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Synthesis of modified potato starches for aqueous solubilization ofbenzo[a]pyrene

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Abstract

For soil rehabilitation, the surfactant-enhanced remediation has emerged as a promising technology. For this purpose, starch derivatives were difunctionalized by 1,4-butane sultone (BS) and 2-octen-1-ylsuccinic anhydride (OSA). Eight distinct products were obtained under different synthesis conditions. The chemical structural characteristics were investigated by ¹H NMR spectroscopy. The compounds were evaluated for their apparent aqueous solubility and their ability to increase the solubility of a hydrophobic pollutant such as benzo[a]pyrene (BaP), used as a polycyclic aromatic hydrocarbon model. In comparison with native starch, the best obtained compound increased starch apparent aqueous solubility by a factor of 10 (up to 3.50 g/L) and also stimulated 77-fold BaP aqueous solubilization (up to 232.97 µg/L) underlining its very high surfactant property. In this study, the right balance between hydrophobic character (octenyl succinate group (OS) grafted) of starch derivatives and starch apparent aqueous solubility (BS grafted) was highlighted.

Highlights

- Modified starches were evaluated for their benzo[a]pyrene desorption capacities
- The best starch stimulated 77-fold the aqueous solubilization of benzo[a]pyrene

Hydrophobic/hydrophilic balance was highlighted for surfactant-enhanced remediation

Keywords:

Potato starch, alkylation, benzo[a]pyrene, surfactant

Chemical compounds used in this article

Potato starch (PubChem SID: 135306210); Sodium hydroxide (PubChem CID: 14798); Dimethyl sulfoxide (PubChem CID: 679); Benzo[a]pyrene (PubChem CID: 2336); 2-octen-1-ylsuccinic anhydride (PuBChem CID: 5362689); 1,4-butane sultone (PubChem CID: 15411); Deuterium oxide (PubChem CID: 24602); Dichloromethane (PubChem CID: 6344)

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) pose a real threat to environment and human health due to their high toxicity. These pollutants are mainly released into the atmosphere from anthropogenic sources before being deposited into the soil. They tend to persist in the environment because of their high chemical stability, low water solubility and tendency to be adsorbed onto soil organic matter (Cerniglia, 1993; Juhasz & Naidu, 2000). For soil rehabilitation, the surfactant-enhanced remediation has emerged as a promising technology, especially for biodegradation involving saprotrophic soil fungi (Goltapeh, Danesh, & Varma, 2013). Indeed, in contaminated soils, PAHs need to be transferred from the solid soil phase to the aqueous one in order to be bioavailable to fungi and consequently be biodegraded (Flotron, Delteil, Padellec, & Camel, 2005; Rafin, De Foucault, & Veignie, 2013).

For this purpose, surfactant like nonionic ones or cyclodextrins could be used to increase the PAHs biodegradation rates in soils (Bardi, Mattei, Steffan, & Marzona, 2000; Ortega-Calvo et al., 2013). Previous studies (<u>Molnár</u> et al., 2005; Veignie, Rafin, Landy, Fourmentin, & Surpateanu, 2009) have demonstrated the interest of using cyclodextrins which are cyclic oligomers composed of α -1,4-linked glucose units (Szejtli, 1988). For most non-polar contaminants, formation of inclusion complex

between the hydrophobic cavity of cyclodextrin and the contaminant increases the apparent solubility of pollutant (Frömming & Szejtli, 1994; Fava, Di Gioia, & Marchetti, 1998).

The existence of similar hydrophobic cavities was also highlighted in starches in our previous study (Rosu et al., 2011). These natural polysaccharides represent a material of choice for surfactant-enhanced remediation approaches owing to several advantages: they are non-toxic, abundant, cheap, renewable, biodegradable and easily modifiable (Liu & Budtova, 2013; Richardson & Gorton, 2003; Rodrigues & Emeje, 2012). Nevertheless, the weak aqueous solubility of native granular starches is also known to be a major constraint that could limit the development of such starch-based materials and derived applications (Bai, Shi, Herrera, & Prakash, 2011; Waterschoot, Gomand, Fierens, & Delcour, 2014). In our previous research, modified starches were synthesized using 1,4-butane sultone (BS) which stimulated starch solubility in water thanks to charged sulfonate groups (Rosu et al., 2013). Moreover, the high molecular size of starch must be preserved in order to avoid a side effect of the surfactant-enhanced remediation i.e. the pollutant discharge in the environment. These previous syntheses allowing a right balance between hydrophobic and hydrophilic characters of starch derivatives were promising. This strategy was used as our guideline for the present research.

