Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Chemica Sinica, 2013, 4(5):96-101

Pelagia Research Library

ISSN: 0976-8505
CODEN (USA) CSHIA5

Synthesis, characterization and application of novel biopolymeric Schiff base from chitosan and 5-chloro-3-methyl-1-phenyl-*1H*-pyrazole-4-carboxaldehyde

Amit B. Dholakia[1*], Jignesh B. Dholakiya[2] and Devyani Y. Mehta[1]

[1]*Department of Chemistry, Shri A. N. Patel P. G. Institute, Anand, Gujarat, India*
[2]*Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India*

ABSTRACT

Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde was prepared by reaction of chitosan with 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde in the presence of acetic acid as a solvent. Some reaction parameters have been investigated such as temperature effect, time effect and mol ratio and optimize the product. The product has been ascertained by FT- IR spectroscopy. Antifungal activities of the Schiff base against Aspergillus niger (A. niger) and Penicillium were measured through the agar well diffusion method.

Keywords: Chitosan, Schiff base, reaction parameters, characterization, antifungal activity.

INTRODUCTION

Chitin is natural polysaccharide usually obtained from the exoskeletons of shellfish and insects. Although, chitin is the second most abundant natural polysaccharide next to cellulose. It is a copolymer of 2-acetoamido-2-deoxy-Dglucose (N-acetyl-glucosamine, GluNAc) and 2-amino-2-deoxy-D-glucose (N-glucosamine, GluN) units randomly or block distributed throughout the biopolymer chain depending on the processing method used to derive the biopolymer [1].

When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media and is called chitosan. The solubilization occurs by protonation of the –NH2 function on the C-2 position of the D-glucosamine repeat unit, whereby the polyelectrolyte in acidic media. Chitosan is the only pseudonatural cationic polymer and thus, it finds many applications that follow from its unique character. Since, last decade so many modifications have been carried out due to presence of free –NH2 and –OH groups on chitosan. Such as preparation of complexes [2-6], drug delivery [7-10], coating [11] hydrogel [12], cross linking polymer [13], nanoparticles and nanocomposites [14, 15], and quaternary salt [16]. Due to presence of both reactive amino and hydroxyl groups that can be used to chemically alter its properties under wild reaction conditions.

Among these substituted biopolymers there are the Schiff's bases obtained by the reaction of free amino groups of chitosan with an active carbonyl compound such as aldehyde and ketone [17-20].

In the present work, we prepared biopolymeric Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde and it has been ascertained by FT-IR spectroscopy. Antifungal activities of synthesized Schiff base were investigated against *Aspergillus niger (A. niger)* and *Penicillium* were measured through the agar well diffusion method.

MATERIALS AND METHODS

Chitosan was kindly supplied as a gift sample by Mahtani chitosan Pvt. Ltd., Veraval, Gujarat. The degree of deacetylation was 90%. 3-Methyl-1-Phenyl-2-pyrazolin-5- one was purchased from Aldrich Chemical Co. and used without further purification. Phosphorous oxychloride **(**S.D.Fine Chemicals Ltd. Bombay) was used as received. Acetic acid, dimethyl formamide (DMF) and ethanol was supplied by S.D.Fine Chemicals Ltd. Bombay. Dimethyl formamide was distilled at 152-154^{0}C and used. Analytical grade acetic acid (Qualigens, Glaxo India Ltd.) was used as received. Absolute ethyl alcohol of a high degree of purity (99.5 %) was supplied by S.D.Fine Chemicals Ltd. Bombay. Ammonia (40%, S.D.Fine Chemicals Ltd. Bombay) was used as received.

Infrared (IR) spectra were scanned using potassium bromide disc method. According to the procedure of this method about 4 mg of sample was mixed with 1 g of potassium bromide. An intimate mixture was obtained by grinding the sample and KBr in a pulverize. The mixture was placed in a disc which was then assembled and evacuated to 3 mm of Hg. A pressure of 18,000 psi was subjected to it for five minutes. For the blank set, standard disc was prepared under similar conditions without sample. The IR spectra of Chitosan, 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde, and Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1Hpyrazole-4-carboxaldehyde were taken in KBr pellets using Nicolet Impact 400 D Fourier Transform Infra Red Spectrophotometer.

