WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 9, 1054-1062. Research Article ISSN 2277– 7105

SYNTHESIS AND FORMULATION OF NEW FUNCTIONALIZED CITRIC ACID BASED DENDRIMERS FOR DRUG DELIVERY USING AN ANTI BACTERIAL AGENT

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Article Received on
21 June 2015,

Revised on 12 July 2015,
Accepted on 05 Aug 2015

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ABSTRACT

The aim of the study is to synthesize G1, G2, G3, G4, G5 citric acid dendrimerse for targeted release by using an anti bacterial drug. The citric acid dendrite structure was synthesized and characterized by using IR. Clindamycin Hcl an anti bacterial drug is effective to both aerobic gram+ve and anaerobic gram –ve bacteria is currently used to show its effect through citric acid. Dendrimer Clindamycin Hcl was efficiently loaded in to citric acid dendritic structure using dissolution process. Entrapment of Clindamycin Hcl drug into the citric acid dendrimer for safe and specific targeting of the drug in to the cells. Drug loaded dendrimers were assessed with their physicochemical properties such as UV, IR, SEM, drug loading and invitro release study of the drug were evaluated.G5,the optimized formulation have to be evaluated for toxicity to ensure the safety and efficacy of dendrimer.

KEYWORDS: Noveldrugdelivery, Citricacid, Clindamycin HCL, Dendrimer, DMF (dimethyl formamide).

INTRODUCTION

Dendrimers were introduced in mid 1980's, this novel class of polymeric materials due to their unique structure and properties they attracted more attention. Their unique structure have high degree of control over molecular weight and shape, this led to the synthesis of unimolecular micelles. These micelles by their compact globular shape, size along with monodispersity and controllable surface functionalities of dendrimers make them excellent systems for targeting cities. Their potentiality can be seen in two different ways; almost the drug molecules can be physically entrapped inside the dendritic structure and in other way is

the most popular way is the drug molecules can be covalently attached onto surface .owing their unique structure, citric acid have been evaluated for improvement of drug solubility and drug permeation and as drug delivery systems foe bio active substances, including anti-bacterial drug.

The citric acid synthesis using polymer as PEG400 (polyethylene glycol) a typical clear, colourless, odourless substance soluble in water, stable to heat and inert to many chemical agents.PEG is considered to be bio compatible that to say it is capable of co existence with living tissues. The covalent attachment of poly ethylene glycol to proteins decreases their immunogenicity and increases their circulation time. So such molecules are expected for to encapsulate drug in dendritic architecture biocompatibility of their hydrophilic shell consisting of poly ethylene grafts.

The present study was aimed to develop and explore the use of citric acid dendritic structure for targeted delivery of an anti-bacterial drug, clindamycin hcl. The citric acid dendritic structure was synthesized and characterized by using IR spectroscopy Clindamycin hcl was efficiently loaded into citric acid dendritic structure using dissolution process.various physicochemical parameters such as UV, SEM, drug loading and in vitro release were evaluated.

MATERIALS

Clindamycin Hcl (Yarrow chemicals, Mumbai), Polyethylene glycol 400 (Finar chemicals., Ahemedabad). Ltd, Ahmadabad), Sodium carbonate, Potassium permanganate, Thionyl chloride, Citric acid, Dimethyl formamide (Sd fine chemicals Ltd, Mumbai), Pyridine, Diethyl ether and were used for synthesis of drug loaded dendrimers. All the chemicals and API were of analytical grade.

METHOD

Citric acid dendrimer were synthesized by divergent method.

SYNTHESIS OF 5thGENERATION CITRIC ACID DENDRIMER

PEG 400(poly ethylene glycol) was weighed accurately and transferred in a round bottom flask sodium carbonate dissolved in water and potassium permanganate which is dissolved in water is taken. Both the solutions were mixed and added to the round bottomed flask containing poly ethylene glycol. The mixture was stirred vigorously on a magnetic stirrer for

3-5 hrs and then cooled to 4-5^{0}C by immersing on ice bath. The reaction mixture was allowed to room temperature. The reaction mixture is filtered, the obtained filtrate is heated continuously by immersing on ice bath randomly to get a concentrate filtrate.The obtained filtrate was cooled and covered with a layer of di ethyl ether and separation of ether is done by removing aqueous layer.

