Formulation and Development of Surface Functionalized Hybrid Nanoparticles for Alveolar Macrophage Targeting

SUMMARY

SUBMITTED IN FULFILMENT FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

PHARMACEUTICAL SCIENCES

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

Submitted By

Priyanka Maurya

Enrollment no. 1371/16

Supervisor

Prof. (Dr.) Shubhini A. Saraf

DEPARTMENT OF PHARMACEUTICAL SCIENCES
SCHOOL FOR BIOSCIENCES AND BIOTECHNOLOGY
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAIBARELI ROAD, LUCKNOW-226025
U.P., INDIA

2022

Summary

Macrophages produce interleukins to avoid inflammation when subjected to antigens, which affect homeostasis in the lungs. Alveolar Macrophages(AM) mediate inflammatory responses by encouraging the release of reactive oxygen species (ROS) and inflammatory mediators. The granuloma so formed harbours *Mycobacterium tuberculosis*(M.tb) until dysregulation of immune response occurs. Targeting strategies include selecting functionalising biocompatible agents specific to the macrophage populations intended to be treated.

Various functionalising agents such as mannose, tuftsin, labrafil, folic acid, and hyaluronic acid are used to stabilise, and surface decorate the formulations for better targeting efficiency. Mannosylation of the formulation is of utmost importance to deliver drugs to macrophages. Macrophages play an essential role in mediating an extensive range of diseases, making them a prime target for nanoparticle-mediated therapies. The nanoparticles, once internalised by the AM, reach the phagolysosome.

This process minimises the side effects and maximises the therapeutic effect. Nanoparticles are slowly cleared from the lungs and escape mucociliary and phagocytic clearance. Pulmonary administration of nanoparticles reduces the drug dose to about 5% of the initial dose. Nanoparticles increase the solubility of drugs and aid in dose reduction by improving the absorption of drugs. When a NP utilises the multifunctionality given to it by way of different materials of construction, it becomes hybrid. Hybrid nanoparticles were developed to reduce the limitations of lone lipidic or polymeric systems. Lipids provide an endless opportunity as an excipient enabling the formulation of solid lipid nanoparticles. The bio-analytical profile of the two drugs is established on the terms of ICH Q2R1 parameters. It was necessary to establish a complete analytical profile as the drug was to be estimated in different samples at different conditions. A robust method was required to minimise the errors in analysis.

For Levofloxacin, a Box-Behnken design was chosen to develop the process parameters for the estimation of Levofloxacin hemihydrate. The design was selected as it reduces the number of runs of a full factorial without compromising on the effectiveness of the factors. 3 factors at 3 levels with 6 centre points were selected for the study. The factors and levels

selected for optimisation were pH (pH 4-5), column oven temp (30-40°C) and flow rate (0.8-1.2ml/min). 18 experimental runs were performed randomly and HPLC areas, Number of theoretical plates (NTP), tailing factor and retention time were the dependent factors for the study. The analysis was carried out on HPLC (Shimadzu Corporation, Japan) equipped with a PDA detector SPD M20A with a D_2 lamp. Chromatograms were recorded by LC solutions software. Analysis was performed on Waters X Bridge C_{18} column (250X4.6mm, 5 µm). Autosampler SIL 20AC was used for sample injection with a needle stroke of 52mm at a sampling rate of 15µl/sec. Injection volume was set at 25µl and detection wavelength at 295 nm. The oven was maintained at 30°C, and samples were maintained at ambient temperature. The mobile phase selected for the separation of components comprised a mixture of 100mM ammonium acetate buffer and a 1:1 mix of acetonitrile and methanol. Binary gradient mode was utilised with 35% phase B at an overall flow rate of 0.93ml/min for 6 min run time.

The developed RP-HPLC method was quite efficient in estimating LVF content in nanoformulations, plasma samples and marketed conventional formulations with fast RT, resolution and sufficient specificity. The RP-HPLC method was entirely validated as per ICH Q2R1 guidelines. Since the method requires a short run time, it can quickly assess several samples in a short period. The method is robust, selective and simple, with increased sensitivity for LVF estimation. BBD used in the development of the method allowed better identification of significant factors affecting the resolution. Until now, there has been no novel method for the estimation of LVF in nanoformulations as well as in plasma samples for pharmacokinetic studies.

