AN EFFICIENT ESTERIFICATION OF PULLULAN USING CARBOXYLIC ACID ANHYDRIDES ACTIVATED WITH IODINE

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Acylation of alcohols (esterification) with anhydride is an important and routinely utilized transformation in organic synthesis. Iodine is a cheap and one of the most powerful anhydride activating agents for the esterification of alcohols. Therefore, an efficient and cost effective method was developed for the acylation of pullulan polysaccharide by using different acid anhydrides activated with iodine. A series of organo-soluble pullulan esters were successfully synthesized, purified and characterized. Under solvent free conditions, it was feasible to fabricate pullulan nonaacetate and pullulan stearate. Attachment (covalent) of the pendant groups onto polymer backbone was verified by spectroscopic techniques. All of the products were characterized by using thermal analysis, FTIR and ^{1}H NMR spectroscopy.

Keywords: Acid anhydrides; Esterification; Acylation; Iodine; NMR spectroscopy; Polysaccharides; Pullulan.

Water soluble extracellular polysaccharide pullulan is produced by strains of *Aureobasidium pullulans*[1,2] consisting of a linear and flexible chain of D-glucopyranosyl units[3] that alternate regularly between one α-(1,6) and two α-(1,4) linkages representing the repeating unit. Pullulan is non-toxic, non-irritating, non-ionic, blood compatible, biodegradable, non-mutagenic, non-immunogenic and non-carcinogenic[4]. Therefore, in last decade, pullulan successfully entered in daily life, pharmaceutical, medicinal and biotechnological fields. Nowadays, pullulan is being widely used in food industry. It provides bulk and texture to food as it is tasteless, odorless and

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low calorie diet. It shows oxygen barrier property as well hence it inhibits fungal growth in food items[5].

Pullulan and mainly its ester derivatives are being used for liver targetting of plasmid DNA [6], as biosensors, as artificial muscles and for drug delivery purposes[7]. A number of pullulan derivatives have been synthesized for their potential uses, e.g. chloroalkylated[8], sulfinylethylated[9], etherified[10], cyanoethylated[11], carboxylated[12], cationized[13], sulfated[14] and acetylated[15]. Literature showed that esterification of pullulan was carried out in different solvents, i.e. dimethylformamide (DMF), dimethylacetamide (DMAc) and *N*-methylpyrrolidone. Tezuka has performed synthesis of pullulan nonaacetate using acetic anhydride in pyridine as base and solvent by using 4-dimethylaminopyridine as a catalyst[16]. As the maltotriosyl unit of pullulan contains nine hydroxyl groups in a geometrically unique environment that shows the structural diversity of pullulan derivatives. By introducing different functional groups into the pullulan, fields of its potential applications can be extended.

Chemical modification of pullulan may be performed, as with cellulose[17–21]. Regarding the esterification of pullulan, few methods are available in literature by using acetyl chloride with pyridine[22], acetic ahydride, pyridine and 4-dimethylaminopyridine[16], carboxylic acids *in situ* activated with tosyl chloride, iminium chloride and 1,1′-carbonyldiimidazole[23] and acetic ahydride activated with strong acids or metalic catalysts[24,25].

In present study, our interests are focused to search efficient reaction methodologies for the esterification of biologically important biopolymer pullulan, hence we are going to exploit the worth of conventional reagent acetic anhydride by using iodine as a catalyst[26]. This esterification method aims to appear more advantageous regarding cost effectiveness because of the fact that other esterification methods available till today need expensive acylation reagents, i.e. *p*-toluenesulfonyl chloride and 1,1′-carbonyldiimidazole, etc. Secondly, nowadays esterification of polysaccharides under homogeneous reaction conditions is the current research area where this fact cannot be neglected that solvent systems used to dissolve polysaccharides are quite expensive, hence cannot be commercialized easily and same is true for low molecular weight organic conversions[27]. Another important aspect of commercial synthesis of polysaccharide acetates is that it is carried out by using acidic catalyst which significantly destroys the glycosidic linkages of polysaccharides. Hence, we are aimed to exploit the use of mild catalyst, i.e. iodine. Iodine, a cheap, commercial available, environment friendly and most importantly powerful reagent, is currently being explored extensively for its catalytic properties on various substrates in different low molecular weight organic transformations[28,29]. Likewise, our focus is to explore acid anhydride/iodine for acylation of pullulan as well.

EXPERIMENTAL

Chemicals

Pullulan (70074, Fluka) was dried under vacuum at 110 °C for 8 h prior to use. Analytical grade organic solvents and other reagents obtained from Fluka were used without further purification.

