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   1.1 Nanthiya Hansupalak, Parichart Kitsongsermthon, Ratana Jiraratananon, Immobilized Flavourzyme on Chitosan Beads for Seasoning Sauce Production: Covalent Binding VS Entrapment, a chapter in ACS Book entitled "Contemporary Science of Polymeric Materials", 2010, **In press**. (ดูภาคผนวก)

   1.2 Sorotsiri Srithong, Ratana Jiraratananon, Nanthiya Hansupalak, A Simple Postsulfonation of Poly(Arylene Ether Sulfone) Radel® R, Journal of Applied Polymer Science, 2010, **In press**. (ดูภาคผนวก)

2. การนำผลงานวิจัยไปใช้ประโยชน์

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Chapter

Immobilized Flavourzyme on Chitosan Beads for Seasoning Sauce Production: Covalent Binding VS Entrapment

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Flavourzyme was immobilized on chitosan beads using two methods, covalent and entrapment, in which beads were identically prepared. Optimum conditions for covalently binding enzyme on beads activated by glutaraldehyde were examined prior to the study of pH- and thermal- stabilities of immobilized enzymes. The quality of seasoning sauce produced by using immobilized enzymes was measured in terms of the total amino acid nitrogen amount. Both immobilizing techniques improved pH- and thermal-stabilities of free protease. Though the covalent immobilization showed the highest loading efficiency, it yielded the lowest enzymatic activity. Nevertheless due to being the most operational stabilities, covalently bound enzymes gave the highest amount of amino acid nitrogen, which was also greater than that from the similar process where free enzymes (Flavourzyme and amylase) were used, suggesting the potential of using immobilized Flavourzyme for the process.

Introduction

Commercial Flavourzyme is a fungal protease/peptidase complex produced from nongenetically modified *Aspergillus oryzae* and contains both endoprotease and exopeptidase activities, which can digest peptide bonds. The enzyme mixture also contains other substances such as salts and stabilizing agents, but it is nontoxic to human. Traditionally brewed soy sauce is made by mixing soybeans and *Aspergillus oryzae* strains, which then release protease enzymes to breaks down soybean proteins into shorter peptide chains and amino acids, contributing the meat-like, unique flavor of soy sauce. To shorten the processing time from months to days, hydrochloric acid is used to hydrolyze proteins, but an inferior taste and undesired and carcinogenic byproducts, i.e. chloropropanols, occur (*1-3*). Recently it has been found that protease enzymes from crude (or commercial) Flavourzyme can replace hydrochloric acid and yields the product called 'seasoning sauce', containing very small (acceptable) amount of carcinogenic byproducts, but having a similar flavor to the brewed soy sauce (*4*). Pure enzyme is, however, intrinsically unstable and costly due to its production and complicated purification. Immobilizing enzyme onto suitable supports, stable in the medium and large enough to prevent the enzyme loss, is one way to lower the production cost as it facilitates the separation of enzyme from the product and allows the enzyme to be used repeatedly in addition to enhancement of enzyme stability.

Immobilization technique is not new to the soy sauce production. For instance, immobilization of protease, protease-producing fungi, glutaminase, and glutaminase-producing yeast in the soy sauce and seasoning sauce productions havae been investigated (*5-10*). In addition, protease immobilization via covalent, entrapment, or physical adsorption on several supports have been examined widely for various purposes such as production of casein hydrolysates, peptide purification, and protein digestion in proteomics (*11-15*). There are also reports on Flavourzyme immobilization via encapsulation, covalent, and electrostatic adsorption on numerous supports such as sodium alginate/starch mixture, Lewatit R258-K, and glyoxyl-agarose (*16-18*). Nonetheless, none of those works are related to the immobilization of crude Flavourzyme on weak-acid and high-ionic-strength resistant chitosan beads, specifically designed for the seasoning sauce making. In addition this current work prepared chitosan beads for covalent and entrapment in the exact same way, employing ionic interactions between chitosan and sodium tripolyphosphate to solidify chitosan beads (*19*). Therefore, comparison of the immobilized enzyme activities could be made without interferences from the bead preparation. The chitosan is food grade and thus safe for utilizing in the sauce production.

