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Formulation and Evaluation of Pyrazinamide loaded Pegylated Polypropylene imine dendrimer for treating Tuberculosis

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ABSTRACT: The purpose of the contemporary research was to construct a Pyrazinamide loaded PEGylated fifth generation (5.0G) Poly (Propylene)-Imine (PPI) dendrimer for the treatment of tuberculosis. A PEGylated poly(propylene-imine) dendrimer was prepared, and the drug pyrazinamide was loaded. Tuberculosis is a deadly contagious disease that affects the respiratory systems of the human. Pyrazinamide (PZA) is used to treat Tuberculosis (TB). The dendrimers exhibit superior performance in the process of targeted drug delivery. It increases the drug loading capacity and minimises haemolytic toxicity. The physical as well as chemical properties of the drugs are analysed through UV-spectrophotometric analysis. The free 5.0G PPI dendrimer, synthesis pyrazinamide loaded PEGylated fifth-generation PPI- dendrimer and PEGylated 5.0G PPI dendrimer are considered subjects of enhancing the drug loading capacity investigation. Several research findings have revealed that PEGylation is suitable for modifying the core Ethylene Diamine (EDA)-PPI dendrimers of ethylene diamine initiator. In this work, the transportation of the drug will be carried out at a controlled rate, thereby increasing therapeutic value and minimising fluctuations in plasma concentration. This feature helps to improve the therapeutic intervention in the patients affected by TB.

Keywords: Pyrazinamide, Ethylene diamine, PPI dendrimers, PEGylation, drug delivery.

INTRODUCTION

Tuberculosis (TB) is the second most infectious disease affecting public health worldwide (*Ong et al.*, 2020). It causes severe complications in the respiratory system of humans. Several kinds of research are conducted to identify the optimal drug for treating TB. Artemisinin and doxycycline are the two dominant drugs used to treat TB, but the treatment time is longer, i.e. up to 24 months (Moniz *et al.*, 2020). Therefore, novel methods are required to minimise the treatment time of TB. Nanotechnology is an emerging field in the 21st century (Fernandes *et al.*, 2021). It has wide applications in the pharmaceutical, healthcare, biologics and chemical industries (Kumar *et al.*, 2023).

The polymeric nanocarriers were advanced to use as the drug carrier. The polymeric nanosystems can be categorised in the nanoparticles poised through nanocapsules or nanospheres, with a diameter ranging from 10 to 1000 nm (Bernal-Chávez *et al.*, 2021) even though most of the polymeric nanoparticles recognised are in the average size of fifty to three hundred and fifty nm. The broad range of drugs can be compressed in the core, overloaded inside a matrix, or covalently associated with the carrier polymeric surface. The polymers utilised for the production of those systems can be of either natural (Selvaraj *et al.*, 2019), like gelatin, alginate, chitosan and albumin, or it can be of synthetic, like polyamides, polyesters, and polyalkyl-cyanocarbonates and poly amino acids (Jain *et al.*,2020).

Dendrimer originates from Greek words such as Meros and Dendron (Gajbhiye *et al.*, 2009). Meros means tree, and Dendron means tree (Chis *et al.*, 2020). The highly regular branching units are systematised in layers termed the generations and exhibit the repetitive monomer unit of those synthetic macromolecules. The dendrimers are non-immunogenic and non-toxic, with excellent drug-loading capability (Rai *et al.*, 2021). Persistent and maintained drug release can be attained with the advancements in controlled release technology (Pandey *et al.*, 2016). There are various approaches for sustained drug release and several reasons contributing to ineffective drug discharge, which has made an effective drug delivery system essential (Firoz *et al.*, 2020). The controlled drug delivery release is painless (Das & Saha 2017). The alteration of the dendrimer's surface can improve physicochemical properties and enhance penetrability and biocompatibility in a cellular atmosphere (Irfan *et al.*, 2020). The properties like excellent branching, greater water solubility, polyvalency, nanoscale size and availability in internal cavities, and immunogenicity deficiency make the dendrimers safe. PPI dendrimers are available until the fifth generation and have various uses in material science

and biology (Pedziwiatr-Werbicka *et al.*, 2019). Several types of research are being conducted to enhance the drug release rate in dendrimers by introducing various chemical modifications upon the dendrimer surface (Wang *et al.*, 2019).

The well-constructed 3D model and variety of functional group ion dendrimers can adopt therapeutic drugs inside and outside the surface of the dendrimer (Tang *et al.*, 2021). The compounds act as drug carrier agents by interacting with the drugs at terminal organic groups through electrostatic interactions. There are two main methods involved in the process of drug delivery. The initial method consists of degrading the drug dendrimer and conjugates in the presence of enzymes and a degrading environment (Singh *et al.*, 2021). The second method requires drug release in response to external environmental variations like pH and temperature. This method does not require external factors. If the action occurs inside the dendrimer, it is called an endo receptor. The movement outside the dendrimer denotes an exoreceptor (Scicluna & Vella-Zarb 2020). A dendrimer is a safe, reliable and selective agent for drug delivery. It is responsible for targeting the affected tissue in the body (Le *et al.*, 2019). The characteristic features of dendrimers are small size, homogeneity, bulkiness and stability. This, in turn, makes it an ideal drug carrier agent for optimal drug delivery.