Measurements

FTIR spectra (ν, cm^{-1}) were measured on IRPrestige-21 (SHIMADZU, JAPAN) using the KBr pellet technique. ^{1}H NMR spectra (δ, ppm; *J*, Hz) of the products were acquired on Bruker 400 MHz machine in acetone-d_6 and DMSO-d_6 at 40 °C. Thermogravimetric analysis (TGA) and derivative thermogravimetric (DTG) analysis were recorded on a SDT Q 600 (TA Instruments, USA) thermal analyzer. Thermal decomposition temperatures (T_d) of the pullulan esters were determined from DTG analysis. The T_d was reported as onset of significant weight loss from the heated sample. Samples (10 mg) were measured under air with a temperature increase of 10 °C/min from 35 up to 600 °C.

Dissolution of Pullulan in Dimethylacetamide (DMAc)

Pullulan (1 g) was dissolved in 20 ml DMAc by stirring at 80 °C for 30 min to obtain optically clear solution.

Esterification of Pullulan Using Acetic Anhydride and Iodine.
Typical Example (Sample **2**)

Iodine (0.5 g, 1.96 mmol) was taken in a pre-dried round bottomed flask. Acetic anhydride (4 ml, 42.3 mmol) was added and reaction mixture was stirred for 15 min without using any solvent. Pre-dried pullulan (1 g, 6 mmol) was added and the resulting mixture was heated at 50 °C for 3 h. Excess of iodine (catalyst) was removed by adding saturated aqueous sodium thiosulfate to the reaction mixture. The white precipitates of pullulan acetate thus formed were filtered. Product was washed three times with water and then re-precipitated from acetone into water. The precipitates of sample **2** were then dried under vacuum at 50 °C. Yield 1.30 g (73%). Degree of substitution (DS): 3. FTIR (KBr): 3390 (O–H), 2935 (C–H), 1752 (C=O ester), 1244 (C–O–C ester), 1432 (CH_2). ^{1}H NMR (400 MHz, acetone-d_6): 3.2–5.5 (anhydroglucose unit-H); 2.02 (acetyl-H).

Esterification of Pullulan Using Acetic Anhydride and Iodine.
Typical Example (Sample **6**)

Iodine (0.5 g, 1.96 mmol) was added to the solution of pullulan (1 g, 6.15 mmol) dissolved in 25 ml DMAc. Acetic anhydride (4 ml, 42.3 mmol) was added and reaction mixture was stirred and refluxed at 50 °C for 3 h under nitrogen. Excess of catalyst was removed by adding saturated aqueous sodium thiosulfate to the reaction mixture. The white precipitates of pullulan acetate thus formed were filtered and washed three times with water. The sample was re-precipitated from acetone into water. The precipitate of sample **6** was dried under vacuum at 50 °C for 24 h yielding the pure product as a white powder. Yield 0.97 g (63%). DS: 2. FTIR (KBr): 3390 (O–H), 2931 (C–H), 1751 (C=O ester), 1246 (C–O–C ester), 1431 (CH_2). ^{1}H NMR (400 MHz, acetone-d_6): 3.2–5.5 (anhydroglucose unit-H); 2.02 (acetyl-CH_3-H).

Analytical Data of Pullulan Stearate **8**

Yield 2.54 g (50%). DS: 2.5. FTIR (KBr): 3400 (weak O–H signal), 2940 (C–H), 1736 (C=O ester), 1248 (C–O–C ester), 1432 (CH_2). ^{1}H NMR (400 MHz, DMSO-d_6): 3.2–5.3 (anhydroglucose unit-H); 0.92 (stearate-CH_3-H); 1.31–2.30 (stearate-CH_2).

Calculations of Degree of Substitution. Typical Procedure

For the purpose of DS, 100 mg of sample was dissolved in 50 ml of 1 M NaOH solution kept under stirring over night and its pH was noted. A 0.01 M HCl was added to maintain pH at 7 followed by a known quantity of 1 M NaOH (20 ml). Excess of base was finally titrated with 0.1 M HCl till neutralization. Degree of substitution of the free acid moieties was calculated.

