Synthesis and Optimization of Acrylic-N-Acryloxysuccinimide Copolymer Microspheres

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Abstract—**A micro-sized poly(*n*-butyl acrylate-co-*N*-acryloxysuccinimide) [poly(nBA-co-NAS] copolymer bio-carrier matrix has been synthesized from n-butyl acrylate (nBA) and *N*-acryloxysuccinimide (NAS) monomers through a single step emulsion photo-polymerization protocol. Narrower size distribution of the copolymer microspheres having a size in the range of 0.31-1.04 µm was obtained using nBA-SDS composition ratio of 0.25:5.00 (mL : mg). The FTIR analysis result confirmed the presence of the useful succinimide functional groups on the poly(nBA-co-NAS), which rendered the copolymer microspheres a feasible alternative to other polymers for use as biological immobilization carrier for enzymes and DNA molecules in the fabrication of advanced biosensor devices. The low glass transition temperature (T_g) of the as-synthesized copolymer microspheres signifies the soft and good adhesion properties of the matrix to be attached to the solid support to form the miniature solid-state biosensing devices**.

Keywords— **microspheres; copolymer; immobilization matrix; photo-polymerization**

I. INTRODUCTION

Emulsion polymerization method has been widely used to synthesize various types of polymeric microspheres. It is usually employed for polymerization process involving hydrophobic monomer, which is insoluble or sparingly soluble in water. Generally, there are four main components for emulsion polymerization reaction to take place [1]. The first component is the monomers, which are slightly soluble or insoluble in the solvent phase. The second component is the initiation material, which can dissolve in the monomer but does not dissolve in the solvent phase. The third component is the surfactant moiety having both hydrophilic and hydrophobic portions as a stabilizer. The fourth component is the solvent such as water.

Several factors may affect the size of the sphere diameter such as the polarity of the emulsion polymerization medium, the amounts of initiator or the monomer used [2] and the

stability of the emulsion droplets formed. The stability of the polymer emulsions can be maintained by adding surfactant stabilizer into the emulsion polymerization formulation. The surfactant is necessary to create the micelles for microbeads formation by trapping between the surface of the emulsion droplet and the solvent in order to lower the interfacial tensions and provide stability through electrostatic and steric hindrance mechanisms over the emulsion droplets in the solution, thus prevents agglomeration of the emulsion droplets [3]–[5]

Photo-polymerization method offers a fast and easy way of polymerization because of the single step polymerization process involved. The fundamental principle of photopolymerization is based on the absorption of UV light by the monomer that results in the generation of a few types of active monomers such as free radicals, cations, and anions. Free radicals of the monomer are then joined together by

chemical bonds to form a polymer chain [6]–[8]. A UV sensitive initiator material such as 2,2-dimethoxy-2-phenylacetophenone (DMPP) [9]–[11] is usually required to be added to the monomer to initiate the photo-polymerization reaction. The photo-initiator will form free radicals upon irradiated with UV light and subsequently reacts with the monomers to form free radicals in the monomer. Consequently, a polymer is formed as a result of the reaction between free radicals in the monomers.

Polymeric microspheres have been extensively used as biomaterials ranging from the medical application in tissue engineering to analytical biosensor applications owing to the nature of the microspheres, which are physically stable, cellular biocompatibility and enable in vivo detection with sorption methods or binding to the ligand on the particles surface [12]. Biosensors constructed based on the polymer microspheres immobilization matrix for biological molecules, e.g. enzyme, cell, tissue, antibody, and DNA are however possessing certain drawbacks such as loss of enzyme activity and leaching out of biological molecules into the solution, hence reduces the capability of biosensing. These limitations could be overcome by incorporating active biochemical functional groups that can be used for binding of the biomolecule functional groups via robust covalent bond during the synthesis of the biochemically functionalized immobilization carrier, and that the activity of the immobilized biological substances should remain unaltered.

