Preparation and Characterization of Mono- and Di-d-α-Tocopheryl Polyethylene Glycol 1000 Succinate

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ABSTRACT: Mono-d-α-tocopheryl polyethylene glycol 1000 (TPGS 1000) and di-TPGS 1000 were prepared from the synthesized TPGS 1000 mixture. The key separation step was performed by a Simulating Moving Bed chromatographic process. The chemical structures and molecular weight distrubution were characterized by ^{1}H-NMR and MALDI-TOF mass spectroscopy. NMR and MALDI-TOF MS data confirmed the occurrence of di-TPGS. Both NMR and MALDI-TOF MS results showed the degree of polymerization of the two esters and the molecular mass. The melting temperatures of the two polymers were investigated by DSC and the thermal decomposition temperatures have been determined by TGA. The melting temperatures of the two esters were 33 and 15°C, separately. And the two separated TPGS esters exhibited different thermal decomposition courses. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 3026–3033, 2011

Key words: mono-TPGS 1000; di-TPGS 1000; preparation; characterization

INTRODUCTION

d-α-Tocopheryl polyethylene glycol 1000 succinate [TPGS 1000, Fig. 1(a)] is a useful polymer that can be widely applied as nonionic surfactant, solubilizer, emulsifier, and absorption enhancer.[1–4] It was formed by esterifying d-α-tocopheryl acid succinate (TAS) with PEG (Mn 1000) as a water-soluble derivative of Vitamin E. It consists of the polar PEG tail and a hydrophobic α-tocopherol head. The amphiphilic structure of TPGS enables itself much more water-soluble than the traditional Vitamin E agents and also could be used as an excipient to enhance the bioavailability and therapeutic efficacy of some drugs which are difficult to be absorbed by the human gastrointestinal tract.[5,6] People have been interested in applying the intriguing polymer in various aspects to certain purpose and previous studies have assured the effectiveness.

Normally, people use the statement of TPGS that refers to the monoester of TPGS. In most studies the TPGS was applied directly as a commercial product without further treatments,[7,8] however in our previous experiments, we found that the commercial TPGS actually comprised two main substances with different polarity, which were confirmed as mono- and di-ester of TPGS by the experiments subsequently. Di-TPGS [Fig. 1(b)] was a byproduct in the esterification process of TPGS due to the equal activity of both hydroxyl groups of PEG, which inevitably occurred in such reactions.[9] To our concern, the unknown properties of di-TPGS may affect the TPGS activity, after all the mono-TPGS is the target surface-active species, in other words, the already proved properties of TPGS should be attributed to a combined effect of the two polymers. It has been published several characterizations, mainly ^{1}H-NMR and FTIR studies on chemical structure assurance[10,11] and thermal properties of commercial TPGS product,[12] with the exact species and composition unrevealed. Furthermore, there isn't any detailed and specific report on the chemical structures (e.g., the molecular weight distribution) and thermal properties of pure mono- and di-TPGS.

It is a pity that so far few detailed studies have been focused on the properties of the di-TPGS. The most likely reason was the difficulty in the separation and purification of di-TPGS. The average molecular weight difference of the two polymers is only about 500, meanwhile both polymers have a certain molecular weight distribution makes the separation based on molecular size unfeasible. Efforts have been made on the chromatographic separation, mostly on analytical HPLC and column chromatography.[13–16] However,

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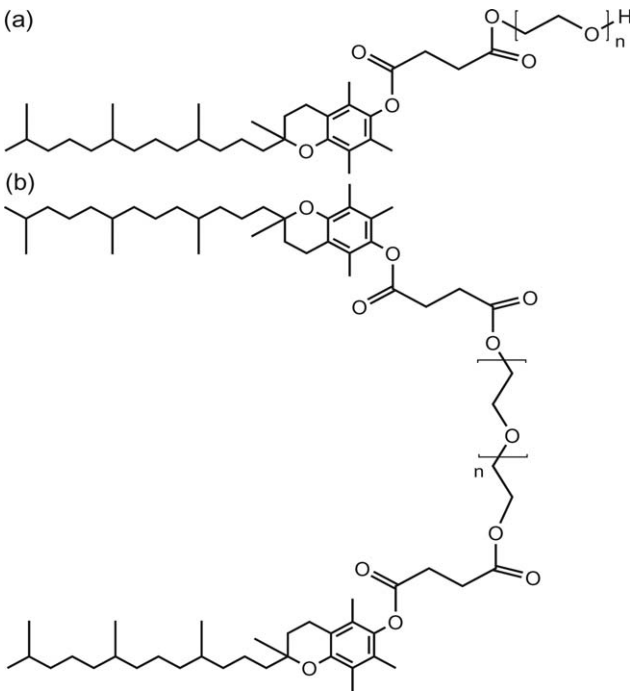


Figure 1 Schematic diagrams of the chemical structures: (a) mono-TPGS, (b) di-TPGS.

the separation of di-TPGS has still not been reported. To the best of our knowledge, it is the first attempt at SMB process design to separate the two esters from a TPGS mixture.