The present research intends to emphasise a synthesis of PPI dendrimers to distribute anti-tuberculosis drugs. Pyrazinamide was preferred to incorporate in PPI dendrimers due to its solubility, anti-tuberculosis activity, and reduced drug half-life. The rate of drug delivery is optimal, and the drug will be released at the measured.

MATERIALS AND METHODS

Materials: The Reney Nickel (RN), Triethylamine, EthyleneDiamine, N, N dicyclohexyl Cellulose dialysis bag, PEG 4000, were the generous offering from the Shasun pharmaceuticals in Chennai, India.

5.0G PPI Dendrimers: It was ensured the procedure stated by Meijer and De Brabender-Van Den Berg using the initiator core as EDA (EthyleneDiamine) (Pedziwiatr-Werbicka *et al.*, 2019). The acrylonitrile is added to the initiator core in the double Michael addition and reaction process to create the partial generation and, subsequently, the heterogeneous hydrogenation utilising the catalyst RN to make the –NH2 (entire generation dendrimers.). The reaction sequence is repeated cyclically to make the PPI dendrimers until the fifth generation. The overall process involved in synthesising 5.0 PPI is depicted in below Fig. 1.

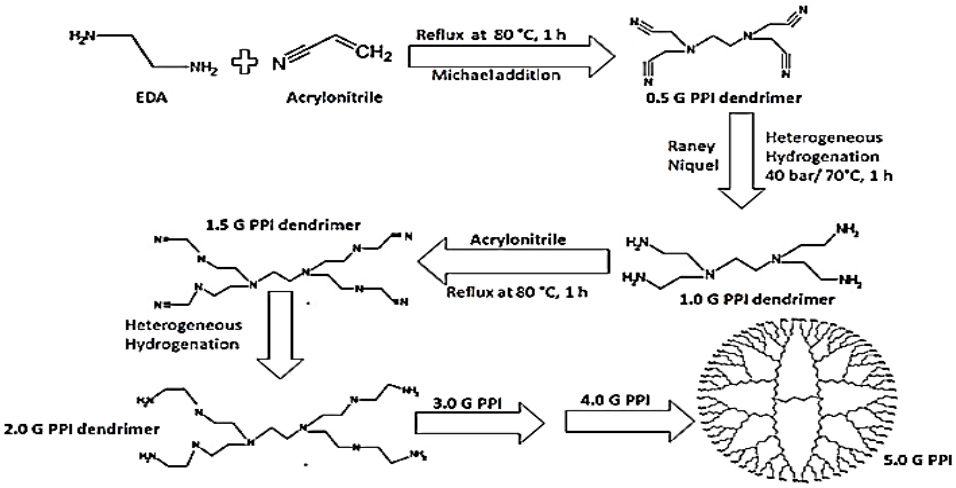


Fig. 1. Scheme for PPI-5G dendrimer- Synthesis(Ordoñez-Benavides & Andrade-Caicedo, 2022).

The N, N dicyclohexyl carbodimide of around 0.64 mmol and pyrazinamide of 0.64 mmol in DMSO of around 10 ml were mixed to the solution of 5.0G EDA-PPI dendrimer of 0.01 mmol upon DMSO of 10 ml. Then, the mixed solution was kept at room temperature for five days. The solution was dialysed in the water to remove the excess chemicals for one day. The precipitate was created by mixing the dialysed solution with the water. After this process, the pyrazinamide-5.0GPPI precipitate was filtered, and the lyophilisation of the supernatant took place. The procedure used to produce 5.0G pyrazinamide conjugated PPI dendrimer is illustrated below in Fig. 2.

PEGylated 5.0GPPI dendrimer*:* The PEGylation (polyethene-glycol) derivatisation is executed after the terminal functional sets of PEG 2000s were initiated-the conversion of Polyethylene glycol 2000 into the derivatives of a dicarboxylic acid and marginally varying the procedure. The chloroacetic acid is utilised to make carboxy methyl PEG 2000 diether. The two carboxylic acid functional groups were formed from the PEG 2000. The 32 mM of potassium tert-butanolate and four mM of PEG 2000 was combined with tert-butanol of 50 ml at 50^{o} C. The mixture was stimulated at the same temperature throughout the entire night. After the evaporation of the solvent took place, 50 ml of dichloromethane was mixed to precipitate. After cleaning and stimulating the mixture using water of 250 cc, the layers are segregated in the separating funnel. The removal of the lower layer of dichloromethane takes

place, which permits the liquid to assemble for an hour; meanwhile, it is strenuous to ten or fifteen ml.

