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Synthesis and characterization of hydroxyethyl chitosan grafted by carboxyl ending DOVOB dendrimer: A novel liquid crystalline polymer

Yaqing Zhao [a,b], Jian Chen [b], Erman Zeng [a], Xiaolan Hu [a], Anhua Liu [a], Yanming Dong [a,b,*]

[a] Department of Materials Science and Engineering, College of Materials, Xiamen University, Xiamen, Fujian 361005, China
[b] Department of Materials Science and Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, Fujian 361005, China

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ABSTRACT

A novel chitosan derivative, hydroxyethyl chitosan grafted 3,4,5-tris[*p*-(*n*-dodecyloxy)-*m*-methoxybenzyloxy]benzoate (HECS-g-DOVOB) was prepared via grafting dendrimer 3,4,5-tris[*p*-(*n*-dodecyloxy)-*m*-methoxybenzyloxy]benzoic acid (DOVOB acid) onto hydroxyethyl chitosan (HECS). The structure of HECS-g-DOVOB was investigated by means of FTIR, ^{1}H NMR, UV and circular dichroism (CD). The degree of substitution was measured to be about 0.39 per glucose unit. The DOVOB acid was able to self-assemble into a thermotropic hexagonal columnar liquid crystalline phase showing fan-like texture, the aqueous HECS solution formed lyotropic cholesteric liquid crystal showing fingerprint texture. Whereas, HECS-g-DOVOB formed lyotropic cholesteric liquid crystal in concentrated DMSO solution with a planar texture, which was different to that of both DOVOB and HECS. The cholesteric pitch of HECS-g-DOVOB is much smaller than that of HECS. The fluorescence spectrum of HECS-g-DOVOB shows excimer fluorescence which is very similar to the dendrimer is a good indication for light-emitting polymeric material.

1. Introduction

Dendrimers are attractive molecules owing to their multifunctional properties (Tomalia & Frechet, 2002; Tomalia et al., 1985). Dendronized polymers, on the other hand, are also attractive because of their rodlike conformation and nanostructure (Karakaya, Claussen, Gessler, Saenger, & Schluter, 1997; Schluter & Rabe, 2000; Zhang, Shu, Bo, & Schluter, 2003). Several groups focused on the self-assembling of dendrimers in liquid crystal and reported many types of liquid crystalline phase like nematic and smectic, cubic, discotic columnar and chiral nematic liquid crystalline phase (Balagurusamy, Ungar, Percec, & Johansson, 1997; Percec, Chu, Ungar, & Zhou, 1995; Percec et al., 2002; Zeng, Jacob, & Polk, 2002).

Chitosan is a polysaccharide formed primarily of repeating units of β(1-4)-2-amino-2-deoxy-D-glucose, although it includes small amount of *N*-acetyl-D-glucosamine. The free amino groups and hydroxyl groups of chitosan offer great potential for further derivation. A variety of chemical modifications have been employed to modify these carbohydrate polymers. Chemical modification of chitin and chitosan include acetylation (Dong, Wu, & Wang, 2002; Nishimura, Kohgo, Kurita, & Kuzuhara, 1991), alkylation (Kurita et al., 1992), etherealization (Delben, Stefancich, & Muzzarelli, 1992; Dobetti & Delben, 1992), graft copolymerization (Jenkins & Hudson, 2002; Liu, Tian, & Hu, 2004) and many other functional polymers (Dutta & Dutta, 2005). However, as per our knowledge there are very few reports on dendronized polysaccharide specifically related to chitin and chitosan backbones. Sashiwa et al. synthesized chitosan-dendrimer hybrid (CDH) according to two methods. In method A, corresponding dendrimers bearing aldehyde and a spacer were synthesized, and those were reacted with chitosan by reductive *N*-alkylarion (Sashiwa, 2005; Sashiwa, Shigemasa, & Roy, 2000; Sashiwa, Shigemasa, & Roy, 2002). The generation of reactive dendrimer is limited owing to steric hindrance. In method B, with binding of chitosan to the dendrimer surface, allows use of commercially available amino-dendrimers such as poly(amidoamine) (PAMAM) or poly(ethyleneimine) dendrimers (Sashiwa & Roy, 2005; Sashiwa, Shigemasa, & Roy, 2001). The stepwise preparation of surface bound functional groups is expected to avoid steric hindrance. Recently, a polyglycerol with dendritic structure (PGLD) was synthesized by ring-opening polymerization of deprotonated glycidol using a polyglycerol as core functionality in a step-growth process. Then, PGLD reacted with *O*-carboxymethylated chitosan to obtain PGLD-chitosan dendrimer (PGLD-Ch) (De Queiroz et al., 2006).