For complete removal of ether from aqueous layer it is heated again on water bath then residual liquid was collected .The obtained polyethylene glycol diacid (G1) was collected. Obtained PEG-diacid was chlorinated with 0.3mol thionyl chloride equipped with a magnetic stirrer with condenser for 45-60 min. Reaction mixture is stirred and heated at 70^{0}C after condensation and thus chlorinated PEG was obtained. To the collected chlorinated product, 0.2mol of citric acid is added and was dissolved in DMF.3mol of pyridine was added ,it s stirred and kept in ice bath for 24 hrs,both the solutions were mixed and kept in an incubator for 6 hrs at 55-60^{o}C. Thus obtained GEN 2 citric acid dendrimer was further purified by using column chromatography technique. Citric acid dendrimers up to GEN 4 were prepared by repetition of all the above steps consecutively, with increasing amount of citric acid and thionyl chloride. The synthesized 5 GEN citric acid dendrimer was further purified by column chromatography.

FORMULATION OF VARIOUS DENDRIMERS

FORMULATION CODE	$KMNO_4$	Na_2CO_3	PEG400	THIONYL CHLORIDE	CITRIC ACID	PYRIDINE	DMF	DRUG
F1(G1)	35.5gm	3.75gm	110ml	4ml	0.01gm	100ml	30ml	100mg
F2(G2)	35.5gm	3.75gm	110ml	5ml	0.03gm	100ml	30ml	100mg
F3(G3)	35.5gm	3.75gm	110ml	6ml	0.05gm	100ml	30ml	100mg
F4(G4)	35.5gm	3.75gm	110ml	7ml	0.06gm	100ml	30ml	100mg
F5(G5)	35.5gm	3.75gm	110ml	9ml	0.08gm	100ml	30ml	100mg

FI= KMNO4+Na2co+PEG400+Thionyl chloride+ citric acid pyridine +DMF+Drug

F2=F1+Thionyl chloride+ citric acid+ pyridine+ Di methyl Form amide+ drug

F3=F2+ Thionyl chloride+ citric acid+ pyridine+ Di methyl Formamide+ drug

F4= F3+ Thionyl chloride+ citric acid+ pyridine+ Di methyl Formamide+drug

F5=F4+ Thionyl chloride+ citric acid+ pyridine+ Di methyl Formamide+ drug

COMPATABILITY STUDIES

Compatibility study of drug and polymer is an important pre requisite before formulation. To develop a stable dosage. A drug-excipent compatibility testing at early stage helps in

increasing the probability of developing a stable dosage form. They were done by mixing drug and polymer.

FTIR SPECTRUM STUDIES

The FT-IR Spectra were done by using UV spectrophotometer and the spectrum was recorded in the region of 4000-400cm-1. The samples (Drugs, plain dendrimers and drug loaded dendrimers) were mixed with 200-400mg of potassium bromide. The sample is compressed as discs by applying pressure of 5tons for 5mins in a hydraulic press. The prepared pellets were placed in the light path and the spectrum was recorded.

DRUG LOADING IN FORMULATION

The citric acid dendrimer synthesized were dissolved in water and mixed with 100 molar times of Clindamycin Hcl and allowed the clindamycin Hcl to dissolve. The mixed solution was allowed to incubate with slow magnetic stirring (50 rpm) using Teflon beads for 24 hrs. The solution was dialyzed twice by using cellulose dialysis bag under strict sink conditions for 10 min to remove free drug from formulation, it is estimated spectrophotometrically at 210 nm to determine indirectly the amount of drug encapsulated with the systems. The dialyzed formulations were lyophilized and used for further characterization.

MORPHOLOGY OF THE GEN 5 CITRIC ACID DENDRIMER

Chem3D ultra 10 (Cambridge soft) was used to study the molecular size and morphology of 5^{th} generation citric acid dendrimer. The external morphology of the lyophilized Clindamycin HCL loaded citric acid dendrimer complex was analyzed by using a scanning electron microscopy. The image was snapped at an accelerating voltage of 15 kv with a chamber pressure of 10.3 mmHg.

SCANNING ELECTRON MICROSCOPY ANALYSIS

Drug loaded dendrimer morphology was observed by scanning electron microscope. Morphology of respective dendrimer image was snapped at an accelerating voltage of 15 kv with a chamber pressure of 10.3 mmHg.