Purification of chitosan :
The purification was carried out by the dissolution of 1 gm Chitosan in 300 mL of dilute 0.5 mol L^{-1} acetic acid solution. The dissolution of the chitosan was assured by stirring the initial suspension during 24 h and precipitated in the hydrogel form by carefully adding concentrated NH4OH. The chitosan hydrogel was washed with water until neutrality followed by ethanol. The final product was dried at 60^{0}C under reduced pressure. The purified sample was kept under reduced pressure in desiccators over silica [21].

FT-IR (KBr, cm-1): 3436 (O-H stretching overlapping the N-H stretching), 2922 and 2875 (C-H stretching), 1642 (amide II band, C-O stretching of the acetyl group), 1592 (amide II band, N-H stretching) 1423–1382 (asymmetrical C-H bending of the CH2 group) and 1155 (anti-symmetric stretching of the C-O-C bridge), 1074 and 1031 (C-O-C stretching bond) and 894 (C-O-C as well as gluasidic linkage) due to sachharide moiety.

Synthesis of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde:
The 3-Methyl-1-Phenyl-2-pyrazolin-5- one in dry dimethyl formamide was cooled to 0^{0}C and add phosphorous oxychloride dropwise, maintaining the temperature between 10-15^{0}C. The reaction mixture was heated on a steam bath, cooled and poured into crushed ice with stirring. The separated product was filtered and washed with water. It was recrystallized from ethanol and occurred as yellow needles. Yield 50-60%, m.p.148 0C.

FT-IR (KBr, cm-1): 3437 (O-H stretching), 3065 (C-H stretching in aromatic ring), 2991 and 2921 (C-H stretching in alkyl group), 2825 and 2715 (C-H aldehydic doublet), 1680 (strong C=O stretching), 1593vw (C=Ncyclic), 740 (C-Cl), 700 and 754 (mono substituted benzene), 1602, 1568, 1466 and 1330 (pyrazole ring).

Synthesis of Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1Hpyrazole-4-carboxaldehyde:
Chitosan Schiff base was synthesized by dissolving 250 mg of previously purified chitosan with 25.0 mL of dilute 0.15 mol L-1 acetic acid solution in the three- necked flask equipped with stirrer, water condenser and the solution was stirred at 35^{0}C, during 24 h, to assure its dissolution in a hydrogel form. Then, a desired amount of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde previously dissolved in 20.0 mL of ethanol was added to the chitosan solution.

This reaction mixture is reacting under the different experimental conditions like mol ratio, temperature and time which are listed below:

- Mol ratio of aldehyde: free amino groups of 1.00: 1.00, 2.00: 1.00, 4.00: 1.00, 6.00: 1.00, 8.00: 1.00 and 10.00: 1.00. The mixtures were stirred upto 24 h at 35^{0}C.
- Reaction temperatures of 35, 40, 45, 50 and 55^{0}C. The ration of aldehyde: free amino groups were kept at 6.00: 1.00 stirring the mixtures upto24 h.
- Reaction time of 4, 8, 10, 14, 18 and 24 h. The ration of aldehyde: free amino groups were kept at 6.00: 1.00 stirring mixtures at 55^{0}C.

Deep yellow gel reveals the formation of the Schiff base on the biopolymer matrix. The resulting gel was collected by filtration, washed several times with ethanol to remove any unreacted aldehyde, dried at 60^{0}C under reduced pressure and obtained yellow powder kept in desiccators over silica gel.

FT-IR (KBr, cm-1): 3428 (O-H stretching), 2924 and 2875 (C-H stretching), 1632 (-C=N vibration of imines), 1589 (-C=C-), 1262 (-C-O) and 753 (-C-H) stretching of aromatic ring, 1423–1382 (asymmetrical C-H bending of the CH2 group) and 1155 (anti-symmetric stretching of the C-O-C bridge), 1074 and 1031(C-O-C stretching bond) and 894 (C-O-C as well as gluasidic linkage) due to saccharide moiety.