The preparation of CDH mostly focused on without self-assembly and liquid crystalline properties. These results encouraged us to synthesize a novel self-assemble and liquid crystalline CDH (HECS-g-DOVOB) using carboxyl ending DOVOB dendrimer by

* Corresponding author. Address: Department of Materials Science and Engineering, College of Materials, Xiamen University, Xiamen, Fujian 361005, China. Tel.: +86 592 2186095; fax: +86 592 2183937.
E-mail address: ymdong@xmu.edu.cn (Y. Dong).

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esterification on hydroxyl groups of hydroxyethyl chitosan (HECS). The liquid crystalline behaviors and self-assemble properties of HECS-g-DOVOB/DMSO in concentrated solution were investigated by polarized optical microscope (POM), circular dichroism and fluorescence.

2. Experimental

2.1. Materials

Commercial reagent-grade chitosan (from crab shell) with degree of deacetylation 91% (determined by acid-base titration method) and viscosity average molecular weight 4.5×10^5, was supplied by Shandong Chitin Powder Factory (China). Vanillin, 1-bromododecane (97%), $NaBH_4$ (99%), methyl-3,4,5-trihydroxybenzoate (97%), ethylene oxide were obtained from Sinopharm Chemical Reagent Co., Ltd and used as received.

2.2. Measurements

The infrared spectra were measured with a Nicolet Avator 360 FTIR by the KBr pellet method. ^{1}H NMR (500 MHz) spectra were obtained with a Varian Unity NMR spectrometer with $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane as the internal reference. Elemental analysis was measured on a Vario EL III elemental analyzer. The UV–vis spectra were recorded on a Beckman DU 7400 spectrometer. The circular dichroism spectra were recorded on a Jasco J-810 spectropolarimeter. The fluorescence emission spectra (FE) were obtained with a Hitachi F-4500 fluorescence spectrophotometer. The micrographs were obtained with an Olympus BH-2 polarized optical microscope.

2.3. Preparation of p-(n-dodecyloxy)-m-methoxybenzaldehyde (**2**)

A mixture of acetone (240 mL), vanillin (**1**, 15.2 g, 100 mmol), K_2CO_3 (14.5 g, 105 mmol) and 1-bromododecane (19.0 mL, 80.0 mmol) was stirred and refluxed in a 500 mL round-bottom flask for 24 h followed by removal of acetone by rotary evaporation. The residue was dissolved in $CHCl_3$, filtered, dried over anhydrous $MgSO_4$, and finally collected after rotary evaporation and recrystallization three times in *n*-hexane to obtain a product, **2** (12.5 g, 49%) as a yellow solid. ^{1}H NMR, δ ($CDCl_3$, TMS, ppm): 0.88 (t, 3H, CH_3, $J = 6.7$), 1.26 [m, 18H, $(CH_2)_9$], 1.86 (m, 2H, CH_2CH_2OAr), 3.92 (s, 3H, CH_3O), 4.09 (t, 2H, CH_2OAr, $J = 6.9$), 6.88–7.36 (overlapped m, 3H, C_6H_3), 9.76 (s, 1H, ArCHO).

2.4. Preparation of p-(n-dodecyloxy)-m-methoxybenzyl alcohol (**3**)

An aqueous 60 mL NaOH solution (1.25%, w/v) containing $NaBH_4$ (3.04 g, 80.0 mmol) was added dropwise into a 250 mL CH_3OH solution of **2** (24.0 g, 75.0 mmol). The mixture was stirred for 4 h at room temperature, then diluted with H_2O (750 mL), and acidified with 5% HCl. After filtration, the resulting precipitate was washed with H_2O and obtained **3** (21.5 g, 89%) with white solid. ^{1}H NMR, δ ($CDCl_3$, TMS, ppm): 0.88 (t, 3H, CH_3, $J = 6.7$), 1.26 [m, 18H, $(CH_2)_9$], 1.83 (m, 2H, CH_2CH_2OAr), 3.87 (s, 3H,CH_3O), 4.00 (t, 2H, CH_2OAr, $J = 6.9$), 6.85–6.92 (overlapped m, 3H, C_6H_3).

