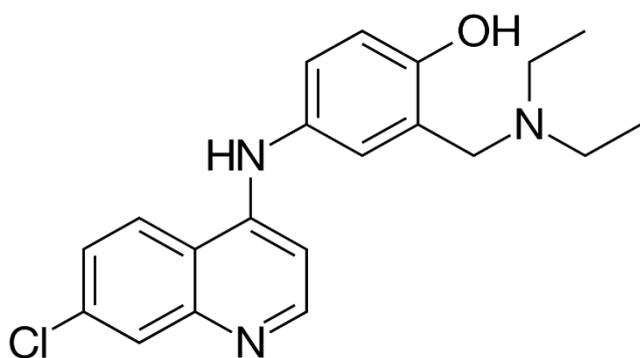


Fig. 1. Chemical structure of Amodiaquine

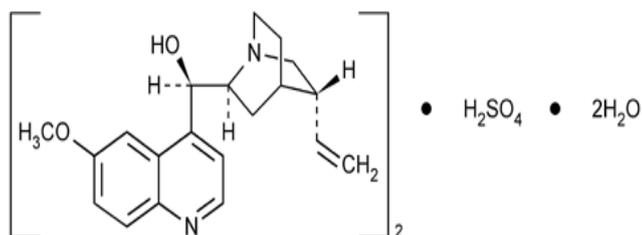


Fig.2. Chemical structure of Quinine Sulphate

## MATERIALS AND METHODS

### Materials:

Amodiaquine and quinine sulphate powder were obtained from Wellona Pharma (Gujarat, India). Bovine Serum Albumin (BSA) was purchased from Aladdin Industrial Corporation, Shanghai (China). Glutaraldehyde Solution was supplied by Muby Chemicals (Gujarat India). Sodium Bisulfite Powder was obtained from Jay Dinesh Chemicals (Gujarat, India). All other chemicals used in the study were of analytical grade.

### Methods:

Amodiaquine microparticles were prepared by the solution cross-linking method as well as the quinine sulphate microspheres.

### Preparation of Amodiaquine and Quinine Sulphate Microparticles Using BSA as a medium for crosslinking (30% drug loading).

1g of the drug was weighed, and 4g of BSA also weighed and added to NaCl (ph 9.3). The BSA was dissolved in 60ml of water, and the amodiaquine in 2ml sulphuric acid and then 8ml of water was added [29]. The two separate solutions were then mixed topped up to 100ml and stirred with a magnetic stirrer to crosslink in solution by adding 400µl glutaraldehyde solution and 1ml sodium bisulfite added to neutralize excess glutaraldehyde. After stirring for an hour, the crosslinked solution was then freeze-dried using Model BILON 6000Y spray dryer to obtain the microparticles [30].

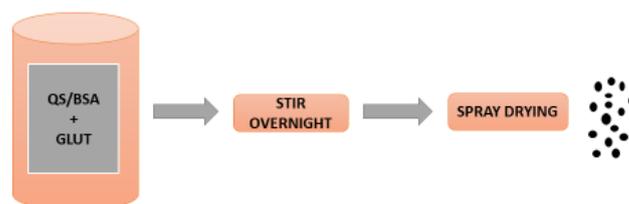


Fig. 3: Schematic Diagram of method Solution Cross-linking

### Characterization of Microparticles

#### Particle Size Determination

About 1mg of each drug microparticles was weighed and dissolved into 20ml of deionized water in two separate tubes. They were vortexed independently to separate particles and the particle size of each tube containing the two different drugs determined in triplicate using the laser particle sizer.

#### Drug Loading

To determine the amount of drug in the microparticles, an amount containing 100mg of each drug was weighed into two separate porcelain mortar and crushed completely using the pestle. 5 ml of PBS was added to each mortar. The suspension made in each mortar was then transferred quantitatively into a dialysis membrane with a molecular weight cut-off 13kD-15kD and secured at each end. Triplicates of dialysis membranes containing the different suspensions were made and placed into dissolution baskets in vessels containing 500ml of PBS and allowed to rotate for two hours. The absorbance and intensity of amodiaquine and quinine sulphate were measured using the JENWAY 6305 UV/Vis Spectrophotometer at 340nm and CytoFluor well plate reader respectively (31). Using the measurements obtained the drug contents of the two drugs were then calculated from standard plot obtained from a twofold dilution of the original drugs used in the preparation of the microspheres. These measurements were done in triplicates and the average value used in the calculation.

