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DEVELOPMENT OF TRIAZINE BASED DENDRIMER OF SALICYLIC ACID WITH INCREASED ANTIBACTERIAL ACTIVITY

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ABSTRACT

The aim of this research work was to develop triazine based dendrimer of salicylic acid with increased antibacterial activity. Surface modification of dendrimer of generation 3 was performed by conjugation with polyethylene glycol (PEG). The amount of drug encapsulated in dendrimer was determined by the separation of drug loaded dendrimer from the suspension containing free drug by centrifugation. The suspension obtained after solvent evaporation was centrifuged. The amount of free drug in the supernatant was measured by UV spectrophotometer. The amount of drug entrapped into dendrimer was calculated as the difference between the drug used for formulation and the amount of drug in supernatant. Antibacterial activity shows promising results as prepared dendrimer drug conjugate shows better results with only 36.05 % entrapped drug as compared to standard drug. 36.05 % entrapped drug gives results more than as compared to standard drug sample. Results reveals that if we assume that the standard drug is completely entrapped, there will be approximately 2.5 fold increase in antibacterial activity. This study also reveals that salicylic acid and prepared dendrimer drug conjugates show better activity for *S. aureus* i.e. for gram positive bacteria.

Keywords: Dendrimers, Triazine dendrimer, Synthesis, Salicylic acid, Antibacterial activity.

1. INTRODUCTION

In 1929, some scientist tried to synthesize polymers in laboratory with some definite structure. The presence of polymers was unavoidable around 1930s [1-3]. For next 20 years researches in polymer chemistry had increased and many novel contributions were added to science and society with the creations of new materials. The ability and desire to manipulate the architecture of macromolecules was now the subject of research. At the time, networked and cross-linked polymers were identified, but most of them resulted in random branching. New classes of polymeric molecular structure are Dendrimers [4-8]. Dendrimers are monomolecular and discrete structures with known composition and atomic connectivity developed from a class of synthetic macromolecules that allowed for the regulated assembly of large assemblies of atoms. Many natural things have three-dimensional architectural molecular symmetrical structures which are highly regular and in nano size. These symmetrical three-dimensional structures, especially macromolecules, could be used as building blocks for molecular devices and nanotechnologies [9]. These words refer to "dendritic macromolecules", which are currently one of the most intensively researched new materials due to their most intriguing special features and characteristics. As scientists explore the interesting characteristics of atomic and molecular constructions developed at the nanometer scale, nanotechnology is becoming one of the most important research efforts of the early twenty-first century. Researchers can sanely design and use nanoparticles for drug delivery, as image contrast agents, and for diagnostic purposes because they can control their physical, chemical, and biological properties [10].

The Greek word "Dendrimer" which means dendrons that is like a tree and meros means units or parts. This shows that dendrimers are tree like in growth, appearance and structure. These structures have at least one branched junction at each repeat unit [11-15] and are considered to be monodisperse macromolecule with perfectly branched regular structure. Here, all chain ends originate from a central core and all repeating unit constitute a branch point. Thus, dendrimers are new class of three-dimensional regular structural macromolecule produced by various synthetic routes. Dendritic polymers are nanometer-sized (10-9 m) spherical type molecules. Dendrimers are formed by

iterative sequence of different reaction steps, each additional iteration leads to high generation dendrimer. These unique structures can be utilized as a tool for desired functions such as internal voids, well defined shape and variable surface functionality. Dendrimer possess many other names such as "starburst dendrimer", "cascade molecules", "arborols", "molecular scaffolds" and "dendritic polymers" [15-20].

With the advancement of new technologies and techniques these drawbacks can be overcame and drugs may be used purposely. One such technique is nano-scale dendrimers. One such established drug is salicylic acid which has been less used systemically due to some toxicity issues of mucosal lining and gastrointestinal tract. But due to its multiple beneficial effects, the drug now is used for local application for acne, eczema, dermatitis, fungal infection and as keratolytic agent [21-24].

We have synthesized a novel surface modified triazine based dendritic molecule of salicylic acid; the wonder drug" using divergent method. Dendrimer was synthesized up to three generation, surface modified with polyethylene glycol and loaded with salicylic acid. The synthesized compound were characterized and tested for biological activity [25-28].

