International Journal of Scientific Research in Chemical Sciences
Vol.4, Issue.3, pp.4-7, JUne (2017)

Research Paper

E-ISSN: 2455-3174

Synthesis and Characterisation of a Hydrophilic Support for Solid Phase Organic Reactions.

K.C. Rohini[1*], V.D. John[2], K.P. Subhashchandran[3]

[1*]Department of Chemistry, Christ College, University of Calicut, Calicut, India
[2] Depatment of Chemistry, Christ College, University of Calicut, Calicut, India
[3] Department of Chemistry, Sri Vyasa NSS College, University of Calicut, Calicut, India

[*]*Corresponding Author: rohinichndrn@gmail.com, Tel.: +00-96056-13695*

Available online at: www.isroset.org

Received 14th May 2017, Revised 26th May 2017, Accepted 15th Jun 2017, Online 30th Jun 2017

***Abstract*—**Glycerol dimethacrylate cross-linked 4-vinyl pyridine (GDMA-4-VP) support with optimum hydrophobic-hydrophilic balance has been introduced as a new class of polymer support in manual solid phase organic synthesis. The synthesis of GDMA-4-VP resin is based on the crosslinking of GDMA with 4-VP (in various cross linking densities) using free radical polymerization, affording a polymer containing secondary hydroxyl groups in great yield and purity. Benzoyl peroxide was used as initiator for the synthesis of polymer either via bulk polymerization or via suspension polymerization in polyvinyl alcohol, the latter yielding a beaded resin. Characterization was done by SEM and FT-IR spectroscopic techniques. The resin was found to undergo good swelling in various solvents, polar and non- polar. The synthetic utility of GDMA-4-VP resin was demonstrated by preparing biologically active Angiotensin II. The purity of the peptide was checked by HPLC.

***Keywords*—** Solid phase synthesis, GDMA-4VP resin, suspension polymerization

I. INTRODUCTION

Solid phase peptide synthesis is the most popular way followed now-a-days to synthesise peptides on small scale and large scale. Commercial anti-HIV peptide, Fuzeon, is a most obvious example which is manufactured in multi kilograms using solid phase synthetic strategy [1]. The success of solid phase technique depends on the properties of solid support [2, 3]. Peptide synthesis using the classical PS-DVB resin meets some drawbacks because of the rigidity, hydrophobicity and physico-chemical incompatibility of the polymer with the growing peptide chain. [4,5].The strong hydrophobic, macromolecular environment of polymer can persuade the growing peptide chain to adopt unfavourable conformation that lead to low yield of purity of target peptides[6]. Peptide chemistry utilises different classes of hydrophilic polymers as support for chemical reactions. The solubility and diffusivity of hydrophilic polymers in water facilitates their biomedical applications. In this work, the Influence of Glyceroldimethacrylate cross-linker in the 4-Vinyl Pyridine support for peptide synthesis was studied by synthesising a biologically active Angiotensin II peptide fragment by improved F-moc strategy.

Angiotensin was individually isolated in Indian apolis and Argentina in the late 1930s (as 'angiotonin' and 'hypertensin', respectively). Angiotensin is a peptide hormone that reasons vasoconstriction and a succeeding rise in blood pressure. It is fragment of the renin-angiotensin system, which is a major aim for drugs that lessen blood pressure. Angiotensin also arouses the discharge of aldosterone, another hormone, from the adrenal cortex. Aldosterone stimulates sodium retention in the distal nephron, in the kidney, which also pushes blood pressure up. Angiotensin II performances on the adrenal cortex, producing it to discharge aldosterone, a hormone that causes the kidneys to recollect sodium and expel potassium. Raised plasma angiotensin II levels are liable for the high aldosterone levels present during the luteal phase of the menstrual cycle [7, 8]. The chemical arrangement of Angiotensin II is presented in figure 1.

