Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2023, Volume 11, Issue 12
ISSN: 2321–3647(online)

Synthesized Dendritic Macromolecule Improve the Solubility of Enzalutamide

Vidhi Patel[1], Princy Patel[2] , Pravinkumar M. Patel[2], Jigar V Patel[1]*
1.Department of Industrial Chemistry, Institute of Science and Technology for Advanced Studies and Research (ISTAR) (affiliated to CVM University), Vallabh Vidyanagar 388120, Gujarat, India
2.Industrial Chemistry Department, V.P.& R.P.T.P. Science College (affiliated to Sardar Patel University), Vallabh Vidyanagar 388120, Gujarat, India.

ABSTRACT

Prostate cancer is an androgen-dependent disease that responds to established therapies that reduce circulating testosterone levels or inhibit androgen binding to the androgen receptor. This has resulted in the development of a variety of new drugs that target this hormone-regulated transcription factor. Oral enzalutamide drug is a potent androgen receptor antagonist inhibitor for the treatment of castration-resistant prostate cancer, but enzalutamide is classified as BCS class II substances with low aqueous solubility. In recent decades, dendrimers have proven to be effective as solubilizers. Due to their special qualities, dendritic macromolecule is an effective drug solubilizing agent. Therefor the current work is to improve the solubility of enzalutamide by using hydroxy-terminated dendritic macromolecules. The potential of nanoscale hydroxy-terminated dendritic macromolecules TG1.0, TG2.0 and TG3.0 as enzalutamide solubility enhancers was investigated. The effect of concentration and generation of synthesized dendritic macromolecules on enzalutamide solubility was investigated. The FT-IR spectra were used to characterize the formation of complexes between drug molecules and dendritic macromolecules. The experimental results revealed that enzalutamide solubility increased with dendrimer concentration and generation. The cytotoxicity and hemolytic potential of synthesized hydroxy-terminated dendritic macromolecules excelled over commercially available polyamidoamine dendrimers (PAMAM) in a cytotoxicity assay using A-549 lung cancer cell lines.

Keywords: Enzalutamide, Cytotoxicity, Dendrimer, Hemolysis, Solubility

*Corresponding Author Email: pramukhprit@yahoo.co.in
Received 20 November 2023, Accepted 10 December 2023

INTRODUCTION

Solubility is critical in achieving the required drug concentration in the systemic circulation for the desired pharmacological effect[1]. More than forty percent of new chemical entities (NCEs)

Please cite this article as: Patel JV *et al*., Synthesized Dendritic Macromolecule Improve the Solubility of Enzalutamide . American Journal of Pharmacy & Health Research 2023.

developed by the pharmaceutical industry are essentially insoluble in water. One of the most difficult aspects of pharmaceutical development, particularly for oral-drug delivery systems, is increasing drug solubility and, as a result, oral bioavailability[2]. Due to its ease of administration, high patient compliance, cost effectiveness, lack of sterility constraints, and flexibility in dosage form composition, oral administration is the most practical and widely used drug delivery technique. As a result, many generic pharmaceutical companies are more likely to produce bioequivalent oral medication formulations[3]. The main disadvantage of the design is the low bioavailability of oral dose forms. Oral bioavailability is influenced by factors such as aqueous solubility, drug permeability, dissolving rate, first-pass metabolism, and sensitivity to efflux mechanisms. The two most common causes of decreased oral bioavailability are poor solubility and insufficient permeability. The solubility issue is a major concern for formulation scientists[4].

Pharmaceutical researchers are always attempting to increase the solubility of poorly water-soluble medicines using innovative drug delivery platforms. Because most new chemical entities are impacted during the development stages due to solubility characteristics, these novel drug delivery platforms demonstrated a potential role in improving solubility and thus bioavailability, increased residence within the biological system, site-specific release, and decreased toxicity. In short, this innovative drug delivery technology has the potential to improve therapeutic outcomes[5-7].

Prostate cancer is the second most frequent cancer in males and the fourth most prevalent cancer in general. In 2020, there will be about 1.4 million new instances of prostate cancer[8]. Enzalutamide (4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide) is a highly effective second-generation androgen receptor inhibitor with molecular weight 464.44 g/mol used to treat metastatic castration-resistant prostate cancer (Figure 1). Medivation and Astellas developed it, and the Food and Drug Administration authorized it in 2012 under the brand name Xtandi[9]. According the Biopharmaceutical Classification System (BCS), enzalutamide is classified as BCS class II substances with low aqueous solubility. Various approaches to improving solubility have been developed over the years. Water-soluble polymers are an important class of excipients that are widely used in the development of new effective pharmaceutical formulations[10]. Polyvinylpyrrolidone, polyvinyl alcohol, polyethylene glycol, chitosan, starch, cellulose derivatives, proteins, polypeptides, and other polymers are employed in polymer-based carriers for pharmaceutical formulations[11-13]. Among other polymeric systems nanostructured dendritic molecules have been successfully applied due to their unique properties for the solubility

enhancement[14].The formation of nanostructured hydroxy-terminated dendrimer with well-defined particle size and globular shape poses great interest in biomedical applications such as solubility enhancement of poorly water-soluble drug[15].

