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Graft Copolymerization and Characterization of Styrene with Chitosan via Radical Polymerization

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ABSTRACT

In this study, styrene was successfully grafted onto chitosan by conventional free radical polymerization technique, using potassium persulphate (KPS) as the initiator. The effect of various reaction conditions including chitosan:monomer weight ratio, polymerization temperature, reaction time and concentration of initiator were studied. The highest percentage grafting (G %) 180% was found at 1:4 chitosan:styrene weight ratio, 0.4 g KPS and 70°C reaction temperature. Fourier transform infrared spectroscopy (FTIR) results showed the presence of polystyrene peaks, indicating the success of the grafting procedure. Thermogravimetric analyses (TGA) revealed that the thermal stability of the prepared copolymer is higher than that of chitosan alone. M_w and M_n of the isolated polystyrene from the graft copolymer were found to be 9.5249×10^4 g/mol and 3.0755×10^4 g/mol, respectively, with a polydispersity index of 3.1

Keywords: Biomaterials, Chitosan, Grafting, Polymers, Polystyrene, Potassium persulphate

INTRODUCTION

Styrene is a synthetic vinyl monomer which is used for various applications. Depending on how the polymerization of styrene is undertaken, the resulting polymer can be hard and transparent like glass or foamed and soft, possessing insulating properties (Kerry, 2016). On the other hand, chitosan is a biopolymer which is an amino polysaccharide resultant from the N-deacetylation of Chitin (Struszczyk, 2002). Chitosan in itself presents reactive functional groups susceptible to chemical modification. The chemical/mechanical properties of chitosan often need to be modified to suit certain application purposes. This modification allows for the properties of the chitosan material to be imparted via the incorporation of a range of substituent groups. In many cases, vinyl monomers are considered to be suitable candidates to help achieve such modifications (Ratanavaraporn, *et al.* 2009).

Modification of chitosan using polymerisation reaction procedures has been widely reported. For instance, styrene was reported to be successfully grafted onto chitosan film through reaction mechanisms that were initiated by γ-rays radiation (Shigano *et al.,* 1982). Also, methyl acrylate and methyl methacrylate were reported to have been grafted onto chitosan using potassium persulphate initiated reactions (Retuert and Yazdani-Pedram 1993). Other reports also presented chitosan films which were modified with acetic and hexanoic anhydrides by complete site-selective N-acetylation and N-hexanoylation at the surface of the chitosan films (Xu, *et al.* 1996). Furthermore, acrylic and methacrylic acids were successfully grafted onto chitosan using ceric ammonium nitrate as the initiator of the reaction (Shantha, *et al.* 1995), while on the other hand a heterogeneous graft copolymerization of chitosan powder with methyl acrylate was reported using trichloroacetyl-Manganase carbonyl co-initiator (Jenkins and Hudson 2002).

In 2007, a novel chitosan-g-poly(acrylic acid)/attapulgite composite was reported where ammonium persulphate initiator was used in the modification reaction procedures (Zhang, *et al.* 2007). In the same year, another team of researchers used the same kind of initiation for processes which allowed the preparation of a novel chitosan-g-poly (acrylic acid)/sodium humate copolymer. In the latter approach ,N,N'-methylene-bis-acrylamide was used as the crosslinking agent, leading to the manufacturer of a materials with desirable mechanical properties(Liu, *et al.* 2007).

Chitosan is a very hydrophilic material. This hydrophilicity often needs to be modified using functionalization techniques. Some hydrophobically modified composites of chitosan were assembled by grafting of the biopolymer with various side chains via Schiff-base reactions with salicylaldehyde and dodecyl aldehyde. These reactions were followed by reductive amination procedures where $NaBH_4$ was used as the reducing agent (He *et al.,* 2012). Another approach to achieving reduced hydrophilicity in chitosan involves its functionalization with nanoparticles. In this regard, some recently functionalized chitosan-coated Fe_3O_4 nanoparticles (CS-coated Fe_3O_4 NPs) were prepared by a non-solvent-aided coacervation procedure which was followed by a chemical crosslinking procedure(Zhou, *et al.* 2014). In 2014, Ye *et al.* employed an electrostatic generator processes which allowed for the enhancement or reduction of the forces of attraction in a novel layer-by-layer electro-assembly process. With this approach, the surface characteristics, wettability of chitosan and its derivatives can be tailored or changed from being hydrophilic to being hydrophobic (- Ye *et al.* 2014).