2. MATERIAL AND METHODS

Triazine trichloride, 1,3 Propane di-amine, Dichloromethane, Epichlorhydrin and Diethanolamine, were received as gift samples from Shree ji Trade International, Indore. Acetone, methanol, ethanol, salicylic acid, EDA, PEG 200 and all other reagents and solvents, chemicals and equipments used for synthesis and analysis were provided by the Institute of Pharmacy, Vikram University, Ujjain. UV spectroscopy was performed on shimadzu 1800, FTIR studies was performed on shimadzu 8400, mass spectroscopy was performed on Waters Micromass Q-Tof Micro.^{1}H NMR was performed on Avance-II (Bruker) spectrophotometer. SEM was performed on JSM 6100 (JEOL).

2.1. General procedure for synthesis of Generation 0.5 dendrimer

Cyanuric Chloride (0.04mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of a 1,3 propane diamine (0.02mmol) dissolved in sodium hydroxide (0.04 mmol) in water was added drop wise in the solution of cyanuric chloride at 0-5°C with stirring. The solution was stirred at 0-5°C for 2 hrs. Then the solution was filtered, washed with methanol and acetone. Remnants were dried under vacuum. A white coloured solid was obtained. Progress of reaction was monitored through TLC.

2.2. General procedure for synthesis of Generation 1 Dendrimer

G 0.5 dendrimer (0.02mmol) was dissolved in an excess of diethanolamine (0.08mmol) which was used as both solvent and reactant. The resulting mixture was refluxed for 2 hrs. After cooling, it was washed by acetone repeatedly to give generation 1 dendrimer. Dendrimer obtained was light brown coloured with honey like consistency.

2.3. General procedure for synthesis of Generation 1.5 Dendrimer

Cyanuric chloride (0.08 mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G1 dendrimer (0.01mmol) containing sodium hydroxide (0.08 mmol) in water was added drop wise in the solution of cyanuric chloride at 0-5°C with stirring. The solution was stirred at 0-5°C for 2 hrs and refluxed for 6 hrs. Then the solution was filtered, washed with methanol and acetone and dried under vacuum. A white coloured solid was formed.

2.4. General procedure for synthesis of Generation 2 Dendrimer

Generation 1.5 dendrimer (0.01 mmol) was dissolved in an excess of diethanolamine (0.16 mmol) which was used as both solvent and reactant. The resulting mixture was refluxed for 2 hrs. After cooling, it was dispersed and washed by acetone repeatedly to give generation 2 dendrimer which was light brown coloured with honey like consistency.

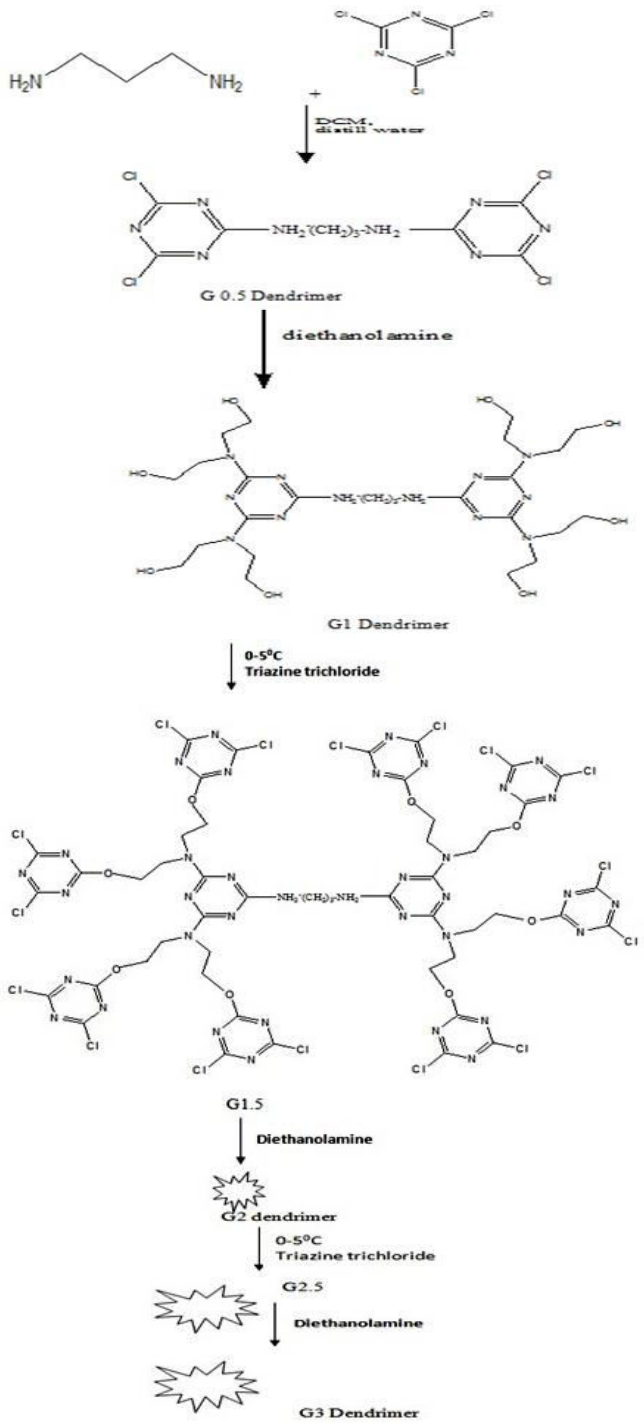
2.5. General procedure for synthesis of Generation 2.5 Dendrimer

