

Thermotropic Behavior of Lithocholic Acid Derivative Linked Hydroxyethyl Cellulose

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ABSTRACT: Various mesogenic lithocholic acid (LA) derivatives [3-acetyl LA (LAAC); 3-propionyl LA (LAP), 3-cinnamoyl LA (LACin); 3-benzoyl LA (LAB), 3-(4-nitrobenzoyl) LA (LANB); and methyl-3-(3-carboxypropionyl) LA, i.e., LA methyl ester monosuccinate (LAMeMS)] were reacted with hydroxyethyl cellulose (HEC) to obtain a series of LA derivatives of HEC. These polymers were characterized by the mesogen content, IR, differential scanning calorimetry, and hot-stage coupled polarizing optical microscopy. The degree of substitution (DS) ranged from 0.27 to 1.11. Polymers HEC-LAB (DS = 1.11), HEC-LANB (DS = 0.80), HEC-LACin (DS = 0.76), and HEC-LAP (DS = 0.27) exhibited a me-

sophase on heating whereas HEC-LAB also showed a mesophase on cooling. The other polymers HEC-LAAC (DS = 0.89) and HEC-LAMeMS (DS = 0.36) did not reveal mesophase formation with either heating or cooling. It is observed that the formation of a mesophase is affected by the type of mesogen that is used rather than the mesogen content of the polymer. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 1995–2001, 2006

Key words: hydroxyethyl cellulose; lithocholic acid; thermotropic

INTRODUCTION

Cellulosic polymers are known to form mesophases.¹ Hydroxypropyl cellulose was the first cellulose derivative reported to exhibit lyotropic liquid crystalline behavior.² Chiroptical properties of lyotropic solutions of mixed ester–ether derivatives of cellulose were investigated by Guo and Gray.^{3–6} A number of cellulose derivatives were shown to exhibit thermotropic liquid crystalline behavior.^{7–12} Hydroxyethyl cellulose (HEC) acetate was also reported to be a thermotropic material.¹³ We have been attempting to synthesize liquid crystalline derivatives of cellulose where the substituent is mesogenic and of biological origin. We are particularly interested in employing cholesterol or bile acid based mesogen to confer liquid crystalline properties to cellulose polymers. These materials might be useful as cholesterol/bile acid sequestrants. The commonly used lipid lowering nonsystemic drugs are anion exchange resins. They immobi-

lize bile acids through ionic interactions, prevent their reabsorption by circulation through the small intestine, and thereby enhance their removal by excretion. A potential approach for treating high blood cholesterol level uses various carbohydrate-derived materials including hydroxypropyl methyl cellulose.¹⁴ These materials act as viscosity builders in the intestine. The result is a decrease in translational diffusion that lowers the absorption of all food ingredients, including cholesterol and bile acids. We feel that a cholesterol/bile acid linked polysaccharide capable of mesophase formation would promote bile acid/cholesterol sequestration, probably through mixed mesophase formation with free bile acid/cholesterol in the intestine. The sequestration in this case would be through an anisotropic interaction rather than an ionic interaction as in the case of anion exchange resins. In our earlier work we employed monocholesteryl succinate as the mesogenic substituent to confer liquid crystalline properties to various polysaccharides,¹⁵ including HEC.¹⁶ To build on this approach, in the present investigation we used a series of lithocholic acid (LA) derivatives as the mesogenic substituents. HEC was selected as the parent compound, considering that the hydroxyethyl group would act as a flexible spacer between the pendent bulky LA derivative and the polymer backbone and may allow the substituents sufficient mobility to achieve their preferred orientation.

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Figure 1 The general structure of lithocholic acid derivatives.

EXPERIMENTAL

Materials

HEC (molar etherification = 2.5) was purchased from E. Merck. The following LA derivatives were synthesized and characterized in our laboratory¹⁷ (Fig. 1, Table I): 3-acetyl LA (LAAC); 3-propionyl LA (LAP); 3-benzoyl LA (LAB); 3-(4-nitrobenzoyl) LA (LANB); 3-cinnamoyl LA (LACin); and methyl-3-(3-carboxypropionyl) LA, which is a monosuccinate derivative of LA methyl ester (LAMeMS).