A Box-Behnken design was chosen to develop the process parameters for the estimation of Ethionamide. 3 factors at 3 levels with 5 centre points were selected for the study. The design was selected as it reduces the number of runs of a full factorial without compromising on the effectiveness of the factors. The factors and levels selected for optimisation were pH (pH 3-4), the composition of the organic phase (30-40%) and flow rate (0.8-1.2ml/min). 17 experimental runs were performed randomly and HPLC areas, Number of theoretical plates (NTP), and retention time were the dependent factors for the study. Analysis was performed on a Phenomenex column (150X4.6mm, 5 µm). Autosampler SIL 20AC was used for sample injection with a needle stroke of 52mm at a sampling rate of 15µl/sec. Injection volume was set at 10µl and detection wavelength at 288 nm. The oven was maintained at 30°C, and samples were maintained at ambient temperature. The mobile phase selected for

separation of components was a mixture of 1% Glacial acetic acid with diethylamine (to adjust the pH) and 1:1 mix of acetonitrile and methanol. The developed RP-HPLC method was quite efficient in estimating Ethionamide content in nanoformulations, plasma samples with fast RT, resolution and sufficient specificity. The RP-HPLC method was entirely validated as per ICH Q2R1 guidelines. Since the method requires a short run time, it can easily assess several samples in a short span of time. The method is robust, selective and simple with increased sensitivity for Ethionamide estimation. BBD used in the development of the method allowed better identification of significant factors affecting the resolution. Until now, there has been no novel method for the estimation of Ethionamide in nanoformulations as well as in plasma samples for pharmacokinetic studies.

The current study aimed to create carubinose-functionalised hybrid sytems that could deliver the levofloxacin payload to alveolar macrophages. The effective delivery of Levofloxacin to AM allows the more efficient intracellular killing of *M.tb*, resulting in lower doses and better patient compliance.

A 17 run no block quadratic response surface design was generated with the help of Design Expert software. A 300-400 nm particle size range was ideal for formulating HF and CHF, along with maximum entrapment and zeta potential. The optimisation was carried out using one-way analysis of variance (ANOVA). The inference drawn from the design expert values indicates that for particle size PVA (p value=<0.001) and soy lecithin(p value=0.0108) play a significant role along with the quadratic factors of soy lecithin (p=0.0044) and PLGA (p=0.0034. For zeta potential (reduced linear model), soy lecithin (p=0.0070) was significant and for entrapment efficiency PLGA (p=<0.0001) and soy lecithin (B^2 p=0.0010) are significant (**Error! Reference source not found.**). The design results were tabulated in the form of equations with coded values. As evident from the equations and design graphs the constraints set up for optimisation yielded a set of 100 solutions. The topmost solution was selected as the optimised formulation. It can be concluded from the data obtained that the size of nanoparticles is controlled linearly by PVA and soy lecithin and quadratically by soy lecithin and PLGA. Carubinose receptors have been found to be expressed specifically on AM surfaces with a high affinity for mannose terminal molecules. A study of AM interactions with various sugar-modified derivatives discovered that carubinose derivatives can be rapidly adsorbed by AM.

The particle size of the optimised formulation was 314.4 nm with a zeta potential of -11.29mV and CHF was found to have a particle size of 471.5nm and zeta potential of 1.21mV. The TEM and SEM figures clearly indicate a bilayer spherical structure. NTA analysis was also carried out on the samples. The results of NTA promulgate the fact that the aggregates are lost upon dilution. The intensity distribution profile indicates that HF consists of smaller particles at a mode of 137.2 nm and CHF had a mode of 222.8nm. The higher range obtained in the Nanoplus can be attributed to impurities, aggregates, and solvent halo around the particles. The ease of carrying outsize and zeta potential analysis via DLS makes it a frontrunner in nanoparticle analysis techniques.

The other physical parameters studied for HF and CHF prove that LVF is completely incorporated in HF and CHF as studied via IR spectra of the formulations and raw materials. The broad band of -OH from 3756 cm^{-1} to 2400 cm^{-1} indicates carubinose linkage with soy lecithin. The overlapping signals of NH with OH of carubinose also indicate the same. The Levofloxacin conjugated carbonyl group is slightly shifted to 1623 cm^{-1}, whereas the acid COOH group is shifted to 1756 cm^{-1}, indicating a strong bond formation with HF. HF and CHF do not particularly express the peaks of LVF, indicating complete entrapment of LVF in the formulation, which is also further extended by the DSC and TGA data. HF also indicates a linkage of LVF, which was also encountered during drug loading estimation. During drug loading estimation, HF and CHF required prior hydrolysis of the formulation in alkaline SDS solution, releasing free LVF. PLGA also undergoes slow hydrolysis *in-vivo,* which explains its sustained release character and also explains the release of drug from the PLGA matrix.