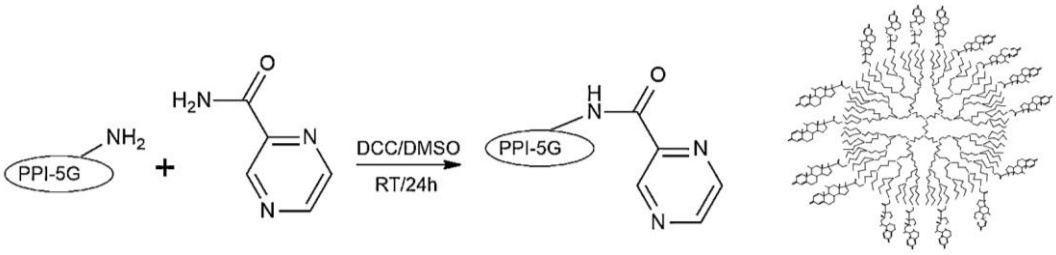


Fig. 2. Structure for synthesis of Pyrazinamide loaded 5.0GPPI Dendrimer.

Followed by it, it was combined with 200 ml of chilled ether. The precipitation will be developed after being kept inside the refrigerator for one day or night. The cold ether is redissolved in the dichloromethane, which is then utilised to filter the distant precipitate. The precipitous dicarboxylic acid PEG 2000 is desiccated in a petri-dish before providing heat in the oven. The 5.0G PPI dendrimer in DMSO (dimethyl sulfoxide) is mixed into the solution of 0.32mmol in DMSO of 10 ml, and PEG COOH2000 of 0.32 MMOL in DMSO of 10ml and the combined mixture was perturbed for five days in room temperature. In order to remove the COOH, Free PEG 2000, partially PEGylated dendrimers and DCC, the product is dialysed in contrary to double distilled water for twenty-four hours. It is then followed by lyophilisation. The product precipitation occurs by adding water (Shukla *et al.*, 2020).

Morphology of dendrimers: The morphology of the dendrimer, which is incorporated by drugs, is investigated. It is examined through a Scanning electron microscope (SEM) (Parham *et al.*, 2021). The nanoparticles are sampled on a metal stick. The counter-foil is coated with gold particles through the ion sputter of Hitachi 1010. This was Followed by examining a sample using the Hitachi 3000N SEM chamber (JSM 5610LV SEM, JEOL, and Japan). The voltage of SEM is kept at 20 V, and the pressure is set to 0.6 mmHg.

Determination of the size of particle and polydispersity index: Zetasizer 300 HS is used to examine the quantity of drug loaded in dendrimers. Samples were dissolved in 2g/ml of refined water and kept at 25°C. The diameter of the sample was determined through the light intensity of scattered nanoparticles. PDI-poly dispersity index is used to measure dispersion homogeneity. The formula evaluated it,

$$DI = [D_{0.9} - D_{0.1}]/D_{0.5}$$

$D_{0.9}$- particle diameter measured at 90th percentile of unwanted particles

$D_{0.1}$-particle diameter measured at the 10th percentile of unwanted particles

$D_{0.5}$- particle diameter measured at the 50th percentile of unwanted particles (Singh *et al.*, 2019).

5. FT-IR and NMR spectroscopy

The plain and PEGylated dendrimers were examined through Bruker DRX-300 for NMR spectroscopy analysis (Jain *et al.*, 2020). The sample is diluted with DO and methanol and subjected to a frequency of 300 MHz. FTIR spectra examination is carried out through the Perkin Elmer RXI system to analyse plain, PEGylated and drug-loaded dendrimers. Sample (1 mg) mixes with potassium bromide (200 mg) at high pressure to obtain pellets. The particles are being scanned at 4 cm. The resolution ranges from 450-4000 cm.

***In-vitro* release of drug:** The release of medicine from PEGylated dendrimers is determined through the dissolution method. The medium is phosphate-buffered saline (0.05 mol). The drug and drug incorporated PEGylated dendrimer of 1000 Da were filled in a dialysis bag and placed in PBS (50 ml and pH-7.4) at a temperature of 37°C. The external solution (1ml) was removed and replaced with an equal capacity of PBS. The spectrophotometer was used to analyse the quantity of medication at the wavelength of 248 nm.

Release kinetic analysis: The outcomes of the *in vitro* medicine release were plotted in zeroth order. It is being calculated from the plot (total percentage of medicine release versus period), Ist order (log overall percentage of drug retention time), and Higuchi matrix system (total percentage of medicine release versus time square root) to recognise the

$$\frac{Mt}{M} = Ktn$$

The mechanism as well as the kinetics of medicine release. The data of drug release were further measured using Peppa's equation, is the magnitude of the drug released at the time "t",

M is the magnitude released at a time "t."