The originality of the present research was to use 1,4-butane sultone (BS) and 2octen-1-ylsuccinic anhydride (OSA) as starting materials for synthesizing difunctionalized starches. These new modified starches were then evaluated for their apparent aqueous solubility and their ability to increase the solubility of hydrophobic pollutants such as benzo[a]pyrene (BaP), used as a PAH model.

2. Material and methods

2.1. Chemicals

Soluble potato starch (**P0** 121096, 0000108507), sodium hydroxide (NaOH) and dimethyl sulfoxide (DMSO) at 99.5% purity were provided by Panreac Quimica SA (Barcelona, Spain). Benzo[a]pyrene (BaP) at 96% HPLC purity and 2-octen-1-

ylsuccinic anhydride (OSA) at 97% purity were purchased from Sigma Aldrich (Steinheim, Germany). The reagent 1,4-butane sultone (BS) at 99% purity was supplied by Acros Organics (Noisy-Le-Grand, France), dichloromethane (DCM) by Fisher Scientific (Illkirch, France), deuterium oxide (D₂O) at 99.8% purity by Carlo Erba Reagents (Val-de-Reuil, France). Cellulose membrane (MWCO of 12–14 kDa) was supplied by Medicell International (London, United Kingdom). Deionized water was used throughout this work.

2.2. Synthesis of difunctionalized starch derivatives

Reactions were performed sequentially (P1, P2, P3, P4, P5, P6) or in one pot (P7, P8). All the reaction parameters are given in Table 1. Representative procedures were described for P1 and P8.

P1: Potato starch (1.00 g, 6 mmol per anhydroglucose unit (AGU)), 5 mL H₂O, 20 mL DMSO and NaOH (0.10 g, 2.5 mmol) were placed in a 100 mL round bottomed flask and were stirred at 20 °C for 24 h. To this mixture, BS (0.92 mL, 9 mmol) was added and the reaction was carried out for 24 h. Finally, OSA (1.90 mL, 9 mmol) was added and the resulting mixture was stirred for 24 h. A total of NaOH pellets (0.40 g, 10 mmol) was added to maintain the pH between 8 and 9.

P8: Potato starch (1.00 g, 6 mmol AGU), 5 mL H₂O, 20 mL DMSO and NaOH were placed in a 100 mL round bottomed flask and were stirred at 70 °C. To this mixture, BS (1.85 mL, 18 mmol) and OSA (1.90 mL, 9 mmol) were simultaneously added and the reaction was carried out for 24 h. A total of NaOH (0.40 g, 10mmol) was added to maintain the pH between 8 and 9.

On completion of the reactions, the modified starches were dialyzed in water during 3 days using a cellulose membrane. The water was changed twice a day and the products were lyophilized (Rosu et al., 2013). Eight products were obtained.

Product	Starch	T (°C)	Duration (h)	DMSO/H ₂ O	NaOH/AGU	BS/AGU
P1	Native	20	48	4/1	1/2	1/2
P2	Dispersed ^a	20	48	4/1	1/2	1/2
P3	Native	20	48	4/1	1/2	1/1
P4	Native	70	48	1/1	4/3	1/1
P5	Native	70	48	1/1	4/3	1/2
P6	Native	70	48	1/1	1/2	1/6
P7 ^b	Native	20	24	4/1	1/2	1/1
P8 ^b	Native	70	24	4/1	1/2	1/1

Table 1Parameters of difunctionalized starches synthesis (OSA/AGU ratio = 1/2).