The TGA curve showed different weight loss regions during the analysis, which can be explained via reference to the thermal behaviour of LVF, PLGA and soy lecithin. The TGA and DSC curves are characteristic for a given material and elicit the thermal character of the materials in question. LVF followed the characteristic profile as mentioned (Gorman et al., 2012) and other studies with an initial 3.5% water loss and a degradation curve beginning at 244.15°C. It also presented a sharp melting peak at 232.77 °C, characteristics of its crystalline nature. However, CHF and HF had a different and completely new show of character with an initial loss of ΔW=17.56% and ΔW=9.59% that indicates water loss and mass loss at lower temperatures as that of body fluids. This can also be an indicator for the initial burst release of the formulation. The second and third regions of weight loss indicate

a breakdown of polymeric structure and loss of functional groups due to pyrolysis. The DSC data for HF and CHF show small endothermic regions initially indicative of glass transition of PLGA and soy lecithin in their native from and presence of unreacted material. No sharp or broad melting peaks or regions can be derived from the data showing complete drug incorporation. The CHF and HF show significant differences in initial weight loss, indicating that the CHF releases drug at body temperature due to melting assisted with diffusion. A decrease in the energy of the initial endotherms indicates that lesser energy is required to release the drug from the system. The individual components do not lose their characteristic peak, which confirms the compatibility of the system.

The change in physical properties of the drug and characteristic endotherms of PLGA and lecithin shows the system is stable and possesses better *in-vivo* performance. The XRD and thermal data reveal that the crystalline LVF is converted to an amorphous form which can be seen in XRD data. Further, weight losses point towards the degradation behaviour of the formulation at higher temperatures which is also seen in stability studies. The XRD data of LVF, HF and CHF were mapped with available literature.

The loss in intensity indicates the conversion of the crystalline drug to an amorphous state. The amorphous state was also confirmed via literature. This conversion indicates that HF and CHF have LVF present but in the entrapped state. The presence of phospholipid bilayer acts as a boundary for delivery systems and resist intracellular targeting. To overcome this hurdle, various endosomal membrane disrupting carriers have been designed. These carriers are hemolytic in nature and thereby are not biocompatible. The hemocompatibility test is designed to rapidly assess the compatibility of the nanocarriers with the cell membrane.

Drug delivery systems that have a long circulation time come directly in contact with RBCs and are required to have a lesser hemolytic activity to remain biocompatible. The reduced hemolytic property of CHF can be attributed to the shielding of amine groups due to Carubinose linkage. The slightly increased but significantly less hemolytic property of HF compared to LVF can be due to the presence of an amine group of soy lecithin on its surface. The FTIR data confirms the presence of these groups. The *in-vitro* dissolution behaviour of HF and CHF point towards a biphasic release of LVF characterised by a small burst release (30% in 5 hr) followed by slow diffusion. The release kinetics curve fitting indicated the best fit for the Higuchi model as well as the Korsmeyer-Peppas model. The Higuchi release kinetics

is governed by the fact that diffusion controls drug release from the system. The small n value for Korsmeyer Peppas model indicates zero-order diffusion. Similar release characteristics were observed for *in-vivo* pharmacokinetic data, which showed that the release continued for 72 h. The core-coat structural characteristic of HF and CHF contribute to the diffusion release mechanism. The coat of carubinose modified HF and unmodified HF acts as a hindrance to drug release. Since LVF is a hydrophilic drug with good permeation characteristics, it was required to be formulated in a manner to control the release as well as to make it target oriented. Modification with carubinose gives it the targeting ability, and the interplay of PLGA and lecithin controls the release.