Calculations of Yield. Typical Procedure

Following formulae were used to calculate the percentage yield of the product

$$\text{WP} + [(\text{WP}/\text{MW AGU}) \times \text{DS} \times \text{MW Sub}] = \text{theoretical yield}$$

$$\text{practical yield}/\text{theoretical yield} \times 100 = \text{\%age yield}$$

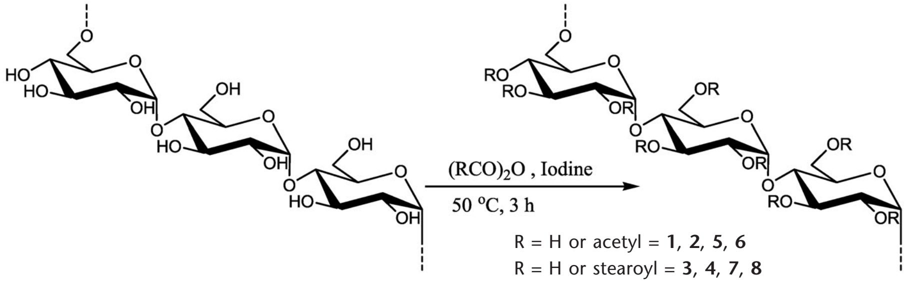
where WP is weight of polymer, MW AGU is molecular weight of anhydroglucose unit, DS is degree of substitution, MW Sub is molecular weight of substituent.

RESULTS AND DISCUSSION

Solvent Free Esterification of Pullulan

Keeping in view the immense importance and efficiency of the iodine as catalyst for acetylation of alcohol under solvent free conditions, it is therefore designed to explore its potential for the esterification of pullulan (a polysaccharide). The pullulan was reacted with different kinds of anhydrides, i.e. small sized acetic anhydride and long chain stearic anhydride (Scheme 1). All reactions were carried out at 50 °C for 3 h using solvent free conditions and different molar ratios of the reactants. Reaction conditions and results are summarized in Table I.

It was found that by increasing the molar ratio of the anhydride DS was also increased which leads to the point that pullulan esters can be engineered with known amount of functionalities on polymer backbone. All samples **1–4** were soluble in organic solvents including DMSO, DMAc and acetone.



SCHEME 1

It is in practice to use excess amounts of esterification reagents along with sulfuric acid as catalyst at elevated temperatures to obtain pullulan nonaacetate at the cost of significant degradation to polymer glycosidic linkages[16]. As aimed, results have significantly indicated that the method is highly efficient as pullulan nonaacetate **2** was synthesized by using 1:6 molar ratio of acetic anhydride to anhydroglucose unit (AGU). On the other hand, pullulan acetate with DS 2.5 was obtained by using only 1:3 molar ratio of acetic anhydride to AGU.

Nevertheless, the method appeared cost effective as one need less molar ratio of reactants and reagents are cheap and commercially available. Iodine catalyst used is non toxic and environment friendly. As this method has carried out with gentle heating (50 °C) for shorter time (3 h) hence it is soft, efficient and energy effective method.

TABLE I
Conditions and results of solvent free esterification of pullulan with acid anhydrides *in situ* activated with iodine

Sample	Acid anhydride	Molar ratio (AGU/anhydride)	Yield, g (%)	DS[a]
1	acetic	1:3	1.22 (74)	2.5
2	acetic	1:6	1.30 (73)	3.0
3	stearic	1:3	1.80 (54)	1.4
4	stearic	1:6	1.95 (45)	2.0

[a] Degree of substitution was calculated by acid base titration after saponification.

In literature, it was found that long chain fatty acids are hard to attach onto polysaccharides as very powerful reagents and harsh reaction conditions are required along with high molar ratios of the reactants. Results have indicated that use of iodine as a catalyst appeared highly efficient to attach such bigger molecules onto pullulan backbone with very good DS and yields. Pullulan stearates **3** and **4** are powdery and pure products free of any unreacted moieties. Purity of the product was determined by ^{1}H NMR and FTIR spectroscopy as therein spectra, no peak of any impurity have been observed.

Esterification of Pullulan in DMAc

It is important to consider that bulky molecules like stearic anhydride when used in higher mole ratio to AGU then, a supporting solvent for better stirring is needed which will allow catalyst and reagents to reach AGU of pullulan for the synthesis of products. Keeping in view this idea, we have designed few reactions to explore the use and strength of iodine as a catalyst by using DMAc as solvent. Results obtained are summarized in Table II. All samples **5–8** were soluble in usual organic solvents including DMSO, DMAc and acetone.

