Synthesis and Therapeutic Performance of Mesoporous Silica Nanocarrier for Antimalarial Drug Delivery System

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Abstract- ***Malaria is endemic and a life-threatening disease been caused by the bite of a female anopheles mosquito which destroys the red blood cells. Nano carrier drug delivery system is of great interest in malaria research for improving the quality of health care delivery. This research work focused on development of inorganic silica nanoparticles as efficient delivery system for antimalarial drugs of Arthemeter (ATM) and Lumefantrine (LFT). The mesoporous silica nanoparticles (MSNPs) both amino modified mobile crystalline matter (aMCM-41) and mobile crystalline matter (MCM-41) were synthesized by co-condensation and sol-gel methods respectively. ATM and LFT antimalarial drugs were loaded in both MCM-41 and aMCM-41 with chloroform as the solvent under varying effects of time (1hr, 3hrs and 6hrs), pH (Neutral and Acidic) and temperature (25ºC and 40ºC) respectively. The synthesized nano carrier (MCM-41 and aMCM-41) and nanodrugs fit well for their expected properties as depicted from Fourier-Transform infrared spectroscopy (FT-IR), Nitrogen Physiosorption Isotherm, UV-Visible Spectroscopy, in vitro kinetic study and in vivo measurement using P. berghei NK65. The drug loading capacities (DLC) and Entrapment Efficiency (EE) of the nano carriers were determined using UV-Visible spectrophotometry. The FT-IR depicts major functional groups of the silanol group (Si-OH) and silaxone (Si-O) which absorbed at 3450 cm^{-1} and 964 cm^{-1} respectively for MCM-41, while after amino functionalization the silanol group was obstructed. The nanodrugs show only the functional groups of MSNPs due to the drugs encapsulation. The synthesized MSNPs (MCM-41 and aMCM-41) have average pore diameter of 5.1617 nm and 2.9778 nm respectively as expected for the mesoporous materials which decreases due to adsorption of ATM encapsulated in MCM-41 and ATM encapsulated in aMCM-41 to 4.395 nm and 2.5551 nm accordingly. ATM encapsulates in MCM-41 and aMCM-41: MCM-41Ↄ ATM and aMCM-41Ↄ ATM have the highest DLC of 79% and 81% and EE of 65% and 67% respectively when compared with LFT with DLC of 77% and 75% and EE of 50% and 54% respectively attributed to the size effect. The in-vitro kinetic studies of the drugs and their nanodrugs showed that MSNPs loaded ATM has the highest percentage of drugs released compared with LFT. The in-vivo measurement of the combination of ATM and LFT loaded MCM-41 and aMCM-41 shows better bio performance for plasmodia clearance in infected mice on the third day compared to other nanodrugs and the parent drugs.Therefore; this shows the satisfactory of the synthesized nano carrier for the delivery of the antimalarial drugs.***

Indexed Terms- ***Antimalarial Drugs (Arthemeter and Lumefantrine), Delivery System, Mesoporous Silica Nanoparticles (MSNPs), Co-condensation and Sol-gel, Encapsulate.***

I. INTRODUCTION

Malaria is endemic and a life-threatening disease been caused by the bite of a female anopheles mosquito which destroys the red blood cells. Many phases has been on ground to fight the problem of resistant to antimalarial drugs but the idea of nano medicine is gaining attention due to its very high impact because the size of drugs can be reduced for better improvement. Also, due to the problem of insolubility, toxicity and instability we need to use nano medicine. Drug delivery systems like Mesoporous silica nanoparticles (MSNPs) control the rate at which a drug is released and the location in the body where it is released. It can be described as a formulation that controls the rate and period of drug delivery (i.e. time-release dosage) and targets specific areas of the body which also helps to overcome the problem of drug insolubility, toxicity and instability. The MSNPs are introduced as chemically and thermally stable nanomaterials with well-defined and controllable morphology and porosity. These particles possess external and internal surfaces that can be selectively functionalized with multiple organic and inorganic groups. Therefore, Mesoporous silica nanoparticles with different surface chemistry were used as drugs (Artemether and Lumefantrine) delivery system to study its influence on drug delivery and antimalarial activity of arthemeter and lumefantrine.