Poly(n-butyl acrylate) [poly(nBA)] is commonly synthesized in the form of membrane for the fabrication of various useful biosensors [9], [13]–[15]. The polyacrylate membrane is hydrophobic, does not require plasticizer, photo-curable and possesses low glass transition temperature (Tg), which renders the acrylic membrane to be easily attached to the other solid support materials such as electrodes. In this study, poly(n-butyl acrylate-co-N-acryloxysuccinimide) [poly(nBA-co-NAS] copolymer microspheres were synthesized. As the n-butyl acrylate (nBA) monomer is insoluble in water, it acts as oil droplet and emulsifies with sodium dodecyl sulfate (SDS) surfactant in a continuous phase of water via emulsion polymerization to form the oil-in-water microemulsion. The N-acryloxysuccinimide (NAS) monomer consists of C=C functional group, and that making it possible to form a copolymer with nBA monomer by the photo-lithography technique in the presence of photo-initiator (Fig. 1).

nBA monomer
NAS monomer
nBA-co-NAS copolymer microsphere
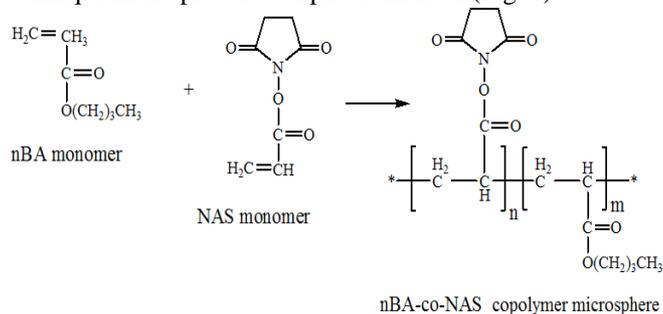
Fig. 1 The proposed chemical reaction between nBA and NAS to form nBA-co-NAS copolymer microsphere

As the copolymer microspheres comprised of succinimide functional groups, it can be used to react with amine functional group to form amide covalent bond [16], [17]. Thus, the proposed plasticizer-free copolymer microspheres have a potential to be used as a bio-immobilization carrier for immobilization of enzyme and aminated DNA molecules in biosensing applications.

II. MATERIAL AND METHODS

A. Chemicals

Aldrich produced 2,2-dimethoxy-2-phenylacetophenone (DMPP) and 1,6-hexanediol diacrylate (HDDA). Sodium dodecyl sulfate (SDS) and N-acryloxysuccinimide (NAS) were obtained from Across and System, respectively. Sigma supplied poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG) (Fig. 2), whilst n-butyl acrylate (nBA) was manufactured by Merck. All the chemical solutions were prepared in deionized water.

(a)   (b)   (c)
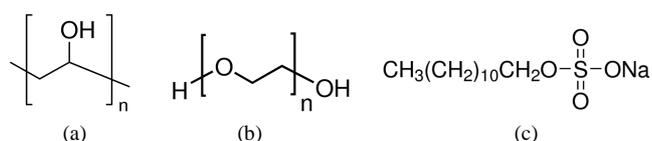
Fig. 2 Molecular structures of the poly (vinyl alcohol) (a) and poly(ethylene glycol) (b) and sodium dodecyl sulfate (c)

B. Synthesis of Poly(nBA-co-NAS) Copolymer Microspheres

Poly(nBA-co-NAS) copolymer microspheres were synthesized by microemulsion photo-polymerization method under UV light treatment [18]. The synthesis of NAS-functionalized acrylic microspheres was optimized by varying the amount of nBA and surfactant in the mixture of 225 mL HDDA, 50 mg DMPP, 3 mg NAS and 7.5 mL deionized water using different types of surfactants (PVA, PEG, and SDS) (Table 1).

