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Synthesis and characterization of a nano fluorescent starch

Huicheng Li [a,*], Bing Zhang [a], Shaoyu Lü [b], Haiyan Ma [a], Mingzhu Liu [b]

[a] College of Chemistry and Chemical Engineering, Longdong University, Qingyang 745000, People's Republic of China
[b] College of Chemistry and Chemical Engineering, Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China

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ABSTRACT

A novel nano fluorescent starch, starch-bearing 3-epoxypropoxy fluorescein (ST-EF) was developed by a simple method. First, 3-epoxypropoxy fluorescein (EF) was prepared via a nucleophilic substitution reaction between fluorescein and epichlorohydrin. Then, ST-EF was synthesized via a ring-opening reaction to attach fluorescein to native cassava starch chains. The degree of substitution (DS) of ST-EF was determined by ultraviolet-visible spectrophotometry. The ^{1}H nuclear magnetic resonance (NMR) spectroscopy, elemental analysis, fourier transformed infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), transmission electron microscope (TEM), dynamic laser scattering (DLS), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) were used to characterize ST-EF. Fluorescent properties of ST-EF in water were studied. The results showed that the nano fluorescent starch shows strong fluorescence as fluorescein, and can be used as a fluorescent polymer in various applications, especially in biomedicine.

1. Introduction

At present, the development of chemical science is to solve the environmental problems and promote the sustainable development of the world. Therefore, materials research has witnessed a paradigm shift into synthesis and application of new materials based on natural polymers. Among them, natural polymer fluorescent material is attractive owning to its unique photophysical and photochemical properties which has been used in photoconductive resin, fluorescent reagent, flashing agent, fluorescence probe, chemical sensor, fluorescence imaging and other fields [1–5].

As early as 1963, Wolf and Pressley [6] had studied the rare earth fluorescent materials based on macromolecules, and from then the studies of polymer fluorescent materials have been reported continuously. In recent years, a number of alternative luminescent nanomaterials, fluorescent polymeric nanoparticles (FPNs) with aggregation-induced emission (AIE) characteristics have successively emerged. Various strategies such as non-covalent self-assembly of AIE dyes and amphiphilic molecules, covalent conjugation of AIE dyes with hydrophilic molecules, polymerization of AIE dyes with other monomers and encapsulation of AIE dyes in silica nanoparticles etc. for fabrication of these AIE nanoprobes have been reported over the past few decades. In these reports, the biomedical applications such as biological imaging, biological sensor, drug delivery and theranostics of these AIE nanosystems were explored [7–16].

Noteworthily, since the 1980s, natural polymer materials based on renewable resources have been developed, becoming one of the frontier fields of polymer science. Natural polymers, compared with synthetic polymers, are abundant, renewable, biodegradable and biocompatible. They contain a lot of hydroxyl and amino functional groups, and can be modified by chemical and physical methods to endow them with fluorescence and other performance [17,18].

Among various natural polymers, starch is an attractive base material. Starch is the product of photosynthesis of plants. It is widely found in seeds, roots, stems and fruits of plants, and is a kind of biodegradable and renewable polymer. It is usually composed of linear macromolecules (amylose) and branched macromolecules (amylopectin) in a proportion of approximately 17:83 in cassava starch [19–21]. In recent years, the development and utilization of starch in non-edible fields have attracted much attention. As far as we know, there are many reports about the preparation and application of starch derivatives, including fluorescent materials. For example, environment-friendly "green" composites based on starch were chemically modified to prepare fluorescence microspheres and fluorescence films, which have been used in chemical sensing, drug capsules, food packaging, fluorescent probes and so on [22–26]. However, as far as we know, few examples of the nano fluorescent starch through covalent linkage with fluorescein via a ring-opening reaction have been reported.

In this study, a nano fluorescent starch, starch-bearing 3-epoxypropoxy fluorescein (ST-EF), was synthesized by native cassava starch with 3-epoxypropoxy fluorescein. Fluorescein (FL) is a classical fluorescence reagent with strong and stable fluorescence [27,28]. It was introduced into starch, affording starch excellent fluorescence

* Corresponding author.
E-mail address: lihuicheng0901@163.com (H. Li).

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performance. Compared with small molecule fluorescence materials, this nano fluorescent starch has better processing performance and better long-term stability fluorescence properties. The fluorescein molecules were immobilized on starch backbones, therefore, they are not easily leaching. In addition, the nano fluorescent starch is easy to be compatible with organisms, non-toxic, harmless, coupled with good water solubility and dispersibility. Therefore, the remarkable optical properties of the ST-EF FPNs making them promising for various biomedical applications including drug delivery, biosensing and bioimaging etc. and other aspects.

2. Materials and methods

2.1. Materials

Native, unmodified food grade cassava starch (average molecular weight of 3.35×10^6, dried at 95 °C for 12 h before use) was provided by Shengdafangzhou Agricultural Products Co. LTD (Gansu, China). Fluorescein and epichlorohydrin were purchased from Tianli Chemical Reagent Factory (Tianjin, China). Ethanol was distilled before used. Potassium iodide, anhydrous alcohol, sodium hydroxide and other chemicals were all analytical grade and used as received. Ultrapure water was used in all experiments.