^a Native starch was dispersed into water and heated at 70 °C during 30 min prior to synthesis

^b One pot reaction

2.3. Structural ¹H NMR characterization of starch

¹H NMR spectra were measured with a 400 MHz Spectrospin NMR spectrometer. The NMR spectrometer is an Avance III 400 spectrometer and the supplier is Bruker, France. Spectra were recorded in D₂O with the following spectra acquisition parameters: 25 °C or 70 °C, relaxation delay 1s, pulse angle 30°, acquisition time 6 s with a total of 16 scans. The routine NMR conditions chosen did not yield quantitative NMR spectra and the ratio values (R) determined in this work were thus apparent R values. This still enabled a qualitative comparison between samples, provided the NMR spectra were recorded in the exact same conditions for the samples being compared.

The apparent ratio (R) between octenyl succinate groups (OS) and BS , which represents the apparent ratio of substituents grafted, was determined from ¹H NMR spectra. R is calculated by the equation:

 $R = (I_{OS}/3) / (I_{BS}/4)$ Eq (1)

The integration of the peak (I_{OS}) was determined at 0.89 ppm matching to (CH_3)_a protons in the alkyl residues as referred to Fig.1. The integration of BS at 1.70

- 1.95 ppm corresponding to $(CH_2)_{B-C}$ protons in the alkyl residue of BS (Fig. 1) was used to determined I_{BS}.

2.4. Determination of the apparent aqueous solubility of modified starch

A saturated solution of each modified starch was prepared in deionized water at room temperature and pH 6 (21–22°C). This solution was stirring during 1 hour. After 24 hours of decantation, the saturation was confirmed by the presence of undissolved material. A precise volume (10 mL) of supernatant was lyophilized and weighed. Apparent solubility experiments were done at three replicates for each modified starch to evaluate repeatability (Rosu et al., 2013).

2.5. BaP solubilization in the presence of modified starch

A BaP solution was initially prepared in DCM at a concentration of 40 mg/L. Then, 375 μ L (15 μ g) of this solution was deposited into a hemolysis tube allowing DCM solvent to completely evaporate at 60 °C. An aliquot of 3 mL of water (as reference) or of aqueous starch solution (at a concentration of 0.45 g/L) was added into the BaP coated hemolysis tube (Rosu, et al., 2013). Tubes were incubated in the dark for 24 h. BaP molecular fluorescence in carbohydrate polymer solutions was analyzed into a quartz cell on a Perkin Elmer LS50B spectrometer (excitation 295 nm, emission 406 nm, time integration 10 s) (Veignie et al., 2009). BaP solubilization was evaluated at three replicates for each compound.

2.6. Specific activity of modified starch and maximum BaP solubilization

For each modified starch solution (0.45 g/L), a specific activity (SA) for BaP solubilization was calculated. It represented the amount of solubilized BaP (μ g) per gram of modified starch. It allowed us to calculate, for each modified starch, the maximum BaP solubilization by the equation:

Maximum BaP solubilization = specific activity X apparent aqueous solubility Eq (2)

3. Results and discussion

3.1. Synthesis conditions

Syntheses were adapted from our previous research (Rosu, et al., 2013). Due to its poor water solubility at room temperature, starch was firstly dissolved in DMSO, an aprotic polar solvent, prior to the addition of the base catalyst (NaOH) and of the reagents (Wei et al., 2011). Even if water addition was not indicated to yield an optimal dissolution of native starch in DMSO (Dona, et al., 2007), it was a necessary condition for optimizing the compound reactivity. Indeed, this pretreatment aims to disorganize the crystalline structure of starch molecules and consequently to enhance their reactivity, favoring a better uniformity of substitutions (Richardson & Gorton, 2003). After starch solubilization obtained, BS and OSA were added simultaneously or sequentially into the reaction medium. Amounts of BS and OSA were based on the presence of the three free hydroxyl groups per AGU.

In order to optimize the process, different synthesis conditions were tested: pretreatment of native starch, temperature, reaction duration, DMSO/H₂O ratio and BS/AGU ratio. Eight products were synthesized (Table 1).