2.5. Preparation of p-(n-dodecyloxy)-m-methoxybenzyl chloride (**4**)

To a 100 mL round-bottom flask containing dry CH_2Cl_2 (100 mL) solution of **3** (10.0 g, 30.9 mmol) was added freshly distilled $SOCl_2$ (3.00 mL, 42.0 mmol) dropwise. After stirring for 2 h at room temperature, the mixture was washed with H_2O, 10% NaOH, again with water and finally dried over anhydrous $MgSO_4$ which results the isolation of **4** (6.42 g, 61%) as a yellow solid after rotary evaporation. ^{1}H NMR, δ ($CDCl_3$, TMS, ppm): 0.88 (t, 3H, CH_3, $J = 6.7$), 1.26 [m, 18H, $(CH_2)_9$], 1.83 (m, 2H, CH_2CH_2OAr), 3.88 (s, 3H, CH_3O), 4.00 (t, 2H, CH_2OAr, $J = 6.9$), 4.56 (s, 1H, CH_2Cl), 6.85–6.92 (overlapped m, 3H, C_6H_3).

2.6. Preparation of methyl 3,4,5-tris[p-(n-dodecyloxy)-m-methoxybenzyloxy] benzoate (**6**)

A mixture of DMF (80.0 mL) and K_2CO_3 (14.0 g, 101 mmol) in a 250 mL three neck round-bottom flask was purged with N_2 for 3 h at room temperature. After adding methyl-3,4,5-trihydroxybenzoate (**5**, 1.84 g, 10.0 mmol), the temperature was rised to 70 °C, followed by adding a DMF (10.0 mL) solution of **4** (10.2 g, 30.0 mmol) quickly through an addition funnel. The mixture was kept at 70 °C for an additional 4 h, and then poured into H_2O, acidified with 5% HCl. After filtration, the residue was recrystallized from EtOH to give **6** (6.80 g, 62%) as an orange solid. ^{1}H NMR, δ ($CDCl_3$, TMS, ppm): 0.88 (t, 9H, CH_3, $J = 6.9$), 1.26 [m, 54H, $(CH_2)^9$], 1.82 (m, 6H, CH_2CH_2OAr), 3.61 and 3.81 (ss, 3H + 6H, CH_3O), 4.00 (tt, 6H, RCH_2OAr, $J = 6.6$), 4.36 (s, 3H, CH_3), 5.06 (ss, 6H, $ArCH_2OAr$), 6.70–7.0 (overlapped m, 9H, C_6H_3).

2.7. Preparation of 3,4,5-tris[p-(n-dodecyloxy)-m-methoxybenzyloxy]benzoic acid (**7**, DOVOB acid)

In a 100 mL round-bottom flask, a 90% ethanol–water solution (30.0 mL) of KOH (1.68 g, 30.0 mmol) and **6** (3.29 g, 3.00 mmol) was refluxed for 1 h, then poured into H_2O (56.0 mL) acidified with 5% HCl. The resulting precipitate was redissolved in a $CHCl_3/CH_3OH$ mixture (95:5 mL) followed by acidification (5% HCl), washing with water, drying over anhydrous $MgSO_4$ and finally rotary evaporation to obtain **7** (2.99 g, 92%) as a yellow solid. ^{1}H NMR, δ ($CDCl_3$, TMS, ppm): 0.89 (t, 9H, CH_3, $J = 6.9$), 1.26 [m, 54H, $(CH_2)_9$], 1.84 (m, 6H, CH_2CH_2OAr), 3.62 and 3.81 (ss, 3H + 6H, CH_3O), 4.00 (tt, 6H, RCH_2OAr, $J = 6.6$), 5.06 (ss, 6H, $ArCH_2OAr$), 6.70–7.02 (overlapped m, 9H, C_6H_3). Fig. 1 outlines the synthesis of DOVOB acid.