The main objectives of this work was to investigate the possibility of using immobilized Flavourzyme to produce the seasoning sauce. Two immobilization techniques, Covalent and entrapment, were compared. For covalent immobilization, optimum conditions for activating chitosan support using glutaraldehyde and for binding protease on the activated support were examined first so that optimally immobilized Flavourzyme could be employed in the sauce making. Glutaraldehyde is used as a crosslinking molecule to form Schiff bases with a chitosan mer at one end and with an enzyme molecule at the other end (*20*). Also total amino acid nitrogen was used as a key parameter reflecting the sauce quality.

Experimental

Materials

Flavourzyme protease (500 unit/g or U/g) from Novozymes was a gift from East Asiatic (Thailand) and chitosan (deacetylation degree of 90 and MW~500k) from Elan Corp. (Thailand). All chemicals were used directly without further purification.

Protease Immobilization

Optimum chitosan concentration for entrapping enzymes has been investigated (*21, 22*) and the 2%w/v chitosan solution is found to be appropriate because the fully formed bead is not too dense to obstruct mass transfer of substrate and products or too loose to hold enzymes. *Covalent Method*: 10 ml of the chitosan solution, made of 2%w/v of chitosan dissolved in 1%v/v acetic acid, was dropped into 0.13 M sodium tripolyphosphate (TPP) solution, which was prepared in 0.1M sodium phosphate buffer (pH7), through a syringe with a 22G needle. After the chitosan beads being cured for 75 min in the same TPP solution, the solution was decanted, and the beads were washed twice using 0.1M sodium phosphate buffer solution (pH7). Next, cleaned beads were agitated in 20 mL of glutaraldehyde solution at pH 7 (prepared in 0.1M sodium phosphate buffer) at room temperature (~28°C) and 220 rpm and later were washed with 0.1M sodium phosphate buffer solution. They were then soaked in 20.5 ml of 0.5M sodium phosphate buffer solution, containing 0.5 ml of Flavourzyme, and shaken at 160 rpm for 3 hr. pH and temperature for the immobilization were varied between 5 – 9 and 30 – 60°C, respectively. Afterwards the beads were stored at 4 °C for 18 hr and then washed in 2M NaCl and deionized water, respectively. *Entrapment*: The same method was applied,

except that the chitosan solution was already well mixed with 0.5 ml of Flavourzyme prior to the solidification in the TPP solution. After being immersed in the same TPP solution for 75 min., the beads were washed twice using 0.1M sodium phosphate buffer solution (pH7) and ready to use.

Making Seasoning Sauce

The seasoning sauce was made according to (*4*) with slight modification (the total hours of 17.5 hr was still unchanged). Briefly, 20 ml of defatted soybeans were soaked and shaken in 100 ml distilled water at 220 rpm and 50°C for 4.5 hr. Then optimally immobilized protease was added and the shaking was continued for additional 13 hr at the same temperature. This process was performed in distilled water (pH~7) without buffering. The liquid and solid portions were separated by vacuum filtration for further characterization. Amino acid nitrogen amount in the liquid portion was characterized using a FP-528 LECO instrument (USA).

Protease Assay and Protein Amount

Protease assay: A mixture of 1 ml of 1.5%w/w casein in 0.1M sodium phosphate buffer solution and approximately 0.15 g of beads containing enzyme (or 1 ml of the liquid containing free enzyme) was allowed to react for 10 min at 160 rpm and 40°C. The tyrosine occurred in the solution was quantified by adding 5 ml of Na_2CO_3 and 1 ml of Folin reagent prior to the absorbance measurement at 660 nm using a Shimadzu UV-visible spectrophotometer UV160A (*23*). The activity (U/mg) was expressed as the amount of tyrosine (μmol) produced per reaction time (min) and protein amount. *Protein amount*: 0.6 ml of the liquid part containing enzymes, obtained from experiments, was mixed with 1.5 ml of Coomassie Brilliant Blue G. at room temperature. The UV absorbance of the solution was measured at 595 nm (*24*).

pH- and Temperature- Stabilities

pH-stability was performed by soaking (free or immobilized) enzymes in 0.1M phosphate buffer (ionic strength = 0.05M) at 50°C and various pH for 15 min, prior to the activity measurement. Similarly, the temperature stability was carried out by incubating (free or immobilized) enzymes in 0.1M phosphate buffer (ionic strength = 0.05M) at pH 7 and various temperature for 15 min. Note that optimum activation and immobilization conditions were utilized to covalently immobilize Flavourzyme on chitosan beads.