II. MATERIALS AND METHODS

- MATERIALS USED

Artemether active agent, Lumefantrine active agent, Chloroform, Distilled water, Hydrochloric acid, SLS (Sodium lauryl sulphate), Tetraethyl orthosilicate (TEOS), Cetyltrimethylammonium bromide, Sodium hydroxide, 3-aminopropyl-triethoxysilane (APTES), Methanol.

- METHODS

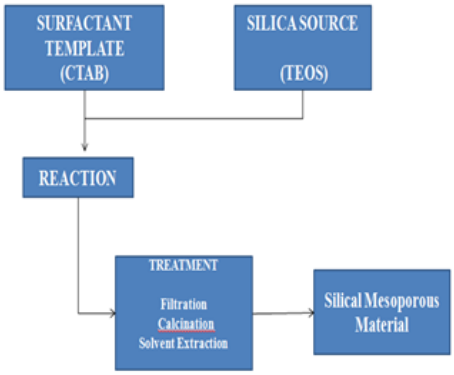

Fig. 1: Basic Reaction Scheme for the Synthesis of Mesoporous Silica Nanoparticles(Beck et al (1992), JACS)

- Synthesis of MCM-41 with Variation in the Ratio of CTAB

480 g of distilled water was accurately measured into a round bottom flask, then 1 or 1.355 or 1.642g of CTAB was weighed and added to the 480g of distilled water as the case may be, 7ml of 2M NaOH was added under stirring for the system to be in alkaline medium. The temperature was set to 80^{o}C, a clear solution was observed at 50^{o}C, at this temperature 6.7mL Tetraethylorthosilicate (TEOS) was added to the solution in drop using a pipette. Once the temperature reached 80^{o}C the mixture was stirred for 2 hours, at this point a milky solution was observed. It was centrifuge and filtered, the residue was washed to neutralize the pH. The washed residue was left to dry over night and placed in a crucible for calcinations, that is, it was heated at high temperature of 550^{o}C for 5 hours. Note that ratio of CTAB was varied to vary the pore size, pore volume and the size of synthesized mesoporous silica nanoparticles.

- Synthesis of Amino Modified MCM-41 with Varied Amount of CTAB

480g of distilled water was accurately measured into a round bottom flask, then 1 or 1.355 or 1.642g of CTAB was weighed and added to the 480g of distilled water as the case may be, 7ml of 2M NaOH was added under stirring for the system to be in alkaline medium. The temperature was set to 80^{o}C, a clear solution was observed at 50^{o}C, at this temperature 6.7ml Tetraethylorthosilicate (TEOS) was added to the solution in drop using a pipette followed by the addition of 0.54ml, 3-aminopropyl-triethoxysilane

(APTES). Once the temperature reached 80ºC the mixture was stirred for 2 hours, at this point a milky solution was observed. It was centrifuge and filtered, the residue was washed to neutralize the pH. The washed residue was left to dry over night. Note that ratio of CTAB was varied to vary the pore size, pore volume and the size of synthesized mesoporous silica nanoparticles. Also, Note: it was not calcined because APTES cannot withstand the temperature of 550°C. Solvent extraction process was used.

- Encapsulation of Antimaria Drugs (Artemether / Lumefantrine) into MCM-41 and Amino Modified MCM-41

40 mg of artemether/Lumefantrine was weighed and dissolved in 10 mL chloroform; the solution was added to a 10mg of the carrier (MCM-41 or Amino Modified MCM-41), the system was covered with cotton wool then shake under different conditions of time (1hr, 3hrs and 6hrs), pH (Basic and Acidic Medium) and temperature (25ºC and 40ºC), this was done to determine the best loading condition for the drug. After shaking, the solution was filtered and the residue was slightly washed. The filtrate is called the supernatant while the residue is called the composite; the absorbance of the supernatant was checked on the uv-specrophotometer to determine the concentration of unloaded and loaded drugs drugs in MCM-41 and Amino Modified MCM-41.

- in-vitro Kinetic Release Study

Antimalarial loaded silica (composite) equivalent of 2 mg of the antimalarial drug were singly weighed and suspended in 5mL of 0.5% Sodium lauryl sulphate (SLS) Buffer. This suspension was then place in dialysis bag with 10KDa molecular weight cut-off and was immersed into 50mL of 0.5% SLS at 37 ^{O}C with continuous stirring at different pH of acidic and neutral medium. At predetermined time intervals of 30mins, 5ml of the samples were withdrawn and immediately replaced with an equal volume of dissolution medium to keep the volume constant. Also, pure antimalarial drugs (Artemether and Lumefantrine) were studied along with silica drug composite to compare the in vitro drug release profile by weighing 2mg of pure antimalarial drugs Artemether and lumefantrine and suspended it in 0.5% SLS similar to that of MCM-41-Art/ Lum. These samples were then properly diluted and analyzed for antimalarial content artemether and lumefantrine at 254nm and 240nm respectively using UV-visible spectrophotometer. The pH effect on the dissolution was studied.