#### Encapsulation Efficiency

The encapsulation efficiency was measured to determine the total drug content of the microparticles. From the drug content determined previously, the encapsulation efficiency was determined by the percentage of the ratio of actual drug concentration to the expected drug concentration,<sup>[32]</sup> Mathematically,

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Actual drug concentration}}{\text{Expected drug concentration}} \times 100$$

**In vitro release studies**

300mg of each drug microparticles were weighed and placed into separate dialysis bags of Mw 13-15D. 5 ml of PBS and trypsin was added to each bag and secured at both ends. After securing both ends of the membranes, they were placed in separate dissolution baskets. Two dissolution vessels into which the baskets would be lowered were filled with 500ml of PBS pH 7.4 each and allowed to rotate at 100rpm at 37°C. At specific time intervals, a 15ml sampling of the contents in the dissolution flask was made at the sampling point with replacement with PBS. The content of the release was done in triplicates for both drugs with the appropriate instruments.

**RESULTS AND DISCUSSION**

**CHARACTERIZATION OF DRUG MICROSPHERES**

*Particle size analysis of drugs*

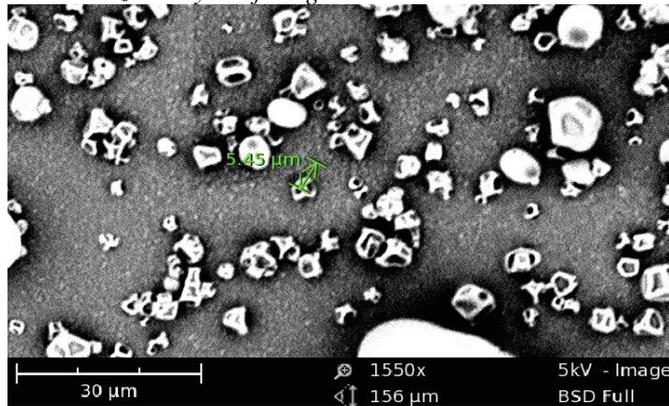


Fig 4. Scanning electron microscope of quinine sulphate formulated microparticles.

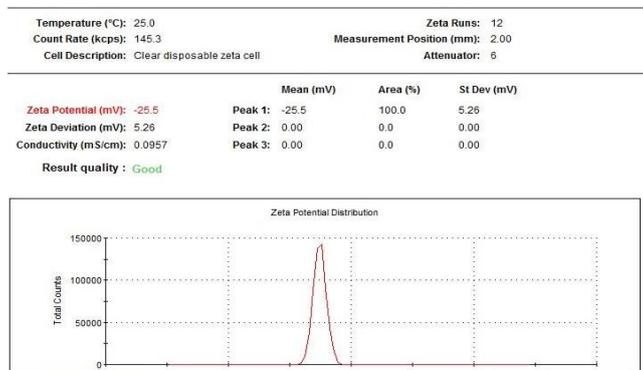


Fig 5. Zeta Particle Analyzer showing the zeta potential and zeta potential distribution of quinine sulphate.

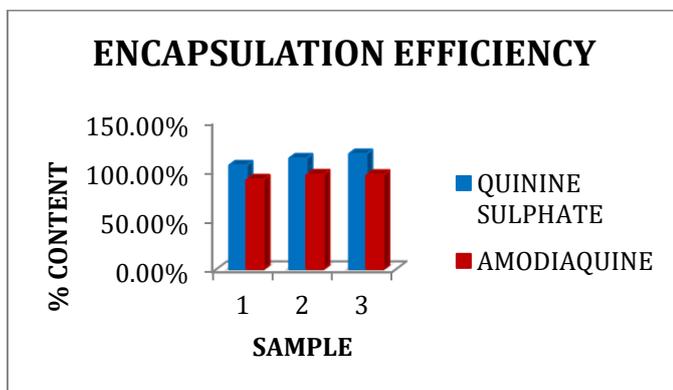


Fig. 6. Bar Graph Showing Encapsulation Efficiency of Amodiaquine and Quinine Sulphate microparticles.