Results and Discussion

Optimum Conditions for Bead Activation

The covalent bonds between a support and an enzyme can strongly affect enzyme conformation and in turn its activity. Thus it is necessary to find optimum conditions to activate chitosan beads with glutaraldehyde at pH 7 before binding with enzymes. Figure 1 shows that enzymatic activity increases with glutaraldehyde concentration when the concentration is less than 0.1%v/v, after which the activity reduction is observed instead. This is typical for covalent immobilization (*25-27*). The number of covalent bonds formed between the support and enzymes through glutaraldehyde molecules plays an important role here. At low glutaraldehyde concentration, the amount of bonds per one enzyme molecule is too low to hold still an enzyme (single point immobilization), resulting in the leakage, which can be prevented by raising the glutaraldehyde concentration. However, at too high concentration, there are too many bonds per one enzyme (multipoint immobilization), distorted conformation, or enzyme denaturation, occurs. In this work external mass transfer effect may be another cause as high enzyme immobilized amount on the bead surface was observed (results are not shown here).

At a fixed glutaraldehyde concentration of 0.05%v/v where the number of bonds per one enzyme is small, the low enzymatic activity is observed. Varying the activation time, or exposure time to the glutaraldehyde solution, can alter the enzymatic activity as illustrated in Figure 1. Similarly, the rise in the amount of covalent bonds formed per one enzyme, resulting from the increment of the activation time, may account for the change in the activity.

In Figure 1 the activation conditions yielding the highest Flavourzyme activity are an activation time of 2.5 hr and 0.1%v/v glutaraldehyde concentration solution. Note that the optimum activation time was found by using polynomial regression, yielding R^2 of 1. The highest specific activity at the optimum conditions (0.089 U/mg protein, equivalent to 3.54 U/ml solution) is much smaller than one reported for thiol protease immobilized on glutaraldehyde-activated chitosan beads (32 U/ml) (*27*). This may be explained by the mass/volume ratio of chitosan to the enzyme solution in the current work being 16 times higher, resulting in much lower enzyme loading on chitosan beads. However the specific activity obtained from this current work is still comparable or somewhat higher than what one reported for crude Flavourzyme immobilized on Diaion HP20, glass beads, and silica gel 60 (*17*).

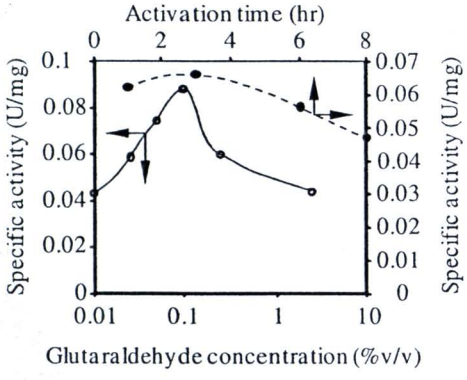

Figure 1. Optimum conditions for activating chitosan beads using glutaraldehyde: activationg time (●; dash lined was obtained by the polynomial regression and R^2 =1; 0.05%v/v glutaraldehyde conc. was used) and glutaraldehyde concentration (○; activation time of 2.5 hr. was used). The immobilization pH and temperature were pH 7 and room temperature, respectively.

Optimum Conditions for Enzyme Immobilization by Covalent Binding

pH and temperature can influence enzyme conformation, and unsuitable conformation of enzyme can cause the lower enzymatic activity (*28*). It is thus necessary to find optimum pH and temperature for a specific immobilization. It should be noted that for the entrapment the polymeric support should entrap free enzyme as it remains in the active conformation and thus the optimum pH and temperature must be the same as those of free enzymes.

For Flavourzyme immobilization on chitosan beads activated using aforementioned optimum conditions, the immobilization conditions yielding the highest activities are pH 7 and 40°C (Figure 2). Note that for this purpose all experiments were conducted in buffer solutions, of which ionic strength was 0.05 M. The optimum pH and temperature found herein correspond to the optimum values suggested by the Novozymes product sheet for neutral free enzymes (*24*), implying the enzyme's conformation unaffected by the present covalent immobilization conditions.