The aim of this article is to report a study on the preparation and characterization of the two esters of TPGS. The separation of TPGS mixture was carried out on a SMB system, and the chemical structure was characterized by ^{1}H-NMR and MALDI-TOF mass spectrometry. The two esters were also characterized through DSC and TGA to assess the thermal properties. To the best of our knowledge, this work is the first attempt at detailed investigating the chemical structures, molecular distribution, and thermal properties of both esters of TPGS.

EXPERIMENTAL

Chemicals and reagents

TPGS 1000 sample was prepared in our laboratory. It was prepared by esterification reaction of d-α-tocopheryl acid succinate and PEG 1000 (1/3, mol/mol) in the presence of *p*-toluene sulfonic acid as a catalyst and toluene as the solvent according to literature.[17] After isolation of the TPGS esters from the reactant, a SMB chromatographic separation was carried out to obtain the mono- and di-TPGS. TPGS 1000 standard was obtained from Sigma-Aldrich (Buchs, Switzerland), and TAS standard (>99%) was received from Sigma-Aldrich (St. Louis, MO). PEG 1000 (CP), toluene (AR) and *p*-toluene sulfonic acid (≥99%) were received from Shanghai Reagent Company (Shanghai, China). The d-α-tocopheryl acid succinate (≥95%) was obtained from Zhejiang Worldbest Pharmaceuticals Science Technic Development. (Lanxi, China), Acetonitrile (Tedia, USA), and isopropanol (Tedia, USA) were HPLC grade.

HPLC analysis

The Waters HPLC system (Milford, MA) equipped with a 1525 binary HPLC pump, a Waters 717 plus autosampler and a Waters 2487 dual λ absorbance UV detector were used for analysis of samples. The analytical column used was Develosil® Rpaqueous C30 column (4.6 mm i.d. × 250 mm, 5 μm, Nomura, Japan). The mobile phase consisted of acetonitrile (A) and isopropanol (B) and carried out with a gradient condition (A: B, v/v): 65 : 35 for 10 min, 70 : 30 for 10 min at a flow rate of 1 mL/min at 35°C. The determination was carried out at 284 nm, which was the maximum absorption wavelength of TAS.

The sample solutions were prepared similarly with the mono- and di-TPGS samples prepared in our laboratory.

SMB separation

A four-zone simulated moving bed (SMB) chromatographic process was carried out to separate the mono- and di-TPGS. The CSEP® C9812 SMB unit (Knauer, Germany) built for the study consisted of 8 packed columns (10 mm i.d. × 150 mm, 10 μm, ODS-AQ, YMC, Japan) with two columns each zone. Four pumps controlled the elution, feed, extraction, and raffinate flow rates. The operating conditions of SMB process was provided by SMB_Guide® software, i.e., the flow rates of feed, raffinate, eluant, extract, zone I, zone II, zone III, and zone IV were 1.00, 3.00, 5.67, 3.67, 10.07, 6.40, 7.40, 4.40 mL/min, respectively. The switching time was 2.40 min. The temperature was 35°C.

^{1}H-NMR spectroscopy

The structures of the separated mono- and di-TPGS were confirmed by measuring ^{1}H-NMR spectra of the polymers in $CDCl_3$ with a Brucker 500 MHz NMR spectrometer. The data were elaborated with MestRec software in the Fourier transform mode and the chemical shift resonances were accurately assigned to the specific protons. The 1H-NMR spectra were used to estimate the degree of polymerisation of the polyoxyethylene block and the average molecular weight of the two polymers.

