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Synthesis and Characterization of Glycerol Dimethacrylate-4-vinyl pyrrole

K. Vijitha, K. Dhanya, Biju Francis, K.S. Vijayalekshmi,
K.M. Sreedhar† and K.P. Subhashchandarn*
Department of Chemistry, Sri Vyasa NSS College, Vyasagiri, P.O. Thrissur-680 623, India
E-mail: kpsubhash@rediffmail.com

The successful application of 10 % glycerol dimethacrylate (GDMA) crosslinked 4-vinyl pyrrole (4-VP) support in manual solid phase organic reactions are reported. The copolymer was prepared using benzoyl peroxide as initiator *via* suspension polymerization in polyvinyl alcohol yielding a beaded resin. GDMA-4VP resin undergoes facile swelling in a variety of solvents both polar and non-polar used in peptide synthesis. The resin was chloromethylated using chloromethyl methyl ether and the chlorine capacity was determined by pyridine fusion method. The chloromethyl resin converted to aminomethyl resin by inter group conversion.

Key Words: Synthesis, Glycerol dimethacrylate, 4-Vinyl pyrrole.

INTRODUCTION

The continued and rapid discoveries of new peptides with interesting biological functions have created an unprecedented demand for chemical synthesis of peptides required for structure-function correlations. Several strategical improvements have been suggested and tested to meet the demand for peptides in high purity and quantity and has approved the method for the synthesis of even large sized peptides. The polymer-supported solid phase approach of peptide synthesis developed by Merrifield has helped to overcome many of the preparative difficulties encountered in the classical solution phase method peptide synthesis. Inspite of these improvements in solid phase method of peptide synthesis, successful peptide assembly is still hampered by inherent problems like poor solvation of the growing peptide chain, often leading to incomplete reaction[1]. Studies have shown that these undesirable problems are related to aggregation due to inter chain interaction behaviour of protected peptide chain leading to the formation of β-sheet structure[2-4]. In solid phase method of peptide synthesis the interchain interaction is known to be dependent on the density of the peptide chains. As the size of the peptide chain increases the swelling of the peptidyl resin decreases because the aggregation of peptide chain mimic higher degree of crosslinking. Construction of a peptide chain on an insoluble solid support has obvious benefits. All the reactions can be carried out to 100 % completion, so that a homogeneous product is obtained. The system is ideally suited for automated

†Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Clappana, Kollam-690 525, India.

operation. The separation of the intermediate peptides from soluble reagents and solvents can be affected simply by filtration and washings with consequent savings in time and labour. The physical losses can be minimized as the peptide remains attached to the support throughout the synthesis and the support can be regenerated by a simple, low cost, high yield reaction.

In this paper, the development of a new crosslinked resin is described, based on glycerol dimethacrylate and 4-vinyl pyrrole and its applications in the synthesis of peptides which have high tendency to aggregate. The efficiency of the polymer was compared with PS-DVB resin[5-8]. The polymer was obtained in regular bead shaped structure. The GDMA-4VP crosslinked polymer support shows rigidity and high mechanical strength.

EXPERIMENTAL

The polymer supports under study were synthesized and characterized in the laboratory. Scanning electron micrographs were taken on a Cambridge S-360 instrument at Materials Research Centre, IISC Bangalore. 4-Vinyl pyrrole (4-VP), glycerol dimethacrylate (GDMA) and polyvinyl alcohol (PVA) were purchased from Sigma Company, USA, benzoyl peroxide was purchased from SISCO, Bombay and was recrystallized before use. Chloromethyl methyl ether (CMME) was prepared according to literature procedure[9]. IR spetra were recorded on a Shimadzu IR 470 spectrophotometer using KBr pellets and the ^{13}C CP-MAS NMR measurements were carried on a Bruker 300 MSL CPMAS instrument. HPLC was performed using Shimadzu SPD-10A UV-Vis detector HPLC. The mass spectral analysis was carried out on a Kratos Analytical (UK) MALDI time of flight mass spectrometer.