Preparation of LA derivative linked HEC

The LA derivatives were first converted to the respective acid chlorides and then reacted with HEC. A mixture of 2.0 g of LA derivative, 30 mL of dry hexane, and 15 mL of thionyl chloride was stirred at room temperature for 24 h. At the end of the reaction time, the excess of thionyl chloride and the solvent were removed under reduced pressure. Dry benzene (15 mL) was added, and the reaction mixture was stirred for 10 min. The benzene was then removed under reduced pressure. Addition and removal of benzene was repeated several times to ensure complete removal of thionyl chloride. The formation of acid chloride was confirmed by IR spectra. The acid chloride was further dried under a vacuum and dissolved in 6–7 mL of dimethyl acetamide. The solution was then added to another flask containing 1 g of HEC in dimethyl acetamide. The total volume of the solvent in the reaction mixture was 20 mL. The reaction mixture was stirred at 70°C for 48 h under a nitrogen atmo-

sphere. The product was precipitated into methanol, filtered, washed extensively with methanol, and air dried. Further purification of the polymer was achieved by Soxhlet extraction with methanol and drying under reduced pressure at room temperature.

Characterization

The LA derivative (mesogen) content of the polymers was determined by saponifying the ester linkages with a known amount of aqueous sodium hydroxide. The excess base was then backtitrated conductometrically with standard hydrochloric acid.¹⁶ The degree of substitution (DS) was ascertained using a value of 2.5 for the molar etherification and considering two ester linkages per substituent, except LAMeMS, which has three ester linkages.

IR spectra were recorded from KBr disks with a Perkin-Elmer 883 IR spectrophotometer calibrated with polystyrene. The phase behavior of the polymers was studied using a Leitz Laborlux 11 POL S polarizing optical microscope equipped with a Leitz microheating stage 350 and a Wild MPS 0.32 X camera. Each sample was heated to isotropization, cooled to room temperature, and again heated to isotropization in a single checking at the rate of 10°C/min. The sample preparation involved sandwiching a very small amount of the powdered compound in between a clean glass slide and a cover glass. The polymers were further characterized by differential scanning calorimetry (DSC) with a Mettler TA 4000 series instrument. It consisted of a DSC 30 cell coupled to a Mettler TC 11 TA processor. Three runs (heating, cooling, and heating) were performed for all the samples at the rate of 10°C/min.

RESULTS AND DISCUSSION

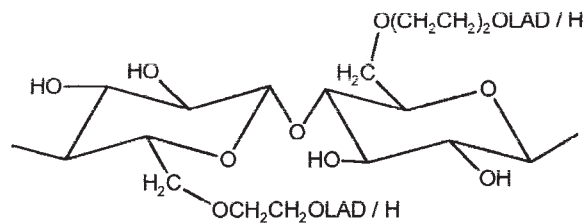
The LA derivatives were reacted with HEC to obtain a series of polymers (Fig. 2, Table II). The substituent (LA derivative) content of the polymers, determined by saponification of the ester linkages as mentioned in

TABLE I
Transition Temperatures of Lithocholic Acid Derivatives

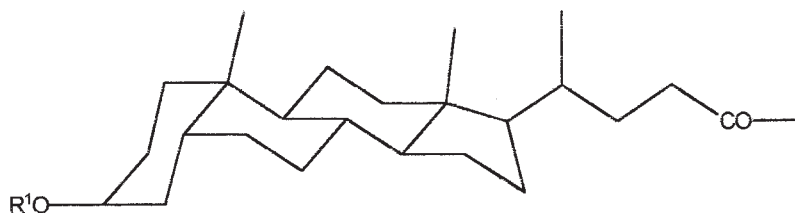
Serial No.	Code	R ¹	R ²	Transition temp. (°C)		Phase
				K-LC	LC-I	
1	LAAC	CH ₃ CO—	H	95, 125 ^a (130) (120 ^a)	176	Smectic
2	LAP	CH ₃ CH ₂ CO—	H	195	220	Smectic
3	LAB	C ₆ H ₅ CO—	H	235 (220)	260	Cholesteric
4	LANB	<i>p</i> -O ₂ N—C ₆ H ₅ CO—	H	180	218	Cholesteric
5	LACin	C ₆ H ₅ CH=CHCO—	H	220	237	Not clear
6	LAMeMS	HOOCCH ₂ CH ₂ CO—	—CH ₃	109	157	Smectic

The general structure of lithocholic acid derivatives is shown in Figure 1.