Calculation of percentage yield:

$P\% = m_2/ M_2 \text{ X } M_1/ m_1 \text{ X } 100\%$

Where m1 is the quantity of chitosan (g); m2 is the quantity of Schiff base (g); M1 is the molecular weight of chitosan unit (161 g/mol); and M2 is the molecular weight of Schiff base unit (g/mol).

Procedure of antifungal activity:
The synthesized compounds were screened for antifungal activity by agar well diffusion method against *Aspergillus niger, Penicillium* using Potato Dextrose Agar Plate. Acetic acid was used as a control for anti fungal activities. Solutions containing 10ppm of the test compounds was added to each well. Incubation period of 24-48 hours at 28^0C was maintained for observation of antifungal activity of test compounds. The zones of inhibition of the fungal growth were measured in millimetres (mm) for different concentrations (0.5% w/v, 0.75% w/v and 1.0% w/v) and reported in table 1.

RESULTS AND DISCUSSION

The synthesized bipolymeric Schiff base is light yellow to yellow colored solid materials. The formation of biopolymeric Schiff base was confirmed by FT-IR spectrums of chitosan and Schiff base. The main evidence of Schiff base formation is certainly the appearance of strong absorption band at 1634 cm-1 attributed to the –C=N vibration characteristic of imine which is not observed in chitosan. The characteristic absorption peak for –NH2 group at 1592 cm-1 decrease in its intensity due to the decrease in its content indicating that the reaction on amino groups in chitosan with aldehyde to give Schiff base. On the other hand there is no evidence of bands characteristic of free aromatic aldehyde near to 1680 cm^{-1}.

Determination of optimum reaction conditions:
In the optimization studies, the percentage yield (%P) was studied as a function of different reaction parameters such as mol ratio, reaction time and temperature.

(a) Effect of mol ratio:
The result of the percentage yield for the preparation of Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde for varying amount of molar concentration are shown in fig. 1.

As shown in fig. 1 it is clearly indicate that initially the value of %P increases with increase in mol ratio of chitosan : aldehyde and reached maximum value of %P at [chitosan : aldehyde] = 6.00: 1.00 mol/L. At the respective optimum value of the mol ratio, the value of %P is found to be 64.24% (cf. fig. 1). Beyond the optimum value of the mol ratio, %P is found to be decreased.

The observed increase in %P within the mol ratio range 1.00: 1.00 - 6.00: 1.00 mol/L(fig. 1) may be due to the fact that within these concentration range, the more avaibility of free amino groups on the backbone and therefore more reactive sites are available for incoming aldehyde groups of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde. The observed decrease yield(%P) at higher mol ratio , i.e. beyond [chitosan : aldehyde] = 6.00: 1.00 mol/L may be attributed to there is no more availability of free amino groups on chitosan backbone and therefore doesn't mining to increase 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde concentration, it means beyond optimum concentration small increase observed for 8.00: 1.00 mol/L is not enough to justify the waste of the aldehyde reagent.

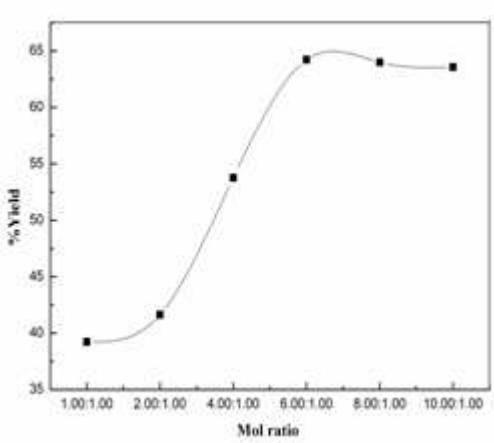

Fig: 1 Effect of mol ratio in biopolymer Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde at various mol ratio concentrations

(b) Effect of reaction temperature:

The effect of the temperature on the biopolymeric Schiff bases obtained with 6.00:1.00 mol ratios during 18 h of reaction is presented in fig. 2. It is clearly indicate that the values of the percentage yield (%P) are found to be increased from 30^0C to 45^0C and thereafter decreases upto 55^0C.The observed increase %P value may be due to upto 45^0C reaction occurs easily in acidic media but after increases temperature from 45^0C to 55^0C could cause hydrolysis of chitosan in acidic media and therefore observed decreased in values of percentage yield.