2.2. Synthesis of 3-epoxypropoxy fluorescein (EF)

EF was synthesized according to a classical organic chemical reaction (nucleophilic substitution) by the following procedure. Potassium iodide (4.15 g, 0.025 mol) and epoxy chloropropane (2.31 g, 0.025 mol) were added into a 250 ml three-necked flask with a mechanical stirrer and a condenser. Then 80 ml of anhydrous ethanol was added into the flask dropwise. The reactor was placed in a water bath, and the mixture was constantly stirred at 50–60 °C for 0.5 h. Then fluorescein (1.67 g, 0.005 mol) and sodium hydroxide (0.6 g, 0.015 mol) dispersed in alcohol were added by dripping slowly. The reaction was allowed to proceed at 60 °C for 2 h. The intermediates were traced by thin layer chromatography (TLC) techniques. After the end of the reaction, the solution was distillated to eliminate alcohol. The crude products were separated by column chromatography with anhydrous ethanol and trichloromethane (1/50, v/v). The resulting product was dried in a vacuum oven at 60 °C for 12 h. The orange colored powdery solid EF was obtained. Yield ≈ 39%. Tm = 146–148 °C. Visible spectra (λ_{max}, nm): 385, 460, 480.

2.3. Synthesis of starch-bearing 3-epoxypropoxy fluorescein (ST-EF)

Cassava starch (5 g), EF (3.88 g, 0.01 mol), NaOH (0.8 g, 0.02 mol) and 50 ml of absolute ethyl alcohol as solvent were added in a round-bottom flask. The mixture was heated to 50 °C and constantly stirred. The reaction was carried out for 4 h at 50–60 °C. The resulting product was neutralized with 0.2 mol/l HCl and cooled, precipitated with a large amount of anhydrous ethanol, centrifugalized and vacuum filtrated, washed with 90% ethanol aqueous solution. The washing and centrifugation procedures were repeated for three times. Finally, the centrifuged solid was put in a dialysis bag and dialyzed with 90% ethanol aqueous solution for 48 h, dried in a vacuum freeze dryer for 12 h. A pale yellow solid ST-EF was obtained. The degree of substitution was determined by ultraviolet-visible spectrophotometry using a literature procedure [29], and it was determined to be 1.5%. Visible spectra (λ_{max}, nm): 460 nm. ΦST-EF ≈ 0.68.

2.4. ^{1}H NMR spectroscopy, component analysis and Fourier transformed infrared (FTIR)

The ^{1}H NMR spectra were obtained from Bruker AV III HD 400 MHz spectrometer using D_2O as the solvents. Contents of carbon, oxygen in native cassava starch, fluorescein and ST-EF were determined by means of combined instruments of X-ray energy dispersive spectrometer (EDS, Oxford instruments Ltd., Britain, resolution: 120 eV) and Low vacuum scanning electron microscopy (JSM-5600LV, Japan, resolution: 3.5 nm). The IR spectra of native cassava starch, fluorescein and ST-EF were recorded by Fourier transformed infrared (FTIR) spectrophotometer (FTIR-8400S, Japan), using KBr tablets.

2.5. Scanning electron microscopy (SEM), field emission transmission electron microscope (TEM) and dynamic laser scattering (DLS)

The morphologies of native cassava starch, fluorescein and ST-EF samples were examined using a JSM-6701F SEM instrument (JEOL Ltd., Japan). The samples were coated with gold prior to observation. For original starch, a small amount of starch samples were dispersed in acetone, and the suspension was taken on the slide. After the acetone was volatilized, the spraying gold was applied. For starch fluorescein samples, the samples were frozen in liquid nitrogen, fractured, and sprayed with gold.

The inner morphology and microstructure of native cassava starch, fluorescein and ST-EF samples were examined by TEM using a Tecnai G2 TEM instrument (FEI company Ltd., America). The samples were dispersed in deionized water, and stirred for 10 min at room temperature. After 10 min of ultrasonic processing, it was dropped in a special copper wire mesh. After drying, the morphology and size of nanoparticles were determined using transmission electron microscopy (TEM).

The hydrodynamic size distribution of native cassava starch, fluorescein and ST-EF FPNs in aqueous solution was determined by dynamic laser scattering (DLS) using a Zetasizer Nano ZS ZEN 3600 apparatus (Malvern Instruments Ltd., Britain).

2.6. X-ray diffraction (XRD)

The X-ray diffraction (XRD) measurements for native cassava starch, fluorescein and ST-EF were taken on X-ray powder diffractometer (PANalytical B.V. Empyrean, XRD-6000, Netherlands) with Nickel filtered Cu-Kα radiation (λ = 1.54056 Å) at a voltage of 40 kV and current of 100 mA.