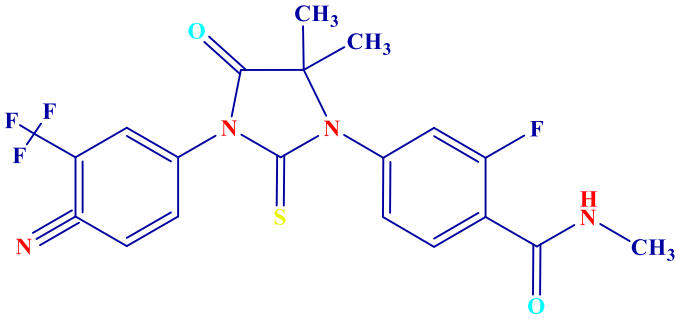

Figure 1: **Structure of the Enzalutamide**

Dendrimers are core-shell nanostructures with precise architecture and low polydispersity that are synthesized layer by layer (in 'generations') around a core unit, resulting in high control over size, branching points, and surface functionality[16]. dendrimer surface, dendrimer generation, dendritic core nature, and dendrimer concentration in solution all of which are capable of impacting solubilization approach and the outcome[17]. Dendrimers are ideal carriers for small molecule drugs and biomolecules due to their ability to personalize their properties to therapeutic needs. Dendrimers have three main properties: (i) nanoscale container properties (i.e., drug encapsulation), (ii) nano-scaffolding properties (i.e., drug surface adsorption or attachment), and (iii) biocompatibility[16]. Poly(amidoamine) (PAMAM), poly(propylene imine) (PPI), and poly(etherhydroxylamine) (PEHAM) dendrimers have been successfully used to create several commercially available small-molecule medicines with anticancer, anti-inflammatory, antiemetics, and antimicrobial activities. Dendrimers are unimolecular micellar molecules with hydrophilic surfaces[18-20]. They combine medicinal molecules and hydrophobes to form covalent and non-covalent compounds that improve solubility. Higher-generation dendritic macromolecules have a greater capacity to encapsulate hydrophobic moieties. A generation-dependent change in the properties and efficiency of a dendrimer is usually observed when used for drug solubilization[21-23].

Considering the premises, the aim of our study is to increase the solubility of antiandrogens enzalutamide using nanostructured dendrimer. This work first investigates the reported dendrimer's hemolysis study and cytotoxicity assays which is performed on the A-549 lung cancer cell lines and demonstrated that synthesized nanostructured dendrimer performed better than commercially available PAMAM dendrimer. Reported nanostructured dendrimer (TG1.0, TG2.0, TG3.0) are used to improve the solubility of Enzalutamide (ENZ) by using higuchi and

conner method. Effect of dendrimer generation and concentration on the solubility enhancement of enzalutamide were studied. The experimental results showed that the dendrimer concentration and generation increased with increasing solubility of the Enzalutamide. The complex formation was confirmed by the infrared spectroscopy.

MATERIALS AND METHOD

Materials

Enzalutamide was generously provided by Indukaka Ipcowala College of Pharmacy, Vallabh Vidyanagar. Triazine trichloride, thiocarbamide, dichloromethane, methanol, and Acetone. All the reagent and solvent used for the synthesis of nanostructured dendrimer and their applications.

Synthesis of dendritic macromolecules

The following procedure was used to create a hydroxy-terminated dendritic macromolecules. At 0-5 ºC, triazine trichloride (0.02 mmol) interacted with thiocarbamide (0.01 mmol) to yield N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)thiocarbamide as the core for dendrimer production. After washing with Acetone and Methanol, N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)thiocarbamide was purified. The reaction of N,N'-bis(4,6-dichloro-1,3,5-triazin-2-yl)thiocarbamide (0.01 mmol) and diethanolamine (0.04 mmol) yielded hydroxyl terminated generation 1 (TG1) dendrimer. Washing and dispersing TG1 dendrimer in dichloromethane (DCM). TG1 dendrimer (0.01 mmol) was reacted with triazine trichloride (0.08 mmol) at 0-5 ºC to generate chlorine terminated half generation TG1.5 dendrimer in the same manner as in the first stage. In a similar manner to the second stage, chlorine terminated half generation dendrimer (TG1.5) (0.01 mmol) was reacted with diethanolamine (0.16 mmol) to produce full generation hydroxyl terminated dendrimer (TG2). The above two procedures were repeated to produce half-generation TG2.5 and full-generation TG3 dendrimers. FT-IR, 1H-NMR, 13C-NMR, and ESI-Mass Spectrometry were used to completely characterize the synthesized dendrimer[24].

Estimation of Enzalutamide λmax

The wavelength at which the active pharma ingredient (API) absorbs the most is referred to as the λ max. For the λ max determination of enzalutamide, weighed accurately 10 mg of Enzalutamide and transferred into 100 ml Volumetric flask, dissolved and volume was made up to the mark with methanol give solutions containing 100µg/ml Enzalutamide. The stock solution of the Enzalutamide was further diluted with solvent to obtain 3, 6, 9, 12 and 15µg/ml solution and scanned over wavelength range of 200-400nm using shimadzu UV-1800 spectrophotometer[25].

Solubility study

The method described by Higuchi and Connors (1965) was used to conduct the solubility research. Excess enzalutamide was added to screw-capped vials holding varying dendrimer generation concentrations (0.6 mmole to 3 mmole). Vials were shaken for 48 hours in a shaking water bath at 37ºC. The vials were centrifuged to remove undissolved enzalutamide and the absorbance of enzalutamide was measured using a Shimadzu UV-1800 spectrophotometer at its characteristic wavelength of 236 nm[26].