Cyanuric Chloride (0.32mmol) was dissolved in dichloromethane and kept in an ice bath. A solution of G2 dendrimer (0.01mmol) containing sodium hydroxide (0.32 mmol) in water was added dropwise in the solution of cyanuric chloride at 0-5°C with stirring. The solution was stirred at 0-5°C for 2 hrs and refluxed for 6 hrs. The solution was then filtered, washed with methanol and acetone and dried under vacuum. A white coloured solid was formed.

2.6. General procedure for synthesis of Generation 3 dendrimer

Generation 2.5 dendrimer (0.01mmol) was dissolved in an excess of diethanolamine (0.64 mmol) which was used as both solvent and reactant. The resulting mixture was refluxed for 2 hrs. After cooling, it was dispersed and washed by acetone repeatedly to give generation 3 dendrimer which was light brown coloured with honey like consistency.

Reaction Scheme



3. RESULTS AND DISCUSSION

Dendrimers were synthesized up to 3 generations and then modified with PEG. Surface modified dendrimer was then conjugated with salicylic acid.

3.1. Characterization of Dendrimers

3.1.1. Generation 0.5 dendrimer

%yield- 79%, M.P-145°C, λ_{max} (nm)-277 Major peaks - 851,878- C-Cl stretching, 1617, 1704- aromatic C=N, 2869- C-H Stretching, 3055- N-H Stretching.

3.1.2. Generation 1 Dendrimer

% yield- 68%, M.P-170°C, λ_{max} (nm)-284, FTIR Major peaks-1020- C-O stretching, 1710- aromatic C=N, 2950 C-H Stretching, 3372- OH stretching.

3.1.3. Generation 1.5 Dendrimer

%yield- 77%, M.P-185°C, λ_{max} (nm)-278 FTIR Major peaks-784,793- C-Cl stretching, 1080- C-O stretching, 1697, 1734-aromatic C=N, 2841- C-H Stretching, 3181- N-H Stretching.

3.1.4. Generation 2 Dendrimer

%yield- 72%, M.P-205°C, λ_{max} (nm)-282 FTIR Major Peaks- 1045-C-O Stretching, 1648, 1741-aromatic C=N, 3072, 2943- C-H Stretching, 3354- OH stretching.

3.1.5. Generation 2.5 Dendrimer

%yield- 74%, M.P-220°C, λ_{max} (nm)-277 FTIR Major Peaks 774- C-Cl stretching, 1039,1079- C-O Stretching, 1716- aromatic C=N, 2890- C-H Stretching, 3074- N-H Stretching.

3.1.6. Generation 3 dendrimer

% yield- 62%, M.P-230°C, λ_{max} (nm)-283 FTIR major peaks 1051, 1082- C-O stretching, 1606, 1719- aromatic C=N stretching,2917,3074 C-H Streching, 3396-OH stretching, calculated ESI mass12044 obtained 12045, δ values ppm ^{1}HNMR - 2.507 Amide-NH, 3.264,3.49-CH_2NH_2 terminal group, 4.70 OH (methanolic).

3.2. Surface Modification of Dendrimer

Surface modification of dendrimer of generation 3 was performed by conjugation with polyethylene glycol (PEG). Polyethylene glycol of molecular weight 200 was used and EDA was used as cross linking agent. PEG (0.5 gm) was dissolved in water/ethanol (1:1, 20 mL) and EDA (0.43 gm) was dissolved in 20 mL water separately. Both the solutions were mixed with continuous stirring.