For similar protease immobilization on chitosan beads, Sangeetha and Abraham (*15*) observed the shift in the optimum pH and temperature to higher pH and lower temperature, which implies the conformational change upon the immobilization. This is probably due to his harsher condition, i.e., high glutaraldehyde concentration (2%v/v) such that all amino groups on the enzyme

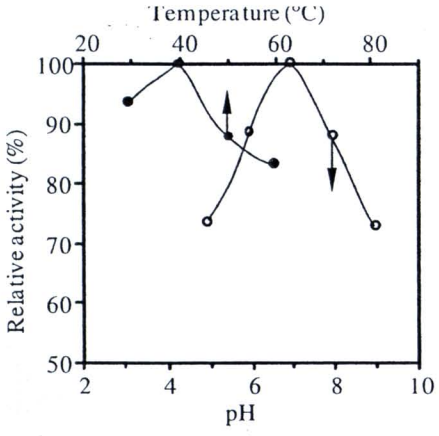

Figure 2. Optimum conditions for Flavourzyme immobilization onto optimally activated chitosan beads: pH (○; constant 30 °C) and temperature (●; constant pH 7).

surface are probably linked with glutaraldehyde. Thus untouched acidic groups ionizing at high pH are responsible for shifting the optimum pH to the alkaline region. In contrast when dilute glutaraldehyde concentration solution(0.05 – 1% v/v) is used, the shifting of the optimum pH towards higher pH is not seen (*27, 29*).

Thermal and pH Stabilities

Enzyme pre-incubation in a phosphate buffer for 15 min at a constant pH or temperature was exercised to observe temperature- and pH-stabilities of enzymes. In Figure 3A, the highest activities obtained from both immobilizations are achieved at the same temperature (50°C) as that of free enzyme, and covalently bound enzyme is the most thermal-stable over a temperature range of 30 – 70°C. In fact both immobilization types improve temperature stability of enzyme, as seen elsewhere (*27, 29*). The greater temperature stability is due to the crosslinks between the support and enzyme and chitosan matrix that protects immobilized enzymes from heat.

Figure 3B displays both immobilizations expanding pH stability of enzymes to both acidic and basic ranges. The covalently attached enzyme shows the greatest pH stability, reflecting the strongest bonds between the support and enzyme that can maintain the active conformation of enzyme in this pH range.

The enhancement of pH stabilities after protease's immobilization on chitosan beads have been observed (*15, 27, 30-32*), but optimum temperature and pH values are different depending on the protease types, chitosan concentration and molecular weight, and immobilization techniques.

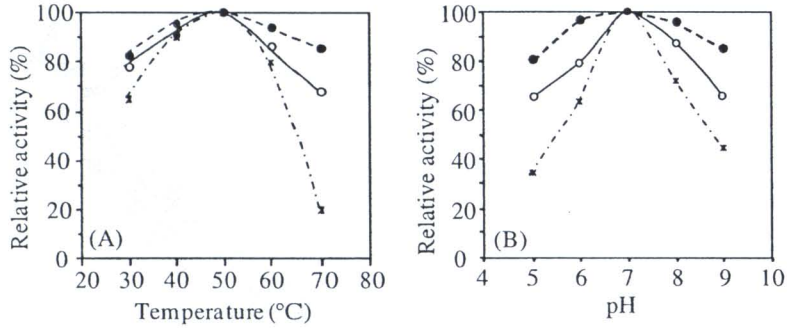

Figure 3. (A) Temperature- and (B) pH-stabilities of free (∗), entrapped (○), and covalently bound (●) enzymes.

Loading Efficiency and Quality of the Seasoning Sauce

Enzyme losses during the covalent and entrapment immobilizations are summarized in Table I as mean ± standard deviation. The covalent immobilization retains more enzyme than the entrapment. In the entrapment process, the greatest enzyme loss occurs during the chitosan solidification in TPP solution because the beads are not fully formed, as reported elsewhere (*21*). Free Flavourzyme expresses the highest activity, followed by the entrapped and covalently bound enzymes, respectively. That is because immobilization, especially the covalent, hinders enzyme's ability to combine with substrate (casein). In addition the existence of external and internal mass transfer of substrates, which has already been proved (*32*), leads to a decrease in enzymatic activity, as reported elsewhere (*33*).