MALDI-TOF mass spectroscopy

The matrix-assisted laser desorption/ionization time-of-flight (MALDI–TOF) mass spectra were

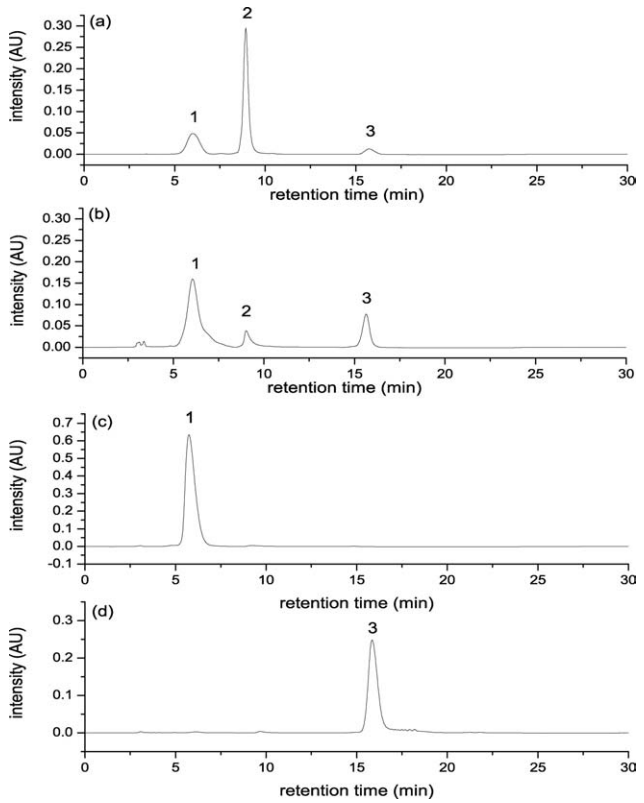


Figure 2 Chromatograms of mixed standards solution (a), synthesized TPGS mixture (b), mono-TPGS esters (c), and di-TPGS esters (d) obtained from SMB separation. Peaks identification: (1) mono-TPGS 1000; (2) TAS; (3) di-TPGS 1000, UV detection at 284 nm, mobile phase: acetonitrile and isopropanol, 10 μL injected.

recorded in linear mode using a Clinprot (Bruker Daltonik, Germany) mass spectrometer. For MS analysis the samples were prepared onto MSP 96 target ground steel (Bruker Daltonik, Germany) using HCCA (α-cyano-*p*-hydroxycinnamic acid; Bruker Daltonik GmbH, Germany) as matrices. The mass range was calibrated with respective peptide standards. The spectra were acquired in the mass range of 100 to 4000 Da.

DSC and TGA characterization

Thermal characteristics of the polymers were measured by differential scanning calorimetry (DSC) using a Pyris 1 DSC (Perkin Elmer, USA). The sample 5–10 mg was accurately weighed and sealed in an aluminum pan. Both heat flow and temperature calibrations of calorimeter were performed following the procedures suggested by the supplier. The heating and cooling circles were set to examine the thermal stability of the polymer samples between −60–60°C with a 10°C/min heating rate and a nitrogen atmosphere was applied for determination.

The second DSC was carried out at 0–90°C with a 10°C/min heating rate and a nitrogen atmosphere was applied for determination.

The degradation temperatures of the two polymer samples were determined by thermogravimetric analysis (TGA) using a Pyris 1 TGA (Perkin Elmer, USA) system. Samples were heated in a nitrogen environment at a heating rate of 15°C/min from room temperature to 800°C.

RESULTS AND DISCUSSION

Separation and analysis of mono- and di-TPGS

As have mentioned previously, we found that the TPGS 1000 comprised two esters, as shown in the HPLC chromatogram (Fig. 2). The molar ratio of the mono-TPGS to di-TPGS was about 11 in the TPGS mixture. According to the different polarity of the two esters, we assigned the elution peaks at around 6 min and 16 min to the mono-TPGS and di-TPGS tentatively. TAS was eluted at around 9 min, the peak of which was sharp, sensitive, and wouldn't be interfered by the elution peaks of the other ester. The typical elution peak of di-TPGS was wide, flat which would be explained by the wide molecular distribution and a stronger interaction with the C30 stationary phase. The di-TPGS has a stronger interaction with the C30 stationary phase and was eluted in a less polarity mobile phase.

To further confirm the structure of the two substances, it was necessary to prepare enough samples to carry out the characterization experiments subsequently. The SMB chromatographic process was known as an efficient and powerful continuous separation technology.[18,19] Here we applied it to the preparation of the two esters with high purity (both >99%, calculated by the integrated peak area in HPLC). The typical eluted solutions of the SMB were examined by HPLC analysis, as shown in Figure 2(c), (d). The obtained mono-TPGS 1000 was

TABLE I
Assignments of the Units and End Groups in the TPGS 1000 Polymers, Relative Areas of Protons

		Proton abundance	
Proton	Chemical shift (ppm)	mono-TPGS	di-TPGS
a	0.8	12	27
c	1.05–1.1	16	32
b	1.2–1.5	8	16
d	1.1–1.2		
e	2.57	2	4
f	2.7–2.9	4	8
g	1.9–2.0	9	18
h	1.7	2	4
i	3.6–3.8	92	78
j	4.25–4.27	2	4

