Synthesis, Molecular and Microstructural Study of Poly-*N*-Vinylpyrrolidone Oximo-*L*-Valyl-Siliconate with IR, ¹H-NMR and SEM

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By reducing PVP with H₂NOH.HCl and NaOH 2:2:1 mass ratios in aqueous ethanol, poly-*N*-vinyl pyrrolidone oxime [PVPO] was prepared with 92% yield. Applying the sol-gel concept, orthosilicic acid [OSA] was made by hydrolyzing TEOS with ethanol in 1:0.5 molar ratios using 1 N KOH aqueous solution as a catalyst. The OSA + PVPO + *L*-Valine (α -amino acid) were mixed with pure ethanolic medium in 1:2:2 mass ratios and refluxed at 78 °C and 6 pH for 6.5 h. A white residue of poly-*N*-vinyl pyrrolidone oximo-*L*-valyl-siliconate [POVS] appeared after 5 h. The heating of reaction mixture was stopped and the contents were brought to NTP. The residue formation of POVS was intensified with lowering a temperature and completely solidified within 5 h, was filtered using a vacuum pump with Whatmann filter paper no. 42. The residue of POVS was washed several times with 20% aqueous cold ethanolic solution and dried in vacuum chamber at 25 °C for 24 h. The MP was noted above 350 °C. Structural and internal morphology were analyzed with IR and ¹H-NMR, and SEM respectively. A drug loading and transporting ability of the POVS in water and at pH = 5 and 8 was determined chromatographically.

Key Words: Poly-*N*-vinyl pyrrolidone oxime, Orthosilicic acid, TEOS, Poly-*N*-vinyl pyrrolidone oximo-*L*-valyl siliconate, SEM

Introduction

Modern research requirements and trends have seen a surge of interests in innovative supramolecular and nanoparticle-based systems with recent developments in synthesis and understanding of chemistry of nanoscale objects. Such approaches are of immense use for analysing broad range of novel phenomena and wide varieties of applications in different fields. This manuscript emphasizes mainly on behavioral properties of newly synthesized POVS molecule. Similar suprastructural molecules were synthesized and reported in our previous paper. The POVS fundamentally, integrates grey areas of molecular research approaches in the fields of supramolecular, dendrimer, biosensor and smart biomolecular chemistry. This manuscript emphasizes on the POVS, a novel molecule which is assumed to have substantial industrial potential because of different hydrophilic and hydrophobic quanta dots within a molecule. The transporting and loading capacities of the POVS with amino acids were studied by paper chromatographic methods and effective results were noted. The aim was to synthesize a molecule which could be an asset as a biosensor or biochip in various clinical areas and tissue engineering. Enormous biophysical, biochemical, biotechnological research and developments are reported where the molecules are applied to offer either catalyzation, or initiating new favorable physicochemical response to generate tissue repairs, synthesis, colloidal formation for heat sinks, food values, fuel additives, applications in textiles, polymers, coatings, biomembrane formation, membrane transport, paints pigments, cosmetics, sol gel, computer technology, liquid crystal devices (LCD), liquid electronic devices (LED) demonstrations, insulators¹⁻⁵ etc.

The POVS is assumed to boost up all above mentioned industrial and biomedical processes in a very effective manner so as to perform similar level of achieved targets with minimum amount of experimental sophistications. Current approaches show that nanoparticles could be developed as fluorescent markers for contrast agents or as optical imaging in magnetic resonance imaging (MRI). For chemotherapeutics, aqueous formulations of supramolecule POVS could be applied by suspending them as nanoparticles. Similarly their bioavailability, biofunctionality and a rate of uptake in body are very much dependent on particle size and morphological applications⁶⁻¹⁰ of supramolecular systems, which can be synthetically improved as per our required properties. Forthcoming decades show the ability of sensing optically, electrically and magnetically to detect the state of biological systems and living organisms could be enormously transformed by the developments in materials sciences.¹¹⁻¹⁴ Thus, an emerging ability to control the patterns of matters on a nanometer scale could be highly expected to have entirely novel types of biosensors and bioactivators. Silicon nanocrystals dispersed in water were widely used as a photosensitizer for a generation of active oxygen and is applied in the photodynamic therapy. The key demand in the current trends of research and development is to develop a highly biocompatible supramolecular dendrimers system especially for biomembrane engineering, body implants, plastic and cosmetic surgery, bio-sol-gels, drug design, cancer cure, biophysical strength to tissues, ligaments and artificial skin patches etc. Thus, synthesis of the POVS, a novel supramolecule using a concept of green and combinatorial chemistry with highly biocompatible units PVPO, silicon (core), and *L*-valine resulting in the formation of abruptly efficient biomolecule with highly biologically active sites and

multifunctional moieties.