**Amodiaquine Release from Microparticles**

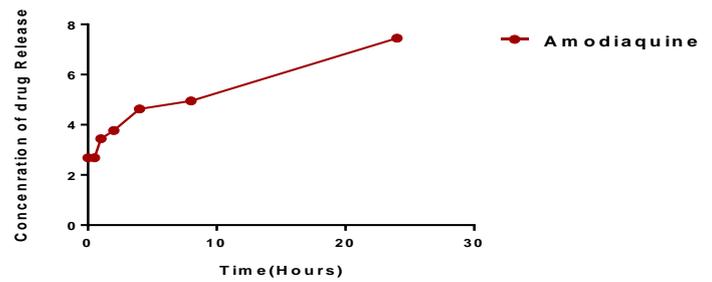


Fig. 7. Graph showing the concentration of release of amodiaquine released from amodiaquine microspheres over a 24-hour period

**Cumulative % Release of Amodiaquine**

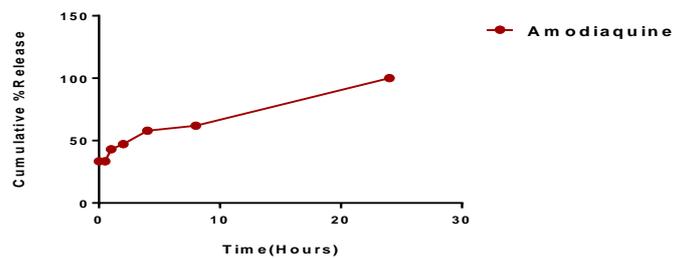


Fig. 8. Graph showing the cumulative release pattern of amodiaquine from amodiaquine microspheres over a 24-hour period

**Quinine Release from microparticles**

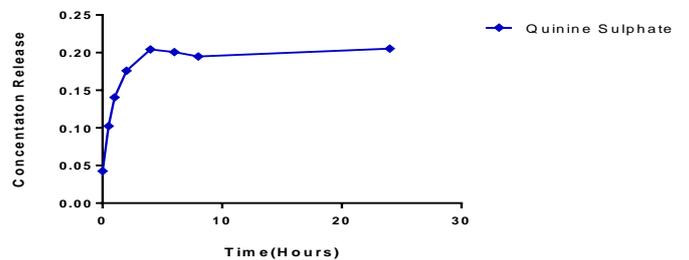


Fig 9. Graph showing the concentration release pattern of quinine sulphate from quinine sulphate microspheres over a 24-hour period

**Cumulative % Release of Quinine**

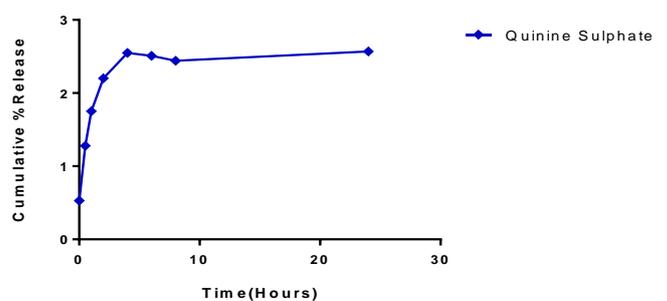


Fig. 10. Graph showing the cumulative release pattern of quinine sulphate from quinine sulphate microspheres over a 24-hour period

Cumulative % Release of Amodiaquine and Quinine

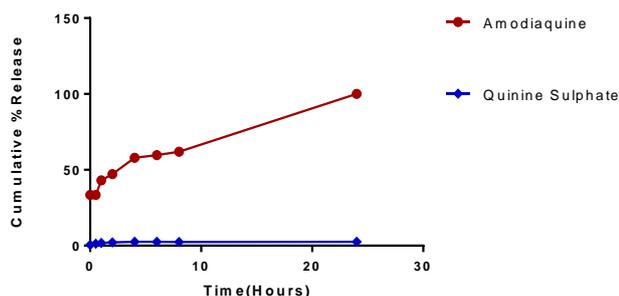


Fig. 11. Graph Showing the cumulative release of both amodiaquine and quinine sulphate over a 24-hour period

## Discussion

Particle size is a key parameter that determines the release of the drug. Mathematically it is inversely proportional to the rate of release of the drug thus a decrease in particle size would produce an increase in the release rate of the drug. As size decreases, the surface-to-volume ratio of the particle increases therefore for a given rate of drug diffusion through the microsphere, the rate of efflux from the microparticle, per mass of the formulation will increase with decreasing particle size [33]. Additionally, water penetration into smaller particles will be quicker due to shorter distance from the surface to the centre of the particle. The particles were between 5-10 $\mu$ m (as illustrated in Fig. 4.) in size, which will adequately facilitate phagocytosis by phagocytic cells. Also, the magnitude of zeta potential from zero will further facilitate phagocytosis. The zeta potential data is shown in Fig. 5.