This work was undertaken with the objective of using conventional polymerization in order to establish the effective combination of factors which can be useful in the grafting of styrene onto chitosan.

MATERIALS AND METHOD

Potassium persulfate (KPS) and ethylbenzene were obtained from Acros Organics (New Jersey, USA). Methanol and chloroform were obtained from QRëc (Selangor, Malaysia). Styrene and Chitosan (with degree of deacetylation 85%) were purchased from Sigma-Aldrich (St. Louis, USA). The chitosan was used after it was dried in oven at 100 °C. Fourier Transform Infrared (FTIR) analysis was conducted using Perkin Elmer Spectrum One FT-IR Spectrometer (Boston, USA). DSC and TGA analyses were carried-out using Hitachi DSC7000X differential scanning calorimeter (Tokyo, Japan). All the chemicals were used without further purification, except for styrene.

Preparation of Soluble Chitosan

Chitosan (1.00 g) was dispersed into 0.10 M HCl (100 mL) and stirred for 5 minutes using magnetic stirrer which resulted in a viscous solution. The chitosan was found to be soluble at lower pH values of 1-3.

Preparation of Chitosan Macroradicals

The chitosan solutions prepared above were transferred into 3-necked flasks, each being equipped with nitrogen inlet tube, condenser, and magnetic stirrer bar. The system was purged with nitrogen and heated at temperatures ranging from 50 to 80 ˚C. Potassium persulphate (0.2 – 1.0g) was added to the above mixtures. Pre-interacting time between potassium persulphate (KPS) and chitosan solution of 30 min was maintained.

Preparation of Chitosan-*graft*- polystyrene

Copolymers of dissolved chitosan grafted with styrene were prepared by considering chitosan:monomer weight ratio, temperature, reaction time, and initiator content. The reaction mixture was poured into methanol and the precipitate was filtered using sintered glass funnel, dried under vacuum at 40 °C as white powdery material, until a constant weight was achieved. The crude graft copolymer was washed with ethyl benzene to remove any polystyrene homopolymer present. The residues were filtered using sintered funnel, washed with methanol and dried under vacuum oven at 40 °C, to achieve constant weight.

The graft true grafted product was hydrolysed by immersing the copolymer (1 g) in HCl (20 mL, 6M), then stirred at 90 °C for 24 hrs. The acid was removed by simple filtration. Chloroform (30 mL) was added to the hydrolysed residue, the residue was concentrated under reduced pressure and poured into methanol to precipitate the polystyrene. Molecular weight of the polystyrene was determined by gel permeation chromatography (GPC).

Thermogravimetric analysis (TGA) was performed by using TA Q500. A temperature calibration test was conducted by running calcium oxalate monohydrate sample as standard. The analysis was conducted at a heating rate of 10 °C/min.

Number average molecular weights, weight average molecular weight and molecular weight distribution (PDI) were measured by gel permeation chromatography (GPC) waters 1515 and waters 2414 RI detectors with a set of Styragel columns (HR3, HR4 and HR5, 7.8 × 300mm). All measurements were carried out at 36 ºC using THF as eluent with flow rate of 1.0 mL/min. The system was calibrated using polystyrene standards with the molecular weight range from 2.95×10^3 to 4.22×10^6.

