SUMMARY
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In the treatment of malaria, combinations of drugs, acting synergistically, are increasingly prescribed in view of the frequency of resistance to single agents. WHO recommends that artemisinin based combinations therapies should be preferred for malaria caused by *Plasmodium falciparum*. Now with the increased use of new combinations, monitoring of potential drug interactions is of great significance to both treatment and research. Therefore, the present work reports the compatibility of various antimalarials (artemether, arteether, artesunate, pyrimethamine and sulphadoxine) in their binary and ternary combinations. These studies are performed both in solid state and in solution phase. Samples with different compositions were subjected to different temperature and humidity conditions and any type of interaction among these drugs was assessed by DSC, FT-IR, XRPD and microreaction calorimeter. DSC and FTIR studies could not detect any interaction or chemical changes in the treated samples. Only small deviations were observed in XRPD pattern in Am:Sulpha (8:1), As:Sulpha (8:1) and As:Sulpha:Pyri (1:1:1) mixtures indicating some incompatibility between these drugs at this composition. Interactions in binary and ternary mixtures of these drugs in solution phase were probed measuring their enthalpy of solution. Excess enthalpies of solution ($\Delta H^E$) were calculated by comparing the enthalpies of solution of pure drugs with those of binary and ternary mixtures under the same experimental conditions. Magnitude of $\Delta H^E$ was further used to calculate the enthalpic interaction coefficient which is correlated with the compatibility/incompatibility. The compatibility results showed that although all the binary systems deviate from ideality but the magnitude of excess enthalpy of solution is not very high especially for the binary mixture of artemisinin derivatives with pyrimethamine and sulphadoxine. The small deviations in excess enthalpies for all the binary systems have been attributed to various non-bonding interactions between different functional groups on both the drug molecules. The results suggest that both the drugs can be co-formulated together in a combination therapy. However, ternary mixtures show somewhat larger interactions suggesting synergetic effects. Magnitude of interaction enthalpy of ternary mixture comprising artesunate, sulphadoxine and pyrimethamine has been calculated to be significant suggesting that the three drugs should not be co-formulated.

Antimalarial drugs not only show resistance but also exhibit poor water solubility and
bioavailability. Therefore, the present study utilizes two of various approaches (alternate crystal forms and complexation with cyclodextrins) developed by biopharmaceutical scientist to improve the physicochemical parameters of these drugs. Different crystal forms of artemether, arteether and artemesuate were prepared and characterized by employing various analytical techniques. Scanning electron microscopy (SEM) revealed differences in the surface morphology of all the forms as compared to each other.

Five different forms of artemether and six alternate forms of arteether were obtained by recrystallizing their commercial samples from various solvents and were found to be morphologically different as suggested by SEM. DSC scans of these forms did not show any desolvation endotherm indicating the absence of solvatomorphism. X-ray powder diffraction (XRPD) pattern of all the forms showed great reduction in intensity of major peaks indicating these to be less crystalline as to the commercial sample. The forms were further differentiated by determining the enthalpy of solution in the mixture of phosphate buffer (pH 7) and acetonitrile (3:1). All the forms behaved exothermically but the magnitude varied from one form to another form. One of the forms obtained by lyophilisation (Form V) in artemether and recrystallizing from mixture of DMSO:H₂O (70:30) (Form V) in arteether were found to be maximum exothermic exhibiting maximum ease of molecular release from the lattice. These forms were most soluble and exhibited highest antimalarial activity with significantly higher survival rate (83.3%) after 30 days as compared to the commercial sample which showed (50.0%) survival rate.

Similarly, five alternate crystal forms of artemesuate were prepared by recrystallizing it from various solvents. Form III and V were found to be solvate as indicated by broad endotherm at 83.04 °C and 76.96 °C prior to melting and accompanied by weight loss in TGA. Weight loss calculations have shown that form III is methanolate and form V is acetone solvate. All the five forms were differentiated morphologically on the basis of SEM. Small changes in XRPD pattern of all the forms were observed. However, few additional peaks were appeared in XRPD pattern of methanol and acetone solvate. Enthalpy of solution measurement showed that all the forms exhibited exothermic behavior in phosphate buffer pH 6.8. The least crystalline form (Form III) was found to be most soluble. The form III also showed least mortality rate (16.7 %) and has shown best antimalarial activity.
Although many alternatives forms of these antimalarials were obtained but no impressive improvement in the crystal lattice was achieved. The data obtained during the characterization of all the alternate solid forms did not indicate the existence of new polymorphic form. All the form was found to be morphologically different from each other with small improvement in solubility in few of them. Therefore, inclusion complexes of these poorly soluble antimalarials with CD’s were prepared to improve their physicochemical parameters.