Hemolysis assay

The hemolytic impact of increasing concentrations of Nanostructure dendrimer on RBC suspension was investigated. In a nutshell, blood samples were centrifuged at 1500rpm for 5 minutes. Following centrifugation, supernatant plasma was removed, and blood cells were resuspended in 0.5 ml PBS and incubated for 1 hour with various dendrimer concentrations (0.01, 0.1, 1.0, 10, 100 and 1000 µg/ml). After incubation, samples were centrifuged, and the supernatant was collected and diluted with an equivalent volume of Phosphate buffer saline (PBS), and absorbance at 540nm was measured. To get the greatest inhibitory effect, distilled water was used as a positive control. The formula was used to calculate the hemolytic effect of various dendrimer concentrations[27].

% Hemolysis= (Absorbance of test/Absorbance of control)*100

Determination of Cell Viability: MTT Assay

Human A549 non-small cell lung cancer cells were grown at 37°C in a CO2 incubator (5% CO2) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Antibiotic-Antimycotic solution. Cell viability was determined by collecting cultivated cells with trypsin and seeding them in a 96-well plate at a density of 10,000 cells per well and incubating for a period of twenty-four hours. Following incubation, cells were treated for 24 hours with various concentrations (10, 100 and 1000, µg/ml) of dendritic macromolecules. The control wells received DMEM. After 24 hours, medium with various dendrimer concentrations was removed from each well, and cells were treated with 0.5mg/ml MTT for 4 hours at 37ºC. Following incubation, MTT was withdrawn and DMSO (100µl/well) was applied to dissolve the formazan crystals. The absorbance of each well was measured at 550nm with an ELISA plate reader, and the % cell viability was determined using the following formula[27].

% Cell Viability= (Mean Absorbance of test/Mean Absorbance of Vehicle control)*100.

RESULTS AND DISCUSSION

Preparation mechanism

Synthesis and characterization of dendrimer TG1.0, TG2.0 and TG3.0 based on thiocarbamide was already reported[20]. Full generation dendrimers TG1.0, TG2.0 and TG3.0 were water soluble whereas half generation dendrimers and core compound were water insoluble. Hemolysis study and cytotoxicity assays of TG3.0 dendritic macromolecules is performed on the A-549 lung cancer cell lines and demonstrated that synthesized dendritic macromolecules performed better than commercially available PAMAM dendrimer. Therefore, only full generation dendrimers were utilized for drug solubilization.

λmax of Enzalutamide

100µg/ml Enzalutamide were diluted with solvent to obtain 3-15µg/ml solution and scanned over wavelength range of 200-400nm using shimadzu UV-1800 spectrophotometer and λmax were found at 236nm. Absorption and overlay spectra of enzalutamide are shown in Figure 2 and Figure 3.

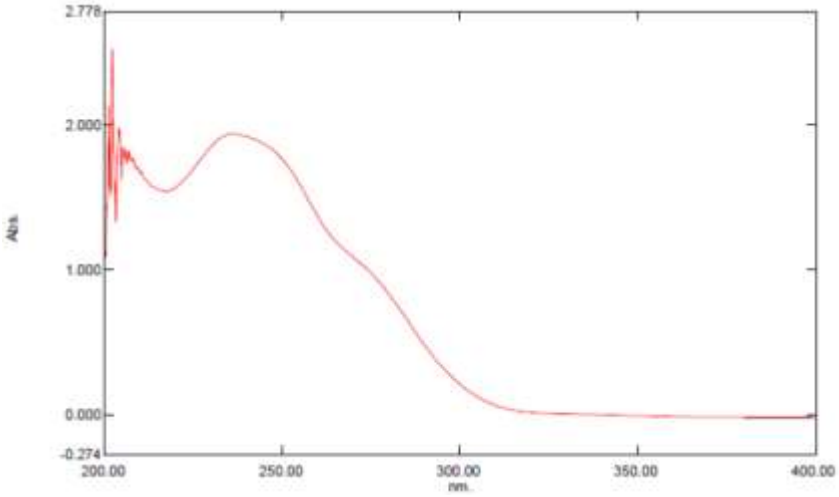

Figure 2*:* Absorption spectra of Enzalutamide

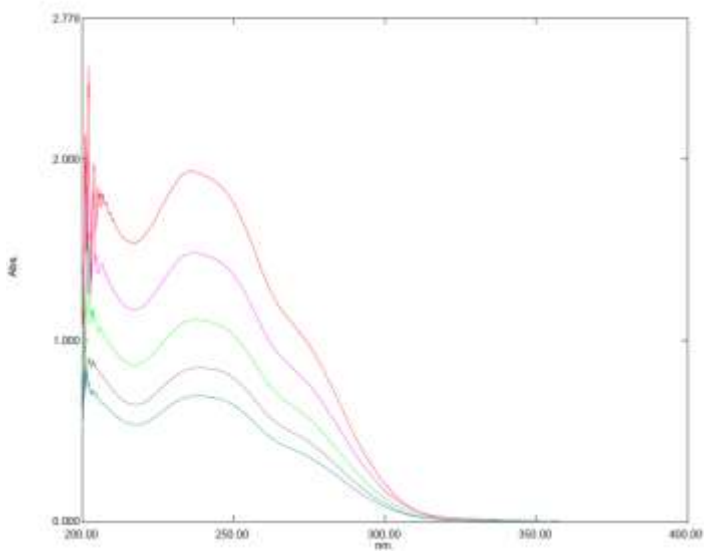

Figure 3*:* Overlay Absorption spectra of Enzalutamide

Drug solubility study

Dendrimers are promising molecular scaffolding for drug delivery due to their unique properties, which include defined size, shape, molecular weight, monodispersity, the presence of a void space, versatile structure, selectivity towards cells and intracellular components, and protection of guest molecules. According to the results shown below, dendritic macromolecules have a higher potential for anticancer medication delivery.