TABLE I
THE COMPOSITION OF nBA MONOMER AND SURFACTANTS (I.E. PVA, PEG, AND SDS) USED IN THE OPTIMIZATION STUDY

Microspheres sample	nBA Volume (mL)	Surfactant weight (mg)		
		PEG	PVA	SDS
A	0.25	5.00	-	-
B	0.25	-	5.00	-
PBSA-1	0.25	-	-	5.00
PBSA-2	1.50	-	-	5.00
PBSA-3	3.50	-	-	5.00
PBSA-4	3.50	-	-	1.00
PBSA-5	3.50	-	-	25.00

Oil-in-water emulsification process was carried out by sonicating the mixture in an Elmasonic ultrasonic water bath for 10 min followed by irradiation with UV light for another 10 min using UV light transmitter unit (RS Ltd.), which consists of four 15-watt light bulbs transmitting UV radiation at the wavelength of 350 nm under continuous nitrogen gas purging. The as-synthesized copolymer microspheres were finally centrifuged (Hermle) to pellet at 4000 rpm for 30 min, and washed thrice with 0.05 M sodium

phosphate buffer solution at pH 7.0 followed by air dried at room temperature (25 °C). Substitution of SDS with PVA and PEG surfactants was also carried out to synthesize the copolymer microspheres to examine the influence of different surfactants used on the copolymer microspheres' size.

C. Physical and Chemical Characterizations of Copolymer Microspheres

The shape of the resulting poly (nBA-co-NAS) copolymer microspheres depends on scanning electron microscopy (SEM, LEO 1450VP). The microspheres size distribution was determined by Mitutoyo digital diameter measuring tool based on 250 to 325 units of copolymer microspheres image captured by SEM. Perkin Elmer GX FTIR spectrophotometer was used to determine the functional groups of the copolymer microspheres in the wavenumber range of 370-4000 cm-1 using KBr disc method. The Tg midpoint value of the copolymer microspheres (3.0-3.5 mg) was characterized by using Mettler Toledo DSC 822e differential scanning calorimetry (DSC) instrument between the temperature range of -60 °C and 0 °C at the heating rate of 10 °C/min under a nitrogen gas flow rate of 40.0 mL/min.

III. RESULTS AND DISCUSSION

D. Physical and Chemical Characterizations of Copolymer Microspheres

As Fig. 3 indicates, the type of the surfactant used significantly influenced the diameter size of the as-synthesized copolymer microspheres but did not affect the spherical shape of the resulting copolymer microspheres. Smaller particles size with a homogeneous size distribution of the copolymer microspheres was obtained using SDS surfactant (0.71-7.07 µm) as compared to the use of PVA (1.88-19.78 µm) and PEG (1.18 to 9.19 µm) surfactants (Table 2). This was attributed to the polar head characteristic and a hydrophobic long alkyl chain of the SDS, which gave better stability to the emulsion droplets in water compared to PVA and PEG surfactants with relatively short alkyl chains. Furthermore, the polymer emulsion droplets appeared to last longer with smaller diameter size using SDS surfactant

compared to when PVA or PEG surfactant was used [3]–[5]
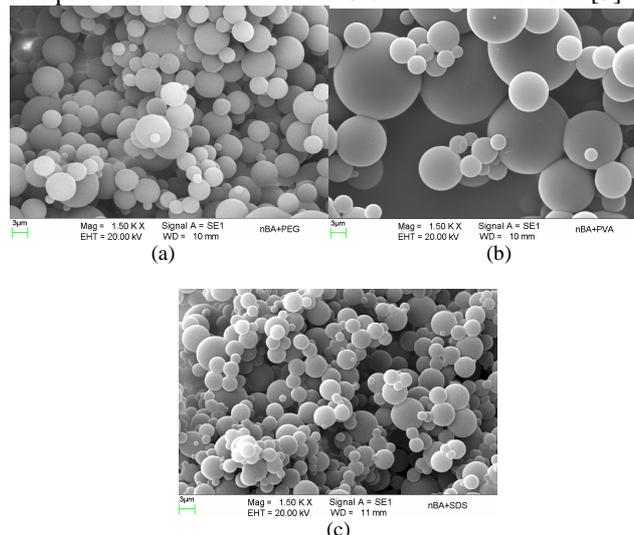
Fig. 3 Scanning electron micrographs of poly(nBA-co-NAS) copolymer microspheres synthesized using different surfactants i.e. PVA (a), PEG (b) and SDS (c)

TABEL II
EFFECT OF SURFACTANT TYPE ON THE SIZE DISTRIBUTION OF THE AS-PREPARED ACRYLIC MICROSPHERES

Surfactant	Microspheres' diameter range (µm)
PVA	1.88-19.78
PEG	1.18-9.19
SDS	0.71-7.07