This combination of nanoparticle degradation for release of LVF gives HF and CHF its unique release character, i.e., the burst release of surface tagged drug and then diffusion-controlled release. The pharmacokinetic data also confirm the same release pattern with an initial burst release followed by a diffusion release pattern. The release depends on the degradation of PLGA *in-vivo*, which was seen in the HPLC estimation of drug content. The drug content values increased drastically when the formulations were placed in alkaline SDS (sodium dodecyl sulphate) solution. The SDS degrades PLGA, which in turn releases LVF. Therefore, the results did not exhibit the accurate drug load when it was attempted to estimate LVF initially by dissolving in dichloromethane(DCM). Whereas, when alkaline SDS was used the LVF content in HF and CHF increased drastically, implying that PLGA and soy lecithin are individually soluble in DCM but, the hybrid formulation with its new configuration renders itself less liable to dissolution in DCM. At this point, alkaline SDS denatures PLGA and releases the LVF for estimation. The same conditions prevail in the AM. The presence of various enzymes and buffering agents help in the degradation of lecithin and PLGA, thereby releasing LVF at the site of action.

Macrophage targeting was achieved successfully, as indicated by the FACS data as well as quantitative uptake studies. The viability assay carried out via MTT exhibited the safety profile of CHF and HF upto 50ppm. The viability of the cells was above 50% till 50ppm, which reduced to 48 and 44 for CHF and HF, respectively. This ensures that at therapeutic levels CHF and HF retain maximum cell viability. The cell viability results also emphasise the fact that the GRAS excipients used in the formulation do not affect the test results. Proper washing of the formulation to get rid of surfactants facilitated good viability results.

Further, the rapid internalisation of CHF as estimated quantitatively through RP-HPLC studies is due to the presence of carubinose on the surface, which is easily recognised by the macrophages and the size of the formulation, which enables quick phagocytosis of the formulation. HF possessed lesser internalisation than CHF but more than LVF because it is internalised by passive diffusion only. The efficacy of quantitative cellular uptake can be further corroborated by FACS results, both qualitative and quantitative. The fluorescence intensity is a time-dependent feature; the formulations were kept under incubation for 6 hrs before taking the readings. The high fluorescence in CHF is due to the receptor-mediated activity of carubinose as well as the passive uptake of the formulation. The uptake of HF is due to non-specific internalisation by endocytosis/phagocytosis. HF also possessed better uptake than LVF, as is evident from the data and can be correlated to a few previous works which indicate that HF is better internalised and uptaken by the cells than the pure drug alone as the presence of lipid in the coating layer promotes rapid internalisation. The enhanced uptake of CHF compared to HF is due to the presence of surface modifier on CHF and it enjoys a two-way uptake of active and passive methods. Fluorescence microscopy results also add up to the concept of better uptake of surface functionalised formulation

The *in-vivo* pharmacokinetic data revealed the controlled release pattern of the formulation. The pharmacokinetic data indicate a long MRT for HF, and CHF indicates better retention of LVF by the two formulations. The increase in AUC is indicative of the better bioavailability of LVF from HF and CHF. The decrease in Cl rate and increased Vss are indicative of better retention of LVF in target organ/AM. These improved characteristics are due to the HS where the lipid coat provides a sheath to the PLGA.

Thus, the reduced available surface area for entry of fluids was a limiting step in drug release. The presence of carubinose on the surface imparted better entry CHF in the AM. The slightly acidic environment of AM leads to a further reduction in drug release from the formulation, which extended its release profile. The stability data obtained after 6 months of study puts forward that CHF and HF are stable under long term storage conditions of refrigeration. When stored at low temperatures, the formulations retained their size with a slight change of 1.7%. The drug loading was reduced by 2.58%. CHF remained stable, as evident from zeta potential values with a 2.58% reduction. HF placed under long term storage conditions exhibited degradation, but the change does not qualify as significant degradation/ change in terms of drug loading (1.59%), particle size (0.93%) and zeta potential (1.24%). ICH guidelines verify

stability in terms of drug content specifically which remained well within limits (<5%) for the entire duration of 6 months. Both HF and CHF showed insignificant changes within 3 months; therefore, the samples were placed under testing conditions for another 3 months. As the formulations are meant to be administered intravenously, the stability studies were done for lyophilised formulations which are meant to be reconstituted just before administration. The stability readings confirm the data obtained by the thermal characterisation that the formulation remains stable at lower temperatures.

The above results conclude that HS is one of the new emerging delivery systems for targeting drugs to AM. They also show sufficient potential to be explored as an alternative for enhanced targeting to the AM resident bacteria. The viability assay ensured that the formulations are safe for normal cells and are not toxic in their therapeutic range.