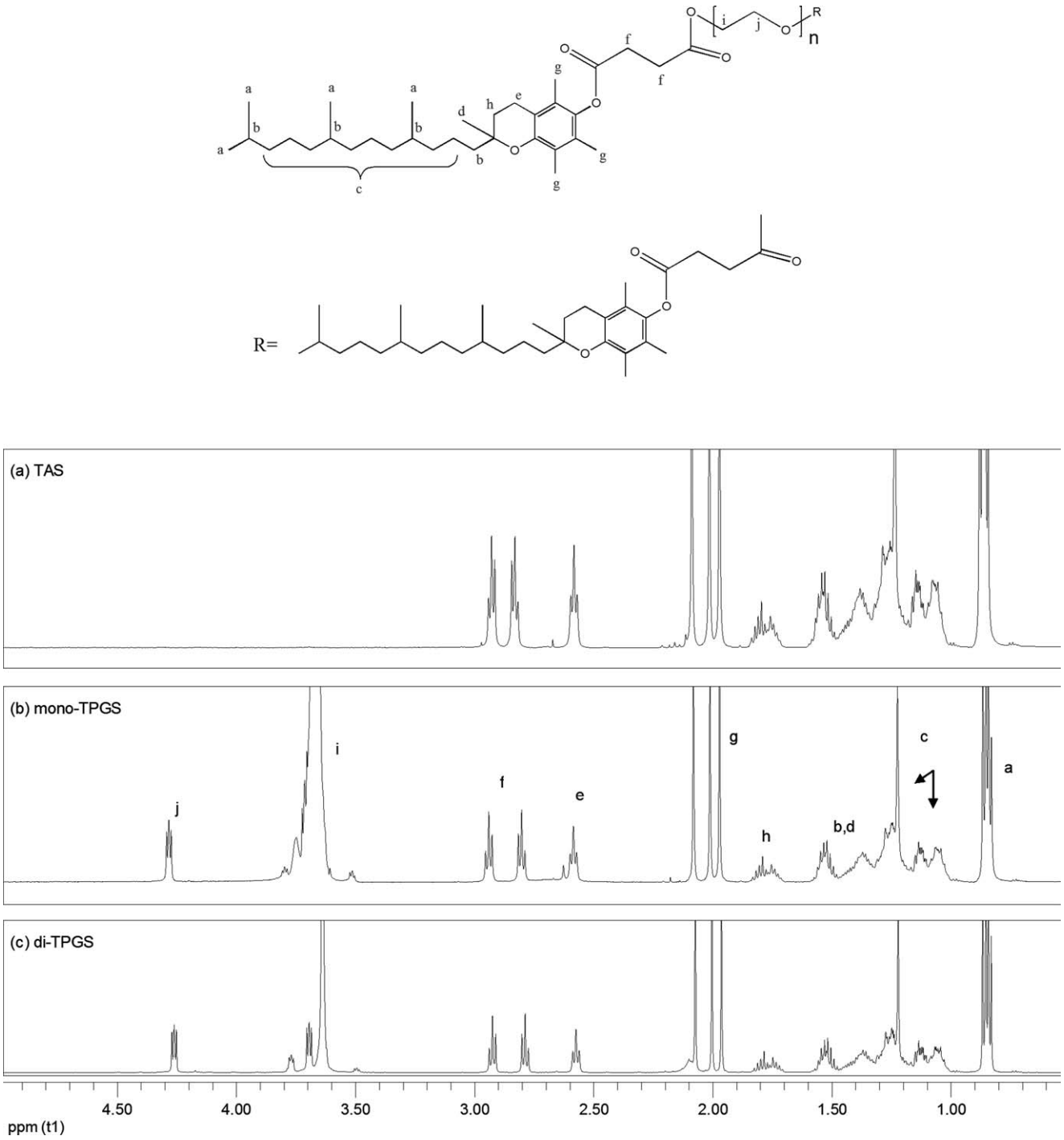


Figure 3 ^{1}H-NMR spectra of (a) TAS, (b) mono-TPGS 1000, (c) di-TPGS 1000 in $CDCl_3$.

a white to light yellow waxy solid, while the di-TPGS 1000 was light brown and in high viscosity liquid state at room temperature.