3.0 Generation dendrimer (1 gm) in 20 mL distilled water was added slowly to the reaction mixture with constant stirring in an ice bath for 30 min. and stirring was continued for another 24 hours. Progress of the reaction was monitored through TLC.

% yield- 71%, M.P-280ºC, λ_{max} (nm)-279 FTIR Major Peaks- 1061- C-O stretching, 1715- aromatic C=N stretching, 2891- C-H Stretching, 3365- OH stretching δ values ppm^{1}HNMR- 2.60 Amide -NH, 3.466,3.635- CH_2NH_2 terminal group, 4.60 OH (methanolic).

3.3. Conjugation of surface modified dendrimer by with salicylic acid

The incorporation of salicylic was carried out by dissolving 50 mg of salicylic acid in 10 ml methanol, 2 ml of this solution was mixed with 50 mg of 3.0 G dendrimers. Both the solutions were mixed with continuous stirring in an ice bath for 30 min. stirring was continued for another 6 hours. Progress of the reaction was monitored through TLC. Drug conjugated modified dendrimer was taken out, washed with methanol and dried which gives light yellow colored product

% yield- 74%, M.P-300ºC, λ_{max} (nm)-264 FTIR Major Peaks- 1676- aromatic C=C, 1716- aromatic C=N stretching, 2552- ,2921- C-H Stretching, 3383- O-H stretching

3.4. SEM Analysis

To obtain a clear insight about the surface morphology of synthesized compound, Scanning Electron Microscopic (SEM) analysis was employed.

From SEM image, stone like morphology was observed. Surface of the particles are spherical and the particles exhibit very uniform and well distributed. This image shows clearly that the surface is composed of macro particle unit and drug was associated with this particle.

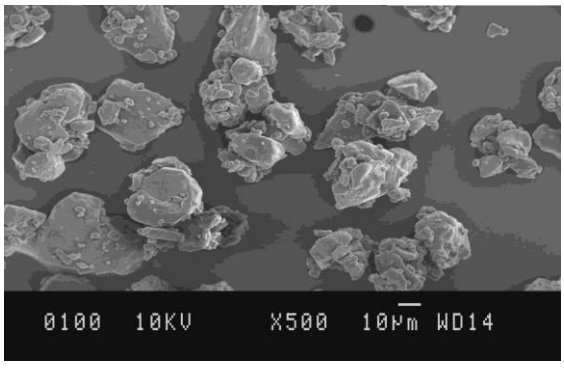


Fig. 1: SEM of prepared denndrimer drug conjugate

3.5. Drug Entrapped Efficiency

The amount of drug encapsulated in dendrimer was determined by the separation of drug loaded dendrimer from the suspension containing free drug by centrifugation. The suspension obtained after solvent evaporation was centrifuged. The amount of free drug in the supernatent was measured by UV spectrophotometer. The amount of drug entrapped into dendrimer was calculated as the difference between the drug used for formulation and the amount of drug in supernatent. The percent of entrapment efficiency was calculated by following formula.

% Entrapment Efficiency = {(Total amount of drug added- Non bound drug)/Total amount of drug} ×100

Drug entrapment efficiency of PEG modified dendrimer and salicylic acid at 234 nm was found to be 36.05±0.5%.

3.6. Antibacterial activity

Antibacterial activity of PEG modified dendrimer salicylic conjugate was tested on *E. coli and S. aureus*. The cylinder method was used to test antibacterial activity, the agar culture media was prepared, sterilized and transferred to a petridish and left to set. After setting up the culture media, two drops (0.1ml) of the sample solution were aseptically put into wells created by scooping out the media with a sterilised glass tube (5mm diameter). These were then incubated for 24 hours at 37±1°C. After that, the zone of inhibition was measured and compared to the standard.

For this study One gram positive (*S. aureus,* L27) and one gram negative (*E. coli* MC410) bacteria has been chosen for activity.

3.6.1. Preparation of stock solution of standard drug

The stock solution of salicylic acid (1mg/ml) in N,N dimethylformamide (DMF) was prepared by dissolving 10 mg of the drug in 10 ml DMF.

3.6.2. Preparation of solutions of synthesized compounds

The stock solution of each synthesized compound (1mg/ml) in DMF were prepared by dissolving 10 mg of the sample in 10 ml DMF.

Antibacterial activity shows promising results as prepared dendrimer drug conjugate shows better results with only 36.05 % entrapped drug as compared to standard drug. 36.05 % entrapped drug gives results more than as compared to standard drug sample.