Ethionamide (ETH) is a second-line antitubercular drug used in multidrug-resistant cases. Ethionamide suffers from poor solubility and is classified as a Class II drug in biopharmaceutical classification. Poor solubility limits its formulation horizon. Macrophages express various surface receptors in response to the infection they encounter. In cases of active tuberculosis, macrophages overproduce mannose and folate receptors. Alveolar macrophages (AM) present in the lungs also show similar characteristic features of macrophages. AM act as first-line defence systems for all types of inhaled pathogens/microparticles. Folate receptors (FR) present on the macrophage surface help in the rapid internalisation of a drug. These receptors are scarcely employed in tubercular targeting, whereas they remain in prime focus in cancer research. A few recent studies have emphasised the presence of folate receptors and their importance in tubercular infections.

The HNP were prepared by a nanoprecipitation technique, a single-step preparation method. Single-step preparation methods reduce the number of steps in a formulation. The response surface design selected in formulation development was a Central composite design based on the quadratic model. It aimed at developing a stable formulation with desired characters; the process aimed at selecting the best combination of formulation components. It is worth noting that on mixing the polymer phase with the aqueous component, a simultaneous arrangement of particles occurs, leading to the formation of a core-coat structure. The prepared batches were characterised for particle size, zeta potential and entrapment efficiency of the drug. The particle size of the optimised formulation was 281.8nm with a

PDI of 0.163, indicating good distribution. The zeta potential of the formulation was found to be -3.49mV. The FC@HNP had a size of 399.4 and a PDI of 0.260, and a zeta potential of 39.12mV, indicating a highly stable dispersive formulation. The particle size and zeta potential data generated by Nanoplus revealed uniformly distributed particles. The folate content was estimated to estimate the folate attached to the surface of FC@HNP. The drug entrapment calculations exhibited a complete range of entrapment efficiency in the formulation from 2.72-45.98%.The TEM image point towards a formulation with a uniform distribution. HNP revealed a powdery character with dispersed particles, and FC@HNP showed a more coated structure with a smooth appearance. The NTA analysis results also pointed towards a uniformly distributed formulation with a smaller size. The smaller size can be due to high dilutions occurring in the formulation and eventual supernatant withdrawal for evaluation.

ETH, HNP, FC@HNP, PLGA, Folic acid, and chitosan were subjected to FTIR studies. The results pointed out that ETH was entrapped in HNP and FC@HNP without any change or modifications in the groups due to processing. Further HNP were evaluated for physical properties, namely. DSC, TGA and XRD. The thermal data indicated that ETH has a sharp melting point followed by degradation. The HNP and FC@HNP showed that after formulating, the thermal resistance increased. The loss of mass was much lower than ETH in the formulations indicated by a broad peak. The HNP and FC@HNP did not possess a sharp melting point as that of ETH. The absence of sharp peaks suggests a change in the character of the drug in the presence of formulation additives from perfectly arranged highly crystalline powder to an amorphous material. It can also be related to the complete incorporation of the drug in the HNP and FC@HNP. The weight loss for HNP from the entire range was 13.33%, and for FC@HNP, it was 30.89%. Soy lecithin, chitosan, and folic acid stuck to their established values with lesser intensity indicating their presence in the formulation. The XRD data also pointed that the organised crystalline drug ETH got converted to an amorphous form characterised by low-intensity peaks at the same values as that of standard ETH with an insignificant shift on the θ scale.

Hemocompatibility tests were conducted to assess the extent of damage that the formulation exerts on RBCs. The tests are also indicative of compatibility between the cell membrane. Since the formulation is administered by the parenteral route, the formulation must be biocompatible with the blood components. The drug release data pointed towards a first-

order release character with no initial burst release. The release was sustained for 5 days at a constant rate. The release is diffusion based as evident from the data. The drug release is dependent on the fact that the erosion of PLGA will lead to drug release. Soy lecithin acts as a barrier layer for the entry of fluid into the nanosystem. ETH is present in the hydrophobic part of the system, which only gets released when PLGA starts degrading and releases ETH in the surroundings.

