INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

CHARACTERIZING DERIVATIZED BOSCH REDUCED CHITOSAN FOR ITS ANTI-OXIDANT ACTIVITY BY DPPH METHOD

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ABSTRACT

Derivatization of a biodegradable polymer 2-acetamido-2-deoxy-D-pyranose attached through β-(1→4) linkages which is N-deacetylated form of chitosan were made by Bosch reduction using aromatic aldehydes viz., Benzaldehyde, Anisaldehyde, 4-Chloro-benzaldehyde, 4-Hydroxy-benzaldehyde, and 4-Methyl-benzalsehyde via Schiff's base intermediate to final Bosch reduced product and characterized to justify the synthesis with ^{1}H NMR. Synthesized derivatives were subjected to antioxidant activity by (DPPH) Diphenyl-2-picryl-hydrazyl method, using ascorbic acid as standard.

Keywords: Bosch reduction, Schiff's base, Aromatic aldehydes, Anti-Oxidant, DPPH.

INTRODUCTION

A hybrid material blended on the molecular scale to form bioactive material by inorganic-organic moieties is a topic of the day[1]. Chitosan is a naturally occurring, bio-degradable polymer; which is the N-deacetylated product of chitin consisting of linear repeating units of 2-acetamido-2-deoxy-D-glucopyranose attached through β-(1-4) linkages. **Fig 1** Chitosan, a white flaky solid, is difficult to manipulate with because of the solubility problems in neutral water, bases, and commonly used organic solvents. The pKa value of the primary amino groups in chitosan is determined to be around 6.5. As a result, even though chitosan and its derivatives are soluble in pH values of lower than 6.0, many of its applications in neutral or basic medium, including those of physiological relevance, may not be realized, for the pH under such situations will trigger an immediate precipitation. On the other hand, acidic solutions, in which chitosan is fairly soluble, may not be desirable in many of its applications, especially those in medicine, cosmetics, and food. There have been two major approaches documented in literature towards improving the solubility of chitosan at neutral pH. Studies aimed at deriving newer applications of chitosan are hence of interest. In the present study, substituted chitosan derivatives were synthesized using aromatic aldehydes viz., Benzaldehyde, Anisaldehyde, 4-Chloro-benzaldehyde, 4-Hydroxy-benzaldehyde, and 4-Methyl-benzalsehyde. However, several of the applications of chitosan cited in the literature utilize some form of chitosan derivative and hence to provide for the solubility[2]. Oxidation is defined as any substances that when present at low concentration compared with those of oxidizable substrates, significantly delays or prevent oxidation of that substrate. There are radical and non-radical processes[3]. There is an increasing interest in antioxidant, particularly in those intended to prevent the deleterious effects of free radicals in human body, and to prevent the deleterious effects of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidant from natural rather then from synthetic sources[4]. Lipid auto-oxidation is a radical process in a chain reaction including induction, propagation, and termination steps.

During the induction period, alkyl and peroxyl radical are formed. These highly reactive chemical species produce hydroperoxides (ROOH) during the propagation phase. Termination is the association of two radicals together to form more stable products. Currently popular method is based upon use of the stable free radical DPPH$^{\cdot}$ method is measured in ambient temperature and thus eliminates the risk of thermal degradation of the molecules tested[5]. The molecule of 1, 1 – diphenyl-2-picryl-hydrazyl (α, α-diphenyl-β-picrylhydrazyl; DPPH: **1**) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that molecule do not dimerise, as would be the case with most other free radicals which gives violet color, characterized by an absorption band in ethanol solution at about 520nm[4].

When a solution of DPPH is mixed with that of a substance that donate a hydrogen atom, then this gives rise to the reduced form (**2**) with the loss of this violet color. Representing the DPPH radical by Z$^{\cdot}$ and the donor molecule by AH, the primary reaction is Z$^{\cdot}$ + AH = ZH + A$^{\cdot}$ where ZH is the reduced form and A$^{\cdot}$ is free radical produced in first step. This latter radical will undergo further reaction which controls the overall stoichiometry, that is, the number of molecules of DPPH reduced by one molecule of reductant.

MATERIALS AND METHODS

Ornidazole was gift sample from Zydus Cadila Health Care Ltd, Ahmedabad. Sodiumborohydride Dichloromethane, Glacial acetic acid, Acetone and sodium hydroxide was purchased from Ranbaxy fine chemicals Ltd. DPPH, Ascorbic acid, etc.