Mt/M is the segment of drug released at a time "t".

The kinetic constant, k, and the diffusion coefficient, n, reveal the crucial mechanism of medicine release. R2 values were measured for the linear curves. It was acquired from the plot regression investigation (Peters *et al.*, 2021).

Stability studies: The pyrazinamide-incorporated PEGylated dendrimer was exposed to various temperatures and light conditions for four weeks. The sample was placed in separate vials and also kept in a thermostatically controlled oven at room temperature (40.2 °C) in the shady (amber-coloured vials) and bright (colourless vials) for four weeks. The samples were verified for colour variation, drug content and release. Data fetched were used to examine physical and chemical degradation, storage conditions and safety measures. At 0°C, samples were transparent and translucent. The drug

loss in the preparation was determined after storage conditions. The known volume of the formulation was dialysed in benzylated cellulose tubes (Sigma, USA). The peripheral medium (10 ml methanol) was spectrophotometrically screened for drug content. The percentage rise in drug release was utilised to investigate the impact of storage conditions on the formulation.

RESULTS AND DISCUSSION

The 5.0G PPI dendrimer production takes place using a method explained by De Brabender – Van Den Berg and Mejer by utilising the initiator core as the ethylene diamine (Pedziwiatr-werbicka *et al*., 2019). The results were reliable with testified PPI dendrimer synthesis. The pyrazinamide was utilised to conjoin the created dendrimers, and the synthesis is illustrated in fig.3 and 4, encouraged by the IR and NMR data. The amide bond creation is the main stage in producing PPI-Pyrazinamide conjugate.

FTIR and NMR spectroscopy: PPI 5.0G dendrimers were produced using ethylenediamine with a slight variation. The synthesis of 0.5G PPI is being validated through IR peaks, and the peak is found at. PPI 1.0G reveals an amine peak in the IR spectrum. It reveals that the nitrile present in 0.5G PPI was transformed to $(NH_2)_4$. The prominent peaks are the bend of C-C at (1116.2cm^{-1}) a stretch of C-N at(1316.7cm^{-1}) the Primary amine at (3369.4 cm^{-1}) and the deflection of amine at (3359.4cm^{-1}) revealing the nitrile group of dendrimers transformed into amine terminals. The outcomes were consistent with the existing synthesis of PPI dendrimer. PEG4000 and DCC were utilised to PEGylate the dendrimers. The IR spectra of the PEGylated PPI 5.0G dendrimer identify a single dominant peak. The significant peak was found due to the presence of ether linkage. The stretch in the amide group has been identified. The substantial amide stretch C-N peaks were found at and, depicted in. Fig. 3 and 4 depict the NMR spectrum, and modification of dendrimers reveals the PEGylation compared to the simple dendrimers. The high integral value reveals an increase in the number of secondary groups in PEG. The abundance of ether linkages resulted in a distinction peak at 3.507 ppm. The other remaining free amine appears around. The unique peak of amide-linkage obtained near 2.504 ppm and 2.496 ppm in the NMR spectra of the carbonyl group.

Drug loaded in PEGylated dendrimers: The drug pyrazinamide was incorporated into the PEGylated PPI dendrimer at the optimal molar ratio of 1:0.5, 1:1, and 1:2 to obtain the formulation. The hydrophobic contact and hydrogen bonding in both pyrazinamide and dendrimer allows the drug to physically bind with micelles and the surface of PEG layers. The dendrimer: drug molar ratio was 1:0.5, and 1:2 was used to generate the formulation. The drug loading percentage is high, and the p-value was 0.0001. It is exceptionally significant. The high value of interaction between the drug and dendrimer resulted in the elevated loading capacity of the drug in the dendrimer.

Drug entrapment efficiency: The moles of entangled medicine in 1 mol of PEGylated dendrimer in the ratio of 1:1 dendrimer: drug is observed to be 92.08±1.2 mol. It is equated to 12.42±0.8 mol in a 1:0.5 molar ratio and 51.1±1.0 mol in a 1:2 ratio. If the entrapped drug is higher than the prerequisite amount, then the host turns toxic and elevated pressure due to increased size resulting in the drug leakage from the system. Fig. 5 illustrates the plot of formulation versus entrapment efficiency in percentile.

Characteristics of dendrimers: Scanning Electron Microscopy (SEM) was used to investigate dendrimers' morphology and surface character. Fig. 6 shows Scanning Electron Micrographs of PEGylated dendrimers and Pyrazinamide and Pyrazinamide dendrimers. It reveals the generation of a spherical form with an uneven surface. The micrographs of drug-encumbered dendrimers of respective drugs revealed that the spherical shape drug-encumbered dendrimers are formed (PEGylated5.0GEDA-PPIdendrimers) and agglomerated.