Enzalutamide's poor water solubility is a major issue in the successful development of effective dosage forms. Solubility measurements were performed according to the method of Higuchi and Connors[23]. In the present investigation, the aqueous solubility of Enzalutamide was found to be 1.955µg/ml. The influence of the dendrimer generation on the apparent solubility of Enzalutamide in water was investigated by phase solubility diagrams. At 37°C temperature, the

effect of triazine based dendrimer concentration on solubility f Enzalutamide was studied, and the findings are presented in Figure 4, Figure 5 and Figure 6. According to our results solubility of Enzalutamide has been significantly improved by dendritic macromolecules. The solubility of enzalutamide increased linearly with increasing dendrimer concentration over the concentration range 0.6–3.0mM. It was discovered that enzalutamide's solubility improved by 3.0mM TG3 dendrimer is 35.983 µg/ml. From the Figure 7 it was clear that the solubility of enzalutamide was affected by the different generation of dendritic macromolecules. The solubility enhancement effect of various dendritic generation used in this study followed the order: TG3 > TG2 > TG1. Dendrimers increase drug solubility most likely due to a cavity, hydrogen bonding, and electrostatic contact between dendrimer terminal functional groups and drug molecules of enzalutamide. Because enzalutamide lacks ionizable groups, its aqueous solubility is unaffected by pH across the physiological range. We also tested the stability of the enzalutamide-containing dendrimer for three weeks and found it to be stable. This research revealed that dendrimer showed better result than □-CD in increasing the solubility of enzalutamide[26].

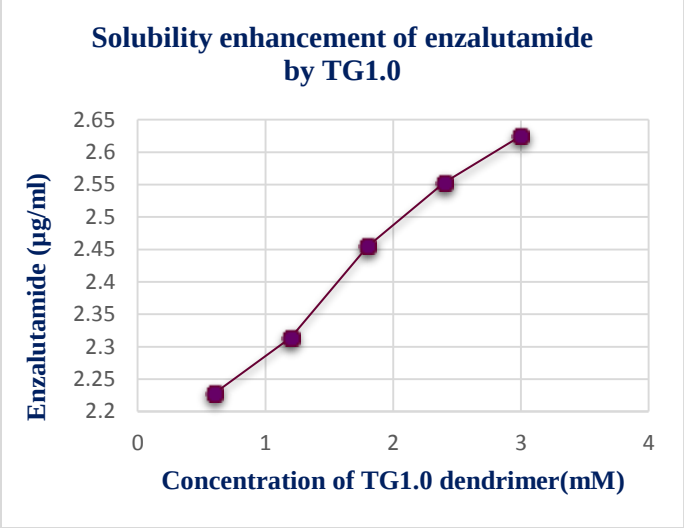

Figure 4*:* Solubility enhancement of enzalutamide by generation 1.0 dendrimer (TG1.0)

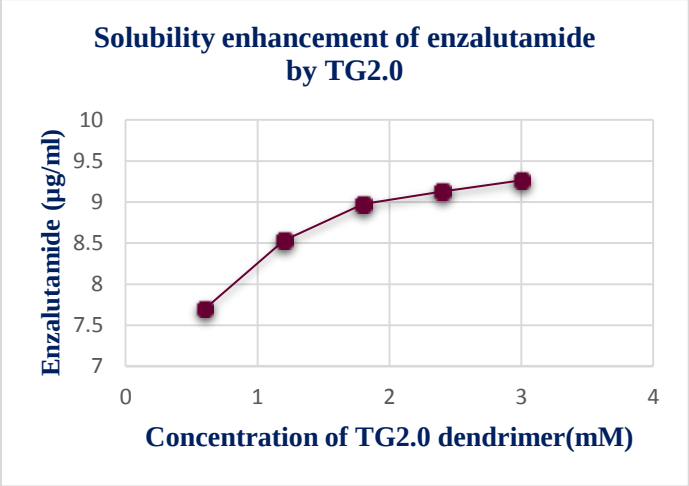

Figure 5: **Solubility enhancement of enzalutamide by generation 2.0 dendrimer (UG2.0)**

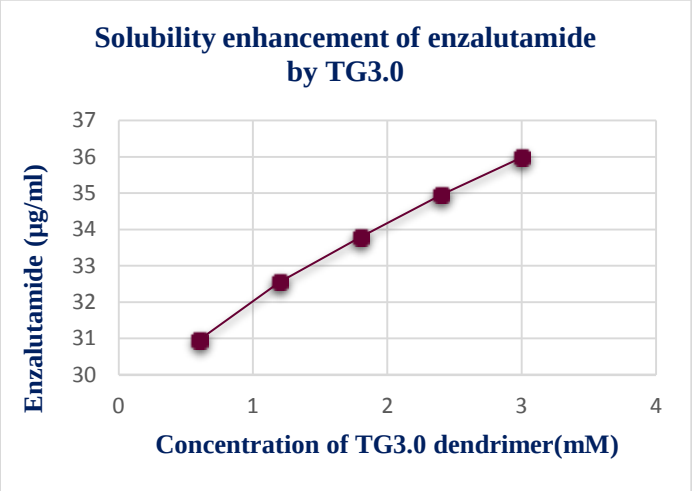

Figure 6*:* **Solubility enhancement of enzalutamide by generation 3.0 dendrimer (TG3.0)**

