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Gel permeation chromatographic method for monitoring the transesterification reaction in a two-step chemoenzymatic synthesis of urethane oil based on vegetable oils

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Abstract

Urethane oils (isocyanate-modified vegetable oils) are used in decorative paints and protective coating formulations. A gel permeation chromatographic method using tetrahydrofuran as mobile phase, an Ultra-Styragel column, a refractive index detector and polystyrene standards has been developed for monitoring the formation of precursor (partial esters) in the first step of a two-step chemoenzymatic synthesis of urethane oil and for the subsequent molecular mass determination of the final product. The method allows the simultaneous determination of precursor and the starting material in the first step and thereby permits the optimization of reaction parameters in the second step to yield urethane oil of the desired molecular mass.

1. Introduction

Urethane materials are used nowadays in decorative paints and protective coating formulations [1], materials providing additional properties like better gloss, high scratch and impact resistance, chemical and solvent resistance, light stability and resistance to degradation from weathering.

Urethane oils are oil-modified polyurethanes prepared by reacting a diisocyanate with partial esters obtained from the transesterification of drying or semidrying oils with polyols. The molecular mass of the urethane oil, an important parameter for the optimization of material properties for a specific application, mainly depends on the nature and composition of the precursor (partial esters) formed in the transesterification step. Gel permeation chromatography (GPC) offers precise information regarding the molecular mass and molecular mass distribution of polymers.

The analysis of polyurethane prepolymers has been carried out by GPC after derivatization with methanol [2]. Several other methods based on HPLC and GC are available for the quantitative determination of digylcerides and monoglycerides [3]. GC requires derivatization of mono- and diglycerides to methyl or propyl esters [4]. GPC analyses were also found to be successful in the analysis of oligomers [5].

In brief, the literature contains the threads of a theoretical basis for understanding how material properties might depend on the reaction

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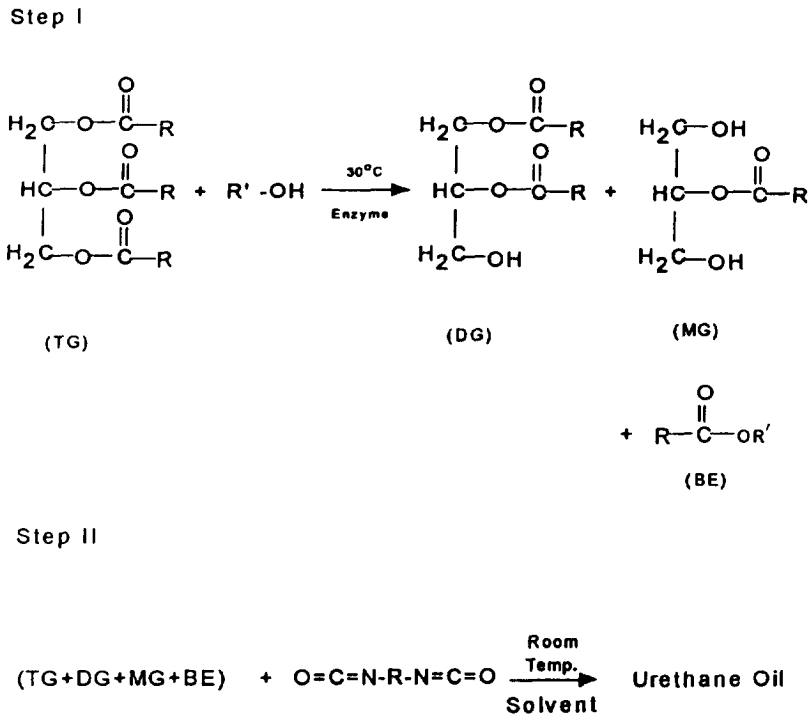

Fig. 1. Reaction scheme for the two-step synthesis of urethane oil.

parameters, but there has not been a practical analytical procedure for monitoring the formation of precursor and simultaneously determining the concentrations of the individual species that are present.

In the present work, we describe an efficient GPC method for monitoring the precursor composition obtained by transesterification of vegetable oil with *n*-butanol in a two-step chemoenzymatic synthesis of urethane oil (Fig. 1).