2.7. Differential scanning calorimetry (DSC)

DSC measurements were performed with a sapphire DSC (Perkin-Elmer, America). About 10 mg of the dried samples were placed in an aluminum pan and sealed. The samples were heated from −40 to 100 °C with a heating rate of 10 °C/min under nitrogen atmosphere. The sample pan was dried to a constant temperature at 105 °C. Glass-transition temperature was estimated. All samples were tested twice. The samples were placed at room temperature with a relative humidity of 55%.

2.8. Fluorescence analysis

Fluorescence test of ST-EF sample was carried out via fluorescence spectrometer (F-7000, Japan). A 10 × 10 mm quartz cell was used for detection. The solvent was deionized water, and the sample concentration was 1.6 mg/ml. Fluorescence scans were obtained using a 5 nm slit width, a 460 nm excitation wavelength, and an emission scan ranging from 480 to 660 nm. Fluorescence quantum yield is an important factor for quantitatively evaluating fluorescent intensity of fluorescein. The quantum yield value of ST-EF was determined using the following equation:

$$\Phi_x = \Phi_{std}\,(A_x/A_{std})\,(E_{std}/E_x)$$

where Φ_x is the quantum yield, it is the total fluorescence emission intensity over all wavelengths, A is the optical density, and E is the molar extinction coefficient determined at 460 nm. The subscript "std"

refers to the reference fluorophore of known quantum yield. Fluorescein (literature quantum yield 0.93) was chose as a standard [29]. To minimize reabsorption effects, the absorption value at the excitation wavelength is required to be smaller than or equal to 0.05.

3. Results and discussion

3.1. Synthesis of starch-bearing 3-epoxypropoxy fluorescein (ST-EF)

To synthesize the nano fluorescent starch ST-EF, 3-epoxypropoxy fluorescein (EF) was synthesized firstly under sodium hydroxide alkaline condition and in ethanol solution. Their synthesis scheme was shown in Scheme 1. In the presence of catalyst of potassium iodide and sodium hydroxide, the nucleophilic substitution reaction occurred between epichlorohydrin and fluorescein molecules. Then, ST-EF was synthesized via ring-opening reaction between EF and cassava starch in ethanol solution, in the presence of NaOH as a catalyst. The reaction mechanism is considered to be the nucleophilic addition of starch to ternary ring under alkaline conditions. As NaOH is a mild alkaline catalyst, therefore, a mild reaction temperature of 50–60 °C was chosen. Compared with the literature methods, the reaction conditions we used were mild, simple, and easy. For example, Guan and Su reported that fluorescent starch was prepared in dimethyl sulfoxide (DMSO) solution, in the presence of NaH as a catalyst, under nitrogen atmosphere for 10 h [29].

3.2. ^{1}H NMR spectra, component analysis and FTIR determination

To verify the successful synthesis of ST-EF, the ^{1}H NMR spectra of starch and ST-EF were first conducted and recorded for comparison. As shown in Fig. 1, after modification via a ring-opening reaction to attach fluorescein to cassava starch chains, a series of typical signal peaks could be observed. For example, the clear signal peaks from 6.5 to 8.5 ppm can be ascribed to the protons of fluorescein linking onto the starch skeleton. The peaks located from 2.5 to 4.5 ppm can be attributed to the protons of methylene, methyl group, related to starch and newly formed group such as —$CH_2CHOHCH_2$— originated from the epoxy group. The results suggested that fluorescein has successfully conjugated with starch. Furthermore, the signal peak at 1.1 ppm is possibly corresponding to the protons of —OH from —$CH_2CHOHCH_2$— of ST-EF, also suggests the successful reaction between epoxypropoxy fluorescein and starch. From the ^{1}H NMR spectra of ST-EF, the signal peaks