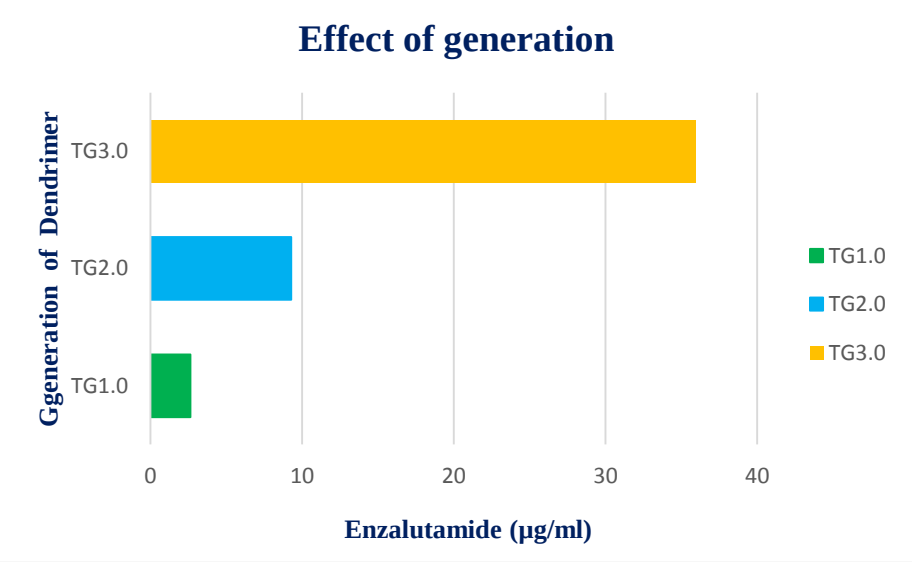

Figure 7*:* **Effect of generation number**

The ability of hydroxy-terminated dendrimers to bind to the enzalutamide drug, the nature of the surface groups of the dendritic macromolecule, and the electrostatic interaction between the dendrimer and the drug are all important factors in their success as drug delivery agents. As a result, the FT-IR spectra were used to characterize the dendrimers containing enzalutamide. FT-IR spectrum of pure TG3 dendrimer showed absorption bands 3314 cm^{-1} for O-H stretching for hydroxyl groups, 1054 cm^{-1} for C–O stretching, 1456 cm^{-1} for C=S stretching, 1652 cm^{-1} for C=N stretching, showed in Figure 8. FT-IR spectrum of enzalutamide showed absorption bands at 3091 cm^{-1} for aromatic C-H stretching, 1665 cm^{-1} for C=O stretching, 1447 cm^{-1} for C=S stretching, 1372 cm^{-1} for C-F stretching, and 1119 cm^{-1} for C-N stretching, as shown in Figure 9. FT-IR spectrum of enzalutamide loaded TG3-dendrimer showed absorption band at 3367 cm^{-1} for O-H stretching, 2935 cm^{-1} for C-H stretching, 1663 cm^{-1} for C=O stretching, 1457 cm^{-1} for C=S stretching, 1363 cm^{-1} for C-F stretching, 1122 cm^{-1} C-N stretching and 1054 cm^{-1} for C-O stretching showed in Figure 10. Thus, in the IR spectrum of enzalutamide-containing dendrimer, the overall characteristic bands for both TG3.0 dendrimer and enzalutamide remained unchanged. As a result, it is clear that dendrimers can increase enzalutamide solubility via hydrogen bonds, hydrophilic interactions, or a combination of the two.