^{1}H-NMR analysis of mono- and di-TPGS

The two substances we found in the HPLC process have to be identified with the exact chemical structure. After SMB chromatographic separation, we obtained the di-TPGS 1000 and mono-TPGS 1000 with high purity. The ^{1}H-NMR characterization was made on the samples obtained and the chemical shift resonances were accurately assigned to the specific protons. The specific assignments of the protons in the mono- and di-TPGS 1000 are presented in Table I. Figure 3 reports the typical ^{1}H-NMR spectra of the TAS and the two esters. The peak at 3.6 was assigned to the $-CH_2-$ protons of the PEG part in the TPGS. In all cases, the spectra were found to be consistent with the expected chemical structure and

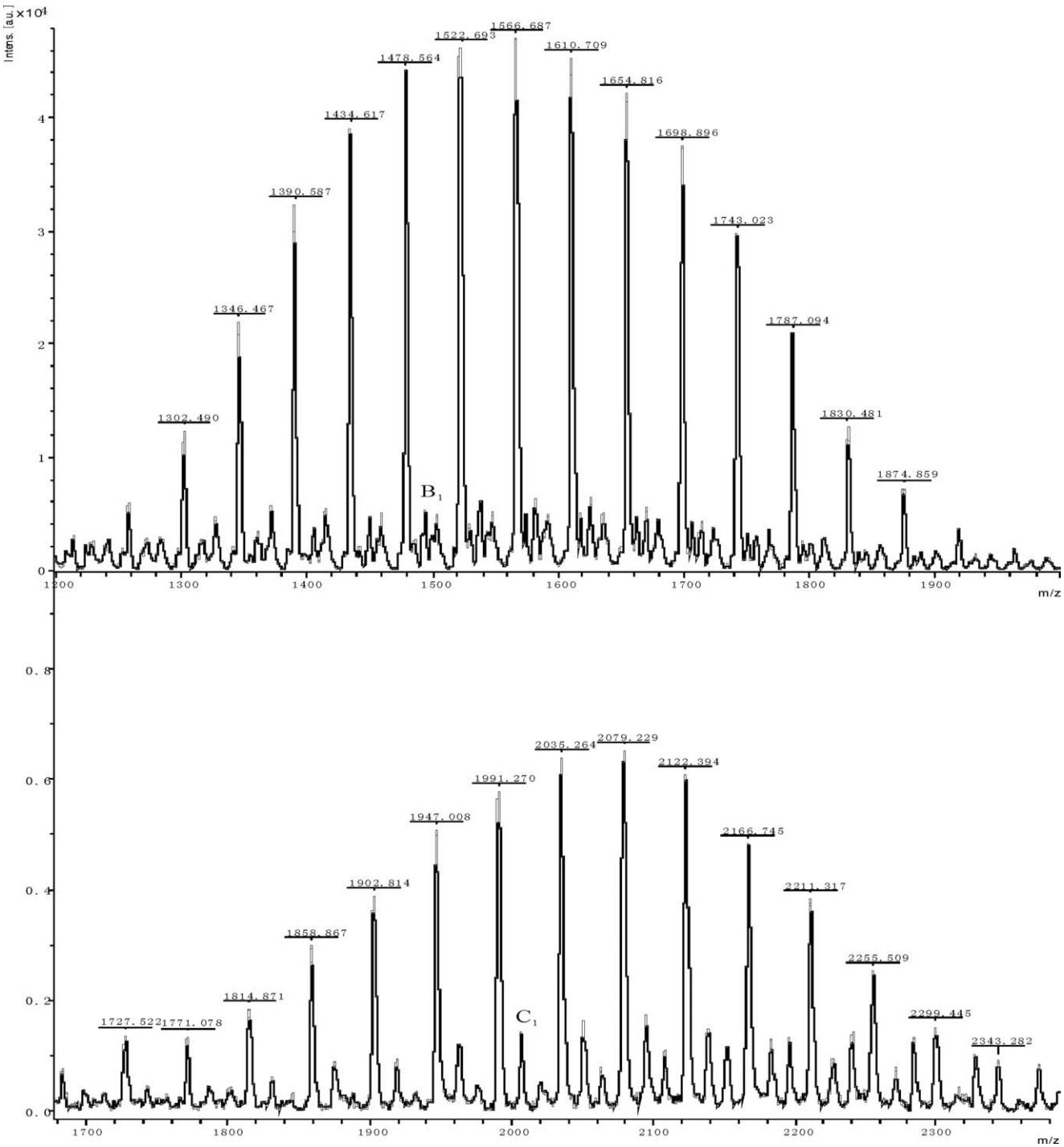


Figure 4 MALDI spectra of the mono- and di-TPGS 1000 polymers: (a) mono-TPGS and (b) di-TPGS.

were in accordance with those found in the literature.[10,11] The spectrum of TAS group exhibits typical peaks between 0.8–3 ppm.[20,21] It was also proved that there are only few differences in the schemes of the two esters. The proton resonances of the two polymers are mostly identical; the only differences were the resonance peak areas of protons at 3.6 (as shown in Fig. 3). Moreover, the integrated chemical shifts of the protons resonance of TPGS 1000 indicated the different average polymerization degrees ($\overline{n}$), i.e., 23.5 for mono-TPGS 1000 and 20.5 for di-TPGS 1000, respectively.