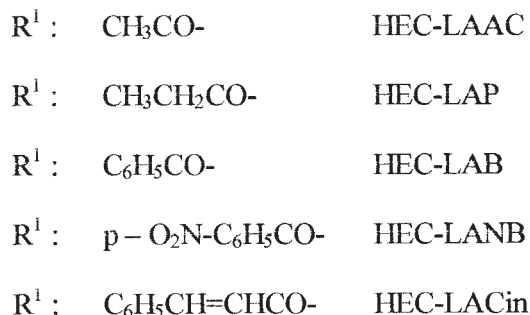
^a Texture change; K-LC, crystal to liquid transition; LC-I, liquid crystal to isotropic liquid transition; cooling.



Where LAD :



and



and

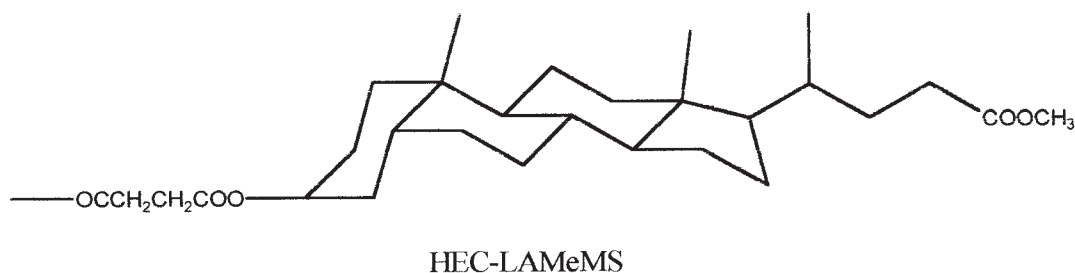


Figure 2 The idealized structure of lithocholic acid derivative linked hydroxyethyl cellulose.

the Experimental section, ranged from 29.3 to 65.5% whereas the highest DS obtained was 1.11 and the lowest 0.27, indicating that the hydroxyls of HEC were partially substituted. The maximum possible DS is 3. The reaction conditions were chosen according to our previous work on the synthesis of cholesterol-linked HEC with varying cholesterol content,¹⁶ and no attempt was made to maximize the extent of the reaction in the present investigation. The IR spectra of the polymers are shown in Figure 3. All the polymers

showed a decrease in the intensity of the peak attributable to hydroxyl stretching at 3500 cm^{-1} and an increase in the intensity of the peak attributable to C—H stretching at about $2800\text{--}3000\text{ cm}^{-1}$ compared to that of the parent polymer (HEC), indicating some of the hydroxyl groups were reacted. A strong peak due to carbonyl stretching at $1720\text{--}1740\text{ cm}^{-1}$ and additional peaks at 1280 and 1260 cm^{-1} due to aromatic and aliphatic esters, respectively, confirm the esterification reaction. The peaks in the range of 700--

TABLE II
Lithocholic Acid Derivative Content of Lithocholic Acid Derivative-Linked Hydroxyethyl Cellulose

Polymer code	Lithocholic acid derivative content (%)		DS ^a
	Saponification	Weight increase	
HEC-LAAC	56.7	57.8	0.89
HEC-LAP	29.3	30.1	0.27
HEC-LAB	65.5	66.9	1.11
HEC-LANB	59.9	61.0	0.80
HEC-LACin	57.7	61.5	0.76
HEC-LAMeMS	39.0	40.6	0.36

^a Calculated from the saponification value.

900 cm⁻¹ may be attributed to C—H deformation of aromatic rings. A sharp peak at 1530 cm⁻¹, the characteristic feature of —N=O stretching, is seen in the IR spectrum of HEC-LANB.

The thermotropic behavior of the polymers was studied by DSC and polarizing optical microscopy. The primary requirements for realization of a liquid crystalline phase, which is sufficient stiffness of the molecules to allow near parallel arrangement of the chains and the asymmetric interactions, are fulfilled

by the semirigid nature of the cellulose backbone and the chirality of the anhydroglucose units, respectively.^{10,18} Flexible substituents have a twofold effect. They hinder crystallization and allow the molecules sufficient mobility to achieve their preferred orientation.¹⁰ The liquid crystalline cellulose derivatives reported thus far¹⁻¹³ contain nonmesogenic substituents, with the exception of our earlier work.^{15,16} In the present investigation all the substituents introduced onto HEC are liquid crystalline (Table I). Figure 4 shows DSC thermograms of the polymers. HEC-LAB exhibited two endotherms from 145 to 190 and 190 to 220°C, suggesting crystal to liquid crystal and liquid crystal to isotropic fluid transitions. The endotherms are much broader than expected for first-order transitions. However, the endotherms represent mesophase formation as evidenced by the microscopic observation. The broad nature of the endotherms, indicative of a transition occurring over a wide temperature range, may be attributed to the high viscosity, high molecular weight, polydispersity, and nonuniform substituent distribution in the polymer.^{19,20} On cooling, the exotherm resulting from the formation of the anisotropic phase appears in the temperature range of 200–60°C. This was also supported by observations under the