PERCENTAGE DRUG ENTRAPMENT STUDIES

Drug entrapment studies were done by using UV spectroscopic method. These studies confirmed the presence of drug in complex structures. To determine the amount of drug entrapped in the sample, drug loaded dendrimer was dissolved in 100ml water. The solution

was centrifuged for 1 hr at 100 rpm in centrifuge. The filtered solution we observed under UV spectrophotometer to determine the quantity of drug inside the complex at 210 nm.

INVITRO DRUG RELEASE

Drug release from known amounts of Clindamycin Hcl loaded citric acid dendrimer was determined using the dialysis bags. The medium comprised of phosphate buffer solution. The dialysis bags were filled with a known amount of Clindamycin Hcl loaded citric acid dendrimers and dialysis bags were n 50 ml of phosphate buffer solution (pH 7.4) at 37^0C with magnetic stirring under sink conditions.1ml aliquots were drawn from the external solution and replaced with the same volume of phosphate buffer solution. The concentration of drug was detected spectrophotometer at 210nm.

RESULTS AND DISCUSSION

DISSOLUTION STUDIES

Invitro release studies of Clindamycin Hcl loaded dendrimers of all 5 formulations were carried out using dissolution apparatus .There was a slow increase of drug release in buffer medium. The formulation showed first order release kinetics mechanism on composite release kinetic studies. Higuchi matrix equation confirmed the release as diffusion Controlled mechanism.

COMPATABILITY STUDIES

Compatibility study of drug and polymer is an important pre requisite before formulation. To develop a stable dosage. A drug-excipient compatibility testing at early stage helps in increasing the probability of developing a stable dosage form. They were done by mixing drug and polymer. These studies found that polymer which is excipient in formulation is compatible with drug.

FTIR SPECTRA FOR PURE CLINDAMYCIN HCL FTIR spectra of pure Clindamycin hcl demonstrated that the characteristic absorption peaks for C-H stretching at 2960.86cm^{-1},C-N stretching at 1421.78cm^{-1},C=O(amide) at 1686.50cm^{-1},C-O(ether) stretching at1079.70cm^{-1},C-CL at 723.28 cm^{-1},C-S stretching a t 686.90 cm^{-1}. This confirms the purity of it.

PERCENTAGE DRUG ENTRAPMENT STUDIES

Drug entrapment studies shown that the drug loaded dendrimer was observed in U.V Spectrophotometer at 210nm, it determines the F5(G5) having a greater entrapment efficiency with 77.4% than the F1, F2, F3, F4 formulations.

SEM CHARACTERIZATION

SEM analysis was carried out to study the morphology of optimized formulation of Clindamycin hcl loaded dendrimer. This image clearly shows the formation of dendrimers with spherical lin shape which is conformed as the dendrimer.

CURVE FITTING ANANLYSIS

The data of drug release from formulated drug dosage forms were subjected to zero order, first order kinetic model. By the linear relationship obtained it was suggested that it fallows first order controlled release. Higuchis model was appilied to drug release data, shown linearity with high R^2 values suggested that drug release from the dendrimer fallows diffusion mechanism. Korsemeyers- peppas model applied shown the exact mechanism with good linearity and the values of 'n' obtained for all the formulations were following drug release with Non-fickian diffusion.