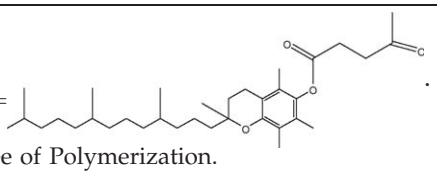
MALDI-TOF mass spectra characterization

To obtain detailed information about the average molecular weight and the distribution of the polymers with different polymerization degree of the

TABLE II
Peak Assignments for MALDI-TOF Mass Spectra of the Two Polymers Shown in Figure 4

mono-TPGS			di-TPGS		
Structure[a]	*m/z* $[M+Na]^+$	DP[b] (*n*)	Structure	*m/z* $[M+Na]^+$	DP (*n*)
$RO(CH_2CH_2O)_nH$	1302.490	16	$RO(CH_2CH_2O)_nR$	1814.794	16
	1346.467	17		1858.764	17
	1390.587	18		1903.223	18
	1434.617	19		1946.848	19
	1478.564	20		1991.052	20
	1522.693	21		2034.783	21
	1566.687	22		2079.562	22
	1610.709	23		2123.960	23
	1654.816	24		2167.107	24
	1698.896	25		2211.054	25
	1743.023	26		2255.162	26
	1787.094	27		2299.190	27
	1830.481	28		2343.282	28

[a] Where: R=



[b] DP: Degree of Polymerization.

two esters, we used MALDI-TOF mass spectra characterization, which was able to analyze the polymer molecules individually. In the preliminary experiment, we tried the ESI/MS spectroscopy to analyze the molecular information of the two esters, it turned out that the ESI/MS spectroscopy was not soft enough to maintain the molecular structure; the spectrum was difficult to identify the mass peaks. The MALDI-TOF MS was known to have the potential to provide not only molecular mass and distribution data for polymers, but also end group and branching information.[22,23] In this article the mono- and di-TPGS 1000 was characterized by MALDI-TOF MS analysis. The spectra of two polymers are shown in Figure 4, and mass assignments are listed in Table II.

Besides the high intensity peaks belonging to the sodiated molecule, low intensity peaks of charged potassium-adduct molecule $[M+K]^+$ was observed, for example, species B_1 (*m/z* 1494.635, $n = 20$) and C_1 (*m/z* 2007.31, $n = 20$), both 16 differed with respect to the $[M+Na]^+$ molecule. As the MALDI-TOF MS analysis was applied without any cationizing agent, it was probably that the alkali metal adduct ions came from the salt in the polymers. Further experiment confirmed the assumptions that the similar adduct molecule, $[M+Na]^+$, $[M+K]^+$ were both observed in the raw material PEG without any treatment (data not shown). It can be presumed that the sodiated molecule had a higher sensitivity in this MALDI-TOF MS.

From the normalized intensity of the individual peak, the number average molecular weight ($\overline{M}_n$), the weight-averaged ($\overline{M}_w$) molecular masses, and molecular mass distributions ($\overline{M}_w/\overline{M}_n$) can be calculated, as presented in Table III.

Previous studies had reported the molecular weight of mono-TPGS 1000 as 1513 approximately, which were obviously calculated according to the esterification process of mono-TPGS 1000, and the average ethylene glycol units content were inconsistent with each other.[8,11,24] Our experimental data shows the factual molecular mass and provides much more detailed information about the structure and the composition of this monoester. Meanwhile, the MALDI spectra data further confirmed that a second TAS group was connected to the PEG chain,

TABLE III
Average Polymerization Degrees ($\bar{n}$), Number-Averaged ($\bar{M}_n$) and Weight-Averaged ($\bar{M}_w$) Molecular Masses, and Molecular Mass Distributions ($\bar{M}_w/\bar{M}_n$) of the Two Esters Calculated from ^{1}H NMR and MALDI Spectra

	$\overline{n}$		$\overline{M}_n$		$\overline{M}_w$	
	^{1}H NMR	MALDI	^{1}H NMR	MALDI	MALDI	$\overline{M}_w/\overline{M}_n$
mono-TPGS	23.5	22.2	1610	1551	1565	1.009
di-TPGS	20.5	21.8	1990	2045	2051	1.003