(NH_2)Asp-Arg-Val-Tyr-Ile-His-Pro-Phe ($CONH_2$)

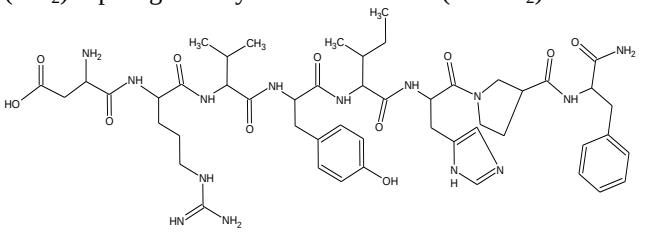


Fig. 1. Chemical composition and structure of Angiotensin II contains 8 amino acid residues (m.wt:1046 g/mol)

After a brief introduction, the synthesis of GDMA-4VP resin by suspension polymerisation is discussed in detail. The experimental part also contains the synthesis of Angiotensin II using Fmoc strategy, on the resin synthesised followed by the result and discussion. In this work we concerned purity which should applicable for biological research and yield for

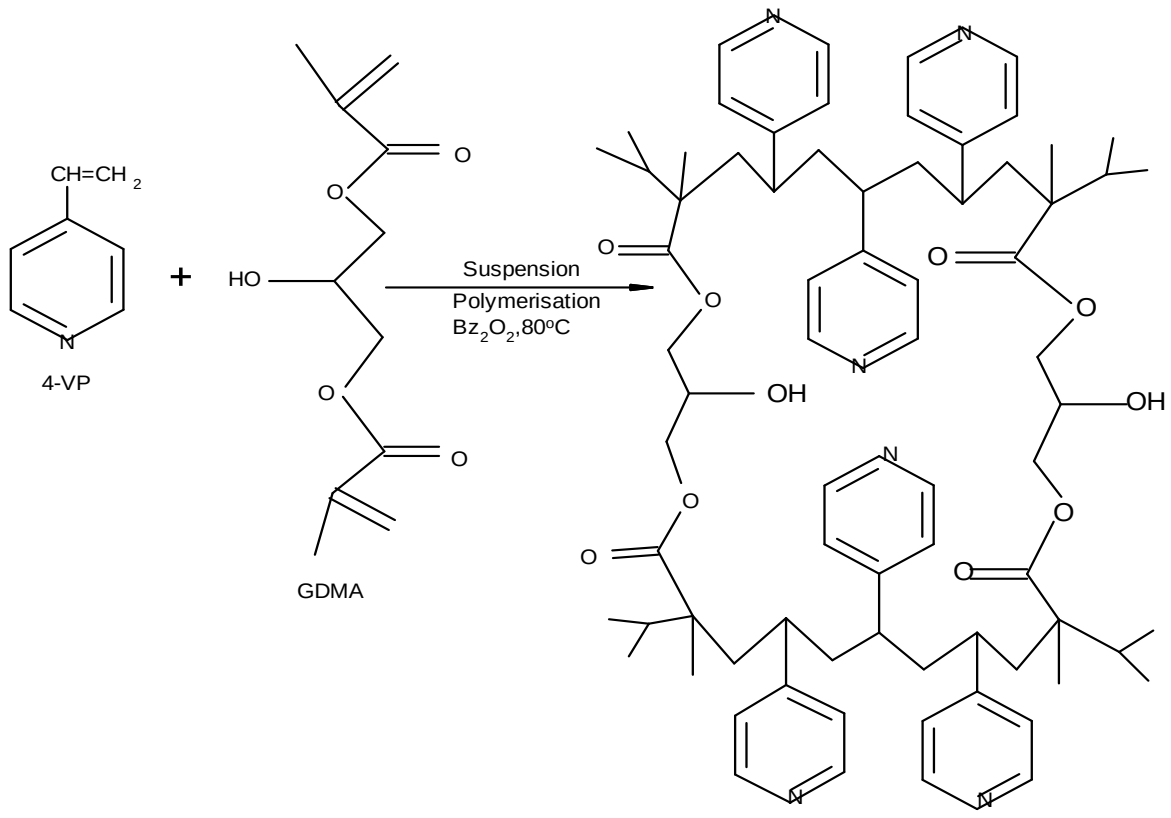
further development. The yield and pureness of the peptides reveals the advantage of the resin.