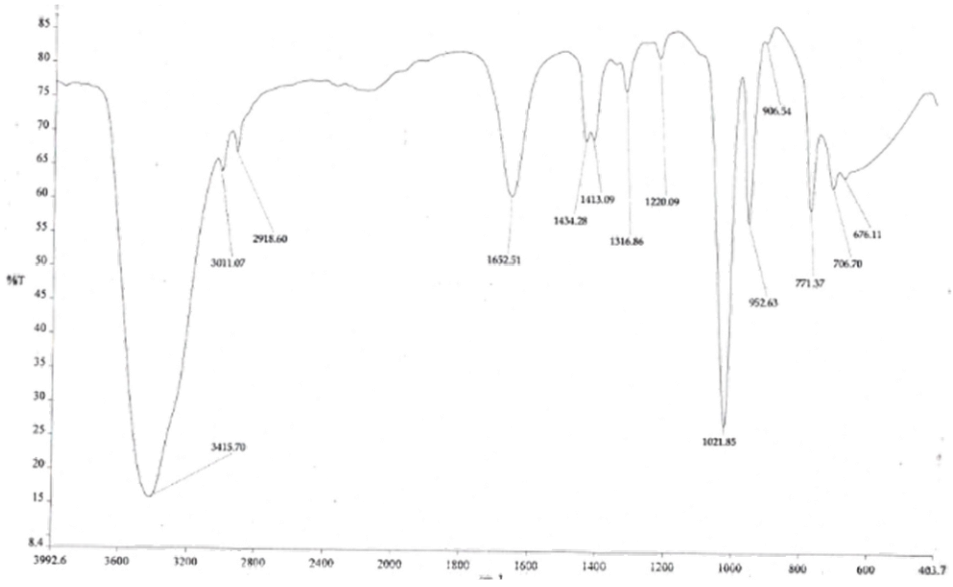


Fig. 3. FTIR spectroscopic data of PEGylated 5.0G PPI dendrimer.

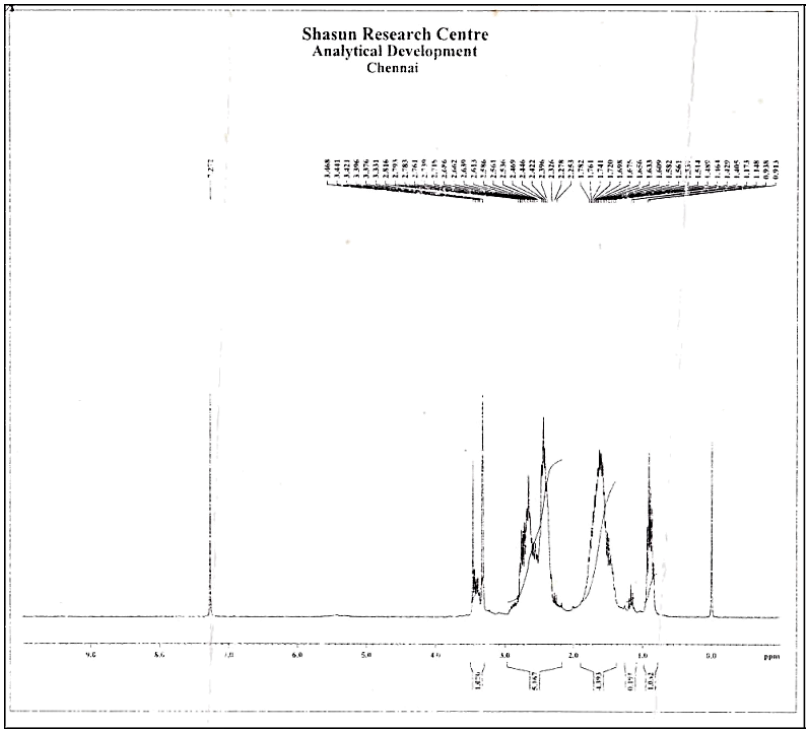


Fig. 4. Schematic representation of ^{1}HNMR spectroscopic data of PEGylated dendrimer.

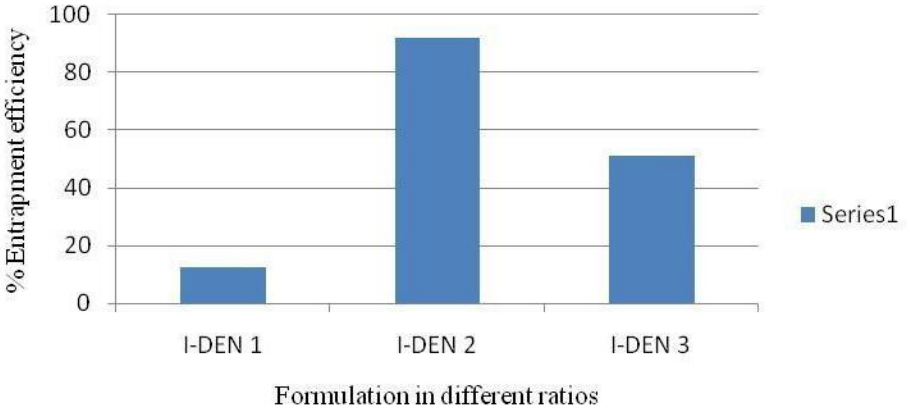


Fig. 5. Formulations in different ratios.