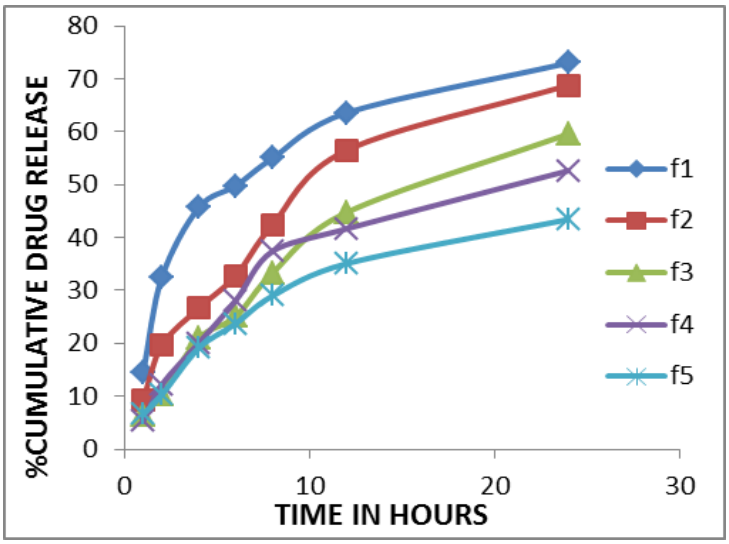

Fig.no.1:Invitro dissolution for G1-G5

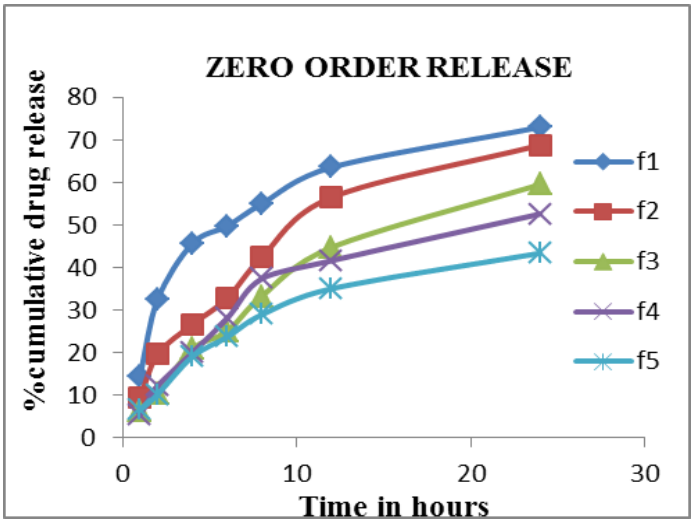

Fig.no 2: Zero order for G1-G5

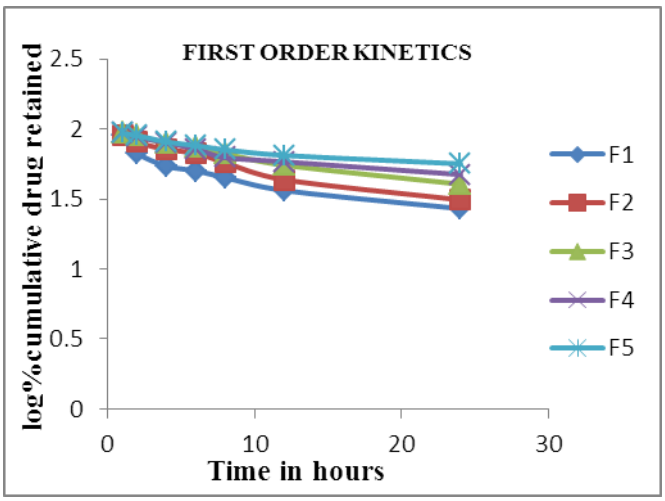

Fig.no.3 First order kinetics for G1-G5

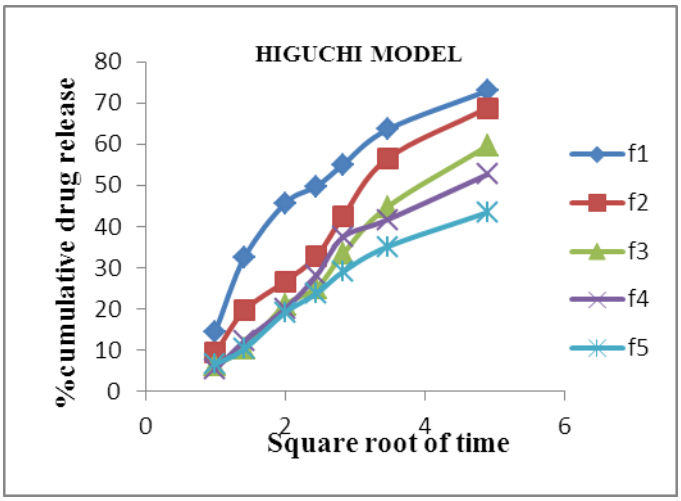

Fig.no.4: Higuchi model for G1-G5

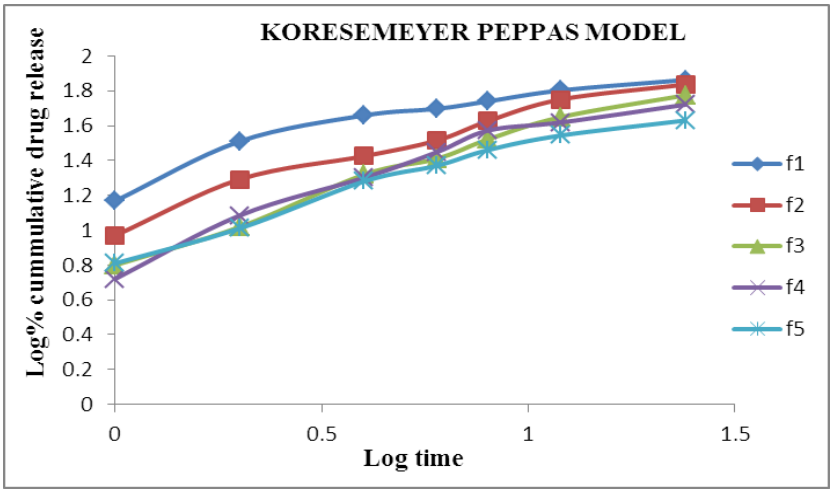

Fig.no.5: Koresemeyer peppas model for G1-G5

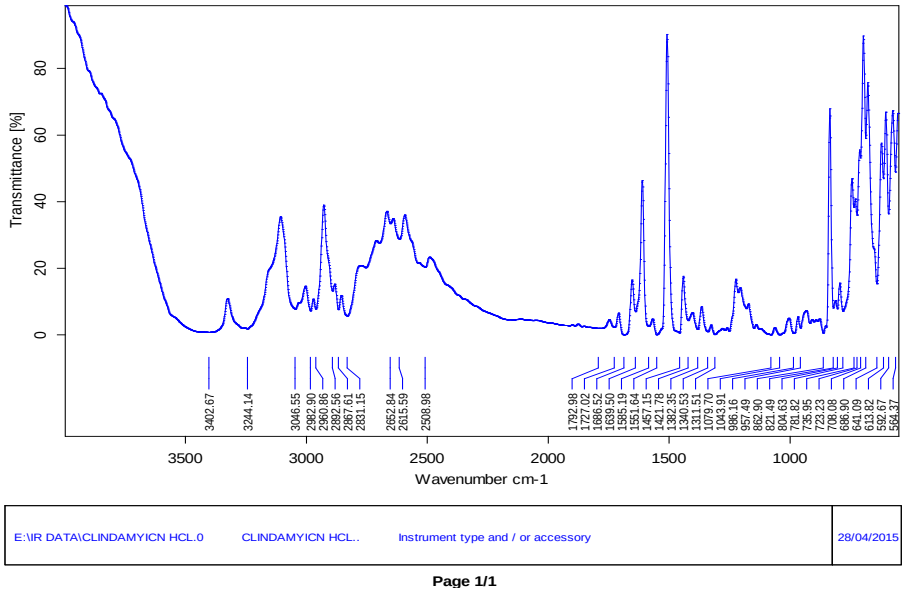

Fig.6. FTIR spectra of ClindamycinHc

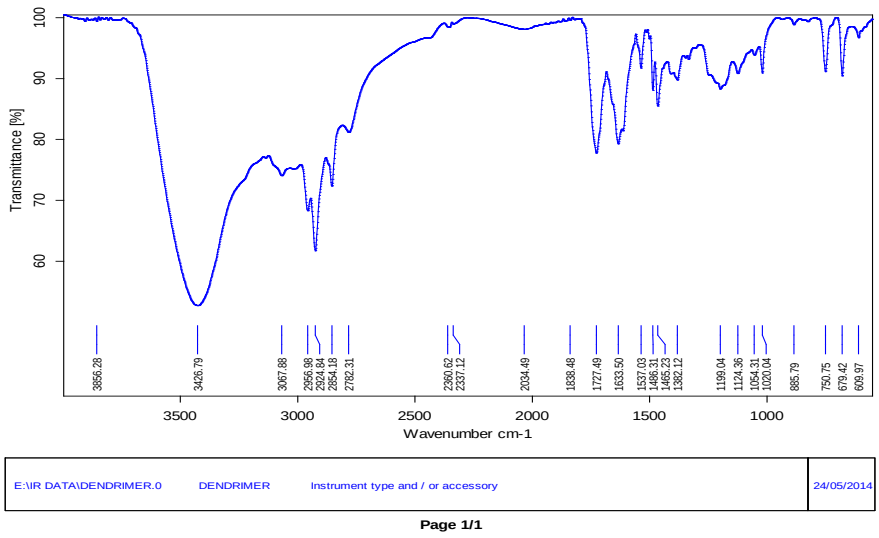

Fig.no.7: FTIR specta of G5 citic acid dendrimer

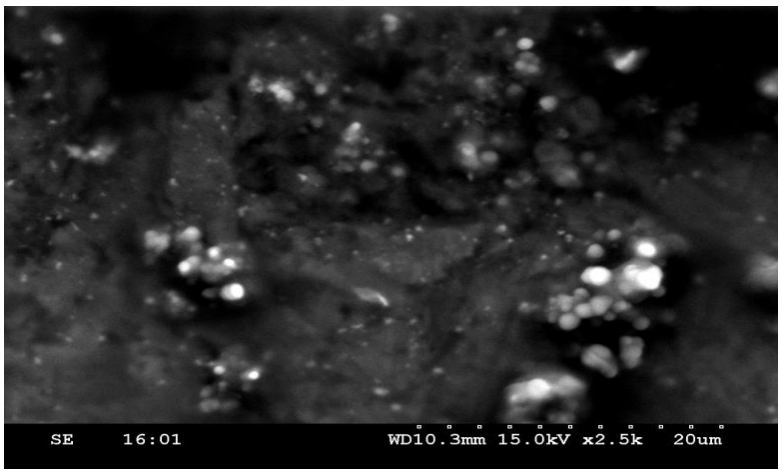

Fig.no.8.SEM image of Clindamycin Hcl loaded dendrimer.

CONCLUSION

From the present study it can be concluded that the prepared Clindamycin Hcl loaded citric acid dendrimer were highly effective than the other novel drug delivery system. The synthesized system was found to be safe and suitable for delivery of drug to any targeting sites.