Table 1: Zone of inhibition (in mm) of standard drug and prepared dendrimer drug

Name of bacteria	standard salicylic Acid	Prepared dendrimer drug sample (36.05 %)
E.coli	4.60	5.00
S. Aureus	10.20	10.30

Table 2: Zone of inhibition in *E.Coli*

Parameter	Prepared drug conjugate	Remarks
% drug entraped	36.05%	Approx 63 % less drug used
Zone of inhibition in E.Coli (in mm)	5.0mm	Approx. 2.5 fold increase in activity.

Table 3: Zone of inhibition in *S. aureus*

Parameter	Prepared drug conjugate	Remarks
% drug entrapped	36.05%	64 % less drug used
Zone of inhibition in *S. aureus* (in mm)	10.3 mm	Approx. 2.5 fold increase in activity.

This reveals that if we assume that the standard drug is completely entrapped, there will be approximately 2.5 fold increase in antibacterial activity. The study also reveals that salicylic acid and prepared dendrimer drug conjugates show better activity for *S. aureus* i.e. for gram positive bacteria.

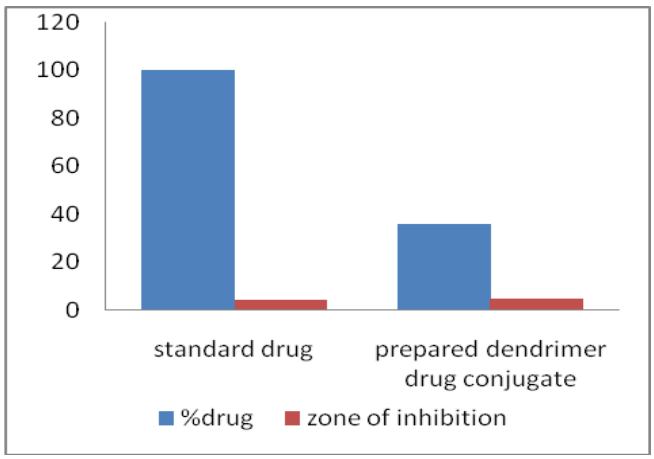


Fig. 2: Graph comparing standard drug and its zone of inhibition and prepared dendrimer drug conjugate and its zone of inhibition in *E. coli*

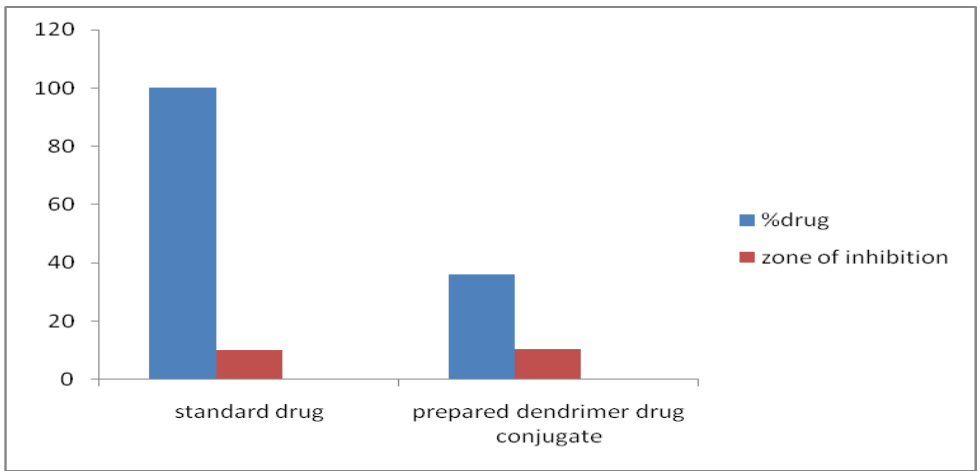


Fig. 3: Graph comparing standard drug and its zone of inhibition and prepared dendrimer drug conjugate and its zone of inhibition in *S. aureus*.

4. CONCLUSION

PEG modified triazine based dendrimer was synthesized and characterized by techniques such as FTIR,UV, H^1 NMR, Mass. SEM analysis revealed that drug particles is attached and entrapped in synthesized dendrimer. Antibacterial activity shows approximately 2.5 fold increase in activity when compared to drug alone. This might open space for novel and better use of drug salicylic acid and increase in its efficiency with reduced side effect due to low drug concentrations.

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Conflict of interest

None declared

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