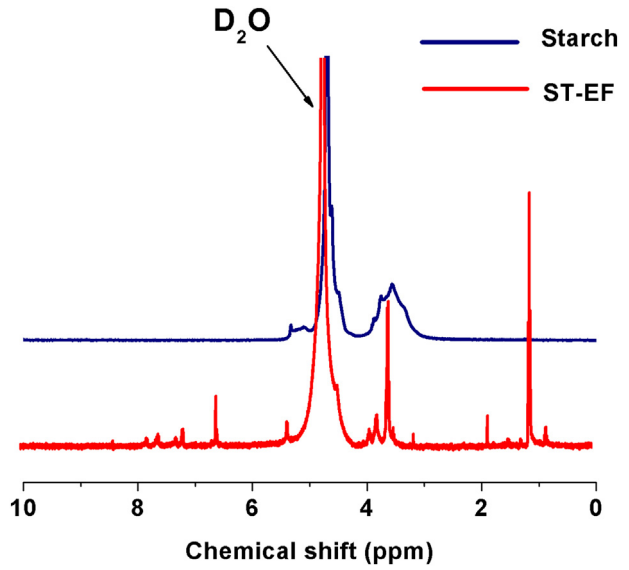
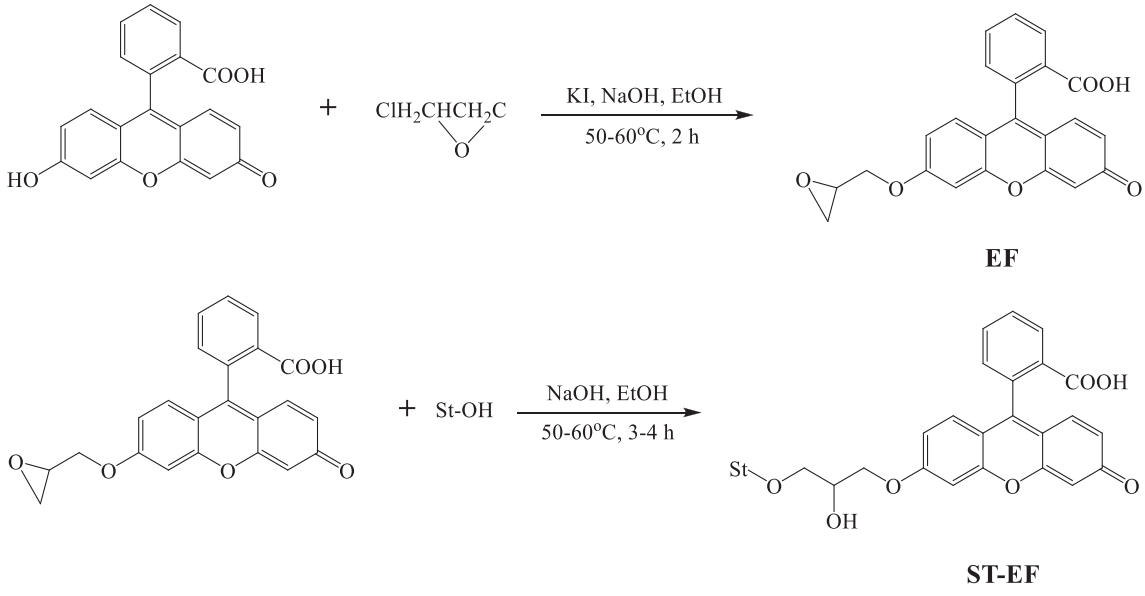


Fig. 1. ^{1}H NMR spectra of cassava starch and ST-EF use D_2O as solvents.

of epoxypropoxy fluorescein and starch could all be observed. The signal peaks located from 6.5 to 8.5 ppm can be assigned to the protons of aromatic rings of fluorescein. This demonstrated the successful construction of the ST-EF LPNs via a ring-opening reaction to attach fluorescein to cassava starch chains.

Contents of carbon, oxygen in cassava starch, fluorescein and ST-EF were determined by an elemental analysis by means of instrument combination, and the content is 47.99%, 52.01% and 71.24%, 28.76% and 34.64%, 65.36%, respectively. The data further confirmed that epoxypropoxy fluorescein was covalently linked to starch.

To further investigate the structure of starch derivatives, FTIR spectra of fluorescein, native cassava starch (ST) and ST-EF were measured, and the results are shown in Fig. 2. In the spectrum of native cassava starch (Fig. 2b), the characteristic absorptions that appeared at 1228 and 990 cm^{-1} are attributed to the α-1,4 glycoside linkage bond (C—O—C) stretching vibration of the anhydrous glucose units. The bands at 1122 and 830 cm^{-1} are assigned to the C—O—C stretching vibrations in the ring. The bands at 945 and 3042 cm^{-1} are the characteristic absorption of the C—OH and —CH_2 stretching vibration. These



Scheme 1. Synthesis scheme of 3 epoxypropoxy fluorescein (EF) and starch-bearing 3 epoxypropoxy fluorescein (ST-EF).

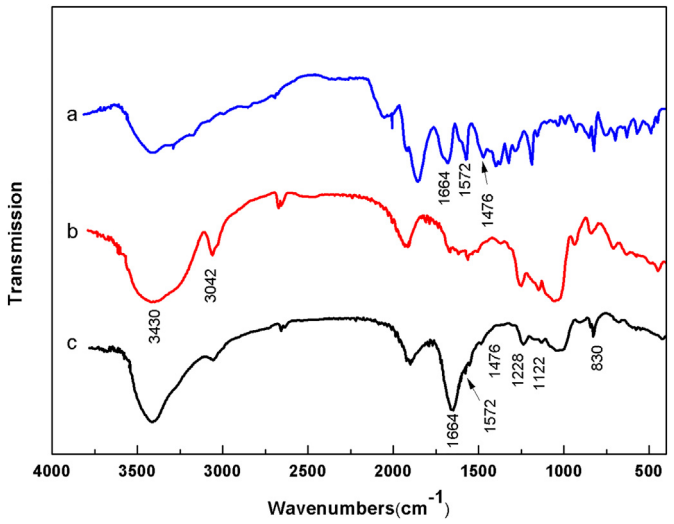


Fig. 2. FTIR spectra of fluorescein (a), native cassava starch (b) and ST-EF (c).

bands also appeared in the spectrum of ST-EF (Fig. 2c), indicating that the backbone of the anhydrous glucose units of the starch was not destroyed in the synthesis process.