The copolymers were weighed before and after washing and later characterized as follows. The degree of percentage grafting G (%) and yield were gravimetrically determined as percentage weight using equation 1 and 2 (Blair and Kam Moon 1982, Kaewtatip and Tanrattanakul 2008):

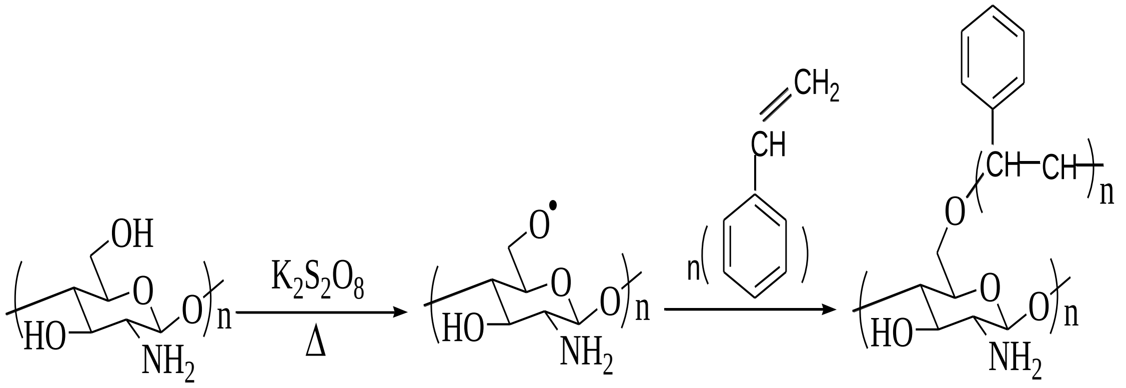
$$G\,(\%) = \frac{W_2 - W_1}{W_1} \times 100 \qquad (1)$$

$$Y\,(\%) = \frac{W_2 - W_1}{W_3} \times 100 \qquad (2)$$

Where, W_1, W_2, and W_3 represent weights of the chitosan, graft copolymer after washing, and polystyrene monomer respectively.

RESULTS AND DISCUSSION

It is well established that free radical polymerization is initiated by the generation of free radical species in the presence of vinyl monomer(s) (Kaewtatip and Tanrattanakul 2008). The active reaction sites are usually generated by addition of initiator. It is believed that water soluble initiator(s) such as potassium persulphate can decompose thermally under suitable temperature, to provide a pair of reactive species($SO_4^{-\bullet}$) (Allcock, Lampe *et al.* 2003). Some of these reactive species could then diffused out of the solvent cage and attack the most easily accessible hydrogen atom on the trunk polymer, thereby generating reactive site(s) on the backbone of the polymer, which could be used to graft vinyl monomer onto the backbone of any cellulose-like polymer (Allcock, Lampe *et al.* 2003, Abu Naim, Umar *et al.* 2013).The generation of chitosan macroradicals and preparation of the graft copolymer are shown in scheme 1.



Grafting was carried out at different fixed temperatures, ranging from 50 to 80 °C. For each set of reaction, the reactive ratios of 1:3, 1:1 and 3:1 of chitosan : styrene weight were used. Also, the KPS content and the reaction time were kept constant (Table 1).

Table 1: **Grafting parameters of Chitosan: Styrene ratio polymerized for 3 hrs with 0.1 M HCl (100 mL) and KPS (0.40 g)**

Chitosan (g)	Styrene (g)	Temp (°C)	(W_2) (g)	G (%)	Y (%)
1.00	3.00	50	1.60	60.00	20.00
1.00	3.00	60	2.80	180.00	60.67
1.00	3.00	70	1.31	31.00	10.33
1.00	3.00	80	1.12	12.00	4.00
1.00	1.00	50	1.20	20.00	20.00
1.00	1.00	60	1.28	28.00	28.00
1.00	1.00	70	1.26	26.00	26.00
1.00	1.00	80	1.23	23.00	23.00
3.00	1.00	50	NR	NR	NR
3.00	1.00	60	1.99	99.00	99.00
3.00	1.00	70	1.63	63.00	63.00
3.00	1.00	80	NR	NR	NR

W_2 represents weight of washed copolymer, G (%) represents percentage grafting,
Y (%) represents percentage yield and NR represents no reaction