PROCEDURE FOR BOSCH REDUCTION

Chitosan 1 gm was first dissolved in 1% (0.2M) of glacial acetic acid followed by the addition of aldehyde (0.132 g 0.2 M) the reaction was stirred for 24 hrs to ensure completion of Schiff's base formation which was marked by change in the appearance of the solution from being clear at the beginning and turning milky white. To this 0.2 gm of sodium borohydride was added this was confirmed by conversion of clear solution and stirred for 24 hrs. To this was added 5% sodium hydroxide to neutralize the mixture. The mixture was precipitated and the precipitate was washed with more amount of water and further with acetone to remove all moisture content and further with dichloromethane to get fine precipitate and dried completely for further use. [**Scheme 1**]

ANTI-OXIDANT ACTIVITY BY DPPH METHOD[6,7]

DPPH solution, 1mmol/L, was prepared by dissolving 31.54 mg of DPPH in 95% v/v buffered methanol (40mL of 0.1 mol/L acetate buffer pH 5.5 with 60 mL of methanol) and made up to 50 mL with buffered methanol. The synthesized compounds at different concentrations such as 0.5 mg, 1, 2mg 4mg and 8 mg. were made up to 4 ml with distilled water. 1 ml of DPPH (1mmol, $3.953x10^{-10}$ µg/ml) was added to each test tube, shaken and the mixture was kept at30^0C for 30 min. The 517 nm is a measuring absorbance of the resulting solution. The effect of ascorbic acid (Vitamin C) on DPPH was also assessed for comparison with that of synthesized compounds. A buffered methanolic dilution (0.2, 0.4, 0.6, 0.8, 1.0 mL) of 1 mg/mL ascorbic acid was made to 4 mL with distilled water. 1 mL DPPH radical (1nmom/L) was added to each test tube and same procedure as in DPPH scavenging experiment was followed. The absorbance measured for the control solution (Buffered methanol with DPPH) was in the range 0.500 ± 0.040. Antiradical activity was expressed as inhibition percentage (1%) and calculated using the following equation: Inhibition percentage = [(Abs control – Abs sample)/ Abs control] x 100.

ANALYSIS TECHNIQUES

The ^{1}H NMR spectra were recorded from Benzyl Chitosan : 1HNMR (Acetic Acid D_4: D_2O): δ 1.80, 2.17 (m, 2H, -CH_2, C-1 and -NH), δ 2.80 (S,3H, -CH_3, C-24), 2.87 (t, 1H, -CH, C-3), 3.26 (m, 1H, -CH, C-11), 3.71 (m, 2H, -CH_2, C-16), 3.6 (m, 1H, C-5), 3.65 (m, 1H, C-15), 3.71 (m, 2H, C-6), 3.78(m, 1H, C-2), 4.01(s, 2H, C-25), 4.35(m, 1H, C-13), 4.52 (m, 1H, C-12), 5.1 (s, 1H, -NH), 5.3 (dd, 1H, C-21), 6.7 (d, 1h, C-10), 7.22-7.29 (m, 5H, Ar-H)

RESULTS AND DISSUSSION

Chitosan can be Derivatized using aldehydes to convert to Schiff's base and Bosch reduction method to get a free flowing powder of chitosan derivatives. Analyzing the product by ^{1}H NMR in acetic acid D_4 in D_2O shows the formation of the Bosch reduced product. The scavenging activity of free radical on DPPH is shown in **Table No. 1** reviling the antioxidant activity; every 0.2 mg of standard ascorbic acid is equivalent to 2mg of the Derivatized product. That is the effective concentration if 10 fold more than the standard.