- Parasite Inoculation

Blood was taken from a donor mouse, previously infected with *Plasmodium berghei* (NK-65) and diluted with isotonic saline. Percentage parasitemia and red blood cell count of the donor mouse was determined using a haemocytometer. To count the red blood cells, blood was suitably diluted with isotonic diluting fluid to prevent lysis of red blood cells and is counted in a Neubauer counting chamber. The dilution factor that was used is 1:200.

4.0ml of the diluting fluid i.e. 3% formol titrate was dispensed into a test tube. A 20 µl pipette was used to draw up blood. This was then introduced into the diluting fluid. The counting chamber was cleaned to settle and a Pasteur pipette was used to draw some of the diluted blood from the tube to fill the counting chamber. The red cells were allowed to settle in the counting chamber for five minutes. The red cells were counted in five groups of 16 small squares in the central ruled areas of the chamber to make a total of 80 of the small squares. An objective lens will be used to count the cells at x40.

- Determination of percentage parasitaemia

To determine the parasitaemia, a drop of blood was collected from the tail of the mice and a glass spreader was used to spread the blood to about 5cm. It was allowed to dry, fixed in methanol and stained with Giemsa stain. The parasitized red blood cells are then counted using the x100 objective (oil immersion).

III. RESULTS AND DISCUSSION

Table 1: Drug Carrier Synthesised with Varied ratio of CTAB (MCM-41 and Amino Modified MCM-41)

S/N	CARRIER	CTAB (g)	TEOS (ml)	APTES (ml)	ACRONYMS	SURFACTANT REMOVED BY
1	A	1.355	6.7		MCM-41	Calcination
2	B	1.355	6.7	0.54	AMCM-41	Solvent Extraction
3	C	1	6.7		MCM-41	Calcination
4	D	1	6.7	0.54	AMCM-41	Solvent Extraction
5	E	1.642	6.7		MCM-41	Calcination
6	F	1.642	6.7	0.54	AMCM-41	Solvent Extraction

- Loading of Drugs (Artemether and Lumefantrine)

In the course of the loading experiment it was discovered that carrier C and D have the best drug loading capacity, which indicates that the smaller the surfactant template (CTAB) the higher the pore volume. Thus, increase in the drug adsorptivity of the carrier. The loading rates of 3hrs have the best drug adsorptivity. That is, the carrier (MCM-41 and aMCM-41) was gradually adsorbing the drugs with time until it was fully absorbed/encapsulated in 3hrs. It was discovered that the systems with pH 3.5 (acidic medium) for both artemether and lumefantrine have the best loading capacity than the systems with pH 7.0 (Neutral Medium).

- FOURIER TRANSFORM INFRARED (FT-IR)

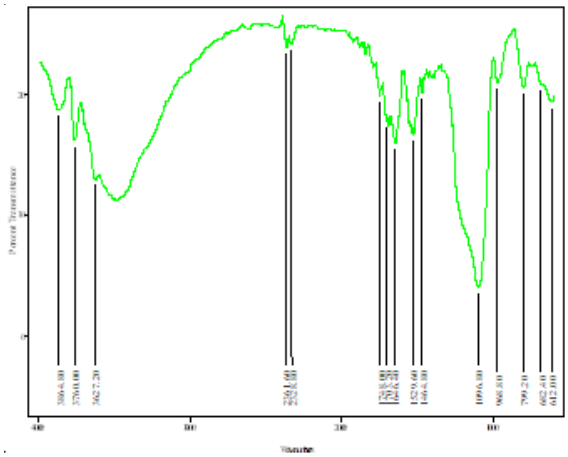

Fig. 2: FT-IR MCM-41

FT-IR characterization of MCM-41 shows the broad band at 3450cm^{-1} may be attributed to surface silanols and the adsorbed water molecule (Si - OH) group. The 1646.40cm^{-1} is attributed to SiO-H bending caused by deformation vibrations of the adsorbed water molecules. The strong 1096.80cm^{-1} band is assigned to internal and external asymmetric Si-O stretching vibrations. The 964cm^{-1} is attributed to Si-O (Silaxone) symmetrical stretching. Also, the 470cm^{-1} is attributed to silaxone (Si-O) bending vibration.