Polymer synthesis

Preparation of 4-vinyl pyrrole (4-VP) crosslinked with 10 % GDMA by suspension polymerization[10]: 4-Vinyl pyrrole and GDMA were destabilized by washing with 10 % NaOH (20 mL × 3) and then washed with distilled water (20 mL × 3). A mixture of 4-VP, GDMA, toluene and were suspended in a 1 % aqueous solution of PVA and kept mechanically stirred at 600 rpm using benzoyl peroxide as free radical initiator at 80 ºC[11,12]. After 6 h the beaded resin was filtered and washed with hot water to remove PVA. The polymer was soxheletted with acetone followed by methanol to remove all linear polymers and low molecular weight products. The beads were meshed to 200 to 400 range using standard sieves. Polymers of 5, 10, 15 and 20 % GDMA cross links were prepared by adjusting the relative amount of the monomer (**Scheme-I**, Table-1).

Functionalization of 4VP-GDMA support with chloromethyl group: General procedure

Preparation of 1 M $ZnCl_2$ solution in THF: Anhydrous $ZnCl_2$ (1.5 g) was placed in a 25 mL Erlenmeyer flask and conc. HCl (3 drops) and distilled water (5 drops)

were added and the contents stirred and heated until the solid dissolved completely. Temperature was gradually raised to evaporate the water and to leave a crust of solid, which was then melted by strong heating. When $ZnCl_2$ became a clear, mobile liquid with no further evolution of bubbles, the flask was placed in a dessicator and allowed to cool. The resulting mass was dissolved in THF (10 mL), freshly distilled from $LiAlH_4$. The exact concentration of the solution was determined by pipetting a sample into water containing several drops of HNO_3 and titrating with 0.1 M $AgNO_3$.

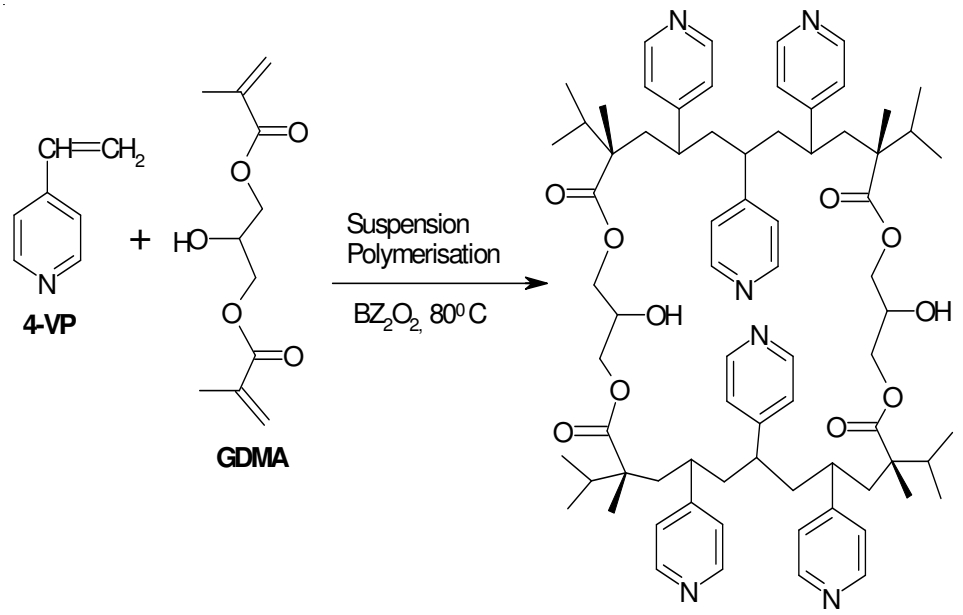
Preparation of chloromethyl methyl ether (CMME): A mixture of methanol (33 mL) and formaldehyde (63 mL) was placed in a two necked round bottomed flask fitted with $CaCl_2$ guard tube. The flask was cooled by placing in an ice bath. Another two-necked flask fitted with a dropping funnel and side tube containing conc. H_2SO_4, kept stirred magnetically. The side tube was placed in a glass washing bottle containing conc. H_2SO_4. Conc. HCl was slowly added to the H_2SO_4 kept stirred in the flask. The HCl gas dried by conc. H_2SO_4 in the drying bottle was bubbled through the methanol-formaldehyde mixture. The slow stream of HCl was continued for 4 h when then first layer of CMME begins to appear. The turbidity which appeared first gradually disappeared separating the aqueous and the ethereal layers. The HCl gas was passed for 4h. more until the solution got saturated. Chloromethyl methyl ether is formed as the upper organic layer. The lower aqueous layer was separated from the organic portion. The ether was dried by using anhydrous $CaCl_2$ and stored in sealed bottles. Yield 40 mL.