Results have shown that this method is more efficient for the synthesis of pullulan stearate as DS 2.5 (sample **8**) was achieved under solvent and DS 2 (sample **4**) under solvent free conditions. This increase of DS under solvent is because of the fact that reagents can easily approach polymer backbone under solvent. On the other hand under solvent free conditions, all reactants in this case were solid and reaction was preceded completely

TABLE II
Conditions and results of esterification of pullulan dissolved in DMAc with acid anhydrides *in situ* activated with iodine

Sample	Acid anhydride	Molar ratio (AGU/anhydride)	Yield, g (%)	DS[a]
5	acetic	1:3	0.80 (58)	1.4
6	acetic	1:6	0.97 (63)	2.0
7	stearic	1:3	2.02 (55)	1.6
8	stearic	1:6	2.54 (50)	2.5

[a] Degree of substitution was calculated by acid base titration after saponification.

heterogeneously. However, pullulan acetates have shown lower DS (DS 2 for sample **6**) under solvent and higher DS (DS 3 for sample **2**) under solvent free conditions. Acetic anhydride is a liquid reagent and it can be easily attacked by iodine. Nevertheless, we can summarize that acetylation is highly favored under solvent free conditions and iodine can catalyze esterification of pullulan with or without solvent. All the products obtained were thoroughly characterized by means of thermal analysis, FTIR and ^{1}H NMR spectroscopy.

Structure Characterization of Pullulan Esters

FTIR spectroscopic studies have shown a distinct ester peak at 1751 cm^{-1} for sample **2** (Fig. 1). The spectrum did not display hydroxyl group absorption at about 3500 cm^{-1} which is indicative of complete substitution of hydroxyl functions with acetyl moieties. It shows purity of the product and success of the reaction. The AGU-CH$_2$ absorptions were recorded at 1390 cm^{-1}. FTIR spectroscopic studies were carried out for all of the samples, however, it is worth mentioning the spectrum of sample **6**. For comparison, spectrum of unmodified pullulan polymer is also shown in Fig. 1. A distinct ester peak appeared at 1759 cm^{-1} along with free hydroxyl groups at 3450 cm^{-1} and CH$_2$ absorption at 1431 cm^{-1}. While comparing spectrum of **6** with **2**, it is

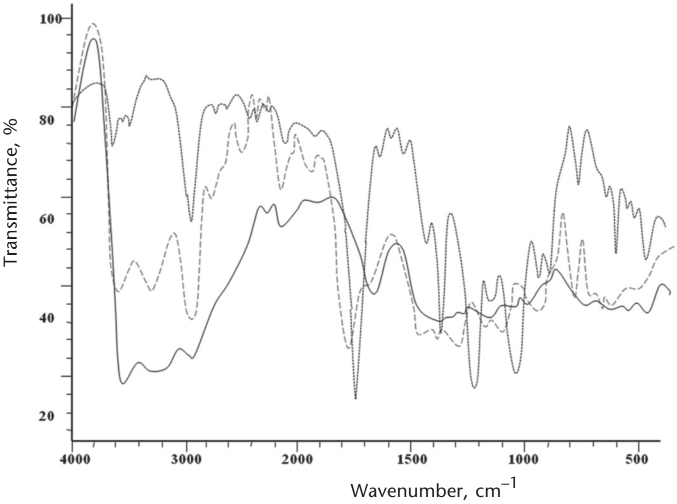


FIG. 1
FTIR (KBr) % transmittance spectrum of unmodified pullulan (——), and pullulan acetates **2** (· · · ·) and **6** (- - - -)

shown that there are free hydroxyl groups available in the sample **6**. However, both reactions were carried out by using similar reaction conditions except sample **6** was synthesized by using solvent. The DS calculation by acid base titration also showed that the DS of acetate for sample **2** is 3 while for sample **6** it is 2.