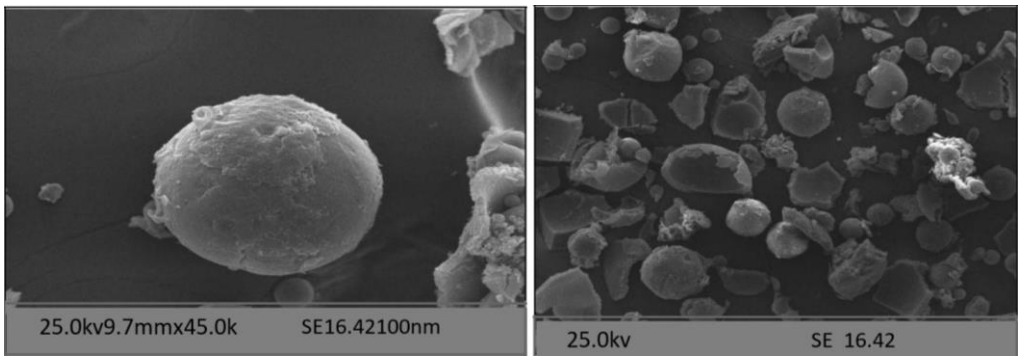


Fig. 6. SEM images of morphology and surface character of dendrimer.

Particle size and PDI: Malvern particle size analyser was utilised to determine the size of synthetic PPI dendrimers that were either plain, PEGylated, or PEGylated with Pyrazinamide. The sizes vary depending on the molar concentration of the medicinal ingredients and PEGylated dendrimer in the formulations, which are used to determine pyrazinamide's size, as shown in Fig. 7.

Zeta-potential: Zeta potential is the primary value in analysing the steadiness of dendrimers (Cacua *et al.*,

2019). The particle could be stabilised broadly when the zeta potential value was around the -30 to + 30 mV range. It is due to electrical repulsion amongst particles. The average zeta potential obtained for formulations P-DEN 1 to P-DEN 3 was observed to be -12.50 ± 1.41mV to -24.17 ± 0.78MV, and for I-DEN 1 to I-DEN 3 was about -16.00 ± 1.00 mV to -30.38 ± 0.02MV. It was concluded that the Pyrazinamide and Pyrazinamide dendrimers obtained in this study were dynamic stable systems. The surface charge of Nanoparticles influences their cell wall penetration. The existing research (Ta *et al.*, 2021) reported that only the negatively charged particles could penetrate the cell wall to reach the inner environment (Fig. 8. a&b).

Differential scanning calorimetry: Plain Pyrazinamide's curve displayed an endothermic peak at its 95.50°C temperature. An endothermic peak was observed in PEGylated PPI 5.0G dendrimers at 60.41°C. No other rise was seen in the blend of pyrazinamide and PEGylated PPI 5.0G dendrimers because two extremes of the compounds were located near 60.70°C. Peak of pure Pyrazinamide emerged at 57.50°C and also PEGylated dendrimers were close to 135.87°C in the DSC plot of pyrazinamide encumbered PEGylated PPI 5.0G dendrimers. The DSC curves thoroughly established and validated the development of the drug–the dendrimer composite (Fig. 9a-c).