3.2. Characterization of the molecular structure of starch by ¹H NMR spectroscopy

3.2.1. Characterization of the native starch

Fig. 1A presents the ¹H NMR spectrum in D₂O of **P0** as reference. The NMR spectrum of native starch was characterized by a broad and complex multiple proton resonance signals between 3.50 and 4.20 ppm assigned to protons H₂₋₆ of the starch AGU. Signals at 4.98 and 5.41 ppm were attributed to the anomeric protons corresponding to the internal α -1,6 and α -1,4 linkages respectively (De Graaf, Lammers, Janssen, & Beenackers, 1995; Gaborieau et al., 2009).





Fig. 1.

Structure of bialkylated starch and ¹H NMR spectra (400 MHz, D_2O , 25 °C for A and B and 70 °C for C) of **A**: native potato starch (**P0**), **B**: sulfobutylated starch and **C**: sulfobutyl-octenyl succinate starch (**P2**).

3.2.2. Characterization of the modified starch

After the sulfobutylation step, an aliquot of the reaction medium was dialyzed and lyophilized in order to obtain a sample ready for NMR spectroscopy analysis. Fig. 1B presents the NMR spectra recorded during the synthesis of **P2** and clearly pointed out the apparition of broad resonance signals in the 1.50-3.50 ppm region attributed to the randomly grafted sulfobutyl groups. Indeed, peaks at 1.70-1.95 ppm results from the 4 protons of the $(CH_2)_B$ and $(CH_2)_C$ while the broad triplet at 2.96 ppm was assigned to $(CH_2)_D$ proton of BS (Qu, Tucker, & Christian, 2002).

At the end of the reaction, NMR spectra of the modified starch derivatives were recorded in D_2O at room temperature. Unfortunately, peaks were too broad and irresolvable to be exploited. Therefore, spectra were recorded at 60 °C according to

Gidley (1985). Modification of starch by OS was confirmed by the apparition of a peak around 0.89 ppm, which included the three protons of the terminal methyl group of OS (Fig. 1C). The others peaks specific of the anhydride groups CH and $(CH_2)_n$ are assigned as follows: 1.30 ppm to $(CH_2)_{b-d}$; 2.00 ppm to $(CH_2)_e$ and between 2.13 and 2.95 ppm to $(CH)_i$, $(CH_2)_h$ and j (Zhang, Zhao, & Xiong, 2013). R was determined by integrating pics at 0.89 ppm and 1.70-1.95 ppm.

3.3. Apparent aqueous solubility of starch derivatives

For each product obtained, the R as well as the aqueous solubility of starch derivatives are expressed in Table 2. In a general way, excepted for **P4** and **P5**, the apparent aqueous solubility of modified potato starches decreased with the augmentation of grafted BS.

P4 and **P5** are characterized by the highest apparent aqueous solubility, respectively superior to 28.00 g/L in comparison with all the products obtained. ¹H NMR spectra of these products showed that these compounds were only grafted by BS. Thus, the **P4** and **P5** reaction conditions only allowed the grafting of BS that could explain their very high water solubility (80 to 137-fold greater than **P0**). Indeed, according to Rogmann, Seidel, & Mischnick (2000), the sulfonylbutylation of ß-cyclodextrins with BS gave appropriate water soluble products due to the ionic character of the sulfonic acid residues. Rosu et al. (2013) also synthesized modified starches with an aqueous solubility 60-fold greater than the native one. The reaction conditions are expressed in Table 1, namely the use of an excess of NaOH, as well as the solvent parameter, promoted the BS grafting, preventing the subsequent OS grafting due to the low number of free hydroxyls as well as steric hinderance.

Table 2 Characteristics of difunctionalized starches.

		Apparent	Specific	Maximum BaP
Product	R ^a	aqueous	activity	solubilization
		solubility (g/L)	(µg/g)	(µg/L) ^b
P0	0.00	0.35 (± 0.07)	15.00	5.25
P1	5.78	1.41 (± 0.07)	589.40	831.05
P2	9.78	1.33 (± 0.19)	618.62	822.77
P3	2.78	3.50 (± 0.13)	517.71	1811.99
P4 ^c	0.00	28.00 (± 0.28)	21.47	601.07
P5 ^c	0.00	47.74 (± 0.47)	16.24	775.51
P6	0.01	8.45 (± 0.14)	12.78	107.97
P7	13.21	0.75 (±0.04)	543.67	409.38
P8	2.53	3.87 (± 0.11)	203.53	787.67