In the spectrum of ST-EF (Fig. 2c), it can be seen that the characteristic absorption of the —OH stretching vibration is at 3430 cm^{-1}, which is indicated that the epoxy groups have been partly substituted. Besides, there are distinct differences between the spectrum of ST and ST-EF. We can see that there are three new peaks in the infrared spectrum of ST-EF at 1476, 1572 and 1664 cm^{-1}. The peak observed at 1664 cm^{-1} is attributed to the C═O stretching of ester or carboxyl in EF. The characteristic absorptions that appeared at 1572 and 1476 cm^{-1} are attributed to the C═C stretching of the benzene ring, which are also observed in the spectrum of fluorescein (Fig. 2a). These results demonstrated that the epoxy groups on EF molecules have reacted with the hydroxyl groups on ST, and EF has been attached to the side chains of starch via chemical bonds.

3.3. Scanning electron microscopy (SEM)

The SEM images of native cassava starch, fluorescein and ST-EF are shown in Fig. 3. Fluorescein (Fig. 3a) shows a pile of scattered small particle of about 1 μm. The native cassava starch granules (Fig. 3b) exhibit spherical shapes with many microcrystalline bundles arranged in a radial manner. Their surface is smooth and the diameter is from 5 to 15 μm. Starch generally exists in the state of particles, and the particle size varies due to the different proportion of linear and branched chain in different starch.

However, the morphology of ST-EF particles (Fig. 3c) is largely changed compared to the native cassava starch. The smooth appearance has become bumpy with irregular wodge like mushrooms. The morphological transformations could be attributed to the fusion of granules caused by the disruption of starch during the heating process [30]. It was reported that starch granules lost their integrity and were split, melted and reformed new structures during the reaction [31,32]. In the study, the introduction of EF groups may disrupt the internal structure and coalesce the starch granules together. Thus, SEM results show that the original structure was disrupted after chemical modification. Therefore, it can be imaged that the crystalline structure of starch may be broken during the reaction.

3.4. Transmission electron microscope (TEM)

The TEM images of fluorescein, native cassava starch and ST-EF are shown in Fig. 4. Fluorescein (Fig. 4a) shows a transparent crystal. Fig. 4b shows the TEM image of native cassava starch. The agglomeration of starch granules is serious. However, for ST-EF particles (Fig. 4c), spherical morphology with a particle size of 30–50 nm and well dispersion were observed. Fig. 3c inset picture shows that after 10 min of ultrasonic dispersion, the ST-EF nanoparticle suspension presents emulsion without stratification, indicating that the prepared starch fluorescein particles are well dispersed.

Native cassava starch shows agglomeration, which is mainly because the surface of starch particle is rich in a large number of free hydroxyl groups. These hydroxyl groups form hydrogen bonds and tend to agglomerate. The modified starch particles ST-EF are more easily dissolved and dispersed in deionized water. This is because the hydrophobic groups —O—CH_2—CH_2— exist. In addition, the rigid plane of the fluorescein molecule destroys the tight entanglement between long chains of polymers. Furthermore, after modified with fluorescein particles, the surface free energy of starch is reduced, and the compatibility of starch molecules with water increases.

The size of ST-EF in SEM figure is disagree with the size in TEM figure, the difference may be caused by different dispersion solvents. ST-EF is slightly soluble in acetone, but is easily dissolved and dispersed in water.

To further investigate the nanometer characteristics, the hydrodynamic size distribution of ST-EF FPNs in water was also determined by dynamic laser scattering (DLS). The particle size distribution of suspensions is shown in Fig. 5. The starch suspension exhibited a broad peak at 1400–4000 nm, and the average particle size of 2304 nm, whereas the particle size range of fluorescein and ST-EF were 845–1165 nm and 60–90 nm, respectively (Fig. 5). The experimental data shows that through chemical modification of starch, the particle diameter is greatly reduced, and the particle size distribution range narrows down, as well as improving the water dispersibility. The results show that size of ST-EF FPNs by DLS is 74.5 ± 19.5 nm. As compared with the TEM

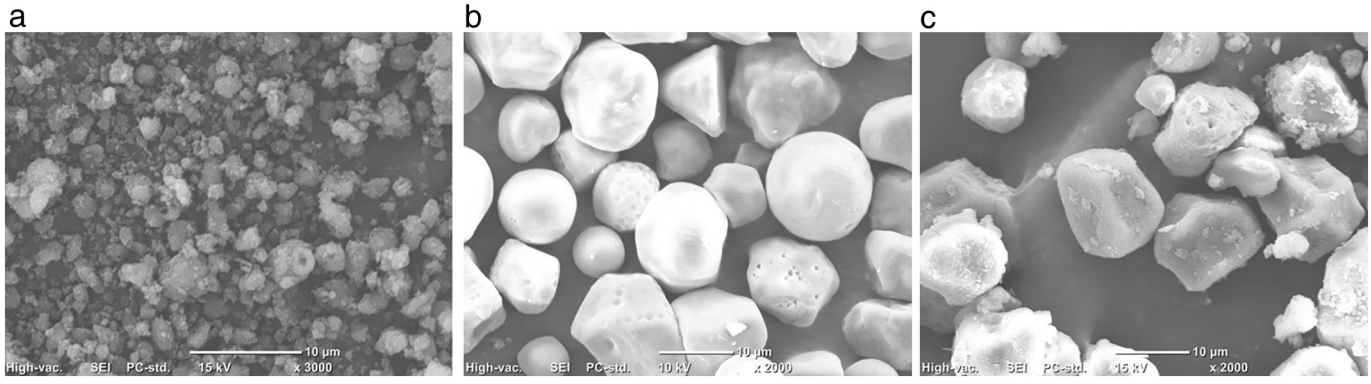


Fig. 3. SEM images of fluorescein (a), native cassava starch (b) and ST-EF (c).