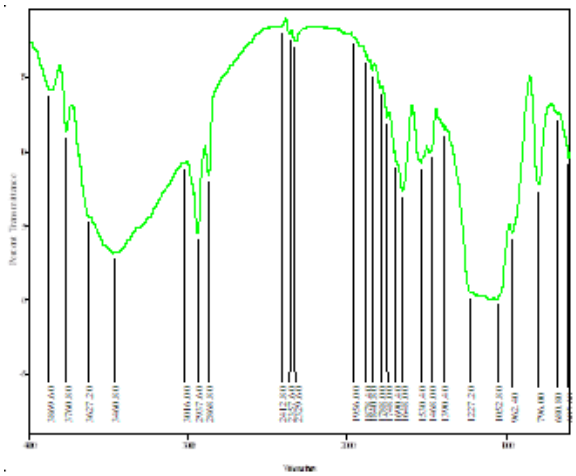

Fig. 3: FT-IR for aMCM-41

Shows the FT-IR characterization of aMCM-41, after amino-functionalization, the band at 3627.20 cm^{-1} for free Silanol (Si–OH) groups was evacuated. The presence of bands at 680.80, 1468 and 2937 cm^{-1} is assigned to N–H bending vibration, N–H asymmetric bending vibration and C–H link, respectively.

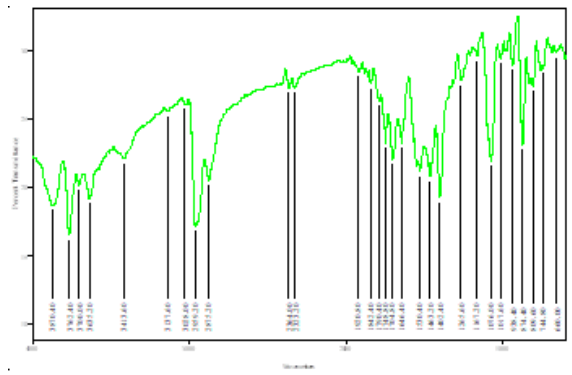

Fig. 4: *FT-IR of Lumefantrine*

shows the FTIR spectra of pure lumefantrine indicated the presence of characteristic peaks of O-H stretching 3413.60, C-H stretching 2950.20 and C-O-O-C bending vibrations 1167.20cm^{-1}, C=O stretching at

1646.40cm^{-1} and C-H bending at 1402.5cm-1, C-Cl stretching 874.40cm^{-1}

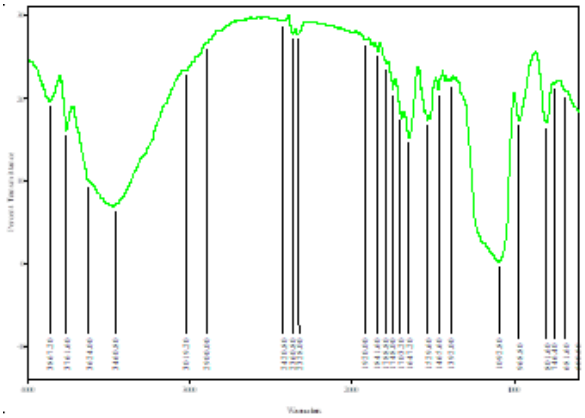

Fig. 5: FT-IR of Lumefantrine Loaded in aMCM-41

Shows the FT-IR of lumefantrine loaded in aMCM-41, it shows the major functional groups of aMCM-41 which is an indication that the lumefantrine had been well encapsulated by aMCM-41

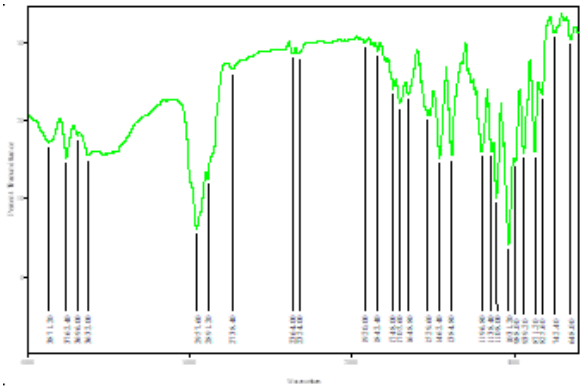

Fig. 6: FT-IR for Artemether (Antimalaria Drug)