Chloromethylation: Chloromethyl[13] functional group was introduced into the resin by CMME in the presence of anhydrous $ZnCl_2$ as the catalyst. To 1 g of resin, which was dried in an oven, added DCM (12 mL) until the resin was swelled well. The swelled resin was then kept for 0.5 h and then added 6 mL $ClCH_2OCH_3$ and 3 mL catalyst dissolved in THF. The mixture was refluxed at 50 ºC with $CaCl_2$ guard tube for 5 h. The mixture was cooled and filtered carefully through a sintered glass funnel (G 2) and washed with (1) THF (30 mL, 3 × 10 min). (2) THF/4N HCl (30 mL 3 × 3 min). (3) THF/H_2O (30 mL, 3 × 3 min). (4) THF (30 mL, 3 × 3min) (5) hot water (6) methanol, the resin was dried overnight under high vacuum at room temperature and the sample was analyzed for chlorine capacity by Volhardt's method[14,15] (**Scheme-II**).

Aminomethylation: The chloro 10 % GDMA-4VP resin (0.14 mmol cl/g, 2 g) was swollen in DMF (50 mL), after 2 h. The resin was filtered and stirred with potassium phthalimide (mg, 2.5 mmol) in DMF (20 mL) at 120 ºC for 12 h. The resin was filtered, washed with DMF (3 × 50 mL) dioxane (3 × 50 mL), ethanol (3 × 50 mL) and methanol (3 × 50 mL). The dried resin was suspended in ethanol (20 mL) and refluxed with hydrazine hydrate (mL, mmol). After 8 h, the resin was filtered, washed with hot ethanol (3 × 50 mL) and methanol (3 × 50 mL) and dried in vacuum. The amino capacity of the resin was estimated by picric acid method[16] (**Schemes III** and **IV**).

RESULTS AND DISCUSSION

Suspension polymerization has been proved to be the most useful technique for synthesizing crosslinked polymer support principally because of the extremely convenient physical form of the beaded product which leads itself to further conversions[17,18]. The chemical nature of the monomer, mole percentage of the crosslinker, type of diluents, geometry of the reaction vessel and organic phase to aqueous phase ratio are the various parameters which determine the yield and topography of the polymer support. If the support and the substrates are compatible, favourable interaction between the polymer and the substrate molecules occur which improves the rate of the reaction.