Inclusion of all the antimalarial drugs (artemether, arteether, artesunate, sulphadoxine and pyrimethamine) in the cavity of β-cyclodextrin (β-CD) as well as its methyl and hydroxypropyl derivatives was investigated experimentally and by molecular modeling studies.

Binary complexes of artemether, arteether, artesunate, pyrimethamine and sulphadoxine with β-CD, M-β-CD and HP-β-CD were prepared using physical mixing, kneading and lyophilized method and characterized in both solid state and solution phase. Phase solubility studies proposed a 1:1 stoichiometry of these prepared complexes which is further supported by NMR, mass spectrometry and confirmed by solution calorimetry. Inclusion of drug in CD cavity in solid state was confirmed by DSC, PXRD, FT-IR studies.

In case of artemether, arteether and artesunate, insertion of trioxane ring with endoperoxide group was suggested by NMR studies. While, in pyrimethamine inclusion of chlorobenzene ring over aminopyrmidine ring into the cyclodextrin cavity is found to more favorable. This mode of inclusion is supported by docking studies. However in sulphadoxine, NMR studies revealed down field shift in protons of aniline ring as well as pyrmidine ring suggesting the co-existence of two 1:1 complexes. Stability constants along with all thermodynamic parameters were determined by solution calorimetry utilizing one class binding model for artemether, arteether, artesunate and pyrimethamine inclusion complexes. Whereas, in case of sulphadoxine two class binding model was used to determine the concentration of drug: CD complex. Numerical value of stability constants for all the complexes increases in the order M-β-CD> HP-β-CD>β-CD. This observation is supported by the in vitro dissolution rate which was found to be maximum for M-β-CD lyophilized complexes of all the drugs. Therefore, all the inclusion complexes prepared by lyophilization were subjected to animal studies and significantly less mean percentage
parasitaemia were observed as to commercial samples and M-β-CD lyophilized complexes have shown maximum antimalarial activity.

However, it is mandatory that the pharmaceutical dosage forms should contain as little CDs as possible, because excess CDs can create problems of formulations bulk or parenteral toxicity as well as reduce drug bioavailability. To overcome these difficulties, water-soluble polymers were incorporated in the drug-CD complexes to improve their solubilizing and complexing abilities. Out of various polymers, 0.25% PEG and 0.20% PVP was found to be the best for enhancing the solubilizing efficiency of artesunate and arteether respectively. Only β-CD was used in ternary system because of its low price, easy availability, suitable cavity dimensions and the least toxicity. Increase in the magnitude of stability constant in the presence of PEG and PVP suggested a significant improvement in the complexation efficiency of β-CD with artesunate/arteether. Besides this, enhancement in vitro dissolution rate and in vivo antimalarial activity against P. berghei infection was also observed in both the ternary complexes.

The ternary systems clearly signify superiority over binary complexes in terms of solubility and reduction in the formulation bulk. Thus, encapsulation of artesunate or arteether by cyclodextrins in presence of PEG or PVP is found to be a good alternative to enhance the bioavailability of the drug as well as to enhance its antimalarial activity.

The work was further extended by entrapping complexed drugs in the lecithin/chitosan nanoparticles as pharmaceutical nanotechnology has unlimited opportunities for enhancing permeation and prolongation of drug in the blood circulation. The rational was to implement simultaneously the CD drug complexation power as well as their potential for enhancement of bioavailability and the inherent properties of nanoparticle formulation. Nanoparticles of artesunate, arteether and their binary complexes were prepared by solvent evaporation method. All prepared nanoparticle formulations lie in nano range (150-350 nm) and have spherical shape which was supported by TEM. Out of all the formulations, 100 mg of drug containing formulations have shown maximum entrapment efficiency. Release rate and antimalarial activity was shown by complexed drug with β-CD loaded nanoparticles as to commercial sample and pure drug loaded nanoparticles suggesting the success of the formulations.