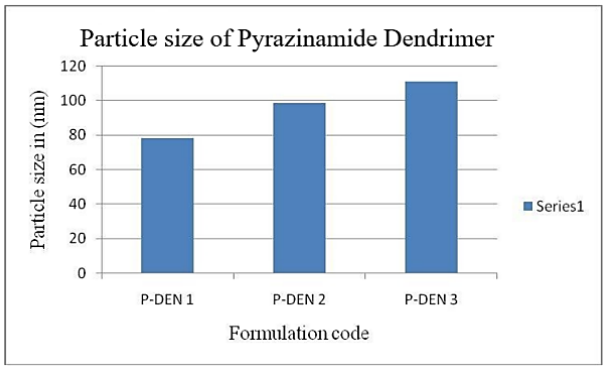
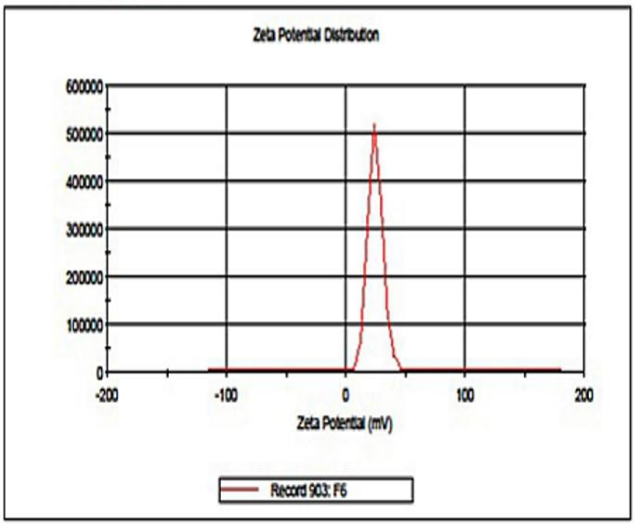
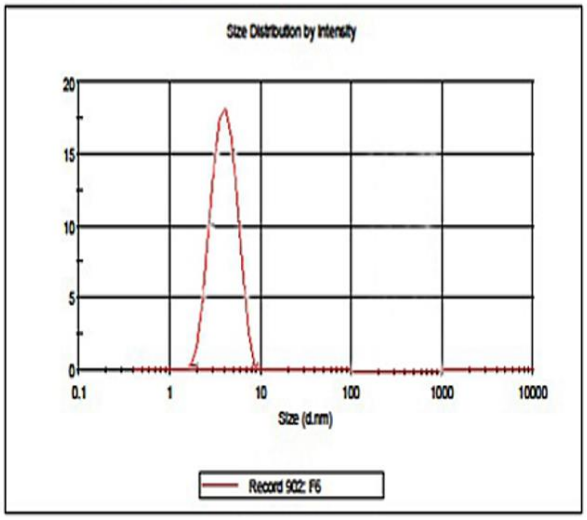


Fig. 7. Particle size of various formulations of Pyrazinamide loaded dendrimers.

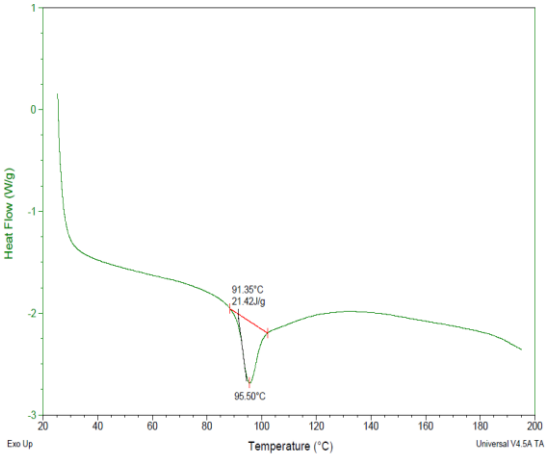


(a)

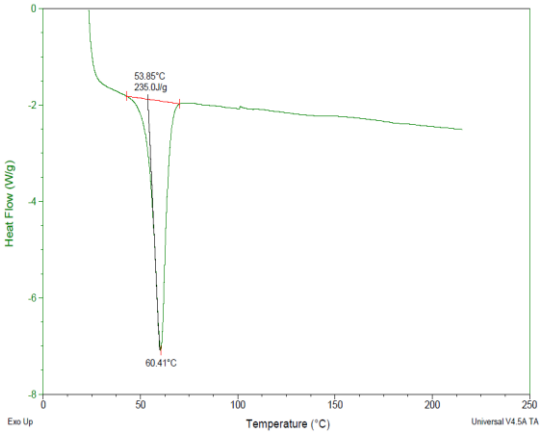


(b)

Fig. 8. (a&b) Zeta Potential of Pyrazinamide.



(a)



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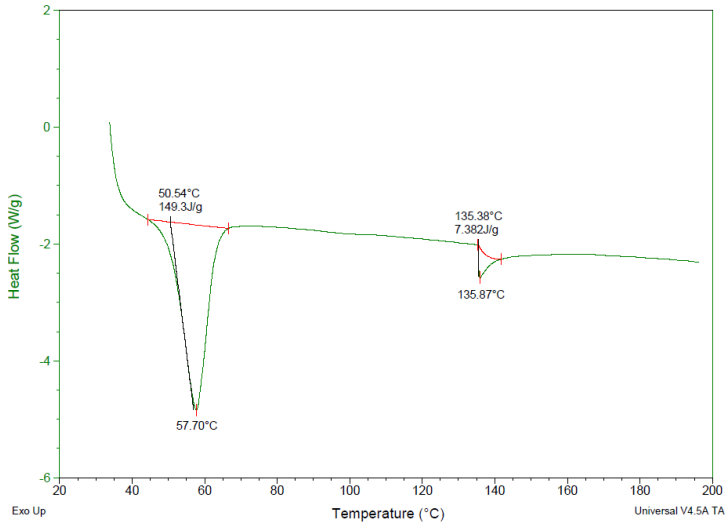

(c)

Fig. 9 (a). DSC curve of Plain Pyrazinamide drug, (b) PEGylated dendrimer and (c) Pyrazinamide loaded dendrimers.