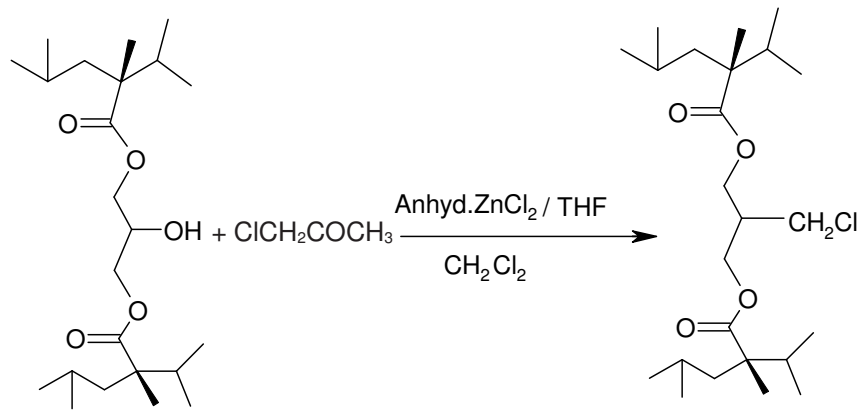


Scheme-I: Suspension polymerization of GDMA-4VP resin

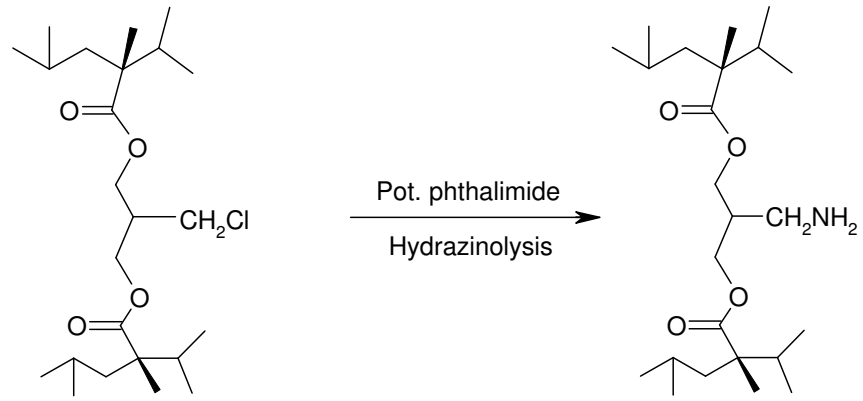
The insoluble polymer support was obtained as uniform spherical beads. Various crosslinking densities of polymer support like 2, 5, 10 and 20 % were synthesized.

TABLE-1
PREPARATION OF GDMA CROSS LINKED 4VP

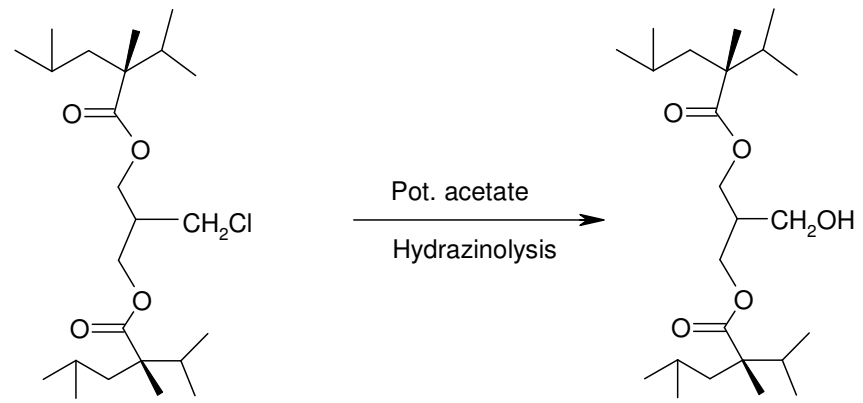
GDMA (mol %)	Amount of GDMA (mL)	Amount of 4VP (mL)	Yield (g)
2	0.506	8.62	7.1
5	0.260	8.55	7.5
10	2.530	8.10	8.2
20	5.066	7.20	9.3