2.8. Synthesis of hydroxyethyl chitosan (HECS)

Hydroxyethyl chitosan (HECS) was synthesized in alkali system (Dong, Wu, & Wang, 2001) (showed in Fig. 2). A (1.00 g, about 6.20 mmol) amount of chitosan powder was added to an aqueous NaOH solution (50%, w/v) and stirring at room temperature for 1 h, then kept at −18 °C overnight. After filtration, the resulting precipitate was added to 15.0 mL iso-propyl alcohol and 10.0 mL ethylene oxide. The mixture was stirred at 0 °C for 24 h. After neutralization by HCl, the product was precipitated by pouring into acetone, filtered and dried to obtain the HECS as a white solid.

2.9. Synthesis of HECS-g-DOVOB

HECS (0.067 g, 0.20 mmol) was added to 10.0 mL methanesulfonic acid with stirring at $-2 \sim 0$ °C for 1 h. A DMF (10.0 mL) solution of DOVOB acid (0.87 g, 0.80 mmol) was added slowly through a dropping funnel to the gel of HECS in methanesulfonic acid. The mixture was stirred at 0 °C for 24 h, and kept at −18 °C overnight. It was extracted with $CHCl_3$ for removal of excess DOVOB acid. Thereafter, excess ammonia solution was added, dialyzed and finally freeze-dried to obtain HECS-g-DOVOB as grey solid. Fig. 3 outlines the synthesis of HECS-g-DOVOB.

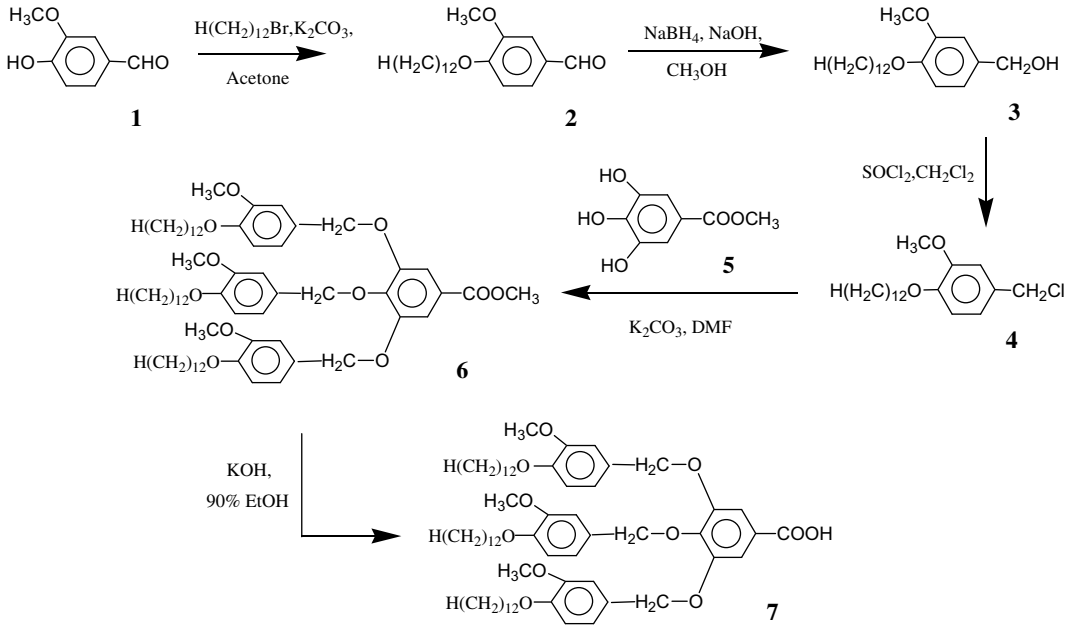

Fig. 1. Synthesis process of DOVOB acid.

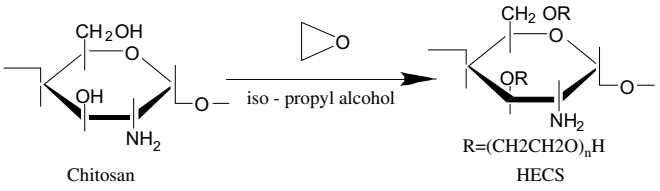

Fig. 2. Synthesis process of HECS.