In order to determine the amount of drug loaded and encapsulated in the microparticles formed, the drug content and encapsulation efficiency were determined. The actual drug content for both amodiaquine and quinine sulphate were 6.13 and 1.11 $\mu$ g with a theoretical content of 6.5 and 1.0 $\mu$ g yielding an encapsulation efficiency of 94.5 and 111% respectively as shown in Fig. 6.

In the *in vitro* release studies, phosphate buffer solution (PBS) was used as the physiological solution and serum added to the contents of the dialysis bag to simulate *in vivo* conditions [34]. Enzymes present in serum degraded the polymer matrix to release the drug. Serum was used to increase the rate of the amount of drug released from the Bovine Serum Albumin (BSA), the polymer matrix. It destroys the covalent bond formed during the cross-linking step between the carboxyl group of the glutaraldehyde and the amino group of the albumin [35]. The release of the drug from the polymer matrix occurs by two main mechanisms: first, an initial burst of the drug owing to some drug particles of the microparticles on the surface of the microparticles left after encapsulation and a slower continuous release phase due to the diffusion of drug through the dialysis bag.

The release of amodiaquine was biphasic with an initial release of about 20-50% of the drug in two hours and a continuous steady release of the drug over 24 hours. At the end of the 24 hours, about 100% of the drug had been released. This is shown in Fig. 7. and 8. On the other hand, quinine sulphate

microparticles demonstrated more of a monophasic release pattern where it took about 4 hours for about 2.6% of the drug to be released. This is illustrated in Fig. 9 and 10. There was a continuous steady release over the 24-hour period, but the release did not increase significantly over the 24-hour period.

Both drug microspheres were however formed by solution crosslinking where the covalent bond was formed in solution overnight before being subjected to spray drying. A reason for the poor release of quinine sulphate from the polymer matrix could be the possibility of the polymer matrix-bound tightly to the quinine sulphate probably due to the amount of glutaraldehyde added to stabilize the bond. Also, there is a possible reaction between the BSA and the quinine sulphate, which would require an alternate choice of polymer matrix, a more compatible polymer matrix. Spray drying is a simple method used to dry fine pharmaceutical chemicals, food, dairy products, blood plasma, numerous organic and inorganic chemicals, rubber latex, ceramic powders, detergents and other products [36]. BSA was used as the polymer matrix because of its non-antigenic property, biodegradability, and biocompatibility and most importantly its ability to control the physicochemical properties of microspheres produced [37]. The spray dryer has the advantage of effectively controlling the product properties and quality and permits high-tonnage production in a continuous or large-scale production. Glutaraldehyde, a cross-linking agent, was employed to stabilize the microspheres formed [38]. In the surface cross-linking method of microsphere formation, butanol was used to absorb excess surface moisture before it was subjected to drying. Amodiaquine and Quinine sulphate was used for the formulation because they have activity against protozoans and since leishmaniasis is a protozoan disease, these formulations are most likely to be effective in the treatment of leishmaniasis [39].

## CONCLUSION AND FUTURE PERSPECTIVES

The spray drying method was used to formulate microparticles of good quality and high encapsulation efficiency of 94.31% and 111% for amodiaquine and quinine sulphate respectively. Amodiaquine microparticles exhibited a biphasic release pattern whilst quinine sulphate microparticles showed a monophasic release pattern with about 100% and 2.6% drug release respectively over the 24-hour period. Further formulation studies should be conducted by altering formulation parameters to improve the release of drug from quinine sulphate microspheres.

There should be screening of other anti-infectives and those found to be good candidates for the treatment of leishmaniasis should be formulated into microparticles. The antimicrobial activity of the microparticles should be analyzed *in vitro* on tissue culture.

## CONFLICT OF INTEREST:

Conflict of Interest: The authors declare no conflict of interest.

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## ABBREVIATIONS

- Th-1- T-helper 1
- Th-2- T-helper 2
- IL- Interleukin
- TGF- $\beta$ - Transforming growth factor  $\beta$

HIV- Human Immunodeficiency Virus  
HAS- Human Serum Albumin  
BSA- Bovine Serum Albumin  
PBS- Phosphate Buffer Solution  
QS- Quinine Sulphate  
Glut- Glutaraldehyde

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