The PVOS is rich in electronic sites and probability of maximum electron dense regions could be noted between the O and N atomic tiers. The electronic rich sites could be in accordance with the Heisenberg's uncertainty principle where the position and momentum of the charge cannot be determined simultaneously and as per Schrödinger wave equation, the charge density cloud is spread over in a form of wave function ψ^2 with higher probability of electron density. Thus, the POVS has free electronic and binding sites for initiating the chemotherapeutic response to get encapsulated, covalently attached or adsorbed onto the surface. The PVOS could be applied for optical nanosensors in a field of bioanalytics. The synthesis of the POVS could be a new direction in nanodevices for analysis of living systems on a molecular level. One of the important combining units in the POVS is L-valine, an essential amino acid that has stimulant activity and promotes muscle growth and tissue repair.¹¹⁻¹⁴ The POVS could be applied as a precursor in penicillin biosynthetic pathway, protein synthesis, muscle metabolism, tissue repair, and for maintenance of proper nitrogen balance in the body. Thus, the POVS is with multifunctional moieties and highly electron rich sites and could be applied with improved efficiency, quality and performance in various fields such as biomedical, pharmacogenomics, protein synthesis, biotemplates, biochips, bioactuators and biosensors.

Experimental Methods

The PVP (mol wt 40,000, K-30, CDH, batch no. 04079, product no. 029579), tetraethyl orthosilicate (E. Merck, Mol wt 208.33, Batch no. S 4953958), hydroxylamine hydrochloride (CDH, batch no. 09043, product no. 010129), α -amino acid L-valine (CDH, batch no. 02034, product no. 037160), and absolute ethanol (E. Merck) were used as received. The chemicals were dried in a vacuum chamber and stored in vacuum desiccator filled with P_2O_5 till use. The POVS was noted weakly water soluble and aqueous solutions, w/w, were prepared with Millipore water of 10^{-7} S cm^{-1} conductivity with 0.01 mg accuracy balance model 100 DS, Dhona Instruments. Pvt Ltd. Calcutta, India.

Synthesis of PVPO. The POVS synthesis was accompanied in a 3 step process and a synthesis of PVP-Oxime was made with the PVP + hydroxylamine hydrochloride by dissolving in 1:1 ratio w/w with 50% aqueous ethanol respectively in a RB flask (250 mL) fitted with magnetic stirrer. The 5 mL of 1 N NaOH solution was added to change the pH for smooth and quick reaction. The reaction mixture was kept over rotamantle attached with magnetic stirrer and stirred for 30 min. A white precipitate of PVP-Oxime was noted and filtered through a Whatman filter paper no. 42 with vacuum pump filtration followed by washing several times with 20% cold aqueous ethanol solution. The precipitate was kept in vacuum chamber and stored. The reaction mechanism of PVPO preparation is illustrated in Figures 1(a) and (b).

Synthesis of orthosilicic acid from TEOS by Sol-Gel technique. Ethanolic TEOS solution of 1:2 ratio (v/v), 20 mL ethanolic TEOS and 60 mL KOH solutions were taken in 250 mL RB flask. The reaction mixture was refluxed for 60 h at 78 °C using

Figure 1. (a) Reaction for preparation of PVP-Oxime. (b) Reaction mechanism of preparation of PVP-Oxime.

Figure 2. Synthesis of orthosilicic acid from TEOS by Sol-Gel technique.

water condenser of 25 inches long followed by cooling the reaction mixture for 48 h. The reaction mechanism is noted in Figure 2. Initially, the product appeared in colloidal form but with the passages of time it settled at the bottom as a white precipitate of OSA. A complete precipitate was formed in almost 3 days time which was filtered, washed several times, dried and stored in vacuum chamber. An absolute dryness was checked with anhydrous $CuSO_4$. The dried sample did not give blue color on mixing with the $CuSO_4$ but the sample which was not completely dried gave blue color with $CuSO_4$.