3. Results and discussion

3.1. Structural characterization of HECS

Compared with completely deacetylated chitosan, the structure of HECS was verified by the enhancement of υ (C–O–C) 1092 cm^{-1} and υ ($-CH_2$) 2850–2922 cm^{-1} (Fig. 4). The degree of molar etherification of HECS that was tested by ^1H NMR and element analysis was about 4 and the etherification occurred mostly on the hydroxyl groups of chitosan (CS) (Dong et al., 2001).

3.2. Structural characterization of HECS-g-DOVOB

We choose HECS instead of chitosan, because introduction of the flexible chain destroy the strong inter-molecular and intra-molecular hydrogen bonds of chitosan and it facilitates the reaction of grafting dendrimer onto polysaccharide. HECS-g-DOVOB was prepared by a similar homogenous reaction of chitosan and DMF solution of DOVOB acid using methanesulfonic acid as solvent and catalyst in low temperature. Acidic condition (methanesulfonic acid as solvent) is disadvantageous for the nucleophilic displacement reaction of amino group that has been protonated (Dong et al., 2006). On the other hand, introduction of the flexible chain reduce the problem of steric hindrance inherent. Therefore, the etherification is supposed to happen preferentially onto the hydroxyl groups. The structure has been confirmed by FTIR results (showed in Fig. 4). Compared with completely HECS, the structure of HECS-g-DOVOB was verified by 1731 cm^{-1} (the esterified carboxylic groups, –COOR), 3034 cm^{-1} stretching vibrations of the C–H on benzene, 1503 and 1605 cm^{-1} vibrations of backbone of benzene ring, and 828 cm^{-1} out-of-plane bending vibrations of C–H on benzene.

The ^1H NMR ($CDCl_3$ + $(CF_3CO)_2O$, ppm) spectrum of HECS-g-DOVOB shows resonance signals at δ 0.88–1.8 ppm corresponding to the protons of aliphatic saturated hydrocarbon, δ 3.0–5.0 ppm (m, 6H) corresponding to the protons of HECS and protons which connects with oxygen protons of the dendrimer, and δ 6.5–7 ppm corresponding to the protons in benzene ring. The degree of substitution was about 0.39 per glucose unit via the calculation of the integral ratio of δ 3.0–5.0 and δ 6.5–7 ppm.

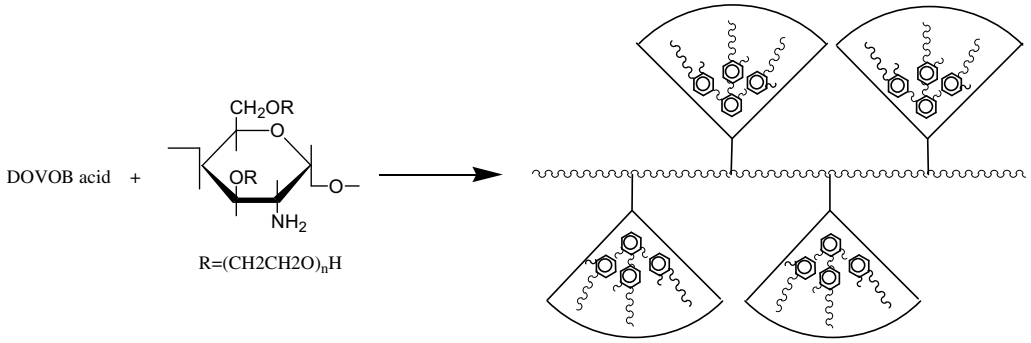

Fig. 3. Synthesis process of HECS-g-DOVOB (the fan-shape structure represents a DOVOB side group).

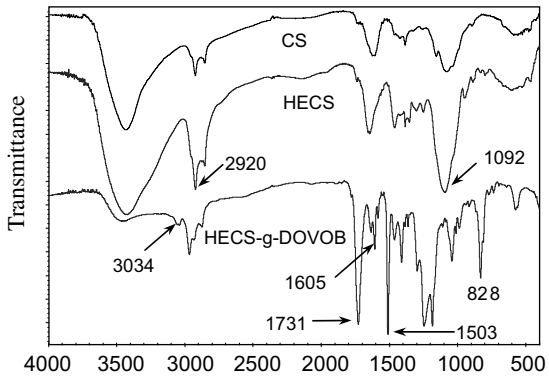

Fig. 4. FTIR spectra of CS, HECS and HECS-g-DOVOB.