The covalently bound enzyme can, however, produce the seasoning sauce which contains the largest concentration of amino acid nitrogen, which represents the quality of the seasoning sauce (*2, 4, 10*). It is attributed to the ability of the covalently bound enzyme to withstand the operation conditions better than the entrapped and free enzymes, as discussed previously.

The amino acid nitrogen amount obtained from employing immobilized enzymes is greater than that obtained from free enzymes (*4*). It should be noted

Table I. Enzyme Loading Efficiency and Amino Acid Nitrogen Amount

Immobilization Method	*Initial Protein Amount (mg)*	*Protein Losses (mg)*			*Loading Efficiency (%)*	*Specific activity (U/mg)*	*Total amount of amino acid nitrogen produced (g/dm^3)*
		In TPP	*Unbound enzymes*	*In buffer wash*			
Covalent	17.44±0.94	-	3.64±0.18	0.49±0.03	76.3±0.90	0.086	21.5
Entrapment	17.44±0.94	9.19±0.24	-	1.15±0.03	40.7±1.40	0.093	15.1
Free enzyme[a]	-	-	-	-	-	0.48	4.1

NOTE: All experiments were repeated thrice.

[a] obtained from (*4*).

that (i) Sahasakmontri (*4*) utilized both free Flavourzyme and amylase to produce the sauce and the soaking time of 30min before adding enzymes and (ii) defatted soybeans and protease in his work and the current paper came from the same sources. Therefore, the advantage of the current work is that only Flavourzyme is applied (though the longer soaking time before adding protease was needed) and yet can produce higher amino acid nitrogen amount.

Conclusions

Optimized entrapment conditions reported elsewhere were applied herein. For the covalent method, to obtain a high activity value chitosan beads must have been activated using 0.1%v/v glutaraldehyde for 2.5 hr. before immobilization. The optimal immobilization pH and temperature were pH 7 and 40°C, respectively, identical to optimum values of free enzyme, suggesting unchanged conformation during the immobilization. Both covalent and entrapment immobilizations boosted pH and thermal stabilities.

It was found that the covalent method enabled beads to retain enzymes more than the entrapment, because the latter took a few seconds for a chitosan bead to be firm enough to effectively hold enzymes, during which the loss of enzyme occurred. However covalently bound enzyme showed the lowest activity, but due to its most operational stability it could produced the largest amount of amino acid nitrogen, indicative of the better quality seasoning sauce. Surprisingly, the nitrogen amount found in this work, using immobilized Flavourzyme protease alone, was higher than that when using both protease and amylase in the free form, suggesting the potential of applying immobilized Flavourzyme on chitosan in the real sauce production. Nonetheless, to approach the real practice conditions, immobilization of more enzyme amount on chitosan beads should be examined further in order to obtain higher activity.

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Separate Table Caption List

Table I. Enzyme Loading Efficiency and Amino Acid Nitrogen Amount

Table I

Immobilization Method	*Initial Protein Amount (mg)*	*Protein Losses (mg)*			*Loading Efficiency (%)*	*Specific activity (U/mg)*	*Total amount of amino acid nitrogen produced (g/dm^3)*
		In TPP	*Unbound enzymes*	*In buffer wash*			
Covalent	17.44±0.94	-	3.64±0.18	0.49±0.03	76.3±0.90	0.086	21.5
Entrapment	17.44±0.94	9.19±0.24	-	1.15±0.03	40.7±1.40	0.093	15.1
Free enzyme[a]	-	-	-	-	-	0.48	4.1

NOTE: All experiments were repeated thrice.

[a] obtained from (*4*).

Nanthiya Hansupalak

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A Simple Postsulfonation of Poly(Arylene Ether Sulfone) Radel® R

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ABSTRACT: Commercially available Radel® R, a poorly acid resistant biphenol-based poly(arylene ether sulfone), was successfully sulfonated by a simple and rapid postsulfonation reaction using oleum as the sulfonating agent. Polymer degradation due to contact with acid could easily be minimized by adjusting reaction conditions. The suitable reaction conditions, when the 1 : 3 molar ratio of the polymer to the sulfonating agent was used, were: 10% of oleum in chloroform, 10% concentration of Radel® R in chloroform, and temperature between 0 and 50°C. Under these conditions, the reaction was complete within 45 min. Thermal properties, degradation characteristics and the viscosity of the postsulfonated polymer are reported. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2010