2. Experimental

2.1. Reagents and solvents

Castor and soybean oils were locally purchased and their purity was checked by GPC, prior to use. 2,4-Toluene diisocyanate was obtained from Fluka (Buchs, Switzerland). HPLC-grade tetrahydrofuran and reagent-grade *n*-butanol were from S.D. Fine-Chem (Bombay, India) and used as received without any further purification. Lipozyme IM 20 (41 IU g^{-1}), a commercially available lipase from the fungus *Mucor miehei*, immobilized on a microporous anion-exchange resin was obtained from Novo Nordisk (Denmark).

Authentic samples of diglyceride, monoglyceride and butyl esters were synthesized in our laboratory, by lipase-catalyzed transesterification of triglyceride oil with *n*-butanol. The reaction mixture was analyzed by GPC in the LC mode to identify and detect the corresponding peaks for diglyceride (DG), monoglyceride (MG) and butyl ester (BE) and triglyceride (TG, starting material). All the components were separated by column chromatography using hexane–chloroform (95:5). The separated components were again analyzed by GPC in the LC mode. A distinguished single peak confirmed

them as single-spot compounds. These components were further characterized by NMR and IR spectroscopy.

2.2. Two-step chemoenzymatic synthesis of urethane oil

In the first step, the precursor (partial esters) has been prepared by lipase-catalyzed transesterification of triglyceride oil with *n*-butanol.

In the second step the partial esters were further reacted with diisocyanate to obtain urethane oil.

2.3. Instrumentation

The GPC system consisted of a Model 6000A pump, a Model R403 refractive index (RI) detector, a Model U6K injector and a 730 datamodule integrator (all from Waters, Milford, MA, USA). Ultra-Styragel columns of different pore sizes, viz. two 500 Å, two 100 Å and one 1000 Å columns, were used.

2.4. Chromatographic analysis

The chromatographic conditions for monitoring step I are as follows: The mobile phase was HPLC-grade tetrahydrofuran. The flow-rate was 1.5 ml/min and Ultra-Styragel columns (500, 500, 100, 100 Å) were used.

The chloroform solution of partial esters formed in step I was treated with 2,4-toluene diisocyanate at room temperature to obtain urethane oil.

The molecular masses were determined by GPC using HPLC-grade tetrahydrofuran as the mobile phase. The flow-rate was 1.5 ml/min. μStyragel columns (10^3, 500, 100 Å) and polystyrene standards of molecular masses 35 000, 8500, 4000 and 2900 were used.

The calibration and quantification of the TG, DG and MG of castor and soybean oil was done by GPC using the conditions mentioned for step I monitoring. Calibration mixtures containing TG, DG and MG in different ratios for each oil were prepared and analyzed. Calibration graphs were constructed and response factors were determined from the slope.

3. Results and discussion

The typical gel permeation chromatogram of a reaction mixture for step I in the two-step synthesis of urethane oil is shown in Fig. 2. The transesterification of TG oil with *n*-butanol gives rise to three peaks corresponding to partial esters, viz. DG, MG and BE respectively. These three peaks are well separated from each other and from the starting material (TG).

Consequently, the GPC analysis was also carried out for monitoring step I on the basis of the increase in the peak height of partial esters and the decrease in the peak height due to consumption of TG (Fig. 3).

The linearity of response for TGs, DGs and MGs of castor and soybean oil was checked over

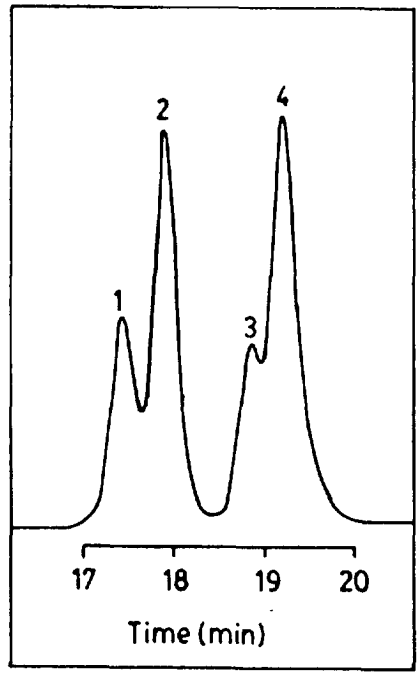

Fig. 2. Typical chromatogram of the reaction mixture in the synthesis of partial esters from triglyceride oil and *n*-butanol. Peaks: 1 = triglyceride; 2 = diglyceride; 3 = monoglyceride; 4 = butyl ester.