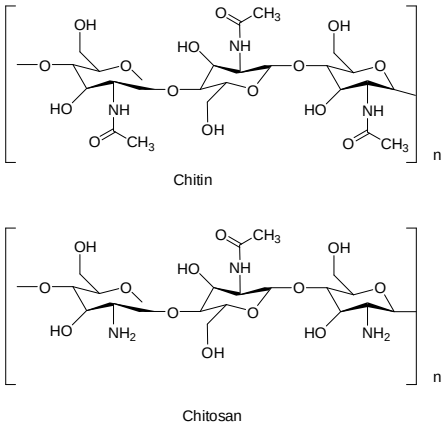
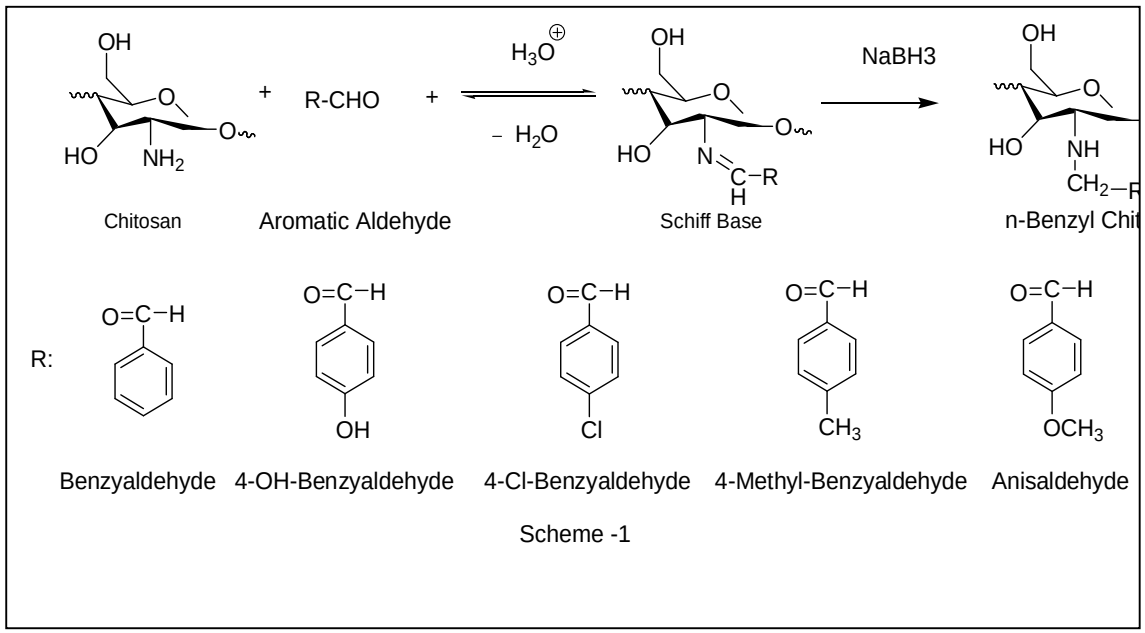


Fig. 1: Chitosan structure

Scheme 1: Scheme of Synthesizing Bosch reduction product



Scheme -1

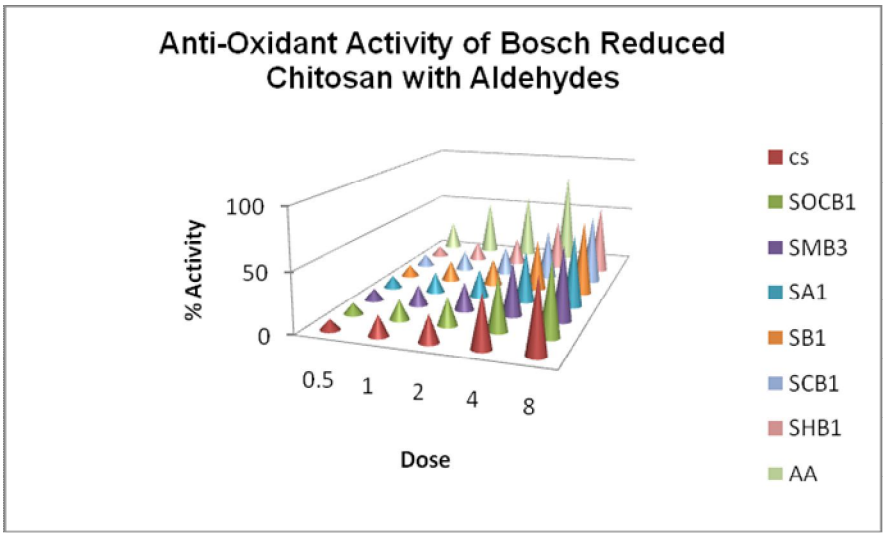


Fig. 2: Anti-Oxidant Activity by DPPH method of Bosch reduced Chitosan with Aldehydes

CS - Chitosan, SCB1- Chloro Benzaldehyde chitosan, SOCB1- 4-O-chloro Benzaldehyde chitosan, SHB1- 4-hydroxy Benzaldehyde chitosan, SMB3- Methyl Benzaldehyde chitosan, SA1- Anisaldehyde chitosan, SB1- Benzyl chitosan, AA- Ascorbic acid,