In-vitro cell line studies were carried out to check the designated goals. The results were encouraging enough and pointed out that the blank formulation was viable at 44% at its maximum concentration of 100ppm. FC@HNP retained viability above 40 % till 25ppm as compared to pure ETH with similar values. The difference in viability was insignificant when the groups were compared. The viability test was followed by quantitative cellular uptake as estimated by RP-HPLC studies. The cellular uptake was found to be much higher in FC@HNP compared to ETH and HNP. The results showed a significant increase in FC@HNP due to the presence of folic acid-chitosan conjugate. The presence of folate receptors on macrophages leads to an increased ETH content. HNP possessed better uptake than ETH, but not more than FC@HNP. The increased drug content in the case of HNP is due to the presence of lipid on the surface and size of the nanoparticles, which facilitates entry into the cells. Phagocytosis is the main mechanism of uptake of large particles by macrophages. This quantitative uptake data is further validated by the FACS studies as well as fluorescence microscopy. The qualitative fluorescence data indicate better results with the surface functionalised formulation. The uptake mechanism remains the same for all three studies (FACS, Fluorescence and quantitative estimation).

The *in-vivo* data adds up to the above results by proving a better pharmacokinetic profile for FC@HNP than HNP and pure ETH. The MRT of the formulation increased by 12.52 times for HNP and 16.99 times for FC@HNP. This was supplemented by an increase in the half-life of ETH due to entrapment in the nanoformulation. Compared to the pure drug, the bioavailability measured by the area under the curve increased by 9.33 and 9.91 times for HNP and FC@HNP, respectively. The increased and improved pharmacokinetic profiles point towards the efficiency of the system to control the release, thereby improving the statistical significance. Therefore, we can infer that the FC@HNP and HNP effectively sustain the release and improve the uptake characteristics of the nanoformulations.

NPs can be formulated to be stable, non-immunogenic, non-inflammatory systems which avoid reticuloendothelial system (RES) uptake. They can reach the target site, bypassing other sites due to specific domains (surface functionalisation) that encourage targeting. Hybrid nanoparticles are a new class of nanoparticles with the beneficial effects of both lipids and polymers. Hybrid nanoparticles possess good loading efficacy, structural integrity, cell targeting properties, and better cellular affinity. Hybrid NPs are also beneficial in the drug delivery of hydrophilic and hydrophobic drugs. The present work aimed to develop stable hybrid nanoformulation and its estimation techniques. A robust RP-HPLC method was established, which minimised the errors in analysis. The developed RP-HPLC methods efficiently estimated Levofloxacin and Ethionamide content in nanoformulations, plasma samples, cytology samples with fast RT, resolution and sufficient specificity. The RP-HPLC methods were validated as per ICH Q2R1 guidelines. It was found to be a robust, selective and simple method with increased sensitivity. BBD used in the development of the method allowed better identification of significant factors affecting the resolution.

A stable surface-modified lipid-polymer hybrid system (CHF) was prepared by selecting lipid and polymer with the desired characteristics. The safety profile of the prepared system and the hemocompatibility offered by them make hybrid systems a safer drug delivery system. LVF entrapped hybrid systems successfully incorporated and controlled the release characteristics of hydrophilic LVF. The enhanced uptake helped in better targeting and achieving higher local drug concentrations. The spherical morphology of the HS aided in better release characteristics. The improved release characters will help in reducing the dosing frequency and advance patient compliance and better therapeutic outcomes.

The hydrophobic Ethionamide was formulated as HNP and FC@HNP. The prepared formulations were meant to be delivered via the parenteral route. This route preferentially escapes the GI intolerance as well as first-pass degradation. The dense surface functionalisation empowers FC@HNP to reach the target readily, circumventing all other possibilities, and release ETH at the desired site. The prepared hybrid formulation showed a sustained release phenomenon due to the polymeric core-lipid shell structure. The positive charge enables better uptake of FC@HNP as confirmed by the uptake studies. The thermal stability was established by DSC and TGA studies. The efficient uptake of FC@HNP is attributed to the folate present on its surface, which binds to the folate receptors present on the surface of AM, thereby releasing ETH in its microenvironment.

The reported hybrid system(s) are expected to prove themselves as attractive alternatives to polymeric/ lipidic nanoparticulate systems regarding their efficacy. The present work accomplishes the hypothesis envisaged at the beginning of research work and helped in building insights into AM targeted drug delivery via carubinose and folic acid receptors. Further studies can be carried out to evaluate the exact mechanism of drug release and the various biochemical processes involved in drug delivery to achieve accurate biodistribution in a disease state; more research can be done in tubercular models. Research into the role of various hybrid dosage forms can be carried out in order to develop better delivery systems for AMs.