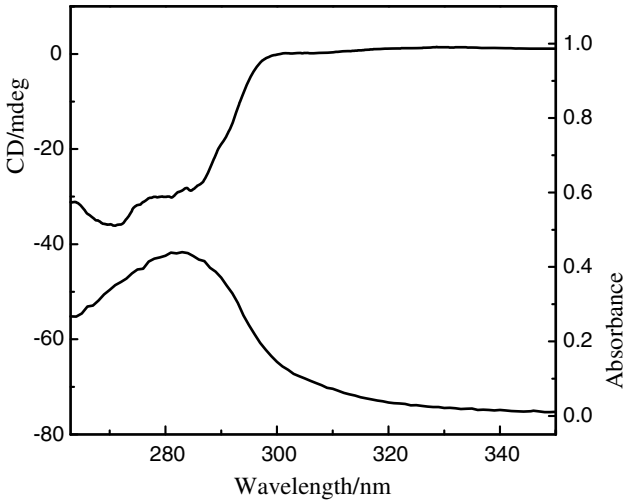

Fig. 5. CD and UV spectra of HECS-g-DOVOB in a DMSO solution. The polymer concentration was 200 mg/L and 20 mg/L, respectively.

Fig. 5 shows that the CD spectrum and the corresponding UV absorbance spectrum of a HECS-g-DOVOB/DMSO solution. In DMSO, HECS-g-DOVOB exhibits a negative CD band from 265 nm to 295 nm. The two peaks centered at 271 nm and 284 nm correspond to the vibrational fine structure of the ^1L_b transition of benzene derivatives as recorded in the UV absorption spectrum. The CD and UV spectra also proved that DOVOB acid dendrimer had been grafted onto HECS bone chain.

3.3. Liquid crystalline behavior of HECS-g-DOVOB

It has been reported that DOVOB acid is a thermotropic liquid crystal, which self-organizes in a columnar hexagonal liquid crystalline phase (Zeng et al., 2002), showing a fan-like texture

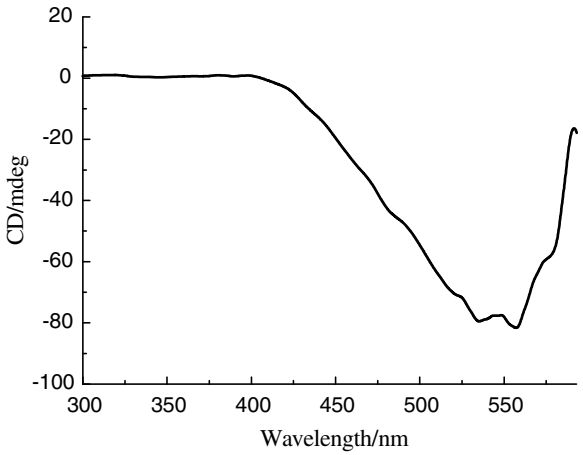

Fig. 7. CD spectrum of HECS-g-DOVOB/DMSO solution (80%, w/v).

(Fig. 6a). HECS formed a cholesteric texture, showing a typical fingerprint texture in an aqueous solution (Fig. 6b). While it was observed that HECS-g-DOVOB solutions in DMSO formed another typical cholesteric texture in very high concentration, i.e. a planar texture (Fig. 6c) in 80% (w/v) HECS-g-DOVOB/DMSO solution. It is well known that the cholesteric phase usually demonstrates a planar texture, while the axes of spirals are almost vertical to the sample plane. HECS and HECS-g-DOVOB both display lyotropic liquid crystalline behavior like others derivatives, whereas the appearance of textures are different.

The study of handedness in cholesteric liquid crystalline phase by CD (Dong, Wu, Zhao, et al., 2003; Dong et al., 2005; Zhao et al., 2005) have been reported elsewhere. On *N*-phthaloylchitosan solutions in several organic solvents, including DMF, DMSO, DMAc and pyridine, CD signals were found at visible light region that correspond to the cholesteric helix of liquid crystalline phase. The CD signals arose from the selective reflection of cholesteric helix pitch. Similar results were also observed on HECS-g-DOVOB/DMSO solution in very high concentration. Fig. 7 shows the CD spectrum of HECS-g-DOVOB/DMSO solution (80%, w/v). When planar cholesteric texture of lyotropic liquid crystal was observed by POM, CD signals at visible light region were observed in the same system. It proved the handedness of cholesteric helix.