Scheme-II: Conversion to chloromethyl resin



Scheme-III: Conversion to aminomethyl resin



Scheme-IV: Conversion to hydroxymethyl resin

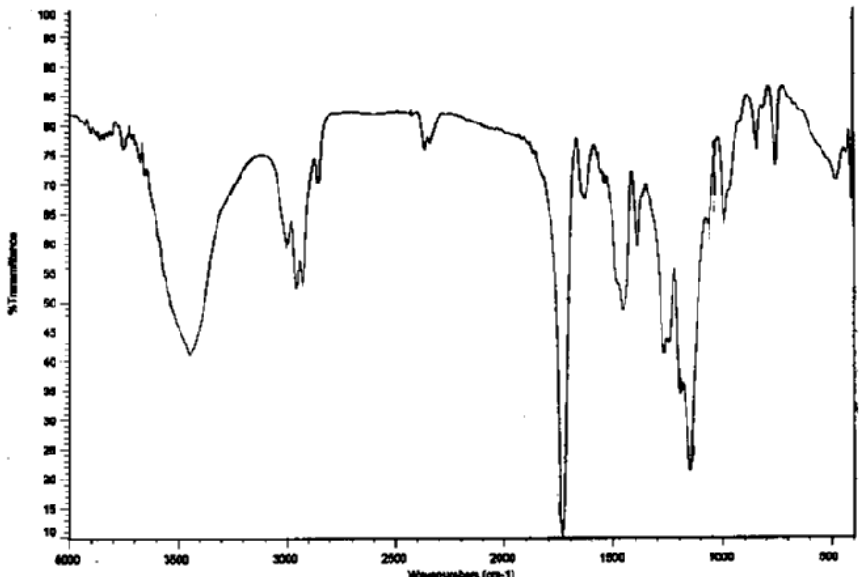


Fig. 1. IR spectrum of GDMA-4-VP

IR and ^{13}C CP MASS NMR spectroscopic techniques are used for the characterization of GDMA-4VP resin. IR spectrum gave a sharp and intense peak at 1732 cm^{-1} corresponding to ester carbonyl group of the crosslinker in addition to the peaks of 4-VP (Fig. 1). The ^{13}C CP MASS NMR peak at 67.469 represents the -OH bearing carbon in the crosslinker and a peak at 180.729 ppm indicates the -CO group of the ester group (Fig. 2). Electron micrograph of GDMA-4-VP resin is shown in Fig. 3.

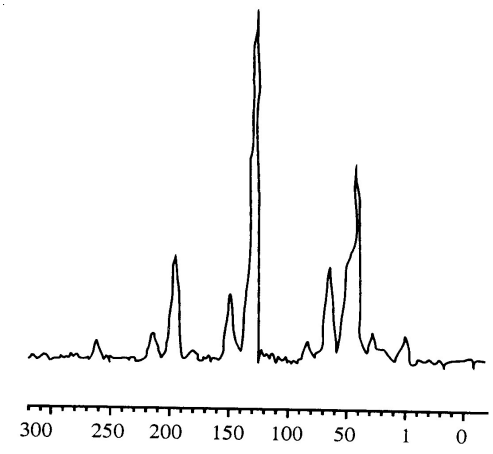


Fig. 2. ^{13}C CP-MASS NMR spectrum of PS-GDMA support

The resin is functionalized with chloro groups using chloromethyl methyl ether and with amino groups by Gabriel phthalimide synthetic strategy.

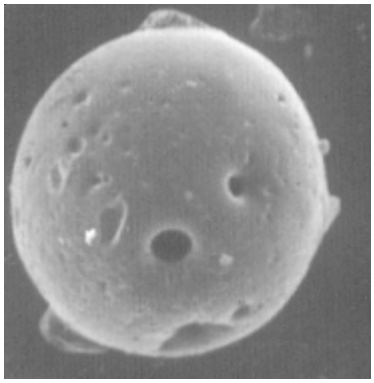


Fig. 3. Electron micrograph of GDMA-4VP resin

General protocol for functional group interconversion

Swelling characteristics: In solid phase synthesis the accessibility of the resin bound substrate to reagents and solvents is very important. The extent of swelling of the resin was a measure of its solvation by a given solvent[19,20]. The GDMA-4VP resin showed extensive swelling in various solvents compared to 1 % PS-DVB (Fig. 4).

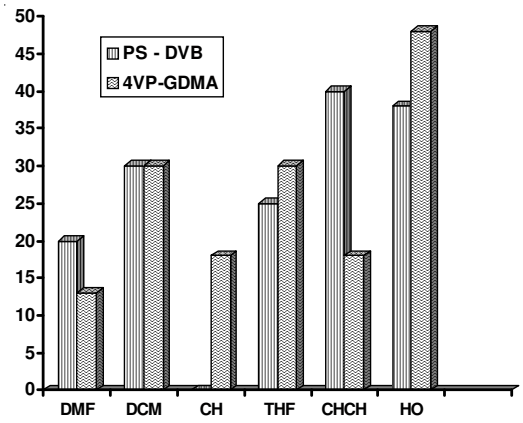


Fig. 4. A comparitive study of swelling characteristics of PS-DVB resin with 4VP-GDMA

Conclusion

The GDMA-4VP resin serves as a new class of polymeric support for solid phase peptide synthesis. The optimum hydrophobic-hydrophilic balance of the resin causes the high swelling in different polar and non-polar solvents. The ease of preparation, functionalization, workup procedures are the advantages of the resin over conventional polymer supports. Peptide synthesis using GDMA-4VP resin showed that the support is flexible and suitably adopted for the conventional synthetic procedures.

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