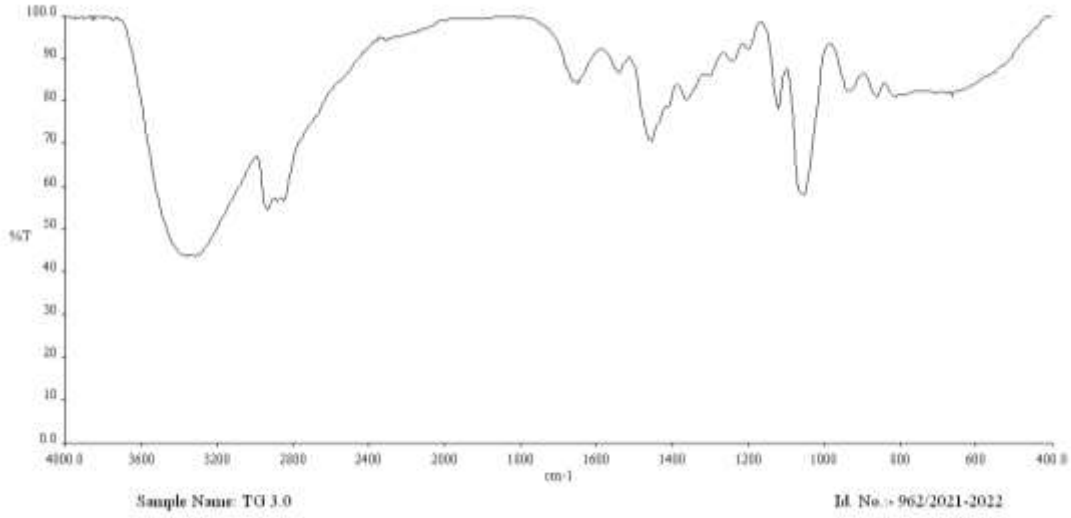

Figure 8*:* FT-IR spectra of TG3.0 dendrimer

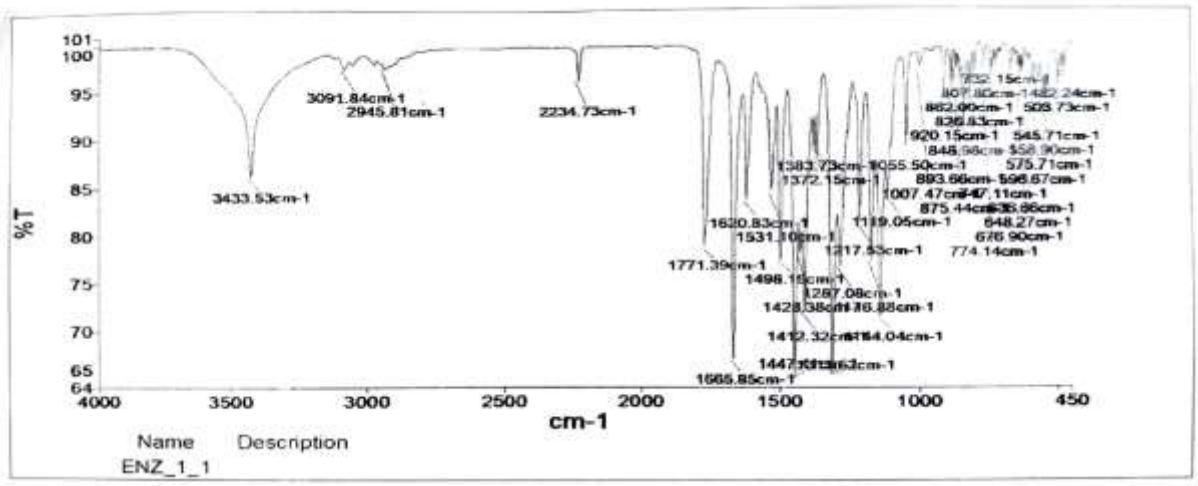

Figure 9*:* FT-IR spectra of enzalutamide (ENZ)

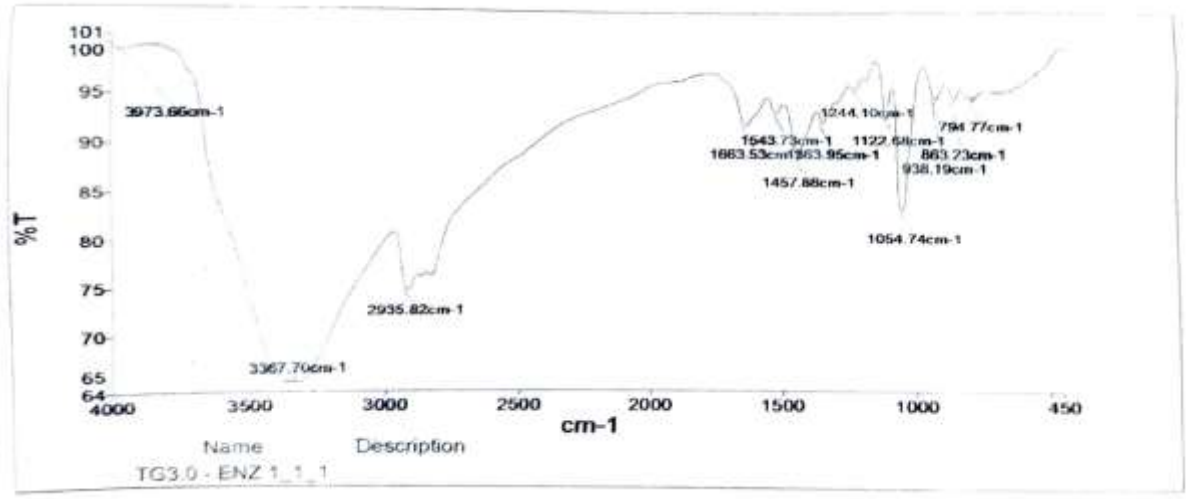

Figure 10: FT-IR of enzalutamide containing generation 3 dendrimer (TG3.0-ENZ)

Hemolytic potential

Effects of hydroxyl-terminated dendritic macromolecules on the erythrocytes are shown in Figure 11. The results indicated that hydroxyl-terminated nanostructured dendrimer caused hemolysis in a concentration-dependent manner. However, triazine based TG3 dendrimer were significantly better hemolysis result compared to PAMAM dendrimer[24]. The positively charged amine groups of PAMAM dendrimer interact with the negatively charged surfaces of erythrocytes (RBCs), resulting in hemolysis[25]. In comparison, TG3.0 dendrimers have anionic hydroxyl groups on the surface, which reduces interaction with erythrocytes (RBCs) and results in significantly lower toxicity.

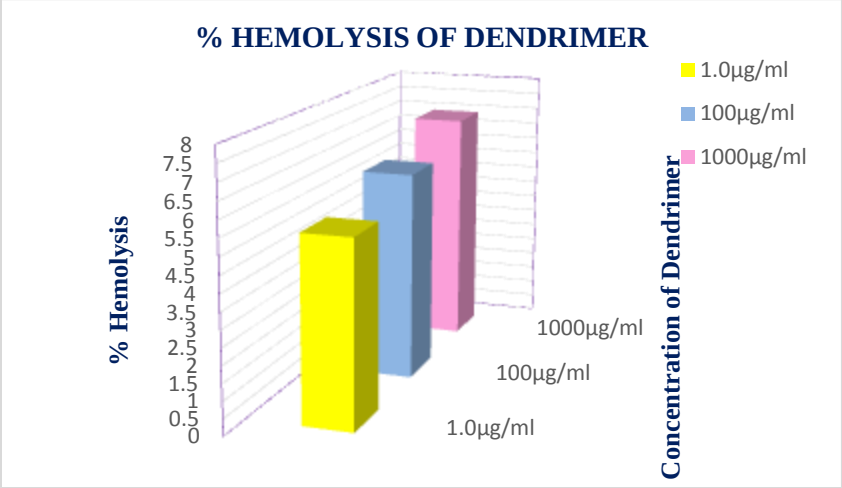

Figure 11:%Hemolysis of dendritic macromolecule (TG3.0)