Furthermore, the average pitch of cholesteric helix could be calculated through the wavelength of selective reflection. Maximum wavelength of selective reflection (λ_{max}) connect to the average pitch of cholesteric helix by the formula: $\overline{P} = \frac{\lambda_{max}}{n}$, where, n is average refractive index. The refractive index of HECS-g-DOVOB/DMSO solution which was measured in Abbe refractometer is 1.465. From Fig. 7 it was found that λ_{max} = 560 nm, so the average pitch of cholesteric helix is 382 nm. In addition, from the fin-

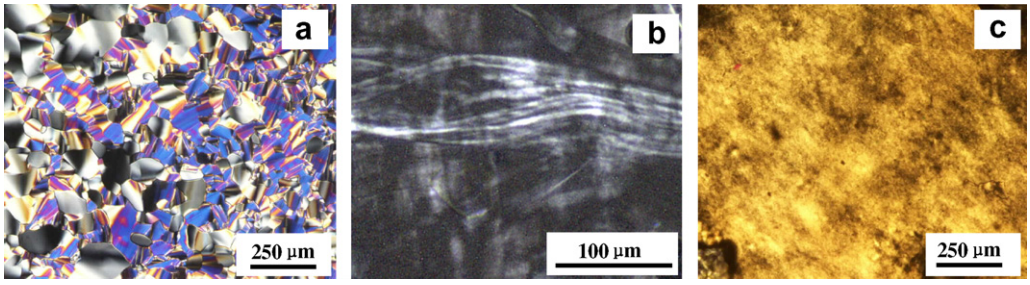

Fig. 6. Photomicrographs of the fan-like texture for DOVOB acid (a) the fingerprint texture for aqueous HECS solution (b) and the planar texture for HECS-g-DOVOB/DMSO solution (c).

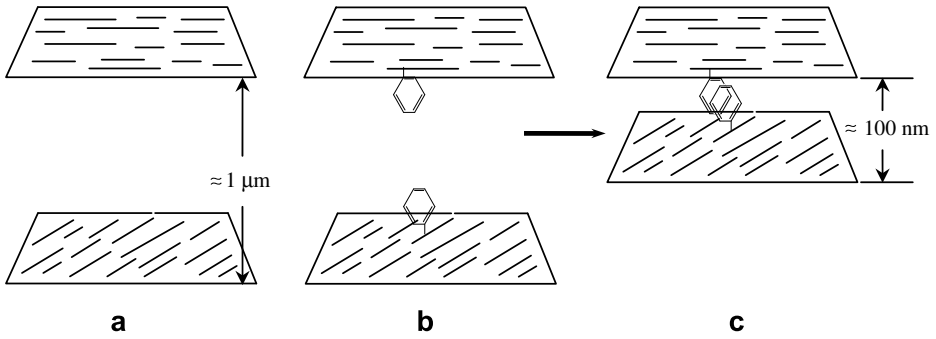

Fig. 8. Schematic diagram showing the interaction between benzene rings of adjacent cholesteric layers which causes the decrease of layer space. Only a benzene ring of HECS-g-DOVOB chain is illustrated in each layer. (a) HECS, (b) HECS-g-DOVOB (after substituted), (c) HECS-g-DOVOB (practical layer space).