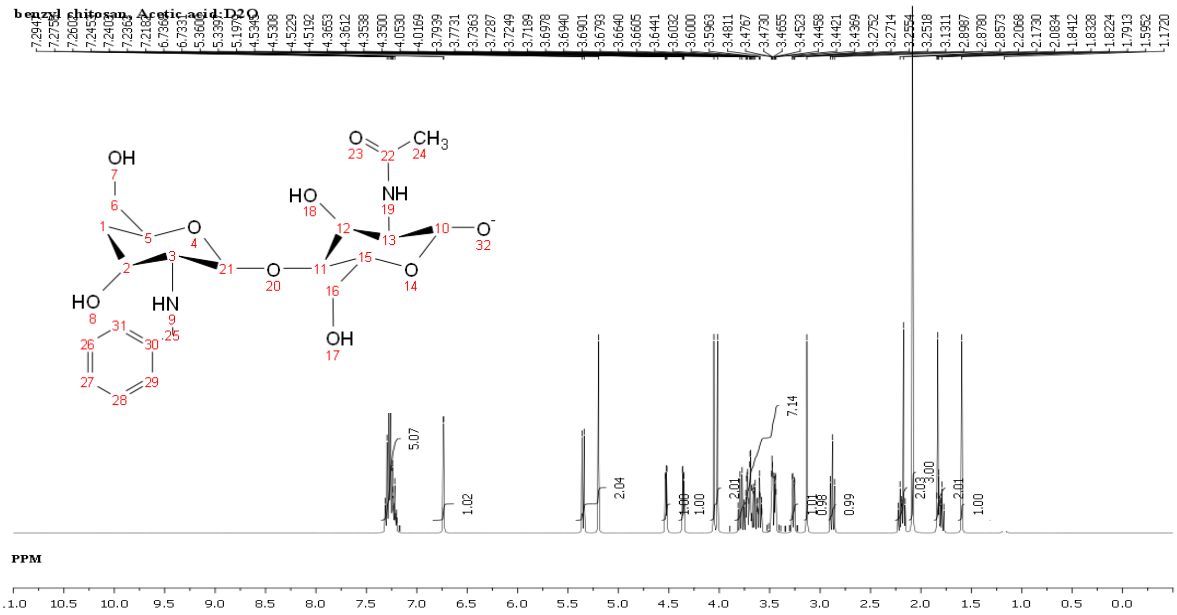


Fig. 3: Characteristic Absorption in H^1 NMR OF Benzyl chitosan

Table 1: Anti-Oxidant Activity by DPPH method of Bosch reduced Chitosan with Aldehydes

Name of the compound	Concentration	% of activity
CS	0.5mg/ml	8.95
	1.0mg/ml	17.05
	2.0mg/ml	22.32
	4.0mg/ml	43.54
	8.0mg/ml	60.66
SOCB1	0.5mg/ml	9.35
	1.0mg/ml	16.56
	2.0mg/ml	23.28
	4.0mg/ml	44.41
	8.0mg/ml	61.39
SMB3	0.5mg/ml	10.01
	1.0mg/ml	17.42
	2.0mg/ml	23.99
	4.0mg/ml	45.21
	8.0mg/ml	62.18
SA1	0.5mg/ml	10.58
	1.0mg/ml	17.62
	2.0mg/ml	24.11
	4.0mg/ml	44.17
	8.0mg/ml	61.00
SB1	0.5mg/ml	10.21
	1.0mg/ml	17.87
	2.0mg/ml	23.22
	4.0mg/ml	44.05
	8.0mg/ml	63.98
SCB1	0.5mg/ml	10.45
	1.0mg/ml	17.29
	2.0mg/ml	24.74
	4.0mg/ml	43.02
	8.0mg/ml	60.23
SHB1	0.5mg/ml	9.24
	1.0mg/ml	17.11
	2.0mg/ml	23.56
	4.0mg/ml	43.88
	8.0mg/ml	60.48
Ascorbic acid	0.01mg/ml	25.39
	0.02mg/ml	49.20
	0.04mg/ml	58.73
	0.08mg/ml	82.53

CS - Chitosan, SCB1- Chloro Benzaldehyde chitosan, SOCB1- 4-O-chloro Benzaldehyde chitosan, SHB1- 4-hydroxy Benzaldehyde chitosan, SMB3- Methyl Benzaldehyde chitosan, SA1- Anisaldehyde chitosan, SB1- Benzyl chitosan, AA- Ascorbic acid.

CONCLUSION

Chitosan can be Derivatized using aldehydes to sciff's base to Bosch reduced state to get a powdered product and sustainable anti-oxidant activity can be achieved by DPPH method comparing with ascorbic acid as standard which shows near activity with 10 fold increase in concentration of the present product which can be used as a lining material for fruit stability and protect the food materials against oxidative degradation and preserve for a longer period.

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