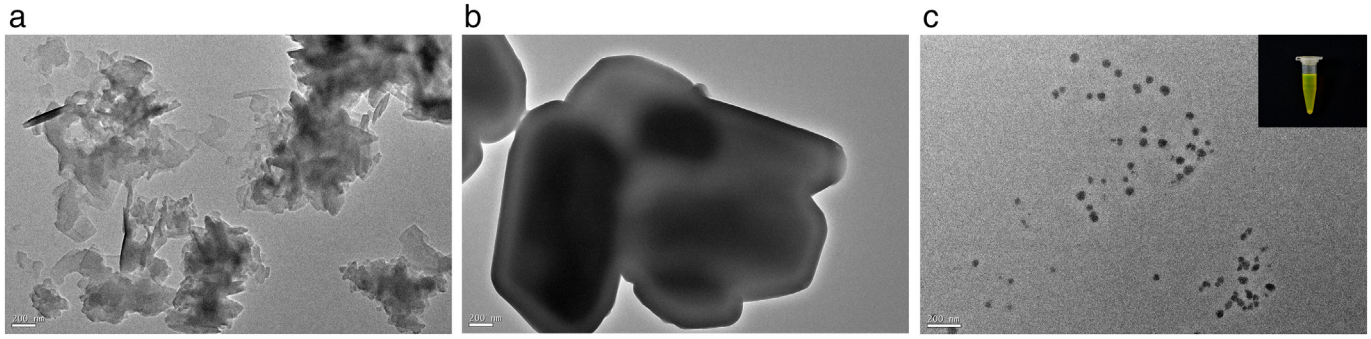


Fig. 4. TEM images of fluorescein (a), native cassava starch (b) and ST-EF (c).

characterization, the size from DLS is relative large. The difference maybe originated from the shrinkage of particles during the preparation of samples for TEM characterization [12]. Therefore, these results give the direct evidence that we have successfully prepared starch based nanoparticles.

3.5. X-ray diffraction (XRD)

To determine if the crystalline structure has been changed after etherification, the X-ray diffractograms of fluorescein, native cassava starch and ST-EF were examined by XRD, as shown in Fig. 6. It can be seen from the figure that fluorescein (Fig. 6a) in 10°–30° appears a large number of dense sharp peaks, indicating that fluorescein is a highly crystalline substance. Native cassava starch (Fig. 6b) exhibits an A-type crystalline pattern, characteristic of tuber starch, with strong reflections at 2θ of 15°, 17°, 18° and 23° [31]. This result is in accordance with reported literature [32,33]. For ST-EF (Fig. 6c), its structure has been totally changed. After being modified by EF, the four reflections of starch in Fig. 6b completely disappeared and only a low and broad 2θ at around 17° was found. However, the intensity of the diffraction peaks reduces significantly, which suggests that most of crystalline structure of the starch was probably converted into amorphous shape. This result shows that the ordered crystalline structure of native starch was destroyed after modified, as suggested by the SEM results. Starch is a polyhydroxy polymer, which contains three independent hydroxyl groups in each monomer, therefore, there is a large number of intramolecular and intermolecular hydrogen bonds. Starch is generally found to be 15%–45% crystallization, equivalently semi-crystalline in nature. The crystallinity is essentially owing to the amylopectin fraction. Therefore, it can be inferred that the crystallinity of cassava starch was destroyed due to covalent linkage of EF on its side chains.

In addition, we can see that when the fluorescein was grafted onto the starch side chains, their sharp peaks disappeared, indicating that its crystalline structure was destroyed. However, when EF was immobilized on the macromolecule chains, ST-EF also shows some sharp peaks because EF is rigid.

3.6. Differential scanning calorimetry (DSC)

The glass transition temperature (Tg) is an important characteristic parameter of polymer, and it is the transition temperature of polymer from relatively brittle "glassy" state into a high elastic state. Below Tg, the molecular chains and the chain segments of polymer cannot relax, only the atoms (or groups) of the molecules vibrate at their equilibrium positions. Above Tg, chain segments start to motion, although molecular chains are unable to move. When the temperature is increased, the whole molecular chains relax. DSC can be used to determine Tg and numerous studies have previously reported for Tg determination of starch [34,35]. In this study, the DSC thermograms of fluorescein, native cassava starch and ST-EF are recorded and shown in Fig. 7.