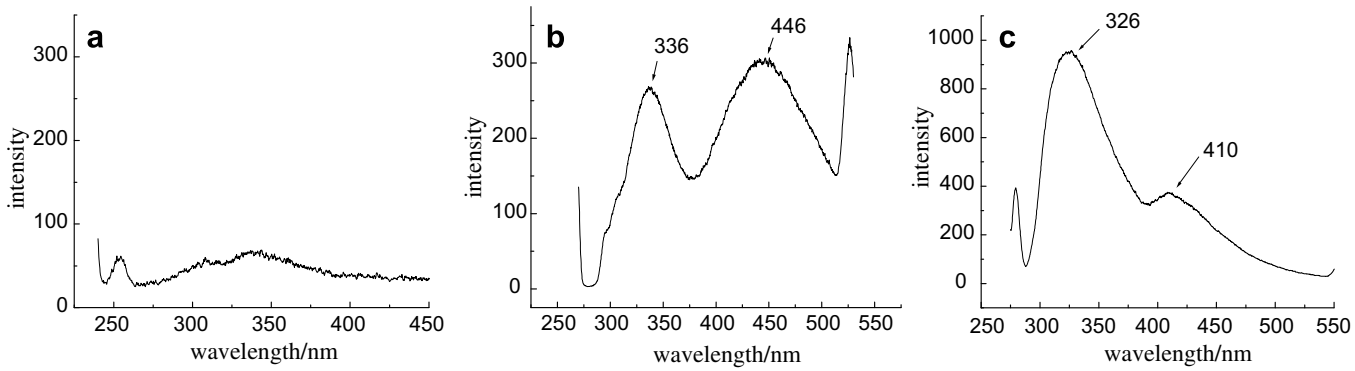

Fig. 9. FE spectra of (a) aqueous HECS solution with the concentration 20 mg/L, (b) DOVOB in a $CHCl_3$ solution with the concentration 50 mg/L, (c) HECS-g-DOVOB in a DMSO solution with the concentration 20 mg/L.

gerprint texture of aqueous HECS solution (see Fig. 6b), the average pitch of cholesteric helix of the system is estimated to be about several microns, directly from the double spaces of bands of fingerprint texture.

From the results mentioned above, it was found that the cholesteric pitch of HECS-g-DOVOB is much smaller than that of HECS, which is very much relevant to the reported results. For the concentrated solution of chitosan and aliphatic chitosan derivatives, the value of average pitch observed to be several micrometers by means of both small angle light scattering and polarized microscopy (Dong, Yuan, Xiao, Wang, & Mei, 1999; Wang, Dong, Liu, Yuan, & Mei, 1999). But for the concentrated solution of aromatic chitosan derivatives, cholesteric pitches were measured to be in the visible wavelength region by CD spectrum (Dong, Wu, Ruan, et al., 2003; Rout, Barman, Pulapura, & Gross, 1994; Rout, Pulapura, & Gross, 1993). It is known that cholesteric helix structure is a kind of twisted layer structure at the supermolecular level, so we consider that cholesteric pitch of aromatic chitosan derivatives was affected by interaction of benzene rings on adjacent cholesteric layers. In the next section, more other proof on fluorescence emission spectra was found. On HECS-g-DOVOB/DMSO dilute solution, excimer fluorescence that attributed to the formation of stable parallel benzene structure was observed. The benzene ring packing between adjacent cholesteric layers is thought to be the driving force of the decrease of the cholesteric pitch (Fig. 8).

3.4. Fluorescence emission of HECS-g-DOVOB

Fig. 9 shows the fluorescence emission spectra of HECS, DOVOB and HECS-g-DOVOB in corresponding appropriate solutions. No fluorescence emission appears in HECS solution. The fluorescence spectra of a $CHCl_3$ solution of DOVOB and a DMSO solution of HECS-g-DOVOB consist of two emission bands, the former (about 330 nm) is attributed to the benzene group, and the later excimer fluorescence (higher then 400 nm) indicates the formation of stable parallel benzene rings. The more significant excimer fluorescence has been observed on $CHCl_3$ solution of DOVOB acid rather than HECS-g-DOVOB because the self-assemble of the dendrimer makes it parallel and stable packed benzene structure. In the case of the polymer solution, the reducing of the proportion of dendrimer causes the excimer fluorescence weaken, however the obvious excimer fluorescence shows the self-assemble of the dendrimer in HECS-g-DOVOB. The CDH, HECS-g-DOVOB is expected to be a light-emitting polymeric material with further increasing of degree of substitution (DS).

4. Conclusions

A novel chitosan-dendrimer hybrid HECS-g-DOVOB was successfully synthesized by esterification. HECS-g-DOVOB formed typical cholesteric phase in concentrated DMSO solution, which is similar to HECS, but the cholesteric pitch is much smaller than that of HECS. The indication of excimer fluorescence in fluorescence spectra may be due to the interaction of benzene rings between adjacent cholesteric layers. This interaction is the driving force for decreasing of the cholesteric pitch into HECS-g-DOVOB.

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