It was observed that the percentage grafting, G (%) and percentage yield, Y (%), increase from 50 to 60 °C, and then decreased afterwards with increasing the temperature. This could be due to decrease in solution viscosity (that could ease monomer penetration) and increase in rate of decomposition of initiator with increasing temperature, resulting in the generation of more free radicals that could lead to the consumption of more monomer. In the system containing 1:1 chitosan: styrene weight ratio, both G (%) and Y (%) have similar values. For 3:1 chitosan: styrene weight ratio, there was no grafting at 50 °C. This could be due to viscosity of the chitosan solution. Both the monomer and KPS could not diffuse freely toward chitosan backbone to effect the copolymerization. There was also the absence of grafting at 80 °C. This could also be due to rapid decomposition of KPS at this elevated temperature which promoted the chain transfer reaction and early termination of the growing radicals (Nishioka, Matsumoto *et al.* 1983). The results indicate that the styrene-rich system was more suitable as it offered the highest G (%) as shown in Table 1.

The amount of chitosan and styrene monomer showed noticeable effects on percentage grafting, G (%). Hence, the styrene-rich system

(3.00 g) was more suitable, as it offered the highest G (%), 180.00 % and Y (%) 60.67 % as shown in Table 1. This phenomenon was also reported by other researchers (Fang, *et al.* 2005, Kaewtatip and Tanrattanakul 2008).

To optimize the initiator content, the polymerization reaction was conducted at 1:3 chitosan: monomer weight ratio, at 60 °C for3 h duration. Table 2shows that the maximum grafting G (%) of 180.00 % and the Y (%) value of 60.67 % were obtained from the system containing 0.40g of KPS. It was observed that there was no grafting following the use of KPS at a concentration level of 0.20 g. This might be ascribable to the insufficient amount of the initiator required to diffuse through the viscous chitosan solution. As such, the KPS could not diffuse freely to the chitosan backbone and hence the initiator would participate out through primary or secondary recombination, resulting in early termination of the growing radicals due to premature termination by chain transfer to either the monomer or the solvent (Matyjaszewski and Gaynor 2000). The G (%) and Y (%) decrease as the KPS content increases, as shown in Table 2. When the concentration of radical species is very high, chain transfer and reaction conversion are low due to the generation of lower chain length species, as the rate of chain propagation decreases. It should be noted that the maximum initiator efficiency is achieved when the initiator contents and temperature are low (Allcock, *et al.* 2003).

Table 2 Effect of initiator content on the percentage of grafting of chitosan, G (%), and yield of copolymerization, Y (%), of samples polymerized at 60°C for 3 h with chitosan (1.00 g) in 0.1M HCl (100 mL) and styrene (3.00 g)

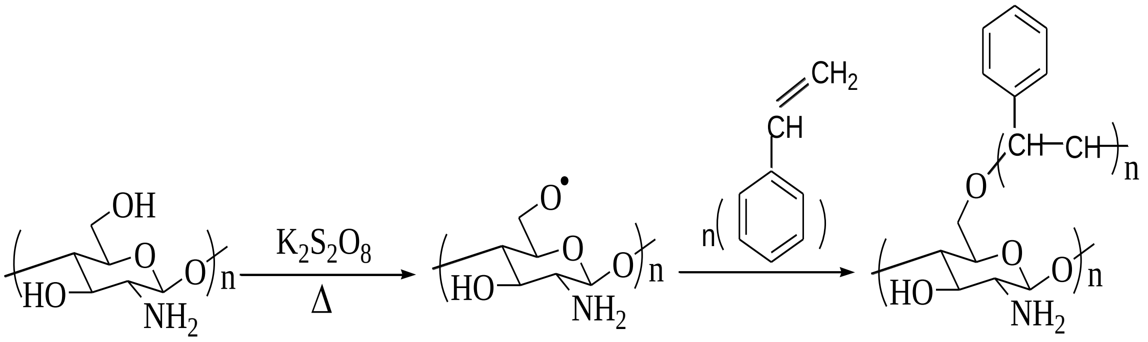
APS (g)	W_2 (g)	G (%)	Y (%)
0.20	NR	NR	NR
0.40	2.79	179.00	59.67
0.60	1.95	95.00	31.67
0.80	1.50	50.00	16.67
1.00	1.16	16.00	5.33