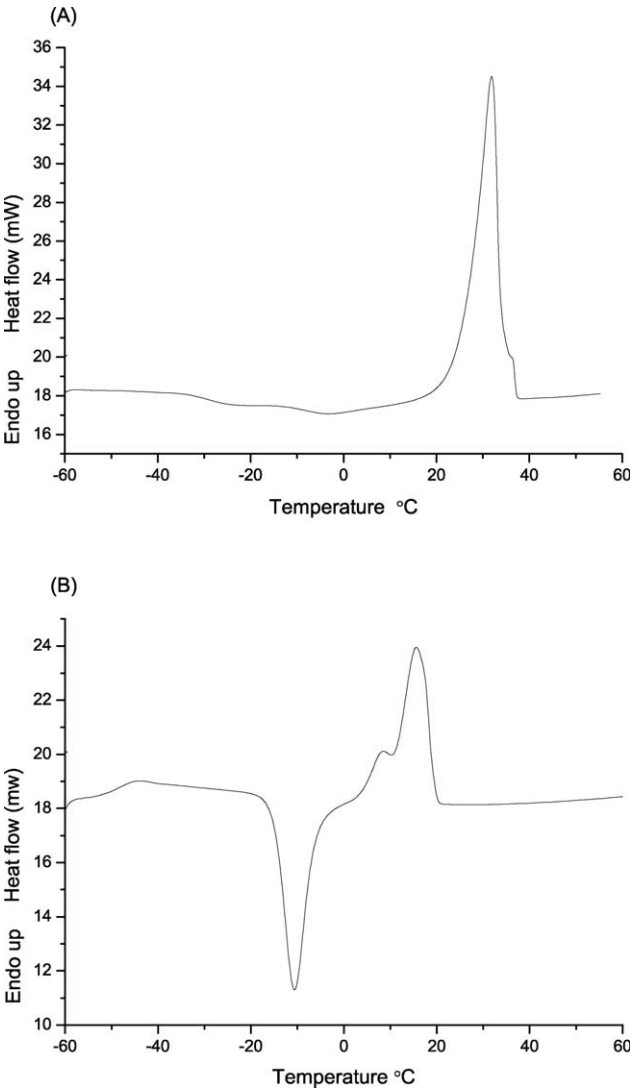


Figure 5 2nd cycle DSC curves of two esters of TPGS 1000: (A) mono-TPGS and (B) di-TPGS.

the molecular mass around 2000 was consistent with the expected structure.

DSC and TGA analysis

The thermal behavior of the mono- and di-TPGS 1000 was investigated by DSC and TGA. Figure 5 shows the 2nd thermogram of the mono-TPGS and di-TPGS. Both exhibited thermal stable property during repeated phase transitions. The melting temperature of mono-TPGS 1000 was ~ 33°C, which was lower than the data reported by Eastman Vitamin E TPGS NF applications and properties.[24] This inconsistency was most likely caused by the different thermal history of mono-TPGS and the different operation condition in the heating and cooling procedure. To verify this, we designed a similar DSC scan procedure accordingly,[12] and the result (shown in Fig. 6) clearly confirmed our prediction, the melting point at ~ 38°C was in accordance with the data reported in the previous studies.[4,12,24]

The similar thermal behavior was observed with di-TPGS 1000, with a melting temperature at 15°C. Note that the 2nd heating curve of di-TPGS exhibited a sharp cold crystallization peak at about −10°C, which revealed that relatively small hydrophobic moieties of PEG-TAS affected the crystallization behavior of TPGS segment. The both ends of the polyoxyethylene block were capped with TAS, making crystallization of the polyoxyethylene block more difficult.

The degeneration courses of the two polymers were determined by TGA analysis. The decomposition temperatures of mono- and di-TPGS 1000 were 428°C and 418°C, respectively, as shown in Figure 7.

Based on the results obtained above, it can be seen that the mono-TPGS 1000 had a weight loss of 10% from room temperature to 300°C, while there was no obvious weight loss for di-TPGS 1000 during the same temperature region. And the little undulate in the derivated weight curve at 200°C indicated the starting of decomposition of mono-TPGS 1000, which can be postulated that the weight loss before 300°C was due to intramolecular dehydration associated with the hydroxyl group in the PEG tail of mono-TPGS, the dehydration produced a serial alkene group which were thermal unstable, and caused a continuous weight loss of the polymer. With reference to the information revealed by Wu et al.,[12] the degradation temperature of commercial TPGS 1000 product was 199°C by a DSC scan, which was also supportive to the results we obtained that the decomposition of mono-TPGS 1000 started at 200°C. Meanwhile, we might find that the di-TPGS 1000 was quite stable when compared to mono-