Key words: functionalization of polymers; ionomers; ion exchangers; sulfonation; Radel® R

INTRODUCTION

Sulfonated poly(arylene ethers), or sPAE, has excellent thermal and mechanical properties. It is therefore commonly used as a thermostable supportive matrix for conductive polymers and as ion-exchange membranes for separation applications.[1,2] sPAE can be produced by sulfonating poly(arylene ethers). This is the postsulfonation method as sulfonation occurs postpolymerization. Sulfonation of the monomer before polymerization is known as presulfonation, or direct-polymerization. Compared to presulfonation, postsulfonation produces sPAE with a lower degree of sulfonation (DS) because only one sulfonate group can be attached to each monomer unit of the polymer;[3] however, postsulfonation is much simpler, faster, and easier than the sulfonation of the monomer followed by a polymerization step.[3,4] Furthermore, a high DS is not always desired because it leads to water-swelling of the polymer membranes in applications such as fuel cells and filtration of aqueous media.

Radel® R is a commercial biphenol-based poly(arylene ethers) that has good mechanical properties, but is less resistant to acids than poly(arylene ethers) such as polyethersulfone (PES) and poly(ether ether ketone) (PEEK). Because of their acid resistance, PES and PEEK can be readily postsulfonated using concentrated sulfuric acid and oleum.[5–7] All chemical agents suitable for sulfonating poly(arylene ethers) are acidic and, therefore, only mild sulfonation conditions can be used so that the polymer chains are not degraded. Several successful postsulfonation methods have been reported for Radel® R,[3,8] but they all involve complex reaction systems and long reaction times (>10 hours). Furthermore, all these methods cause a significant reduction in chain length which may affect mechanical properties.

This work reports on a simple, mild, and rapid postsulfonation method for sulfonating Radel® R using oleum, a readily available sulfonating agent. Chain degradation and thermal properties of the sulfonated polymer obtained from the postsulfonation were examined at two reaction temperatures and compared with those reported in literature for sulfonated Radel® R produced from similar technique, but using different sulfonating agent, and that from the direct-polymerization of presulfonated monomers. Note that same conditions were applied to test our product.

EXPERIMENTAL

Materials

Radel® R (M_n = 24,000), or poly(arylene ether sulfone), was a gift from Solvay advanced polymers.

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Oleum (65% w/v SO_3) purchased from Merck was used as a sulfonating agent. Chloroform and the other chemicals mentioned were analytical reagent grade or higher and used directly.

Postsulfonation of Radel® R

A molar ratio of the polymer to the sulfonating agent was 1 : 3 in all experiments. A three necked round bottomed flask equipped with a mechanical stirrer in the center neck and glass stoppers in the other necks, was used to dissolve 2 g of oven-dried Radel® R in 20 mL of chloroform at room temperature overnight while stirring. A dropping funnel was used for drop-wise addition of a dispersion of 1 mL of 65% oleum and 10 mL of chloroform into the agitated flask over a period of 30 min, during which temperature of the polymer solution was set at 0°C (or 50°C). The reaction was then allowed at that temperature for a further 15 min and stopped by adding a sufficient quantity of methanol to cover the precipitate formed in the reaction flask. The precipitate was recovered by filtration and neutralized by rinsing with deionized water. The precipitate was dried in an oven at 60°C for 24 h and then at 80°C in a vacuum oven for a further 24 h. Sulfonated Radel® R, or sRAD, was thus obtained.

Characterizations

Thin sheets of the polymer samples prepared by solution casting technique were used to measure the FTIR spectra in a Perkin–Elmer Spectrum GX FTIR Spectrometer (Perkin–Elmer). The DS of synthesized products was calculated from the ^{1}H-NMR spectra, obtained from a Bruker Avance-300 NMR spectrometer, as the ratio of the peak areas for the protons adjacent to the pendant ionic groups to that of the other aromatic protons.