The characteristic FT-IR peaks of the pure artemether occurred at C-H stretching at 2957.60 cm^{-1}, C-H bending at 1462.40 cm^{-1}, C-O bending at 1031.20 cm^{-1}, C-O-O-C bending vibration at 1196.80 cm^{-1}, O-O-C stretching at 871.20 cm^{-1} and O-O stretching at 742.40 cm^{-1} respectively.

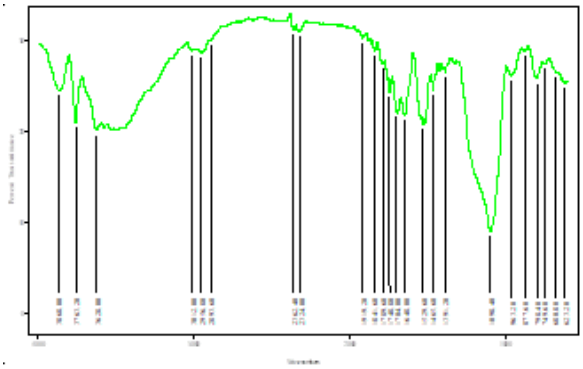

Fig. 7: FT-IR of Artemether Loaded MCM-41

shows the FT-IR of arthemeter loaded in MCM-41, it shows the major functional groups of MCM-41 which is an indication that the arthemeter had been well encapsulated by MCM-41.