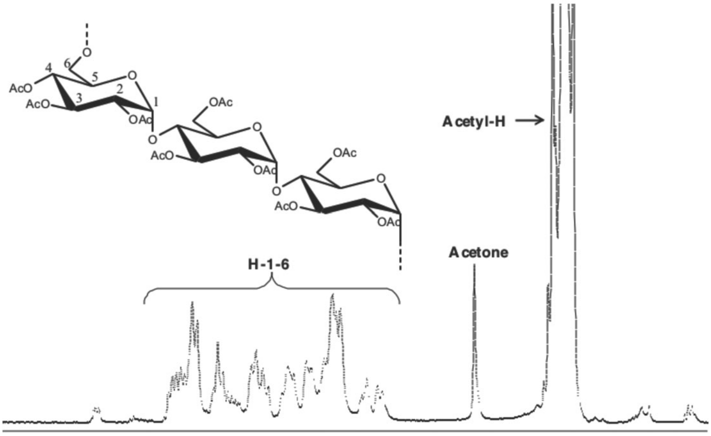


FIG. 2
^{1}H NMR spectrum (400 MHz, acetone-d_6) of pullulan acetate **2**

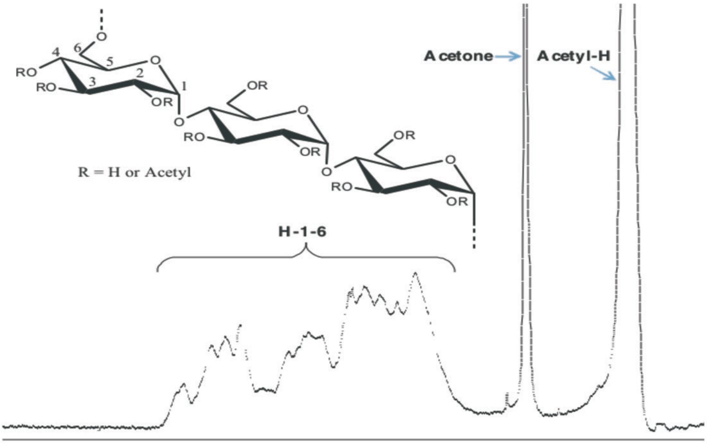


FIG. 3
^{1}H NMR spectrum (400 MHz, acetone-d_6) of pullulan acetate **6**

^{1}H NMR spectroscopy was thoroughly carried out to check the success of reaction and purity of samples. A typical ^{1}H NMR spectrum (acetone-d_6) of pullulan acetate **2** (Fig. 2) has revealed the success of reaction. Methyl protons of acetyl moiety appeared at 2.02 ppm while well resolved AGU-H signals appeared 3.5–5.7 ppm. Nevertheless, spectrum has shown that the sample is highly functionalized with almost no free OH groups. In case of free OH and their hydrogen bonding in polymer, AGU-H region becomes highly overlapped which can be observed in case of spectrum of sample **8**. The sample **8** has DS 2 hence there are free hydroxyl groups in polymer backbone which undergo hydrogen bonding to give signal overlapping in spectrum (Fig. 3).

T_d 369 and 357 °C were obtained from TGA for pullulan acetate **2** (DS 3) and **6** (DS 2), respectively. Spectra have indicated that pullulan acetate **2** (DS 3) with higher DS is thermally more stable as compared to pullulan acetate **6** (DS 2) with low DS. Typical thermal analyses (TGA and DTG) for sample **2** are shown in Fig. 4. T_d 368 °C was obtained for pullulan stearate **6**. All of the thermal analysis spectra have revealed that products obtained were thermally stable.

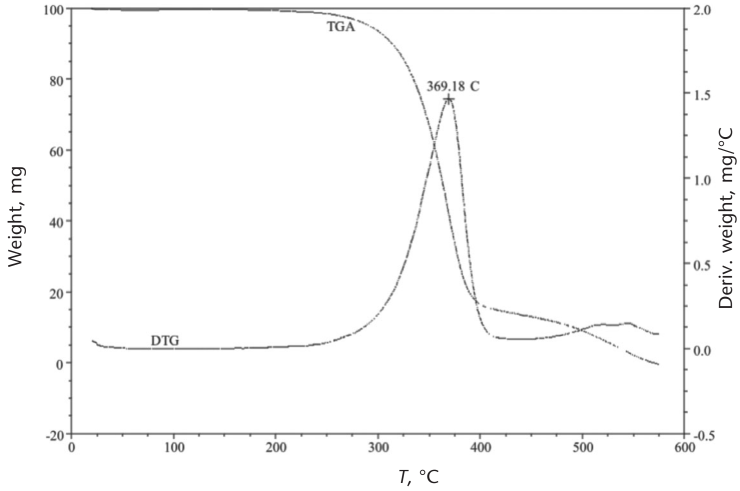


FIG. 4
Simultaneous TGA and DTG spectra of pullulan acetate **2**

CONCLUSIONS

In present research work, a new and efficient method for the esterification of pullulan was successfully designed, i.e. pullulan was allowed to react with *in situ* activated acid anhydrides using iodine as a catalyst. Pullulan was successfully esterified with small sized acetic anhydride and long chain stearic anhydride. Pullulan nonaacetate was synthesized with greater efficiency even without using any solvent. As all reactions were carried out at 50 °C for only 3 h hence present method appeared highly efficient, time saving and energy effective. Because iodine is a cheap, commercially available, non degradative to glycosidic linkages and environment friendly reagent, hence this reaction methodology can be adopted on commercial scale for acetylation of pullulan.

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