Synthesis of POVS molecule. The TEOS, a silicon compound, was hydrolyzed into orthosilicic acid [$Si(OH)_4$] in presence of a stabilizing agent. The $Si(OH)_4$, PVPO and α -amino acid (L-valine), were mixed and the POVS molecule formed which was noted as supramolecular 1st tier dendrimer molecule. The POVS was blended with several materials for wider applications in different fields with respect to desired properties and requirements. The PVP, valine and silicic acid were used as received. The PVP was converted into PVPO. The TEOS was converted into orthosilicic acid by sol-gel technique. Taking L-valine, PVPO and silicic acid in ratio of 2:2:1 (w/w) in aqueous ethanolic medium were refluxed in 250 mL RB flask at 78 °C for 6.5 h at pH 6. A white semi crystalline solid product was formed which on cooling for 4 h, the reaction mixture was filter-

Figure 3. (a) Preparation of the POVS. (b) The atomic tier structure of the POVS molecule.

ed off through Whatmann filter paper no. 42. It was washed several times with cold aqueous ethanolic solution, the yield was 90%. The product was vacuum dried and stored in P_2O_5 filled vacuum desiccator. The detailed reaction mechanism is depicted in Figure 3(a).

Characterization.

Spectroscopy: FTIR spectra were recorded in a form of thin film within KBr disc with Nicollet Protégé-460 Spectrometer (ν_{max} , cm^{-1}). The proton NMR spectra were monitored in deuterium on DPX-dix 300 MHz Bruker Avance Spectrometer with TMS as an internal reference (chemical shift δ/ppm). Scanning Electron Microscopy (SEM) illustrated microstructural properties and surface morphology which were studied by EVO-50

ZEISS Vacuum Pressure Scanning Electron Microscopy. The micrographs were recorded at two different magnifications.

Molecular weight determination. Average viscosity molecular weight of POVS was determined with Mark-Houwink-Sakurada equation⁸ $[\eta] = kM^a$ where k and a , are constants, derived by using different molecular weights $[M]$ of Markers. The $[\eta]$ is intrinsic viscosity and was derived by substituting data in Jones Dole equation.⁹ The viscosities of very dilute aqueous solutions of 0.2, 0.5, 0.8, 1.1 mg/100 mL POVS, were determined with Survimeter¹⁰ and polyvinyl alcohol (PVOH) of different molecular weights was used as markers for analysis of constants. A detailed experimental procedure and data analysis are given in our earlier communication.⁸⁻¹⁰ Average molecular weight of the POVS was $80,425 \text{ g mol}^{-1}$ which inferred an integrated supramolecular system.

Results and Discussion

IR Spectra. FTIR Spectra illustrated a vibrational (stretching) frequency at 2974.78 cm^{-1} and 2954.86 cm^{-1} are of C-H oscillations of polyvinyl side chains $(CH_2-CH_2)_n$ of PVPO. A peak at 2954.86 cm^{-1} showed a percentage transmittance of 12% with the POVS which inferred weaker influence of alkyl chains of the molecule. The stretching frequency at 2627.84 cm^{-1} inferred the -C-H vibrations of valine and the 2106.33 cm^{-1} showed the C-N of the C-N-O which connects the amino acid to Si atom. Though the -OH of valine is at terminal position but its stretching is disrupted by the alkyl chain of amino acid, and a weak folder in the form of a weak band at 3300 cm^{-1} without any further split stretching vibrations. The -OH group of valine was noted with very weak band in its FTIR spectrum where simply a folder is observed. It inferred similar activity of terminal -OH which is illustrated by the NMR studies. Probably the proton of the terminal -OH, may be under a stress caused by different electronic environments.

The carbon chain of valine developed a stronger +I effects and steric effects. The oxygen being more electronegative in nature than the N atom and the lone pair of the N is shifted towards oxygen atom which showed an impact on the stretching vibrations of the O-H bond by shifting its electron pair through -C-C- to the N side, especially in case of Valine. Thus, it did not behave like a free -OH and is very strongly bound, which is evident from FTIR and not seen in the NMR spectra. The C-C=O(OH) showed a band at 1586.28 cm^{-1} as the -C=O belongs to a pure ketonic structure except OH group attachment at one side. In this process, the stretching frequencies of -C=O are influenced and oscillate at 1586.28 cm^{-1} . The bands at 1350.27 cm^{-1} are of $-CH_3$ symmetrical deformations and asymmetric stretching vibrations at 715.95 cm^{-1} inferred the -Si-O- linkage. A sharp band at 1507.91 cm^{-1} inferred stretching vibrations of -C=N- of oxime group. The prominent bands nearer to each other at 1472.64 and 1425.00 cm^{-1} inferred the bending vibrations of -CH- of the pyrrolidone group. Many stretching and bending frequencies at lower frequencies are nearer and closer to each other, showed that the molecule has deformed groups like -C=O(OH). The free non-bonding electrons on oxygen atoms attached to Si and the two binding sites on valine experienced some inter electronic repulsion and affect the variations in molecular vibrational

Figure 4. FTIR spectra of the supramolecule.