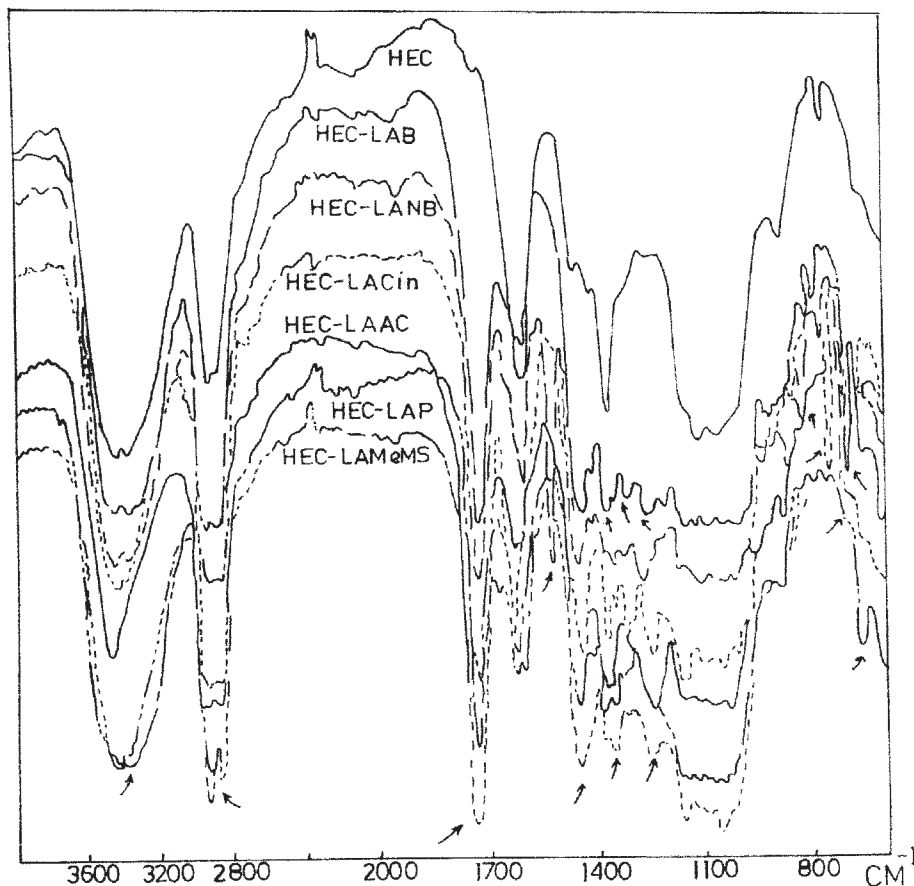


Figure 3 IR spectra of lithocholic acid derivative linked hydroxyethyl cellulose.