W_2 represents weight of washed copolymer, G (%) represents percentage grafting, Y (%) represents percentage yield and NR represents no reaction

The optimum reaction time was found to be 3 hrs, leading to values of G (%) of 180.00 % and Y (%) of 60.67 %, as shown in Table 3, when using the same reaction conditions during the preparation of chitin-*g*-PS copolymer. This longer reaction time in chitosan-g-Polystyrene copolymer could be ascribable to the viscosity of the chitosan in acidic media. As such, both the KPS and polystyrene could not diffuse easily to the surface of the chitosan backbone and this will delay the generation of macroradicals on the surface of the chitosan. The decrease in grafting percentage and grafting efficiency at time longer than 3 h could be due to chain termination brought about by primary radicals of either the trunk polymer macroradicals or the growing grafted chains, or both (Nishioka and Kosai 1981).

Table 3 Effect of reaction time on the percentage grafting chitosan, G (%), and yield of graft copolymerization of chitosan-*g*-PS samples polymerized at 60 °C, KPS (0.4 g), using 1: 3 ratio and of 0.10M HCl (100 mL)

Time (h)	W_2 (g)	G (%)	Y (%)
1	1.14	14.00	4.67
2	1.42	42.00	14.00
3	2.79	179.00	59.67
4	1.65	65.00	21.67
5	1.68	68.00	22.67



Scheme 1: Preparation of chitosan-g-PS copolymer using 1:3 chitosan:styrene weight ratio, 0.40g KPS and 60 °C.

To ascertain the presence of polystyrene functionality in the prepared chitosan-g-PS copolymer, the FTIR spectra of the linear polystyrene, chitosan and the washed (prepared) copolymers with highest percentage grafting were run and compared. Figure1 shows FTIR spectra of (a) unmodified chitosan and (b) the prepared chitosan-*g*-PS copolymer. The spectrum (b) indicates the presence of polystyrene peaks with IR-band intensities at 3150-3000 cm^{-1} (=C-H (aromatic)), 3000-2850 cm^{-1} (-C-H stretching (alkane)), 1660-1500 cm^{-1} (C=C aromatic) in addition to those of chitin and chitosan at 3600-3200 cm^{-1} (O-H and N-H stretching). This is also in accordance with the observations reported by other researchers (Kurita, et al. 1992, Kurita, *et al.* 1996).