Figure 5. ^1H NMR spectra of the supramolecule.

energy levels and oscillations. The energy variations in terms of stretching frequencies (Figure 4) are helpful for study of molecular activities of the POVS.

^1H NMR spectra. The protons of valine are in similar environments with negligible variations and hence the protons of a complete alkyl chain apparently behaved as a single unit with (δ) 2.597 ppm chemical shift (Figure 5). But the chemical shift is influenced by one proton which is in touch of oxygen atoms that is noted at 2.915 ppm and the protons which are directly attached to alkyl groups of valine and PVPO are at 2.597 ppm and 2.366 ppm respectively. The electronegativity of the oxygen deformed a proton of the -OH to weakly bind and easily accessible at a lower magnetic field and hence the proton of -OH resonate at 7.45 ppm. Thus, the protons of both the IL and PVPO are associated with -C-H, are in same environment and intensity of chemical shift was enhanced over each other. A peak at 3.616 ppm is due to the -C-H vibrations of the isopropyl group of valine. The vibrations are comparatively at a lower field, δ = 4.687 ppm.

To bring a highly stabilized sigma bonded protons into gyro-magnetic motions higher energy is required. So the -CH₃ of isopropyl group of valine depicted the chemical shift at 1.203 ppm

and both the alkyl groups are in the same environment. The Figure 3(b) describes the positions of the O and N atoms in the POVS where the O are placed near Si while the N atoms form a tier outside to a tier of the O atoms. The chemical shift of protons of the POVS as compared to the TMS where the tier structures within the POVS showed clearly a presence of electron rich sites and there is continuous delocalization of the electronic charge within the boundaries of the O tier and N tier. There is some complementarity between position and momentum of electronic charge between the boundaries because of wave-particle duality and this is in accordance with the Heisenberg's principle ($\Delta \times \Delta p \geq h/4\pi$) where the velocity and position of electron could not be determined simultaneously. This electronic theory could be in accordance with the Schrödinger wave equation as the e^- densities are confined to the O and N boundaries in the form of wave functions ψ_O and ψ_N respectively and there is high significance of ψ^2 in this condition. We can say that the POVS showed an electronic delocalization within the O and N boundaries and maximum probability of finding e^- charge cloud is within these tiers. The attachment of molecular structures through similar bonds to similar atoms in a similar environment showed similar behavior. Our NMR study seems to be similar to TMS, except

Figure 6. (a) Scanning electron micrograph of POVS at 50 kx. (b) Scanning electron micrograph of POVS at 30 kx. (c) Scanning electron micrograph of POVS at 50 of Ortho Silicic Acid.

Figure 7. (a) Paper chromatography of the POVS + L-alanine at pH 8. (b) Paper chromatography of the POVS + L-alanine at pH 5.

the chemical shift of the POVS at lower field and alternatively arrangement of valine and its O atoms are near Si, has shifted slightly to a lower field due to a tier of N atoms linked to O of the -O-Si- tier. The new environment of the proton attached to N in valine and PVPO units shift the chemical shift (δ) to slightly lower field (2.597 ppm).

It inferred that the alkyl chains of the valine and PVPO behaved as integrated units is an interesting observation about the POVS where the valine and PVPO units acted as methyl groups of TMS. In TMS as the alkyl groups ($-\text{CH}_3$) are at different positions attached to Si through oxygen. Similarly the valine and PVPO are at different positions attached in a similar manner then their effects are additive like methyl groups of the TMS. So a similar behavior between POVS and TMS is remarkable and could be used as an excellent standard. In case of such specific NMR, the molecules need standard field at lower field than that of the TMS. Since the O and N atoms have non-bonding electrons and hence the units of the POVS have electrostatic potential ($-e^2/r$) to bind and kinetic potential ($p^2/2m$) for mobility. In silicic acid, similar to TEOS the protons are not in continuity but are in similar environment. The isopropyl chain of valine is less hydrophobic and must have hindered the vinyl chain of PVPO. So the valine must have influenced the $-\text{CH}_2\text{-CH}_2-$ of PVPO and $-\text{O-Si-O-}$ units. The POVS is also following the same trend as that of silicic acid and TEOS with sharp peak and higher

intensity where the valine has shorter alkyl chains and PVPO has larger alkyl chains. The +I effect of alkyl chains of valine may influence the gyro-magnetic field of the molecule and are effective to slightly weaken the gyro-magnetic motions of PVPO as depicted in the spectra and much broadening of the peak is noted.