For measuring the intrinsic viscosity, the polymer samples were dissolved in dimethylacetamide (DMac) and measured at 25°C using a Ubbelohde U-tube viscometer. To test tensile strength using a Hounsfield H50KS universal testing machine (UK), a 10-by-1 cm thin polymer membrane was subject to a speed of 0.2 mm/min at room temperature (25°C). The thermal properties were determined in an STD 2960 simultaneous DTA/TGA (TA Instruments). Measurements of thermal properties were carried out under a nitrogen atmosphere at up to 800°C with a heating rate of 10°C/min.

RESULTS AND DISCUSSION

Sulfonated Radel® R, sRAD, was successfully produced by postsulfonation with oleum under mild conditions that minimized damage to the acid sensitive parent polymer. The reactor setup is simplified, compared to the other published postsulfonation methods.[3,8] There is no need for a condenser nor a nitrogen atmosphere during the reaction. The polymer produced was in the protonated-form which is suitable for making proton-conducting membranes of a fuel cell or of other applications.[3,4,9]

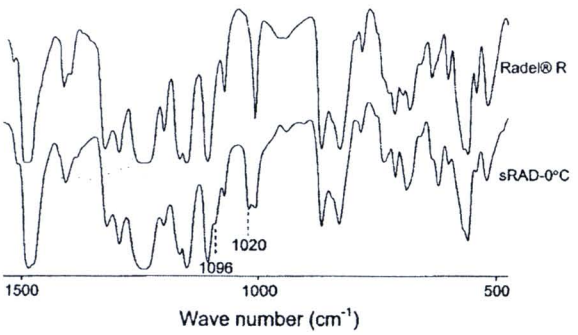

Figure 1 FTIR spectra of Radel® R and sRAD synthesized at 0°C.

The suitable reaction conditions that did not damage the acid-sensitive Radel® R were found after many failed experiments: a dilute solution of oleum in chloroform (~ 10% v/v), the polymer solution in chloroform (~ 10% w/v), and a low temperature (approximately 0–50°C). It is necessary to remind that temperature less than 0°C might not be desirable as it significantly lengthened the reaction time. Use of temperature greater than 50°C or relatively concentrated acidic solutions caused chain degradation, yielding brittle polymer samples that readily dissolved in water. In addition, as reported previously,[3] the acidic solution had to be added to the reactor very slow to prevent polymer precipitation.

Properties of the polymer samples produced under the preferred processing conditions were characterized. Figure 1 shows the FTIR spectra of Radel® R and the product polymer synthesized at 0°C. The absorption peaks at 1020 and 1096 cm^{-1}, corresponding to sulfonate groups, confirming that postsulfonation was achieved. The same peaks were also discerned from the sRad synthesized at 50°C. The DS in Table I was increased with raising reaction temperature due to the enhanced rate of the sulfonation reaction.

The intrinsic viscosity of a polymer solution is indicative of the hydrodynamic volume of the polymer molecules. Postsulfonation was observed to increase the intrinsic viscosity relative to the precursor polymer (Table I). This was because the electrostatic repulsion associated with the ionized sulfonated groups increased the effective hydrodynamic diameter of the polymer molecules. The intrinsic viscosity of the product obtained at 50°C was lower than the product produced at 0°C (Table I). This was because of a greater degradation of the poly(arylene ether sulfone) chains

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TABLE I
Properties of Radel® R and sRAD

Reaction temperature (°C)	DS[a] (%)	$[\eta]^{DMac}_{25°C}$ (cm^3/g)	Ultimate tensile strength (MPa)	$T_{5\%}$[b] (°C)	Char yield at 700°C[c] (%)	T_g (°C)
n/a[d]	0	67	44.96	544	45.7	224
0	48	248	30.16	374	50.5	233, 320
50	56	175	27.46	345	51.3	261, 315

[a] Degree of sulfonation (DS) values were obtained from ^{1}H-NMR spectra (not shown here).
[b] The 5% weight loss temperature ($T_{5\%}$).
[c] Char yield at 700°C was obtained from TGA curves.
[d] The sample was commercial Radel® R.
Note that all experiments were repeated at least twice with similar results.

at 50°C compared to at 0°C,[7,10] which was confirmed by the decrease in the ultimate tensile strength shown in Table I. This degradation notwithstanding, the sRAD samples produced at 50°C could be satisfactorily cast into transparent nonbrittle thin films using the solution casting method.