E. Effect of Monomer and Surfactant Composition on the Size of the Microspheres

Various nBA monomer and SDS surfactant compositions were used to synthesize the poly (nBA-co-NAS) copolymer microspheres without changing the surface morphology of the spherical microspheres. However, the size of the microspheres produced can be regulated by varying the composition of nBA monomer and SDS surfactant in the polymer precursor. Table 3 summarizes the size distributions of the copolymer microspheres with varying nBA and SDS amounts in the polymer formulation.

TABLE III
EFFECT OF NBA AND SDS COMPOSITIONS ON THE SIZE OF THE COPOLYMER MICROSPHERES

Microspheres Sample	nBA-SDS composition (mL : mg)	Micro-spheres' diameter range (µm)	Dominant and smallest microspheres' diameter (µm)
PBSA-1	0.25:5.00	0.31-1.04	0.50
PBSA-2	1.50:5.00	0.32-1.51	0.75
PBSA-3	3.50:5.00	0.68-4.44	2.39
PBSA-4	3.50:1.00	0.87-5.18	4.04
PBSA-5	3.50:25.00	0.52-1.81	1.11

The copolymer microspheres diameter was found to increase proportionally from 0.50 µm to 2.39 µm with the increasing nBA monomer amount from 0.25 mL to 3.5 mL under a solid SDS composition at 5.0 mg. This can be explained by the fact that the increasing nBA monomer used has resulted in the increasing size of the polymer emulsion droplets produced in the photopolymerization reaction. However, the use of high nBA monomer amount at 3.5 mL

with low SDS amount between 1.0 mg and 25.0 mg in the emulsion photo-polymerization process exhibited a broad microspheres size distribution (sample PBSA-3, PBSA-4 and PBSA-5), whereby homogeneous copolymer microspheres with uniform size distribution was unachievable. This was because the presence of a large amount of nBA monomers has caused the emulsion droplets to to become unstable in water and easily coalesced to form

larger emulsion droplets, therefore resulted in a more full microspheres size distribution. The effect of increasing monomer amount on the increasing polymeric microspheres diameter has also been reported [2] [18]The amount of SDS surfactant used to synthesize the copolymer microspheres was further optimized by fixing the nBA monomer loading at 3.5 mL. The copolymer microspheres size was observed to decrease as increasing SDS surfactant amount was added to the oil and water mixture. This was ascribed to the effect of surfactant, which serves to maintain the stability of the emulsion droplets formed by adsorbing at the oil-water interface. The Higher amount of SDS surfactant present in the oil-in-water micro emulsion provided higher stability to the micro emulsion droplets, and that prevented flocculation of micro-sized emulsion droplets into larger droplets [3] [5] [18]. In addition, the emulsion droplets would last longer with a slightly higher SDS stabilizer loading.

F. Chemical Elucidation of Poly(nBA-co-NAS) Copolymer Microspheres

The functional groups present in the as-synthesized poly (nBA-co-NAS) FTIR determined copolymer microspheres are tabulated in Table 4.

TABLE IV
LIST OF FUNCTIONAL GROUPS PRESENT IN THE ACRYLIC MICROSPHERES COMPARED WITH THE REFERENCE SPECTRUM OBTAINED FROM FTIR ANALYSIS (SDS WAS USED AS THE SURFACTANT)

Functional group	Frequency (cm^{-1})	
	Acrylic microspheres	Reference [19]
O-H (strain)	3450.61	3400-3200
C-H (strain)	2961.13-2874.49	3000-2850
C=O (ester)	1736.88	1750-1730
C=O (amide)	1698.85	1680-1630
C-O (strain)	1188.99-1066.13	1300-1000
CH$_3$ (elbow)	1397.19-1376.24	1450-1375
-CH$_2$-(elbow)	1453.53	1465
C-N (strain)	1256.42-1165.58	1350-1000