***In-vitro* study of drug discharge:** A proportional examination of the PEGylation effect on drug discharge from EDA-PPI dendrimer-(NH2)64 was accomplished. In the three preparations of PEGylated dendrimers, the ratio of (1:1) shows a moderately slower release of pyrazinamide when equated with the (1:0.5 or 1:2) ratio of PEGylated dendrimers. Pure pyrazinamide released 86.9% in 24 hours, while the drug-encumbered PEGylated 5.0G EDA- PPI dendrimers released only 30.7% in 24 hours. The drug release rate of pyrazinamide was 54%, and in the case of a drug-loaded dendrimer, it is reduced to 16.57 percentile and 91.4 percentile in 8 hours and 160 hours, respectively. With increased dendrimer synthesis, Pyrazinamide-loaded PEGylated dendrimers' cumulative per cent release is dropped. This might be because the medication interacts more hydrophobically with the dendrimer's core in higher-generation dendrimers (5.0G). The slower drug release profile, in which drug diffusion occurs only through tiny channels in the PEGylated dendrimers and drug dissolution. The differential also influences the number of terminal PEG groups (Fig. 10).

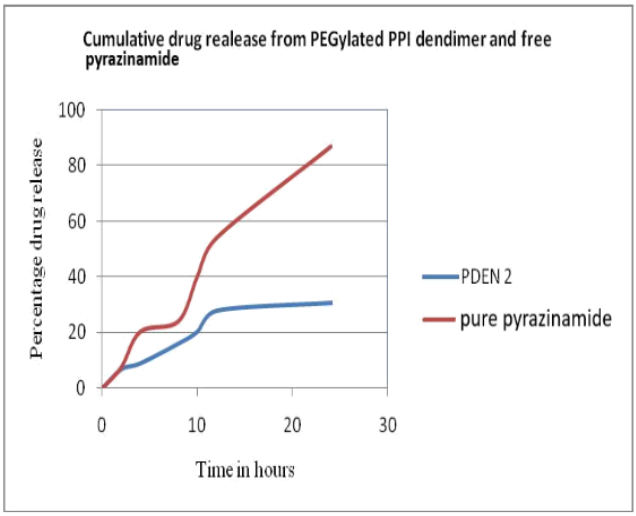

Fig. 10. Cumulative drug release from PEGylated PPI dendrimer and free pyrazinamide.

Release kinetics study: Pyrazinamide dendrimers loaded with PEGylated PPI 5.0G were monitored for 8 hours before the total amount of the drug was released from the system regarding diffusion. A steady release of the medication over a time frame of 48 to 96 hours respectively. It exemplifies the release of Pyrazinamide from the dendrimer system. Both drug molecule diffusion and polymer degradation are involved in drug release.

Stability analysis of drug-encumbered PEGylated PPI dendrimers: The stability studies were conducted on two dendrimer formulations at 250 ± 20 °C, 60% ± 5% RH, 50 ± 20 °C, 60% ± 5% RH and 450 ± 20 °C / 75% ± 5% RH for three months. After three months, particle size, appearance as well as content of drugs were analysed. No significant difference was observed for the above parameters, and the optimised formulation shows good stability over three months in dark and room temperature. An effective delivery system necessitates

competence and strength during storage. These features make stability studies significant for novel pharmaceutical products.

CONCLUSION

Dendrimers emerge as an excellent drug carrier, preferred for treating TB. Among the classes of dendrimers, PPI dendrimer remains the most recognised one. In addition to it, dendrimers can improve drug efficiency (Scicluna *et al.*, 2020). Hence, this research utilised the pyrazinamide drug for treating tuberculosis and loaded it into the PPI dendrimer to enhance its efficacy and control the drug release rate. The PEGylation is discovered as the appropriate surface modification technique to enhance the controlled release of the drug in 5.0G PPI dendrimer. The study utilises the EDA as the originator core for producing 5.0G PPI dendrimer and finally encumbered the Pyrazinamide. Hence, the Pyrazinamide loaded. PEGylated 5.0 G PPI exhibits controlled drug release for a long time compared to the free fifth-generation PPI and PEGylated PPI dendrimer. Also, the study conducted *in vitro* drug release analysis and release kinetic and stability experiments revealed that the prepared Pyrazinamide loaded PEGylated 5.0 G PPI regulates the drug release for a prolonged time and minimises the variation in plasma drug concentration. This novel approach enhances drug therapy management in TB patients and reduces the treatment time.

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