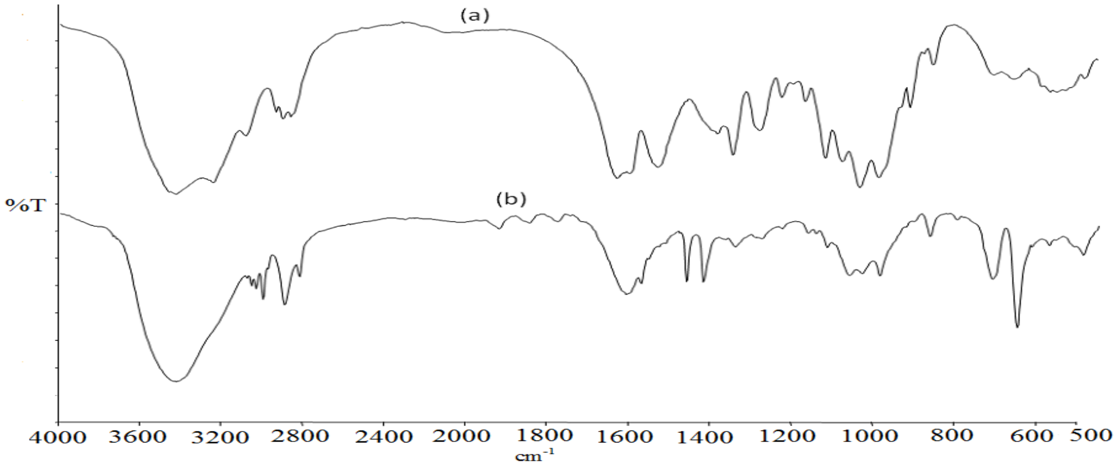
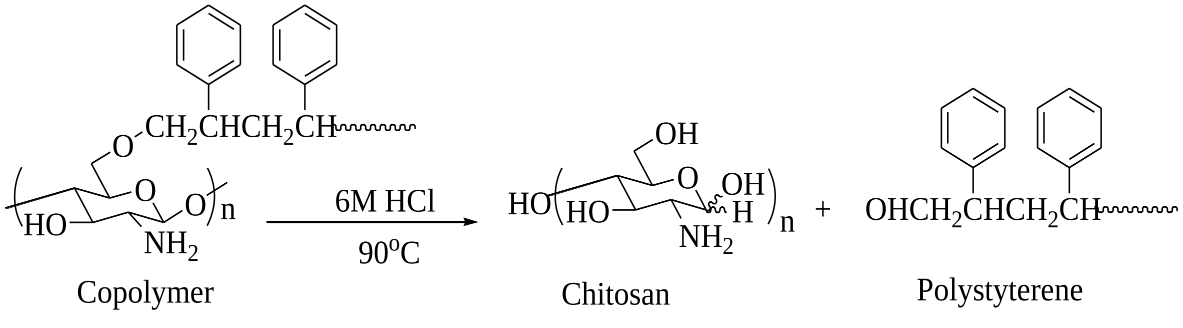


Figure 1: Infrared spectra of (a) chitosan (b) chitosan-g-PS copolymer

The GPC analysis also was carried out to enable the determination of molecular weight distribution and polydispersity indices of the isolated polystyrenes after the copolymers were hydrolyzed with hydrochloric acid, as shown in Scheme 2.



Scheme 2: Reaction scheme for the hydrolysis of the Chitosan-g-PS

GPC analysis of the chitosan copolymer revealed that the number-average molecular weight M_n and weight-average molecular weight M_w of the isolated polystyrene were found to be 9.5249×10^4 g/mol and 3.0755×10^4 g/mol, respectively, giving a polydispersity index of 3.1 The broad molecular weight distribution could be due to uneven distribution of the side chains on the chitin and chitosan backbone (Allcock, *et al.* 2003).

The thermal properties of the original chitosan and the graft copolymer were investigated by thermogravimetric analysis (TGA). The copolymer showed higher stability in comparison to the chitosan, as shown in Figure 2. The initial weight lost from 40 to 95°C (8.39 %) could be due to loss in moisture in chitosan. There was a remarkable weight loss (69.36 %) in chitosan with the temperature from 280 to 389 °C. This could be due to breakage of C-O-C glycosidic bond as reported by other researchers (Liu, *et al.* 2013). While for the graft copolymer, the weight loss was observed at higher temperature range, around 430 to 470 °C, there was further decrease in weight with increase in temperature to 600 °C. The decomposition of the chitosan commenced at around 422 °C with 17.89 % weight loss, while the decomposition of the graft copolymer commenced at approximately 475 °C, with little ash being generated compared to the chitosan decomposition.