II. Experimental Materials

Glyceroldimethacrylate, Polyvinyl alcohol, Benzoylperoxide, 4-Vinylpyridine, toluene, sodiumhydroxide, dimethylformamide, dichloromethane, methanol and chloroform were bought from Merkmillipore (India). Fmoc- Amino acids were purchased from Sigma-Aldrich, Switzerland and Alfa-aesar England. 4-Dimethylaminopyridine (DMAP), Hydroxzybenzotiazole (HOBt), Diisopropylethylamine (DIPEA), Diisopropylcarbodiimide(DIC), Trifluroaceticacid (TFA),Triisopropylsilane (TIS), Pyridine and Piperidine reagents was purchased from Sigma-Aldrich, Germany and china,Alfa-asear England and Merkmillipore (India).Other solvents were pick up from Merkmillipore(India) and lobachemi Mumbai.

Preparation of GDMA cross-linked 4-VP support

The polymerisation was done in a conventional suspension polymerisation reactor. Glyceroldimethacrylate and 4-Vinylpyridine were destabilised using 1%NaOH. 1 g PVA was dissolved in 100 ml distilled water at 80^{o}C to prepare a 1% aqueous solution. A mixture of GDMA, 4VP and benzoyl peroxide were poured to the PVA solution keeping the solution stirred mechanically at 1000 rpm. The temperature of the system was maintained at 80^{o}C and the reaction was continued to 6 hrs. Resin beads began to appear on the wall of the vessel. The system was kept overnight as such. The beaded resin was then filtered and washed with hot water to remove PVA. The unreacted monomers were washed off and the resin beads were dried under vacuum. The polymer was soxheletted with acetone followed by methanol to remove all linear polymers. The beads were meshed to 100-200 range. The solubility and the swelling of resin in various solvents used for solid phase synthesis were conducted. The structure of the resin is given in Scheme 1.



Scheme 1. *Suspension polymerisation of GDMA and 4-VP*

III. SYNTHESIS OF ANGIOTENSIN II

Sequence:

Angiotensin II - NH_2 -Asp-Arg-Val-Tyr-Ile-His-Pro-Phe – COOH

The peptide was synthesised on GDMA-4-VP resin (250mg resin, 0.65mmol/g capacity) using F-moc chemistry. Each coupling was performed with 2 equivalents of HOBt with respect to resin capacity and equivalence of amino acid.

The first amino acid Fmoc- Phe - OH was anchored by esterification to the resin using the following procedure.

The resin was transferred to a clean, dry, sililated peptide synthesiser, added sufficient amount of NMP and kept for an hour for swelling and the excess NMP was removed. Fmoc-Phe-OH (3 eq.), DMAP (0.1 eq.) and DIC (3 eq.) were added to the swollen resin and shaken for 60 minutes. Washing of the resin was performed with NMP (2 times), DMF (2 times), MeOH (1 time). The above esterification reaction was repeated to enhance the reaction. The reaction was monitored by thin layer chromatography.

Second amino acid Fmoc-Pro-OH was coupled to the sequence as follows.

Coupling: Fmoc protection was removed from the resin bound amino acid. Fmoc- Pro-OH dissolved in minimum quantity of NMP in a 25ml RB flask. To that HOBt and DIC were added and shaked it well for 3mins and immediately the content was transferred in to the resin with moisture free atmosphere and shaked it well for 5mins, to that DIPEA was added and shaken well for 45mins. Reaction progress was monitored by TLC. Small pinch of the resin was taken and washed and tested with ninhydrin. In the case of negative result, washing and deprotection was performed. The remaining amino acids were coupled following the above method.

The detailed synthetic strategy, time duration of reaction process and conditions are given in table1.

TABLE-1

Synthesis of Angiotensin-2 (NH_2) Asp-Arg-Val-Tyr-Ile-His-Pro-Phe ($CONH_2$):

Aminoacid (Fmoc-)	**Coupling(mins)**			**Ninhydrin**	**Washing**	**Deprotection 15mins(×2)**	**Washing**
	1st	**2nd**	**3rd**				
Fmoc-Phe-OH	45	40	-	-ve	Done	Done	Done
Fmoc-Pro-OH	30	35	-	-ve	Done	Done	Done
Fmoc-His(trt)-OH	30	30	-	-ve	Done	Done	Done
Fmoc-Ile-OH	30	30	45	-ve	Done	Done	Done
Fmoc-Tyr(tbu)-OH	30	40	-	-ve	Done	Done	Done
Fmoc-Val-OH	30	35	40	+ve*	Done	Done	Done
Fmoc-Arg(pbf)-OH	30	35	-	-ve	Done	Done	Done
Boc-Asp(tbu)-OH	30	40	-	-ve	Done	-	-