NITROGEN PHYSIOSORPTION ISOTHERM

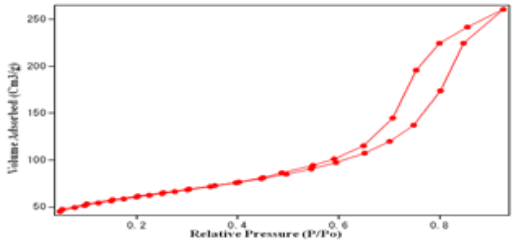

Fig. 8: Nitrogen Physiosorption Isotherm of MCM-41

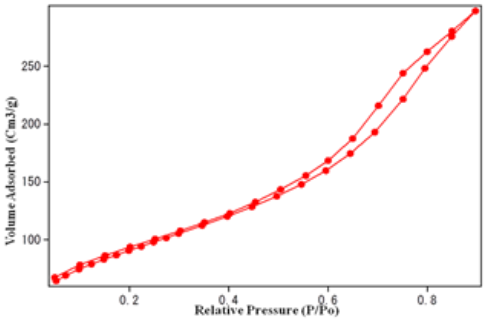

FIG. 9: Nitrogen Physiosorption Isotherm Of Artemether Encapsulated InMCM-41

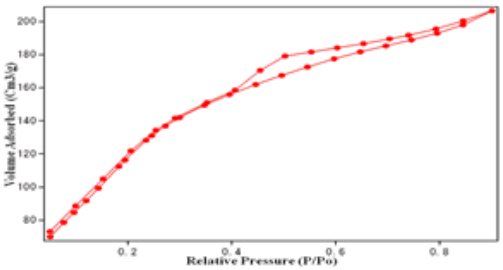

Fig. 10: Nitrogen Physiosorption Isotherm of aMCM-41

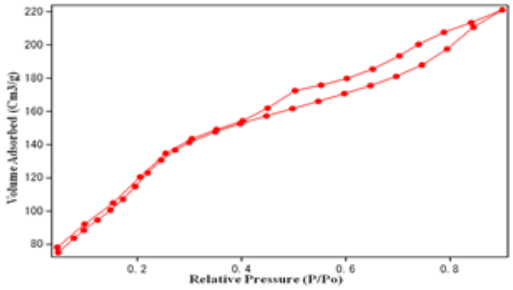

Fig. 11: Nitrogen Physiosorption Isotherm of Artemether Encapsulated In aMCM-41

Figure 8 Nitrogen Physiosorption Isotherm of MCM-41 showing the BET surface areas 131.6077 m^2/g and pore volume of 3.1617 nm which is in accordance with a well synthesized MCM-41. Figure 9 shows the graph of the result for Nitrogen Physiosorption Isotherm of artemether encapsulated in MCM-41 showing the BET surface areas 201.2754 m^2/g and pore volume of 4.3953 nm which shows an increase in the surface area and decrease in pore volume which is an indication that the drug had been encapsulated in MCM-41. Figure 10 show the graph of the result for Nitrogen Physiosorption Isotherm of aMCM-41 showing the BET surface area 159.2630 m^2/g and pore volume 2.9778 nm 2.5551 nm which is in accordance with a well synthesized aMCM-41. Figure 11 shows the graph of the result for Nitrogen Physiosorption Isotherm of artemether encapsulated in aMCM-41 showing the BET surface areas 184.8063 m^2/g and pore volume 2.9778 nm which shows an increase in the surface area and decrease in pore volume which is an indication that the drug had been encapsulated in aMCM-41.

KINETIC RELEASE OF THE ENCAPSULATED DRUGS AND THE FREE DRUGS (IN VITRO STUDY)

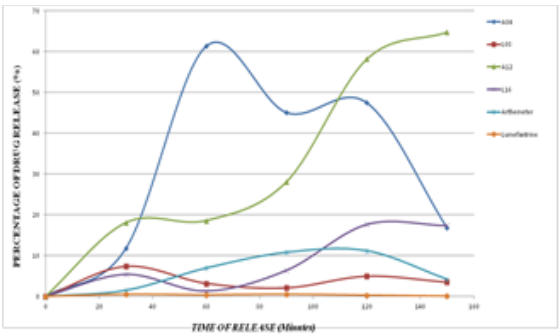

Fig. 12: The Kinetic Release of the Encapsulated Drugs and the Free Drugs (In vitro Study)

The invivo release studies of the artemether and lumefantrine loaded in MCM-41 and aMCM-41 with the free drugs (Artemether and Lumefantrine) from figure 12. It was discovered that artemether and lumefantrine loaded in MCM-41 have the highest percentage of drug release with time followed by the artemether and lumefantrine loaded in MCM-41 which makes it much better than the parent drugs (Artemether and Lumefantrine).

INVIVO STUDIES

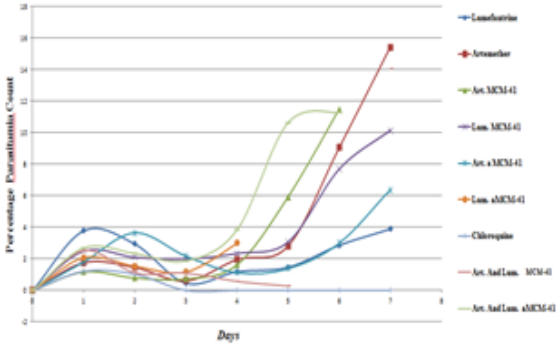

Fig. 12: Chemo suppression analysis using mice

This show the results of chemo suppression analysis whereby Chloroquine was used as positive control which suppresses the antimalarial fully on the third day while water was used as negative control. In the cause of the studies it was discovered that the combination of artemether and lumefantrine in MCM-41 and aMCM-41 compared to the parent drugs (artemether and lumefantrine) have the best suppression on the antimalarial followed by the artemether encapsulated and lumefantrine encapsulated in MCM-41 and aMCM-41.

CONCLUSION

A well ordered MCM-41 and aMCM-41 were synthesised with three ratio of CTAB (1.355g, 1g, and 1.642g) to 6.7ml of TEOS. It was discovered that MCM-41 and aMCM-41 synthesised with CTAB of 1g to 6.7ml TEOS have the best drug loading capacity and were acronyms as C and D. The antimalaria drugs artemether and lumefantrine were loaded in the carriers (MCM-41 and aMCM-41) under the condition of time (1hr, 3hrs and 6hrs), pH (Acidic, Basic and Neutral Medium) and temperature (25 ^{o}C and 40 ^{o}C). It was observed that the drugs were best loaded in the carrier in 3hrs, Acidic medium and at the temperature of 25 ^{o}C.

The synthesised MCM_41 and their nano drugs fit well for their properties as depicted from the FT-IR, N_2 Physiosorption isotherm and invitro kinetic studies. The MCM-41 loaded lumefantrine and artemether gives better therapeutic performance over other nano drugs and their parent drugs (atemether and lumefantrine). These lend credence to the use of nano carrier for high potency effectiveness of antimalarial drugs delivery.

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