Scanning electron microscopy. Scanning electron micrographs showed the features of microstructure and surface morphology of the POVS where the molecular orientations are depicted in the nanometer range (Figures 6 a and b). In case of valine, the amino acid being a small molecule, the crystals appear to be smaller in size. However some accumulations of molecules are seen together due to the hydrophobic forces within the molecule. There is a loss of symmetry in the arrangement of molecules. The internal morphology showed an accumulation or clusters may be bunch of oxime group of the PVPO which is bound by free $-\text{COOH}$ groups along with adjacent isopropyl groups of valine which does not have free binding sites. If the linkage of $-\text{NH}_2$ and PVPO with the Si atom had stronger interactions and interatomic forces. So the SEM micrographs would be more condensed and the crystal appeared to be more compact. The micrographs of pure silicic acid molecules showed more cohesive forces in dominance whereas in the POVS the cohesivity is lost. Thus the POVS showed variation in the internal morphology of the molecule. The more hydrophobic na-

ture of the valine perhaps showed less interactions within the molecule and the micrographs does not showed compactness in the molecule. Thus, the microstructures provided us more valuable structural information on optimizations of biologically active supramolecules.

Paper chromatography. Using column chromatographic technique at pH = 5.0 and 8.0 of citric acid and disodium hydrogen phosphate buffer and water as medium the drug loading capacity of the supramolecule was studied. The POVS, L-alanine, L-isoleucine and glycine solutions were prepared in buffers and water, and were loaded on strips of 14×4.5 cm² dimensions of a chromatographic paper which were dipped in water and respective buffers. The capillary rise on the strips was stabilized within 10 min, and the strips were withdrawn from the media and were kept at 25 °C for about 10 - 15 min. The I₂ + 3% KI solution were prepared with millipore water and were sprayed on the strips which developed a yellow color. The yellow color with iodine solution was stable for about 10 - 15 min. Similar experiments were conducted with spray of 0.1% ninhydrin ethanolic solution which developed a purple color within 30 min, brightened after few min and did not vanish for 1 h. The *R_f* values for the supramolecule and amino acids were determined using (distance of solvent)/(distance of the sample) relation. The ninhydrin stained strips are depicted the Figures 7(a) and (b).

Prospective applications. The above analysis showed that the POVS is a supramolecule with high biocompatibility, highly biologically active sites, with multifunctional moieties and could be encompassed to broad areas of various biomedical, clinical and pharmacological fields. The molecule could be extensively applied in bioengineering, biomembranes, tissue engineering, semiconductors, biochips *etc.* The POVS could be a medical asset to various pharmacological applications such as thermal therapy, absorbing the toxins, capturing the metal ions, drug carriers, drug delivery systems. The molecule could be also applied in the treatment of metal toxicity as the free binding sites are helpful in entrapping the toxic metals. The supramolecule is smart, with different biologically active functional sites, electronic binding sites and can play a major role in improving the therapeutic efficacy of pharmacological agents like drug delivery systems, drug carriers and drug vehicles. The POVS is an excellent molecular model with multifunctional applications in the field of biomolecular, bioclinical, membrane engineering technology, tissue enegineering, nanocarriers, as biosensors and biochips.

Conclusion

Our analysis showed that the POVS is biocompatible, with combining units PVPO and valine are highly biologically active

and with unique multifunctional moieties and free binding electronic sites, could be encompassed to a broad range of various biomedical applications. Considering silicon atom as core, the POVS has the potential to develop various electronically controlled linkages and attachments in the molecule which could also develop interesting biological membranes, enhance the development of artificial skin and develop porous skin, developing the chemical potential within the biological systems. The transporting and binding ability of the POVS with varying pH medium was also studied which showed the dominant cohesive and adhesive forces within and can be applied as nanocarriers within the biological systems. The presence of multifunctional active sites makes the novel POVS to be used as bio-sensor and bio-chip for various clinical applications. The POVS can render valuable information regarding the biomolecular reactions in various biological systems like oxygen intake in blood, decomposition of glucose in blood, combination and permutations of protein molecules for enzymatic processes to release of energy and tissue repair, tissue engineering *etc.* So this is remarkable in the field of biochemistry, biomedical, bioclinical, chemopharmaconomics.

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