The FTIR absorption band of the hydroxyl group (-OH) at 3450.61 cm-1 is due to the presence of water molecules as the copolymer microspheres sample was analyzed in wet condition. The adsorption bands of 2961.13-2874.49 cm-1, 1397.19-1376.24 cm-1, and 1188.99-1066.13 cm-1 are corresponding to the respective C-H (strain), -CH3 (bend), C-O (strain) functional groups of nBA and NAS monomers. The adsorption band indicates the -CH2- functional group (elbow) in the copolymer microspheres at 1453.53 cm-1. The strong and sharp C=O functional group absorption bands of nBA and NAS monomers are observed at 1736.88 cm-1 and 1698.85 cm-1, respectively. C-N (strain) functional group from NAS monomer is perceived between 1256.42 cm-1 and 1165.58 cm-1. The presence of succinimide functional group in this copolymer microspheres can thus be used for covalent coupling with biomolecules such as enzymes and aminated DNAs via succinimide-amine coupling reaction [16]–[18] for robust immobilization of biomolecules to the copolymer microspheres carrier matrix. Besides, the monomer composition and surfactant types are not affected to the functional group of polymer and consistent [18].

G. Glass Transition Temperature of the Copolymer Microspheres

The glass transition temperature (Tg) of the polymer is the temperature at which the polymer changes from a solid (glass) to a rubber state [20]. Tg value may indicate the physical properties of a polymer such as a polymer viscosity [21], [22] For polymer applied in the ion selective membrane, the Tg value of the polymeric membrane is required to be below the room temperature [9] in order to facilitate the membrane attaches to the substrate support i.e. the electrode. The Tg values of the poly (nBA-co-NAS) copolymer microspheres taken at the midpoint (Fig. 5) of the transition at various nBA-SDS compositions are shown in Table 5.
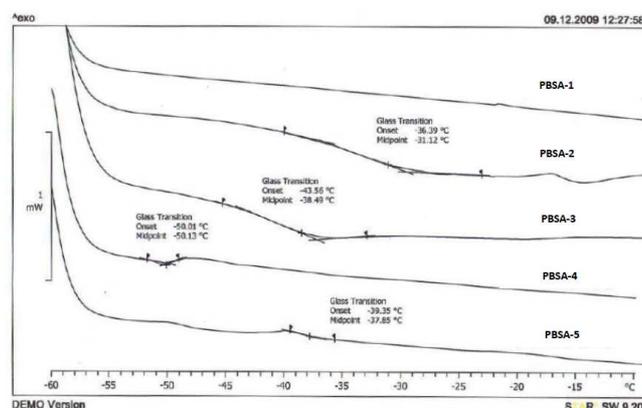
Fig. 5 Glass transition profile of acrylic microspheres

TABLE V
THE TG VALUES OF THE POLY(NBA-CO-NAS) COPOLYMER MICROSPHERES PRODUCED WITH VARYING NBA-SDS COMPOSITIONS

Microspheres Sample	nBA-SDS composition (mL: mg)	T$_g$ midpoint value (^{o}C)
PBSA-1	0.25:5.00	-
PBSA-2	1.50:5.00	-31.12
PBSA-3	3.50:5.00	-38.49
PBSA-4	3.50:1.00	-50.13
PBSA-5	3.50:25.00	-37.85

The Tg value of the copolymer microspheres decreased with the increase of the nBA monomer amount, whereas increasing the amount of SDS surfactant, the copolymer microspheres demonstrated increasing Tg value [2]. The low Tg value of the copolymer microspheres indicates high elasticity properties of the matrix [9], [13], [15], which could serve as a good candidate carrier matrix to be attached to any plastic support materials.

IV. CONCLUSIONS

Copolymer microspheres based bio immobilization matrices has been successfully synthesized from nBA and NAS monomers via micro emulsion photo-polymerization. The type of surfactant and the amount of monomer or surfactant used appeared to influence the size as well as the

T_g value of the copolymer microspheres. The low T_g value of the copolymer microspheres allows strong adherence of the matrix to any substrate supports. Moreover, the available succinimide ester functional group on the copolymer microspheres further allows covalent immobilization of biological molecules to the matrix surface for the development of versatile biosensors.

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