^a R was determined according to ¹H NMR spectra and was calculated by the equation: R = ($I_{OS}/3$) / ($I_{BS}/4$)

^b Maximum BaP solubilization = specific activity X apparent aqueous solubility

Eq (2)

^c Only BS was successfully grafted

P6 had a very low R value (0.01) closed to those of monofunctionalized starches (**P4** and **P5**). Nevertheless, its aqueous solubility decreased dramatically to 8.45 g/L. Synthesis parameters of **P6** differed only from those of **P4** by NaOH proportion and BS/AGU. In those conditions, a small amount of OS was grafted as judged by ¹H NMR spectroscopy. The weak presence of OS grafted could explain the decrease of its aqueous solubility.

P2 differed from **P1** only by the fact that **P2** was dispersed by a heating pretreatment before the synthesis reaction. This supplementary step did not confer to the obtained product the desired properties. In fact, its aqueous solubility was as weak as this of **P1**. For this reason, this pretreatment was subsequently abandoned during the study.

Concerning **P3**, BS/AGU was doubled in comparison with **P1**. This parameter reaction was more effective to graft BS. In fact, **P3** presented a 2.5-fold superior apparent aqueous solubility in comparison with **P1**.

Lastly, both products (**P7** and **P8**) were obtained in one pot reaction. For **P7**, all the reactions parameters were identical as those of **P3**. **P7** having a high R in correlation to a high OS grafted showed an important decrease of water solubility. **P8** differed from **P7** by the temperature reaction and this parameter seems to influence the properties of starch obtained. In fact, **P8** presented a 5-fold superior apparent aqueous solubility in comparison with **P7**. The decrease of OS grafted on **P8** was in correlation with the important BS grafted. BS, as a C4 alkyl group, was more soluble in the solvent than OSA. It could be a better competitor than OSA for hydroxyl groups of AGU. This phenomenon was amplified in one pot reaction because substituents were added simultaneously.

The products (**P1**, **P2**, **P3**, **P7**, **P8**) had water solubility inferior to 4 g/L, that could be explained by the high presence of OS substituted on starch (R > 2.53).

3.4. Solubility of starch derivatives versus BaP aqueous solubilization

Fig. 2 illustrates the aqueous solubility of starch derivatives versus BaP solubility. The starch derivatives synthesized could be classified in 3 groups, labeled from I to III, according to both parameters.





Relationship between the apparent aqueous solubility (g/L) and the BaP solubilization (μ g/L) for the synthesized starch products.

Native potato starch **P0** is characterized by a very low water solubility and also a weak ability to desorb BaP (6.75 μ g/L).

Group I contained three products (**P4**, **P5**, **P6**) characterized by a high apparent aqueous solubility, 24 to 136 times greater (> to 8 g/L) than **P0** and a very weak efficiency to desorb BaP (< to 10 μ g/L). As explained in 3.3., these compounds were highly alkylated by a BS substituent which stimulated their hydrophilic properties, but have a null or a very low rate of OS substitution limiting their BaP solubilization. Nevertheless, the reaction conditions of **P5** permitted to obtain a starch derivative 2-fold more soluble in water than those obtained by Rosu et al. (2013). Such products could be interesting for developing applications in waste water flocculation or as additives in paper manufacturing (Crini, 2005; Sorokin, Kachkarova-Sorokina, Donzé, Pinel, & Gallezot, 2004).

Group II included three products (**P1**, **P2**, **P7**) which presented the highest capacities to desorb BaP (> to 244 μ g/L) and low water solubility (< to 1.5 g/L). In comparison with BaP solubility in water (3.00 μ g/L) or in presence of **P0**, those starch products were able to stimulate up to 93-fold BaP aqueous solubilization underlining their high surfactant properties. Thus, starch modifications with OSA stimulated hydrophobic characters and consequently, the efficiency to desorb BaP as a hydrophobic pollutant model. However, the more grafted OS, the less soluble the obtained products. **P2**, desorbing BaP in aqueous phase at high capacity (278.38 μ g/L) was 2-fold more efficient than those found in our previous study (Rosu et al., 2013).