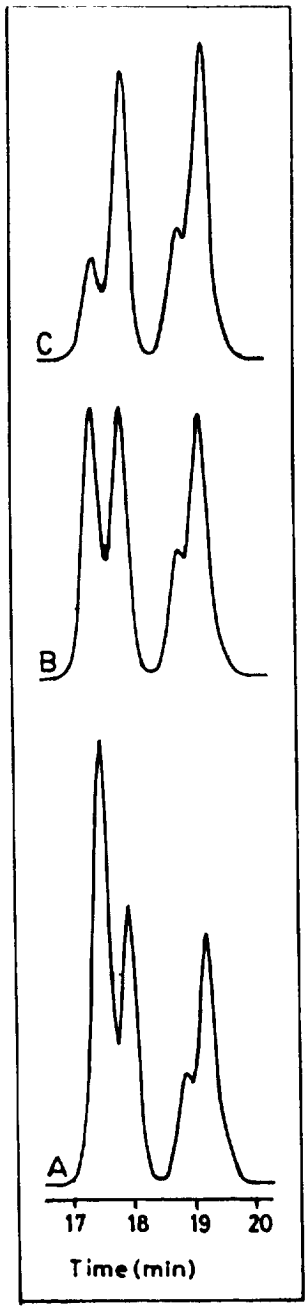

Fig. 3. Chromatograms for monitoring the gradual conversion of triglyceride to partial esters. (A) Conversion after 2 h; (B) conversion after 4 h; (C) conversion after 8 h.

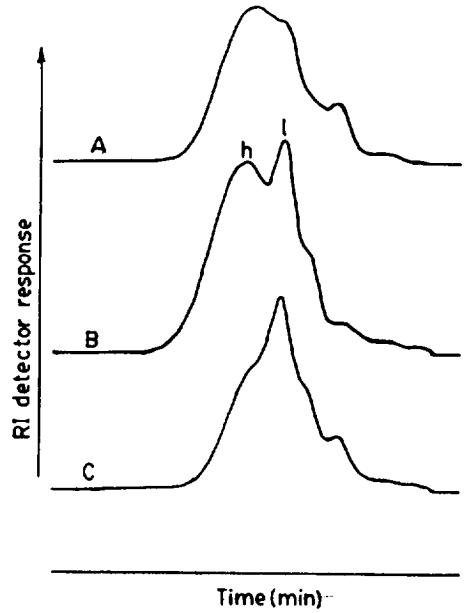

Fig. 4. Molecular mass distribution curves of castor oil-based urethane oils synthesized from partial esters (precursor) of different compositions; h = high- and l = low-molecular-mass species.

the concentration range 0.5–1.6 mg/100 μl. The area/concentration ratios for TG, DG and MG of castor oil were as 254.6, 258.6 and 260.6, respectively, whereas the area/concentration ratios for soybean oil were as 259, 263 and 265 respectively. The detection limit for analytes was found to be *ca.* 10 μg, and hence the injection sample amount was adjusted accordingly for the analysis of actual reaction mixture.

In the present study, the linearity of response of the RI detector was confirmed from the area/concentration ratios of TG, DG and MG. On the basis of these values, the relation between the area percentage and actual percentage was established, which further facilitated the quantification of the actual reaction mixture. Secondly, as the reaction follows a known path with no side products, the mass balance was always near 100%.

As the response factors for TGs, DGs and MGs of each oil are similar, it was not essential to take the response factors into account while determining the concentration of each con-