No Tg is detected for fluorescein samples (Fig. 7a) because fluorescein is a small molecule compound. However, both natural cassava starch (Fig. 7b) and ST-EF (Fig. 7c) show glass transition with the temperature increasing. Tg of natural cassava starch and ST-EF is 80 °C and 75 °C, respectively. Compared with natural starch, Tg of modified starch is lower. Similar results were reported by other modified starch [36–38]. This is because that the modification of starch destroys the crystalline structure and the order of crystallite partially changed to amorphous form. The introduction of the substituent groups inhibits the inter-chain interaction and results in a loosened structure. These results are confirmed by the X-ray diffraction pattern analysis.

3.7. Fluorescent properties of ST-EF in aqueous solution

It is well known that fluorescein emits green fluorescence under blue or ultraviolet light. The maximum absorption wavelength is 490 nm and 460 nm, respectively. In many applications such as fluorescent antibody technology, it is widely used as a fluorescent tracer

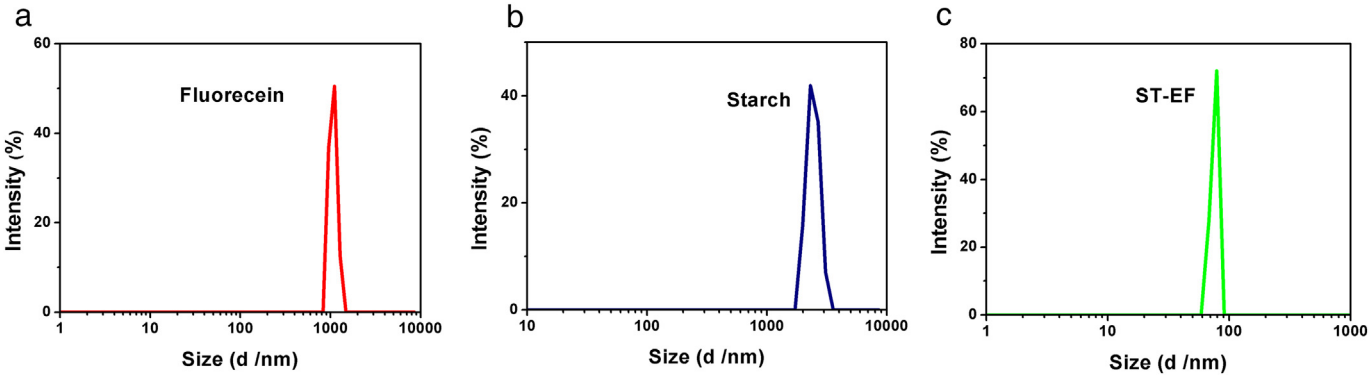


Fig. 5. Particle size distribution of suspensions (0.01%, w/v), fluorescein (a), native cassava starch (b) and ST-EF (c) from left to right.

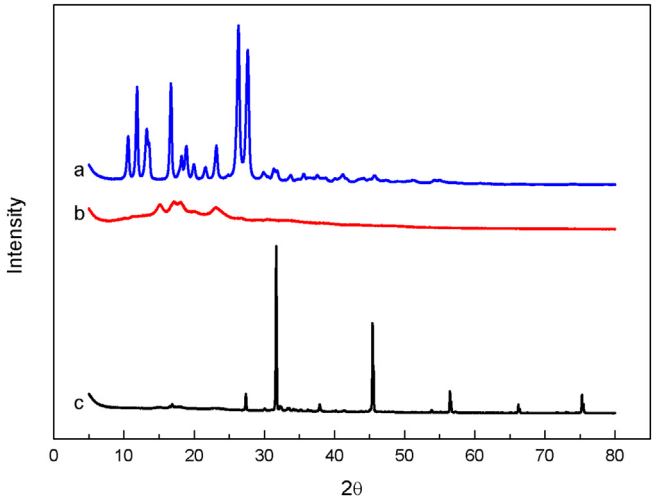


Fig. 6. X-ray diffractograms of fluorescein (a), native cassava starch (b) and ST-EF (c).

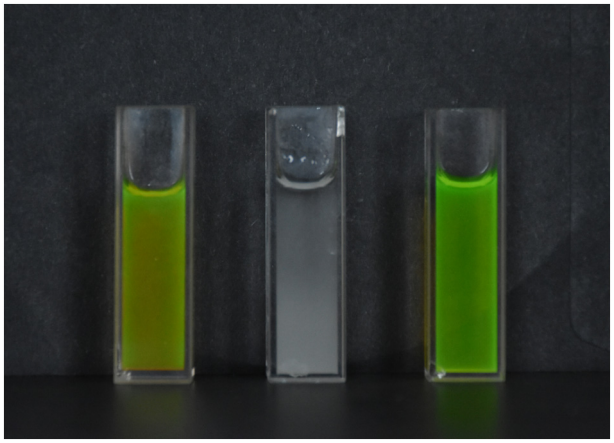


Fig. 8. Photos of fluorescein, cassava starch and ST-EF in aqueous solution from left to right under visible light.