As shown in Table I, the introduction of sulfonate groups affected the thermal properties of Radel® R. On sulfonation, the 5% weight loss temperature ($T_{5\%}$) was reduced by 30% or more, depending on the DS (Table I). As shown in Figure 2, thermal degradation of sRAD at 300°C and 480°C followed a two-step process compared with the one-step degradation of the pristine Radel® R. In two-step degradation, the first step was attributed to a loss of sulfonate groups. Hence, in the first step, the weight loss of sRAD with the higher DS was greater and began slightly earlier than the weight loss of the polymer with the lower DS (Fig. 2). The second weight loss step of sRAD occurred at the same temperature as for the pristine Radel® R and was, therefore, attributed to the decomposition of the polymer backbone.

The char yield for polymer decomposition at 700°C and the glass-transition temperature (T_g) increased with increasing DS (Table I). The higher char yield of the more sulfonated polymer indicates that sulfonation improved the flame resistance of the polymer. An increasing glass transition temperature as a consequence of increasing sulfonation occurred because the presence of the ionized sulfonate groups reduced the flexibility and the mobility of the polymer chain. The two T_g values (Table I) for the sRAD were associated with the hydrophobic and the ionic clusters. Dual T_g values are typical of random amorphous ionomers in which the ionic clusters are large enough to hinder the movement of the nearby chain segments.[11,12]

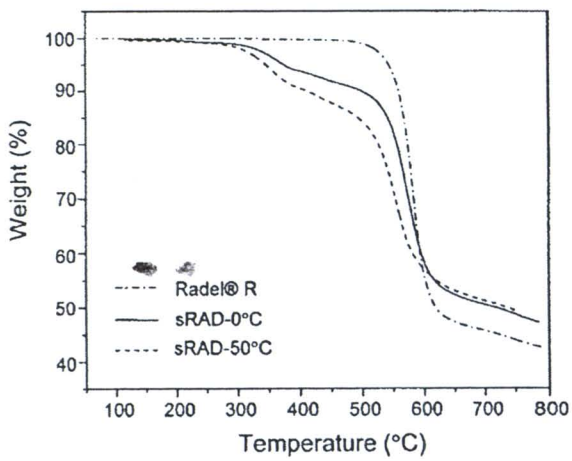

Figure 2 TGA curves of Radel® R and sRAD's synthesized at 0 and 50°C.

Similar effects of the DS on the intrinsic viscosity and the thermal properties of the polymer have previously been reported.[3] In this study,[3] a biphenol-based poly(arylene ether sulfone), that was chemically identical to Radel® R, was postsulfonated using trimethylsilyl chlorosulfonate. Thermal properties of sRad produced in the current work were also found to be comparable to those previously reported.[3] In contrast to the earlier study,[3] for a comparable DS achieved by postsulfonation, the intrinsic viscosity at the same temperature of our sulfonated polymer was about one order of magnitude greater. Our intrinsic viscosity was close to a product produced previously by direct polymerization.[3,4] Normally, a high intrinsic viscosity is expected for sulfonated poly(arylene ether sulfone) produced by direct polymerization because this method does not subject the polymer to acid degradation. Our work confirms that postsulfonation of Radel® R under suitable conditions can be used to produce sulfonated poly(arylene ether sulfone) that is quite comparable to the same product obtained by direct polymerization. In addition, our values of $T_{5\%}$, the char yield at 700°C and T_g for the polymer produced by postsulfonation were comparable to the values reported for the polymer produced by direct polymerization.[4]

CONCLUSIONS

A simple, mild and rapid method for postsulfonation of the acid sensitive commercial poly(arylene

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ether sulfone) Radel® R was established. Postsulfonation always requires acid conditions and the methods reported in the literature are unsatisfactory for use with

Radel® R because they produce severely degraded brittle sulfonated Radel® R. For the molar ratio of the polymer to sulfonating agent being 1 : 3, suitable sulfonation reaction conditions involved the use of readily available oleum at a concentration of 10%, a Radel® R concentration in the range of 10%, and temperature between 0 and 50°C. The reaction was completed within 45 min. Previously, sulfonated poly(arylene ether sulfones) with properties comparable to those obtained in this study, had been produced only by direct polymerization from a presulfonated monomer. This suggests a strong potential of the proposed simple postsulfonation method.

Solvay Advanced Polymers, USA, are thanked for providing Radel® R.

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