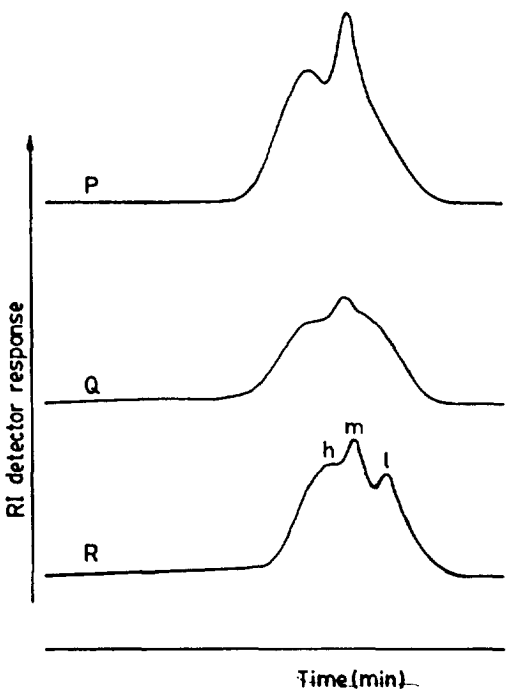

Fig. 5. Molecular mass distribution curves of soybean oil-based urethane oils synthesized from partial esters (precursor) of different compositions; h = high; m = medium- and l = low-molecular-mass species.

stituent of the reaction mixture. The inter-assay precision of the method was established by triplicate analyses of synthetic mixtures of different compositions and the percentage error was found to be generally less than 2%.

The results of the GPC analysis of actual reaction mixtures as shown in Table 1 were in good agreement; the yields were obtained after isolating each constituent of the reaction mixtures by column chromatography. This clearly indicates that the consumption of TG, the formation of precursor (partial esters) and their compositions, could be determined during the course of the transesterification step itself.

Fig. 3, the set of chromatograms of the kinetic study for transesterification of TG oil with *n*-butanol at definite time intervals, shows a gradual decrease in the height of the TG peak, with a corresponding increase in the peak heights of DG, MG and BE. It was observed (Table 1) that the MG/DG ratio in the precursor increased with time, indicating the two-step reaction sequence, where oil was transesterified to DG first and then further converted to MG as expected [6].

Table 2 reports the molecular masses of various urethane oils synthesized by the reaction between partial esters of different compositions formed in step I and 2,4-toluene diisocyanate. It is evident from Table 2 that the molecular mass decreases with increase in the MG content in the precursor composition.

Figs. 4 and 5 show the typical molecular mass distribution pattern of the final product obtained from partial esters of different compositions. GPC chromatograms for castor oil-based urethane oils contain two peaks, one for high-molecular-mass and the other for low-molecular-mass species. However, soybean oil-based urethane oils contain three peaks for high-, medium- and low-molecular-mass species, respectively. The time axis being the same, it is a

Table 1
Results of the analysis of transesterification of various triglyceride oils with *n*-butanol

No.	Type of oil	Oil-to-alcohol ratio	Reaction time (h)	TG (%)[a]	DG (%)[a]	MG (%)[a]	BE (%)[a]
1	Castor	1:1	2	41.3	24.5	08.7	25.5
2		1:1	4	28.7	28.7	11.9	30.7
3		1:1	8	16.9	33.1	12.7	37.3
1	Soybean	1:3	2	42.3	24.4	08.1	25.2
2		1:3	4	29.2	29.3	12.1	29.4
3		1:3	8	10.1	16.7	23.0	50.2

[a] Percentages were obtained by GPC.

Table 2
Results showing molecular masses of various urethane oils synthesised in step II

Type of oil	Oil-to-alcohol ratio	No.	Precursor composition in step I				Molecular mass of urethane oil after step II
			TG (%)[a]	DG (%)[a]	MG (%)[a]	BE (%)[a]	
Castor	1:1	A	12.3	33.9	12.9	40.9	2886
	1:2	B	09.6	16.2	23.8	50.4	2143
	1:3	C	00.0	02.6	42.2	55.2	1827
Soybean	1:3	P	29.2	29.3	12.1	29.4	2057
	1:3	Q	10.1	16.7	23.0	50.2	1920
	1:3	R	00.0	02.4	42.2	55.4	1477

[a] Percentages were obtained by GPC.

direct evidence of the correlation between the composition and molecular mass distribution.

4. Conclusions

The method allows the simultaneous determination of precursor and starting material in the transesterification step, in the two-step chemoenzymatic synthesis of urethane oil. The composition of the precursor and the triglyceride-to-alcohol ratio are functions of time and could be determined at any instance during the course of the reaction (step I); therefore the reaction parameters of step II could be optimized to yield urethane oils of the desired molecular mass and molecular mass distribution.

As the method described is accurate and precise it can, in general, be used for monitoring various types of urethane oils starting from all naturally occurring triglycerides or from the chemically engineered triglycerides like tristearin and triolein.

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