[39,40]. In this study, we have designed and synthesized functional fluorescein with environmentally friendly starch grafts. We can see that ST-EF emits bright green fluorescence like fluorescein under visible light in Fig. 8. The photograph was taken 6 h after the solution was prepared, showing strong photoluminescence characteristics and fluorescence stability. This phenomenon indicated that ST-EF could still achieve better long-term fluorescence stability similar to that of fluorescein.

The fluorescence spectrum of ST-EF in aqueous solution is showed in Fig. 9. The maximum fluorescence excitation peak is found at 460 nm, and the maximum emission wavelength is located at 524 nm. Apparently, the polymer bearing fluorescein conjugate EF shows the characteristic excitation and emission properties of fluorescein. Similar to fluorescein, ST-EF has strong and stable bright green fluorescence in aqueous solution media and both its excitation wavelength and emission wavelength are in the range of visible region. Fig. 9 inset picture is the photo of ST-EF in aqueous solution under visible light. The fluorescence quantum yield is 0.68 in aqueous solution (pH = 7.0) using fluorescein (literature quantum yield 0.93) as reference. Although the quantum yield value of ST-EF is approximately three quarters of that of fluorescein, their brightness is sufficient for the application in bioimaging or biosensors. Compared with small molecule fluorescence materials, this nano fluorescent starch FPNs has better processing performance and more stable fluorescence properties. The fluorescein molecules were immobilized on starch backbones, therefore, they are not easily leaching. In addition, the nano fluorescent starch is easy to be compatible with organisms, non-toxic, harmless, coupled with good water solubility and dispersibility. Therefore, it is suggested that the nano fluorescent starch can be used in the areas of fluorescence tracing technology, water-based fluorescent paint, cosmetic technique, chemical sensor and various biomedical applications including drug delivery, biosensing and bioimaging etc. and other aspects.

4. Conclusions

A novel nano fluorescent starch, starch-bearing 3-epoxypropoxy fluorescein (ST-EF) was developed by the reaction of cassava starch and 3-epoxypropoxy fluorescein (EF). The degree of substitution (DS) of the starch derivative was measured by ultraviolet-visible spectrophotometry and it was determined to be 1.5%. A series of characterization techniques including ^{1}H NMR spectrum and Fourier transformed infrared (FTIR) spectroscopy, etc. confirmed that ST-EF was synthesized successfully. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic laser scattering (DLS), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) determinations suggested that the ordered crystalline structure of native starch was destroyed after modifying. ST-EF particles are well dispersed in aqueous solution and the particle size is within the range of 30–50 nm. ST-EF shows strong fluorescence as fluorescein. Therefore, it is suggested that the nano fluorescent starch we synthesized can be used in

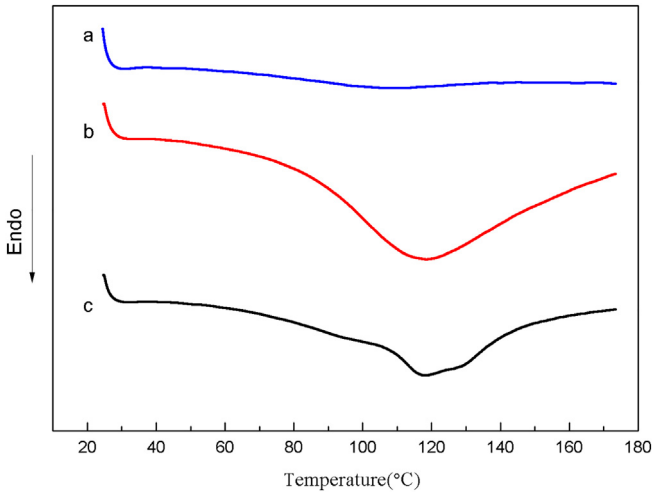


Fig. 7. DSC thermograms of fluorescein (a), native cassava starch (b) and ST-EF (c).

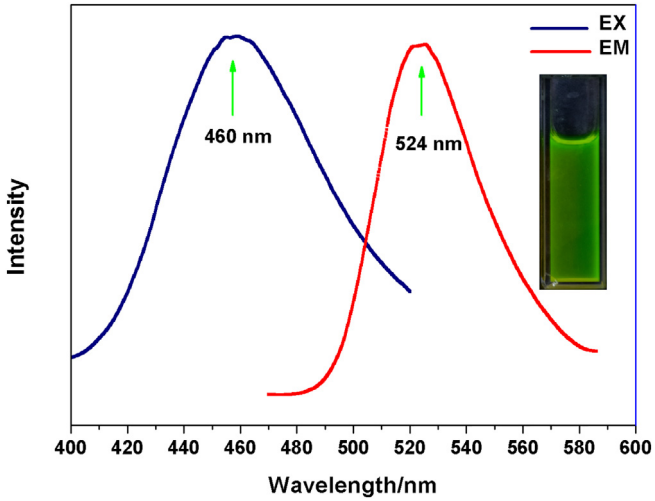


Fig. 9. Fluorescence spectra of ST-EF in aqueous solution.

fluorescence tracing technology, decorative coating, cosmetic technique, chemical sensor and various biomedical applications including drug delivery, biosensing and bioimaging etc. and other aspects.

Acknowledgements

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