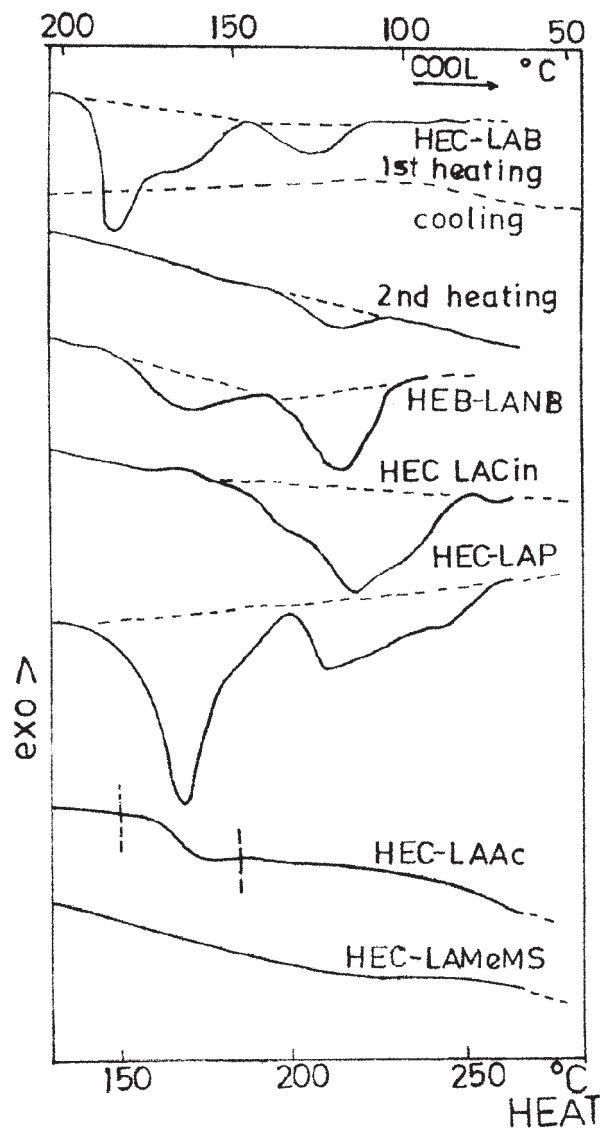


Figure 4 DSC thermograms of lithocholic acid derivative linked hydroxyethyl cellulose.

polarizing optical microscope. On second heating, a single endotherm in the 198–230°C range was observed, suggesting that the anisotropic phase formed during cooling was retained at room temperature. Therefore, the single endotherm observed on second heating may be attributed to a liquid crystal to isotropic fluid transition. HEC-LANB also showed two endotherms from 150 to 193 and 193 to 230°C, which is in line with the observations made for HEC-LAB; and the endotherms may be attributed to crystal to liquid crystal and liquid crystal to isotropic fluid transitions, respectively. This inference was also supported by polarizing optical microscope studies. However, the DSC thermograms and microscopic observations did not reveal mesophase formation on cooling as well as on second heating for HEC-LANB. HEC-LACin, even though mesomorphic (as observed by optical micro-

scope), does not show two distinct endotherms as in the case of HEC-LAB or HEC-LANB. Conversely, it showed a broad endotherm giving the appearance of overlapped endotherms. Optical studies revealed mesophase formation in the range of the broad endotherm, that is, from 185 to 248°C. Huang¹³ reported similar observations for HEC acetate. No distinct transitions were seen on cooling or on second heating; those could be attributed to mesophase formation. HEC-LAP also exhibits two endotherms corresponding to crystal to liquid crystal (140–198°C) and liquid crystal to isotropic fluid transitions (198–255°C), similar to HEC-LAB or HEC-LANB. Thermal behavior similar to that of HEC-LACin was observed on cooling and on second heating; that is, the thermograms were featureless. The other polymers in the series (HEC-LAAC and HEC-LAMeMS) did not exhibit any peculiar transition that could be attributed to mesophase formation either on heating or on cooling. That these polymers are not mesomorphic was further confirmed by observation under the optical microscope.

It is interesting to note that all the LA derivatives used to modify HEC in the present investigation are liquid crystalline in their own right. They exhibit clearly identifiable textures, as is usually the case with low molecular weight compounds. However, clearly identifiable textures could not be seen in the case of some derivatives when linked to HEC. Moreover, the endotherms observed were not distinct, which may be attributed to the high molecular weight, high viscosity, polydispersity, and nonuniform substituent distribution.^{19,20}

The absence of mesomorphic behavior in the case of HEC-LAAC, even though its DS (0.89) is in the range of that for the other polymers showing a liquid crystalline phase and the substituent LAAC is liquid crystalline, may be attributed to its size. The acetyl group on LA is the smallest substituent in the series, which might not be large enough to cause an increase in the length to diameter ratio in order to impart liquid crystalline properties effectively, when bound to the polymer. It may be noted that the crystal to liquid crystal and liquid crystal to isotropic transition temperatures are lowest for LAAC (95 and 176°C) and LAMeMS (109 and 157°C) among the series (Table I). In other cases much bulkier and rigid substituents such as cinnamoyl, nitrobenzoyl, and benzoyl were utilized. Propinoyl substituent is also larger compared to acetyl. The absence of mesomorphic behavior in the case of HEC-LAMeMS may also be due to the mode of linking to the polymer. LAMeMS is linked to the HEC through the C-3 position, which is otherwise C-24 for all the other derivatives. It should be noted that the parent polymer is nonmesomorphic and decomposes before melting, whereas all the modified polymers in the present study melt clearly and in most cases also display liquid crystalline behavior.