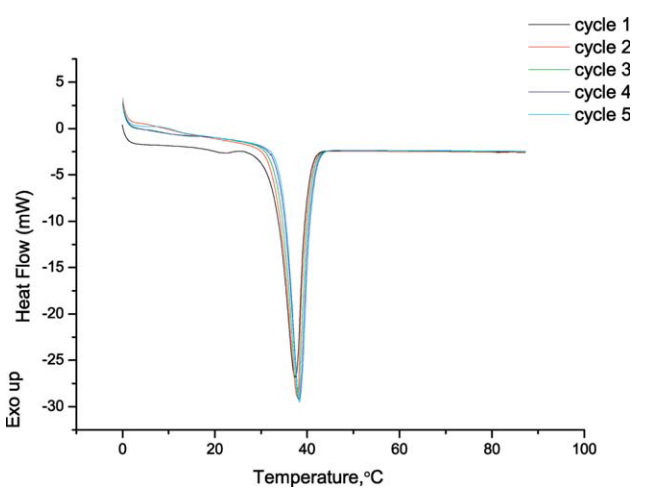


Figure 6 DSC curves of five cycles for the mono-TPGS at 10°C /min from 0–90°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

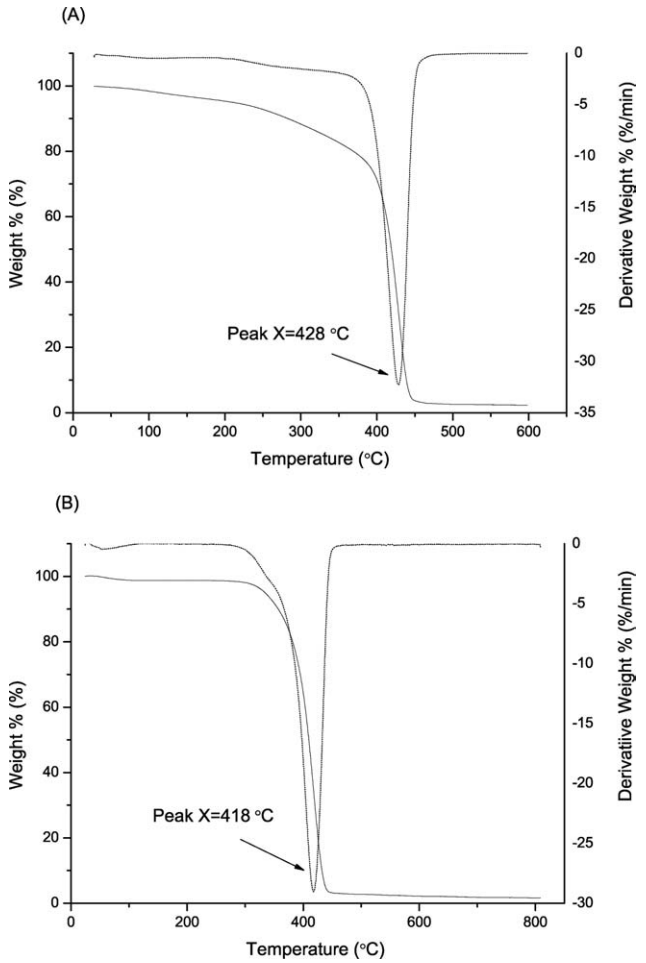


Figure 7 TGA curves of the two TPGS 1000 esters: (A) mono-TPGS and (B) di-TPGS.

TPGS 1000 in the region of room temperature to 300°C until the massive degradation at 418°C.

By comparing the thermal degradation behavior of these polymers it was observed that both TPGS 1000 esters exhibited similar thermal characteristics, and it was also as expected, as there were the similar PEG structure in their framework, with almost the same degrees of polymerization. The different thermal properties of the two esters may still affect the downstream application of TPGS product.

CONCLUSIONS

The combined preparation and characterization of mono-TPGS 1000 and di-TPGS 1000 is reported for the first time. SMB chromatographic procedure was applied to separate the two polymers. Combined ^{1}H-NMR and MALDI-TOF MS analysis was used to a reliable determination of the chemical structure and molar composition and distribution of the two esters. Both ^{1}H-NMR spectroscopy and the MALDI-TOF MS data showed the occurrence of di-TPGS. The thermal properties of the two polymers were investigated by DSC and TGA analysis. The mono-TPGS 1000 polymers could be particularly used as stable matrix for drug modification. The results showed that di-TPGS 1000 is much more stable matrix, and particular properties need a further investigation.

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