Cytotoxicity

The MTT assay technique was used to investigate the cellular toxicity of TG3.0 dendritic macromolecules on A-549 cell lines. MTT is a yellow dye that is easily soluble in water. MTT can be transformed into water-insoluble, blue-colored formazan crystals by reductive breakage of the tetrazolium ring by living cells. Formazan crystals recovered with organic solvents and evaluated at a wavelength of 550 nm, and result are correlated with living cells to determine cell viability. Cytotoxicity revealed that TG3 dendritic macromolecules had greater than 90% cell viability at concentrations ranging from 10 µg/ml to 1000 µg/ml. As a result, TG3 dendrimer was far less cytotoxic. The morphology of A-549 cell lines when treated with control and varied dendrimer concentrations is shown, exhibiting a reduction in cell density with increasing dendrimer concentration from 10 µg/ml to 1000 µg/ml. Figure 12 and Figure 13 are shown microscopic image of control and 1000 µg/ml dendrimer concentration respectively.

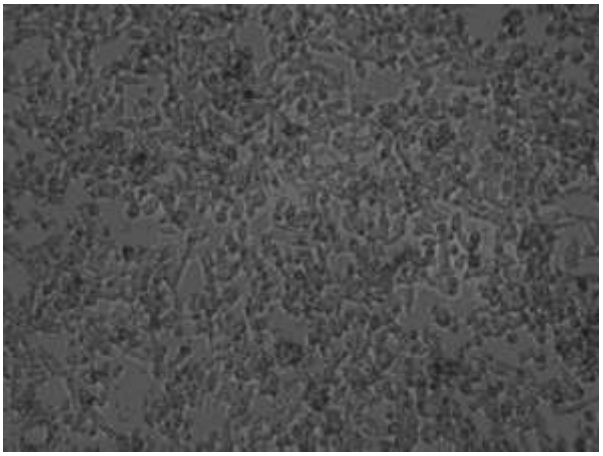

Figure 12*:* Microscopic images of A-549 cell lines treated with Control

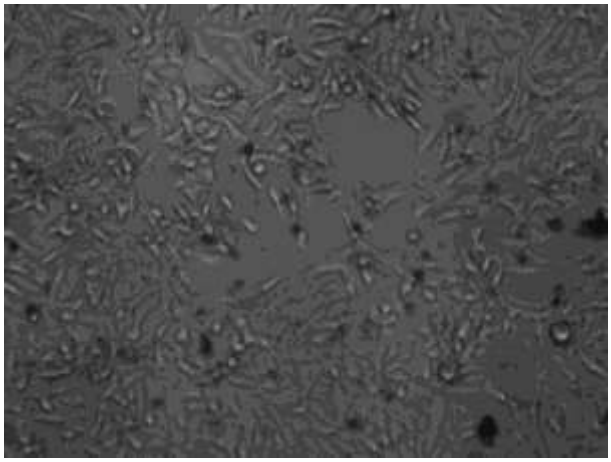

Figure 13 : Microscopic images of A-549 cell lines treated with 1000 µg/ml of TG3.0 Dendrimer

CONCLUSION

To enhance the oral bioavailability of enzalutamide, various generation of dendritic macromolecules were synthesized. The cytotoxicity and hemolytic assays revealed that dendritic macromolecules were substantially less toxic and biocompatible, which indicating its applicability as a possible drug carrier system. Enhancement of the drug solubility in aqueous solution depends on the concentration and the generation of dendritic macromolecules. Present study demonstrates that TG3.0 dendrimer has great potential to enhance the solubility of enzalutamide. Dendrimer was more effective than □-CD in enhancing enzalutamide solubility.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the Principal, Institute of science and technology for advanced studies and research (ISTAR), V.P & R.P.T.P Science College and department of pharmacy, Nirma university. The authors would like to acknowledge the Indukaka Ipcowala College of Pharmacy, Vallabh Vidyanagar for providing the API, Sophisticated Instrumentation Centre for Advanced Research and Testing (SICART) at CVM University, Vallabh Vidyanagar and Centre of excellence (COE) for providing the spectroscopic analysis facility.

DECLARATION OF INTERESTS

The authors have declared no conflict of interest

REFERENCE

1. Vemula VR, Lagishetty V, Lingala S. Solubility enhancement techniques. Int. J. Pharm. Sci. Rev. Res. 2010;5(1):41-51.
2. Sharma D, Soni M, Kumar S, Gupta GD. Solubility enhancement–eminent role in poorly soluble drugs. Res J Pharm Technol. 2009; 2(2):220-224.