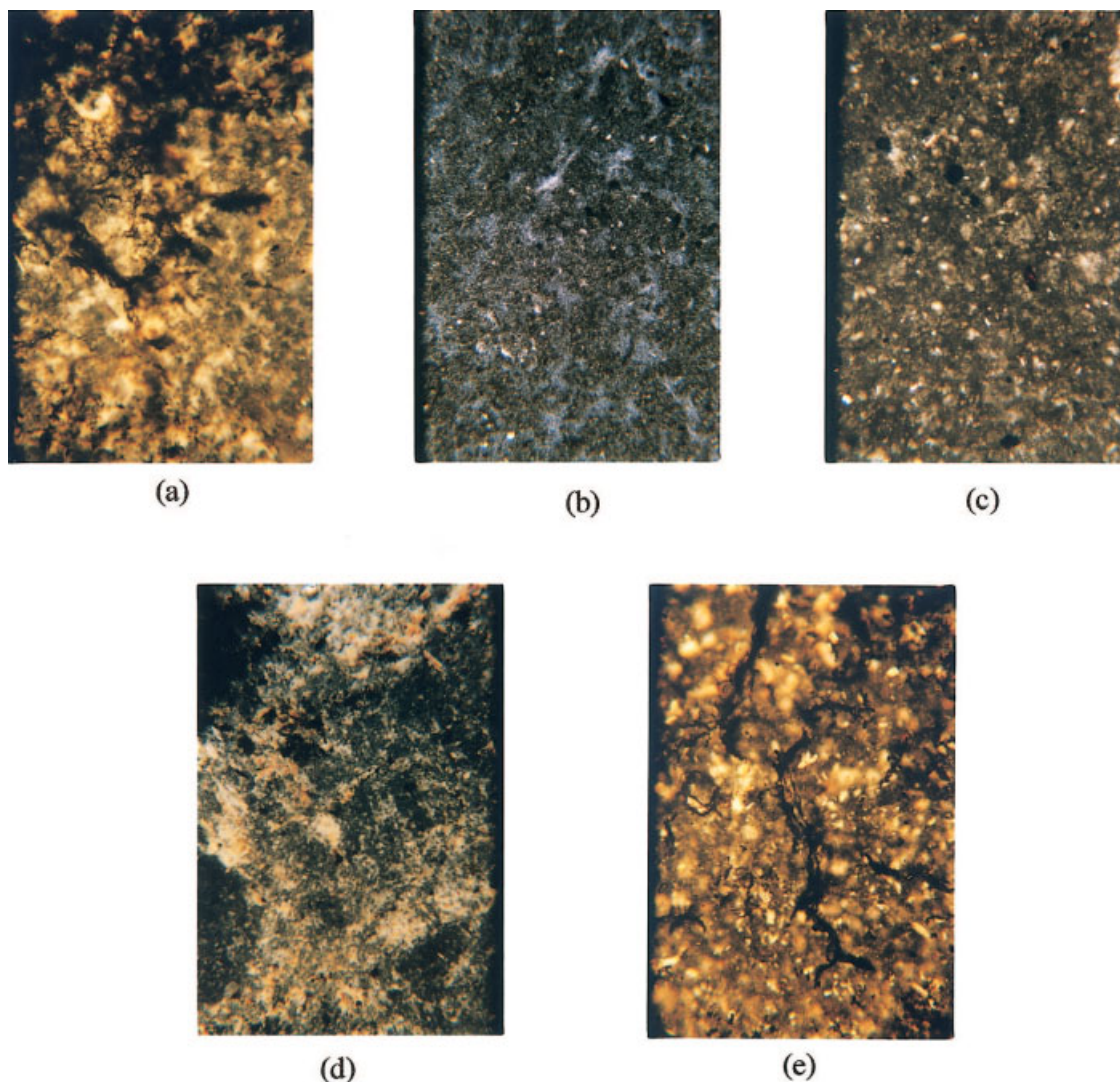


Figure 5 Optical micrographs (crossed polars) of polymers: (a) HEC-LAB at 185°C (heating mode), (b) HEC-LAB at 200°C (cooling mode), (c) HEC-LANB at 170°C (heating mode), (d) HEC-LACin at 210°C (heating mode), and (e) HEC-LAP at 145°C (heating mode).