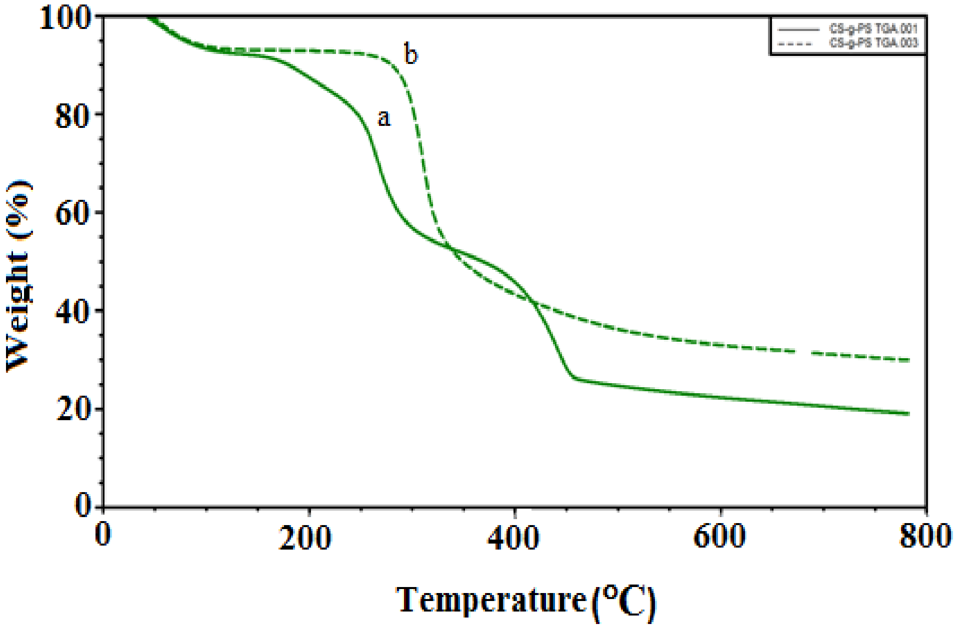


Figure 2: TGA Thermograms of (a) chitosan and (b) Chitosan-g-PS copolymer

CHN analysis was conducted to estimate the elemental composition of the carbon, hydrogen and nitrogen of the prepared copolymers and the polystyrene obtained from hydrolyzed copolymers, in comparison to the theoretical values of the unmodified biopolymers. The CHN results indicated that the elemental composition of carbon, hydrogen and nitrogen were reasonably close to the theoretical values, with highest elemental percentage being that of carbon, as shown in Table 4.

Ideally, the experimental values should be very close to the theoretical values, but there is a bit discrepancy between the values obtained in this study. This could be due to non-specific nature of radical polymerization, as the reaction is so random that it is hard to get exact values(Kamal, *et al.* 2010). This can also be attributed to the broad value of PDI that was obtained in the GPC analysis.

It should also be stated that, this type of polymerization is a faster process, which leads to great chain termination, in comparison to slow radical kinetic reaction. The random/non-specific nature of this type of reaction leads to the preparation of copolymer having broader molecular weight with side chains of un-equal length (Wang and Matyjaszewski 1995, Matyjaszewski and Gaynor 2000, Matyjaszewski and Xia 2001, Matyjaszewski 2012). As such, the CHN experimental values could be higher or lower than the theoretical values.

Table 4: Elemental analysis of the chitosan, graft copolymers and hydrolysed polystyrenes from graft copolymers

Element		Carbon (%)	Hydrogen (%)	N_2 (%)
	Experimental	92.2391	5.4860	0.0000
Chitosan	Theoretical	44.7204	6.8323	8.6956
	Experimental	49.1402	7.9226	7.6533
Chitosan-g-PS	Theoretical	63.6363	6.8181	5.3030
	Experimental	67.1488	6.2835	5.8086
Hydrolyzed	Theoretical	92.3077	7.6921	0.0000
PS from coplymer	Experimental	90.6938	6.4581	0.0000

CONCLUSION

Chitosan was successfully grafted with polystyrene using pottasium persulphate initiator. The optimum condition of grafting was obtained when chitosan (1 g) and styrene monomer (3 g), reaction temperature 70 ˚C and reaction time of 3 hrs were used. The graft copolymer was characterized by some techniques. TGA results showed that the copolymer has higher thermal stability compared to chitosan itself. Hydrolysis of the most grafted copolymer allowed isolation of polystyrene with M_n and M_w as 3.0755×10^4 g/mol and 9.5249×10^4 g/mol respectively, with polydispersity index (PDI), 3.1.

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