Group III contained two products **P3** and **P8** characterized by a similar R as well as a comparable apparent aqueous solubility, i.e. an enhancement by a factor 10 of that of native starch **P0**. **P8** is also characterized by an intermediate capacity to desorb BaP (91.59 μ g/L). This product could be considered as a good compromise presenting both hydrophobic and hydrophilic properties.

Likewise, **P3** stimulated 77-fold BaP aqueous solubilization (232.97 μ g/L) underlining its very high surfactant property. **P3** matches both criteria required to obtain a modified starch matrix to optimize BaP solubilization.

3.5. BaP solubilization in aqueous phase versus apparent OS/BS ratio



Fig. 3 shows the R value of starch derivatives versus BaP solubilization in aqueous phase.

Fig. 3.

BaP solubilization (μ g/L) versus the apparent ratio R (I_{OS}/I_{BS}) for the synthesized starch products.

P4, **P5** and **P6** presented a weak R value. Indeed, these products were not or weakly substituted by OS and consequently were inefficient to desorb BaP (Fig. 2; Table 2). Thus, a minimal amount of grafted OS seems to be required to stimulate BaP transfer to aqueous phase.

In contrast, **P1**, **P2** and **P7** were characterized by a high capacity to desorb BaP between 244 and 280 μ g/L. The R value of these products was between 5.78 and 13.21. In these synthesis conditions, OS was successfully grafted.

The comparison between **P3** and **P8** was particularly interesting for the comprehension of BS and OSA roles. Indeed, both products were characterized by a similar R. But, they differed by their capacity to solubilize BaP with 232.97 and 91.59 μ g/L respectively (Fig. 2; Table 2). The optimal target zone of I_{OS}/I_{BS} seemed to be very tight for obtaining the required criteria, especially high capacity to desorb BaP. If R < 2.53, starch derivatives were soluble but quite inefficient to solubilize BaP. At the opposite, R > 2.78 would conduct to weakly soluble products with an high BaP solubilization. Nevertheless, BaP solubilization reached a limit value, as illustrated by the asymptotic part of the sigmoid Fig. 3. From a structural point of view, the R target zone (2.53 < R < 2.78) would be in line with an optimal repartition of both substituting groups (BS and OS) along the macromolecular structure of the starch.

Therefore, it is necessary to find an adequate compromise between grafted OS and BS to obtain a right balance between hydrophobic versus hydrophilic properties of starch derivatives. Our results showed that OS and BS amounts grafted were also strongly influenced by the reaction conditions. Indeed, starch etherification with BS was more effective with NaOH aqueous solution used as solvent, while starch esterification with OSA was more performant using DMSO solution. Moreover, OSA esterification could be hindered if a reduced number of OH groups was available in AGU according to BS grafted (Heinze, Rensing, & Koschella, 2007). Starch derivatives with different amphiphilic characters conferring them various tensioactive properties could be synthesized by controlling the number of hydrophobic and hydrophilic substituents (Heinze et al., 2007).

All these results are summarized in Table 2 by calculating the maximal theorical value of BaP solubilization for each product. This value was calculated with a saturated aqueous solution of each starch related to their aqueous solubility. According to these calculations, **P3** had the highest capacity to desorb BaP (1811.99

16

 μ g/L) compared with the other products by reaching 604-fold the BaP aqueous solubility (3.00 μ g/L).

Conclusion

To sum up, 8 new starch-based surfactants were successfully synthesized using 2-octen-1-ylsuccinic anhydride (OSA) and 1,4-butane sultone (BS) as reagents. The efficiency of the reaction conditions as well as the apparent OS/BS ratio was monitored using ¹H NMR spectroscopy. The apparent aqueous solubility measurement and BaP desorption were used to evaluate the hydrophilic / hydrophobic balance of each compound. It was found that **P3** possessed the best equilibrium with an aqueous solubility of 3.50 g/L and a capacity to desorb 232.97 μ g/L of BAP. To our point of view, **P3** is the most promising candidate for the elaboration of surfactant-enhanced remediation of PAHs contaminated soils. Further research will be oriented towards the scale up synthesis of **P3** with the aim of its utilization in combination with fungi-based remediation process.