REFERENCES

1. Metin tulu.,Ali serol erturk. Dendrimers as antibacterial agents(6).
2. Harrison's principles of internal medicine (18^{th} edition) volume 1.
3. Health education and community pharmacy, N.S PARMAR.
4. Varun Trivedi, Upendra Patel, Bhavin Bhimani.dendrimer:polymerof 21^{st} Centuary. International of pharmaceutical research and Bio-science., 2012; 1: 1-21.
5. Introduction to novel drug delivery(N.K Jain).
6. Tomalia DA, Baker, Dewald J, Hall M, kallos G, and martin s; A new class of polymer: starburst dendritic macromolecules, polymer. J., 1985; 17(1): 117-132.
7. Basavaraj k Nanjwade, Hiren M Bechra, Ganesh k Derkar, Manvi FV, Veerendra k Nanjwade. Eur j pharm sci., 2009; 38: 185-196.
8. pushkars phillip A, pathak k and pathak D, dendrimer: Nanotechnology derived novel polymers in drug delivery, Indian, J.pharm.Edu.Res., 2006; 40(3): 153-158.
9. New come , G.R., Moorefield, C.N and vogtle, F., 1996. Dendritic molecule concept, synthesis prespective, volt publisher.
10. Svenson, S., Controlling surfactant self-assemble, curr. Opin. Colloid interface sci., 2004; 9: 201-212.
11. M.K khan, S.S. nigavekar, L.D. Minc, M.S. kariapper, B.M. Nair, W.G lesniak, L.P Balogh, Invivo distribution of dendrmer and dendrimer Nanocomposites-implications for cancer imaging and therapy, Technol. Cancer Res. Treat., 2005; 4: 603-613.
12. Mishra Ina. J drug Delhi and thera., 2011; 1(2): 70-74. http:// jddtonline. Info/.
13. Introduction to novel drug delivery (N.K Jain).
14. c.Howker and J.M.J. frechet, J.Chem. 80c chem. Commun., 1990; 1010.
15. Brabander-van den Berg, E.M.M. and Meijer, E.W. Poly(propylene imine) dendrimer:Large scale synthesis by Heterogeneously catalyzed Hydrogenation Angew chem Int Ed Engl., 32; 1308-1311.
16. C.Hawker and J.M.J.Frechet, J. chem. Soc. Chem. Commun., 1990; 1010.
17. C.J Hawker, K.L. wooley and J.M.J. Frechet, J. chem.Soc.perkin. Trans.1., 1993-1287.