3. Krishnaiah YS. Pharmaceutical technologies for enhancing oral bioavailability of poorly soluble drugs. JBioequiv Availab. 2010;2(2):28-36.
4. Di L, Kerns EH. Drug-like properties: concepts, structure design and methods from ADME to toxicity optimization. Academic press, 2015.
5. Kumar S, Singh P. Various techniques for solubility enhancement: An overview. J. Pharm. Innov. 2016;5:23.
6. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. International Scholarly Research Notices. 2012; 2012.
7. Sharma N, Baldi A. Exploring versatile applications of cyclodextrins: An overview. Drug Deliv. 2016;23: 739–757.
8. World Cancer Research Fund International. Prostate cancer statistics. 2020. https://www.wcrf.org/.
9. Bondigalla R, Challa GN, Yarraguntla SR, Bandu R, Alla SR. Characteristics, properties, and analytical and bio-analytical methods of enzalutamide: A review. Sep. Sci. Plus. 2023;6(4):220119.
10. Volkova TV, Drozd KV, Surov AO. Effect of polymers and cyclodextrins on solubility, permeability and distribution of enzalutamide and apalutamide antiandrogens. J. Mol. Liq. 2021;15;322:114937. https://doi.org/10.1016/j.molliq.2020.114937
11. Zhou J, Fu H, Peng G, Cao H, Zhang Y, Liu M, Wu W, Qing X, Zhou J. Solubility and solution thermodynamics of flofenicol in binary PEG 400+ water systems. Fluid Ph. Equilibria. 2014; 25; 376:159-64. https://doi.org/10.1016/j.fluid.2014.05.030.
12. Sanghvi R, Narazaki R, Machatha SG, Yalkowsky SH. Solubility improvement of drugs using N-methyl pyrrolidone. Aaps Pharmscitech. 2008; 9:366-76. https://doi.org/10.1208/s12249-008-9050-z.
13. Bai TC, Yan GB, Hu J, Zhang HL, Huang CG. Solubility of silybin in aqueous poly (ethylene glycol) solution. Int. J. Pharm. 2006; 308(1-2):100-106. https://doi.org/10.1016/j.ijpharm.2005.10.032.
14. Patel P, Patel V, Patel PM. Synthetic strategy of dendrimers: A review. *J*. Indian Chem. Soc. 2022; 99(7):100514. https://doi.org/10.1016/j.jics.2022.100514.
15. Patel V, Patel P, Patel PM, Patel JV. Lead and copper metal ion uptake by a novel nanoscale hydroxy-terminated dendritic macromolecules. *J*. Indian Chem. Soc. 2022; 99(10):100717. https://doi.org/10.1016/j.jics.2022.100717.

16. Svenson S. Dendrimers as versatile platform in drug delivery applications. Eur J Pharm Biopharm. 2009; 71(3):445-462.
17. Choudhary S, Gupta L, Rani S, Dave K, Gupta U. Impact of dendrimers on solubility of hydrophobic drug molecules. Front. pharmacol. 2017; 8:261. https://doi.org/10.3389/fphar.2017.00261
18. Patel P, Patel V, Patel PM. Novel nanostructured dendrimer based on 1, 3-bis (4, 6-dichloro-1, 3, 5-triazine-2-yl) urea as an excellent adsorbent for Pb2+, Ni2+, Co2+ and Zn2+ metal ions. *J*. Indian Chem. Soc. 2022; 99(11):100763. https://doi.org/10.1016/j.jics.2022.100763.
19. Patel P, Patel V, Patel PM. Application of novel nanoscale hydroxy-terminated dendrimer for Cu+2 and Cd+2 ion uptake, Chem. Pap. 2022; 77(3):1287-1297. https://doi.org/10.1007/s11696-022-02541-1.
20. Sohail I, Bhatti IA, Ashar A, Sarim FM, Mohsin M, Naveed R, Nazir A. Polyamidoamine (PAMAM) dendrimers synthesis, characterization and adsorptive removal of nickel ions from aqueous solution, *J*. Mater. Res. Technol. 2020; 9(1)498-506. https://doi.org/10.1016/j.jmrt.2019.10.079.
21. Patel V, Patel P, Patel JV, Patel PM. Dendrimer as a versatile platform for biomedical application: A review. *J*. Indian Chem. Soc. 2022; 99(7):100516. https://doi.org/10.1016/j.jics.2022.100516.
22. Rai DB, Medicherla K, Pooja D, Kulhari H. Dendrimer-mediated delivery of anticancer drugs for colon cancer treatment. Pharmaceutics, 2023; 15(3):801.
23. Kesharwani P. Aptamers Engineered Nanocarriers for Cancer Therapy. Elsevier Science & Technology, 2022.
24. Sheikh A, Kaur H, Abourehab MA, Alam MS, Kesharwani P. Aptamer-functionalized dendrimers for targeted cancer therapy. 2023; 255-275.
25. Patel V, Patel P, Patel PM, Patel JV. Highly efficient novel nanostructured dendritic macromolecules for remediation of aquatic heavy metal ions, Inorg. Chem. Commun. 2023; 148:110381. https://doi.org/10.1016/j.inoche.2022.110381
26. Zamir GK, Shwetal SP, Prashant KD, Praveen OP. Validated UV spectroscopic methods for determination of enzalutamide in pure and pharmaceutical dosage form. Anal. Chem. 2016; 16:111.
27. Higuchi T, Connors A. Phase-solubility techniques. In: Advances in Analytical Chemistry and Instrumentation, John Wiley, New York 1965.

28. Jain S, Kaur A, Puri R, Utreja P, Jain A, Bhide M, Ratnam R, Singh V, Patil AS, Jayaraman N, Kaushik G. Poly propyl ether imine (PETIM) dendrimer: A novel non-toxic dendrimer for sustained drug delivery. Eur J Med Chem. 2010; 45:4997-5005.