Cleavage of crude peptide from resin: After synthesis the resin were washed with hexane, DCM, $CHCl_3$ and MeOH and dried. The cleavage was performed with 95%TFA: 2.5%TIS: 2%water: 0.5% m-cresol. The resin was treated with these solvents for 3hours under nitrogen atmosphere and the resin were washed 4times with TFA. The filtrate was collected in 50ml RB-Flash and all the traces of TFA was evaporated by using Rota vacuum evaporator [9,10].The peptide were isolated with excess of peroxide free pure cold diethylether and the peptide were washed 3times with diethylether and centrifuged. The white powder form of peptide was taken in small tubes and sealed [11].

IV. RESULTS AND DISCUSSION

GDMA-4VP RESIN support was prepared by suspension polymerisation using benzoyl peroxide as initiator. The insoluble polymer support was obtained as spherical uniform beads. The resin has excellent swelling properties and stability and satisfies all conditions of solid phase peptide synthesis. It was quite stable even afterwards vigorous conditions of functionalization. The scanning electron micrograph image of the resin is given in Fig.2

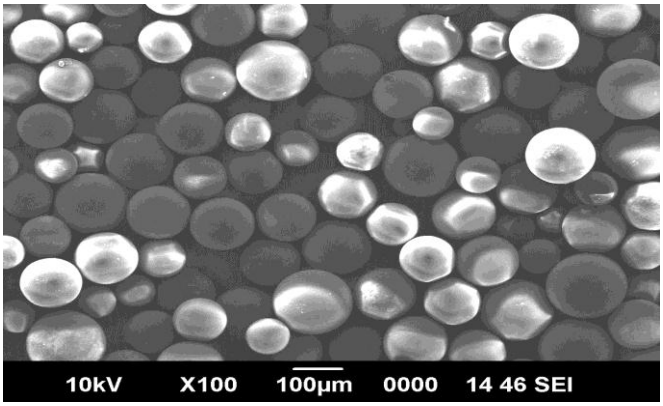


Fig.2. Scanning electron micrograph of GDMA-4VP

The IR spectrum of GDMA-4VP resin gave a sharp and intense peak at 1716 cm^{-1}corresponding to the ester carbonyl group of the cross linker. The peak at 3430 cm^{-1} corresponding to hydroxyl group of the cross linker in addition to those of monomers

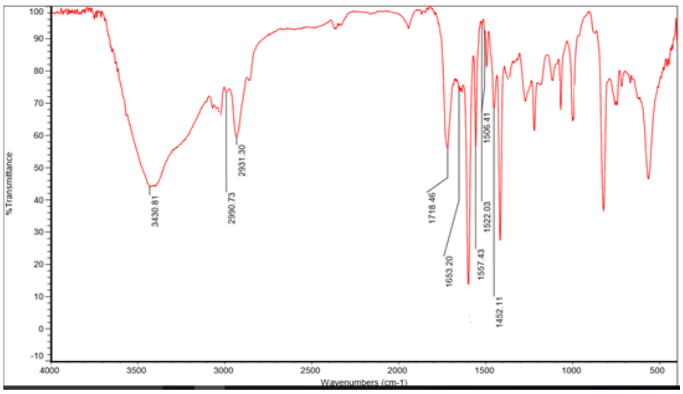
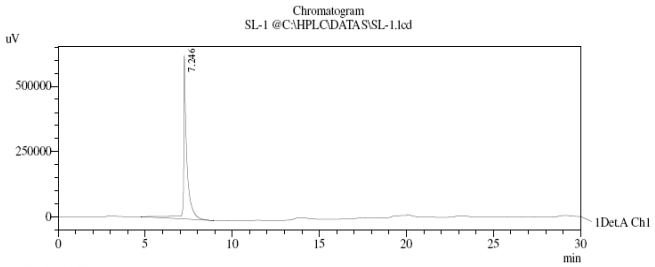


Fig. 3. FT-IR spectral analysis of GDMA-4VP

The accuracy of HPLC assay method was assessed by standard method (the known peptide sample purity were recorded and compared with standard reference). The purity of the peptide is clearly visible in figure 4.



PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.246	8271888	626757	100.000	100.000
Total		8271888	626757	100.000	100.000

Fig.4. HPLC of Angiotensin-2 -NH_2Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-COOH

The single sharp peak at ret.time 7.24mins shows our target peptide purity. Crude peptide yield was found to be 83.67%

V. Conclusion and Future Scope

The hydrophilic, flexible support GDMA-4VP for solid phase organic synthesis developed, shows extra ordinary swelling and stability in the solvents used for solid phase peptide synthesis besides the ease of preparation and functionalization. So the synthesis can be done in a cost effective way by minimising the quantity of solvents used. The biologically active peptide fragment AngiotensinII prepared by improved F-moc solid phase peptide strategy shows hygienic purity and good yield. So the aim of peptide synthesis with low cost, less pollution, maximum yield and purity is achieved. The coupling method is also relatively fast as compared with previously reported procedures. It will helpful for further research and improvement

References

[1]. F. Albericio ,F. G.Martin, " Solid supports for the synthesis of peptides. From the first resin used to the most sophisticated in the market",Chimica oggi,vol .26, Issue.4,pp.29-34,2008.
[2]. P.C.Hodge ,D.CSherrington,"Polymer supported reactions in Organic Chemistry",Wiley:Chichester,1998.
[3]. M.Medal,"Solid phase peptide synthesis;Fields G.Ed,Academi press:New York, pp.83-104,1997.
[4]. S.Zalipzky,J.L.Chang,F.Albercio and G.Barany,React.polym.,22,243,1994.
[5]. H.Hellerman,H.W.Lucas,J.maul,V.N.R.pillai, M.Mutter, "Poly(ethylene glycol)s grafted onto cross-linked polystyrenes,2 multidetachably anchored polymer systems for the synthesis of solubilised peprtides", Macromolecular Chemistry and Physics"Vol.184,Issue12,pp.2603-2617,1983.
[6]. R.C.Sheppered,Peptides 1971,Proc.of the 11 th Europ.pept.Symp,Nesvadba H,Eds,`3vfc j3jNorth Holland,Amsterdam 1973.
[7]. E.L.Boulpaep,W.F. Boron," Medical Physiology: a Cellular and Molecular Approach", St. Louis, Mo: Elsevier Saunders. pp. 771,2005.
[8]. N. Basso , N.A.Terragno, "History about the discovery of the renin-angiotensin system". Hypertension,Vol38, Issue6,pp1246–9,2001.
[9]. E. Atherton,R.C. Shepard, "Solid Phase Peptide Synthesis- A Practical Approach using the Fmoc-Polyamide Techinique" IRL Press, Oxford, 1984.
[10]. M.Bodanszky, "Principles of Peptide Synthesis", Springer Verlag: New York, 1984.
[11]. B. Merrifield,"Solid phase synthesis", Science 232, pp.341-347,1986.

AUTHORS PROFILE

Mrs.Rohini.K.c completed her graduation and post graduation in Chemistry from Sri.Vyasa.NSS College, Thrissur,Kerala. She got B.Ed degree from Calicut University in 2007. She is now working as Higher Secondary School Teacher. Her area of Interest is in solid phase peptide synthesis.

Dr.K.P.Subhashchandran He did his Msc from University of Kerala, M.Phil from Andhra University and PhD from Mahathma Gandhi University, kottayam. He got Excellency Award in research2003 and UGC research award in 2006. He has publications in national and international journals and paper presentations in symposiums. He has 15 years of teaching experience. His areas of interest are Synthetic Organic Chemistry, Polymer Chemistry, Peptide Chemistry and Nano technology

Dr. V.D. John: He completed his MSc from Calicut University. His Ph.D work was in the topic" Synthesis, characterisation and antitumour activity of metal chelates of 1, 7-diarylhepta-1, 6-diene-3,5-dioones". Seven students are doing research work under his guidance now. He is a Member of Indian Academy of Chemistry. He got M M Ghani Award for Best College teacher-2011. He was the Chairman of Board of Studies Chemistry 2009, Chairman of Board of Examinatins-2010, 11, 12,13,14,15. His area of interest is in the biochemical studies of metal complexes of curcuminoids.