It is also seen in the present study that the clearing temperature of the polymer is affected by the DS or mesogen content. The clearing temperature is high for the polymers with low DS whereas it is low when the DS is high. Thus, it is 255°C for HEC-LAP (DS = 0.27), 248°C for HEC-LACin (DS = 0.76), 230°C for HEC-LAB (DS = 0.80), and 220°C for HEC-LAB (DS = 1.11). This can be explained on the basis of the increase in the flexibility with the increase in the DS up to a certain value. Actually the effect of a substituent on the flexibility of the polymer backbone depends on the nature of the substituent, the polymer backbone, and the DS. Introducing substituents onto a flexible backbone (polyacrylates, polysiloxanes) increases the rigidity of the polymer backbone, whereas introducing substituents onto more rigid backbones [polystyrene, poly (methylmethacrylate)] increases their flexibility.²¹ The value of a in the Mark–Houwink–Sakurada

equation indicates that cellulosics have a more rigid backbone than polystyrene.¹ Therefore, it is logical to expect that introducing a substituent onto HEC would increase the flexibility of the backbone. Another plausible reason for the increase in the backbone flexibility with increasing DS is the decrease in interchain interactions due to a decrease in the number of free hydroxyl groups. In our earlier work on cholesterol-linked HEC,¹⁶ we found that the temperature at which the mesophase begins to appear decreases with an increasing DS of the cholesteryl monosuccinate substituent from 0.6 to 1.5 whereas it increases with a further increase in the DS from 1.5 to 2.0, probably because of the crowding of the bulky substituent on the backbone. At the higher DS, chain flexibility decreases as the side group to side group interactions become increasingly severe. In the present case the highest DS was 1.11. The temperature at which the

mesophase appears in these polymers, however, cannot be discussed in reference to the DS because the mesogen that was utilized was different in each case. Each mesogen has its own effect in addition to the effect of the DS on the temperature at which the mesophase appears.

The mesomorphic nature of the polymers was also studied using polarizing optical microscopy. HEC-LAB, a slightly yellowish, nonbirefringent powder, softens on heating and displays birefringence from 145 to 150°C onward. The birefringent field continues to increase with the temperature. Strong birefringence could be seen around 180–190°C [Fig. 5(a)], but a clear texture could not be observed. On further heating, the flow of the birefringent structure was observed at 210°C and it became isotropic at about 220–230°C. It was further heated to 235°C and held at this temperature for 5 min and then cooled. Birefringence was seen while cooling from 200°C, which remained up to room temperature [Fig. 5(b)] and could be seen in the entire view field. Annealing at 200°C helped to improve the birefringence a little. The polymer sample became isotropic on reheating at 230°C. All these observations coincide well with the DSC results discussed earlier. HEC-LANB softened at about 150°C with the simultaneous appearance of birefringence [Fig. 5(c)], which increased slowly with the temperature, exhibited flow from 190 to 200°C, and became isotropic at about 220–230°C. HEC-LACin softened at about 180–185°C and revealed birefringence [Fig. 5(d)], which progressively increased up to 210°C, exhibited flow at around 230°C, and finally became isotropic at 250°C. Polymer HEC-LAP also showed birefringence with softening from 140°C [Fig. 5(e)], a progressive increase in the birefringence, and isotropization at about 255°C. The polymers HEC-LANB, HEC-LACin, and HEC-LAP did not reveal mesophase formation either on cooling or on second heating. HEC-LAAC and HEC-LAMeMS softened at about 145 and 150°C, respectively, and flow occurred at about 230 and 240°C, respectively, without displaying birefringence.

Although most of the LA derivatives of HEC display liquid crystalline behavior, none of the polymers exhibited a clearly identifiable texture under the microscope. However, birefringence was observed in most of the cases and the birefringent pattern could be deformed under pressure or shear (applied by gently touching the cover glass), indicating that the micro-

scopic texture was different from that of normal semicrystalline polymers. The presence of birefringence in LA derivatives of HEC and the absence of birefringence and melting in the parent polymer (HEC) suggests that the polymers HEC-LAB, HEC-LANB, HEC-LACin, and HEC-LAP are liquid crystalline.

CONCLUSIONS

The reaction of LA derivatives with HEC yielded liquid crystalline LA esters of HEC with DS values ranging from 0.27 to 1.11. The formation of a mesophase was affected by the type of mesogen and by the mode of its linkage rather than the mesogen content or the DS.

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