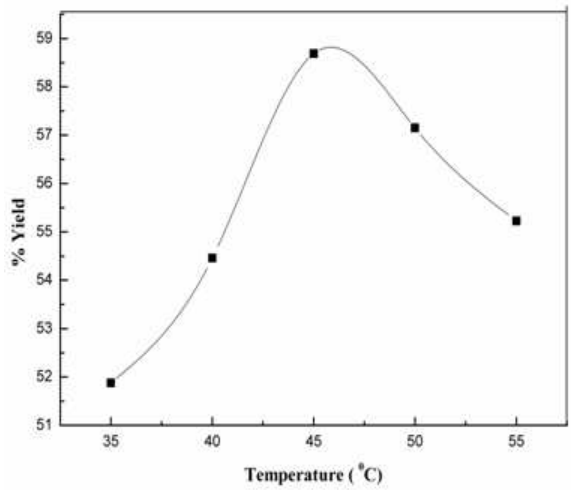

Fig: 2 Effect of mol ratio in biopolymer Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde at various reaction temperatures

(c) Effect of reaction time:

The effect of the time on the biopolyemric Schiff bases obtained with 6.00:1.00 mol ratios during 45^0C of reaction is presented in fig. 3. It is clearly shows that values of percentage yield (%P) are found to be increases from 4 h to 18 h and there after it decreases upto 24 h. The observed increases in %P which is due to free amino groups are available for substitution of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde and after that it decreases due to absence of free amino groups. No change in the acetylate groups was observed during the reaction.

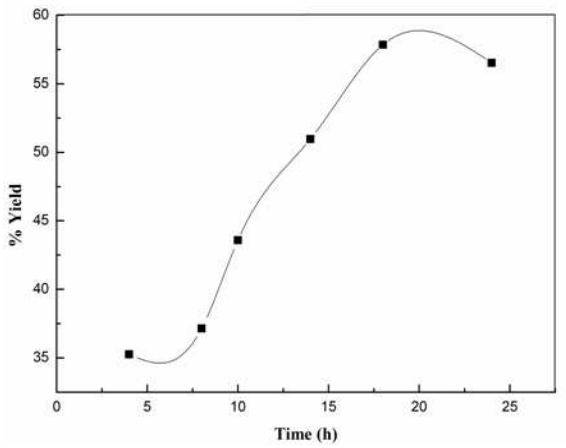

Fig: 3 Effect of mol ratio in biopolymer Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde at various reaction times

Antifungal activity:

The capabilities of chitosan and schiff base of chitosan in growth of zone of inhibition against *A.niger* and *penicillium* is shown in table 1. It clears for *A.niger* and *penicillium* that no zones of inhibition growth were observed for chitosan against both of *A.niger* and *Penicillium*. The schiff base shows better antifungal activity than chitosan, which not only have growth of inhibition effect against both *A.niger* and *Penicillium* but also contributed to the growth of the microbe. The possible reason is that *A.niger* and *Penicillium* are belongs to a growth of fungi whose cell walls contain chitosan. Therefore, *A.niger* and *Penicillium* has acertain resistance to the antifungal performace of chitosan [22].

Sample	0.5% (w/v)	0.75% (w/v)	1.0% (w/v)
Control	-	-	-
Chitosan	-	-	-
Schiff base of chitosan against *Penicillium*	08	12	14
Schiff base of chitosan against *A. niger*	12	13	18

Table: 1 The zone of inhibition of chitosan and schiff base of chitosan against *Penicillium and A.niger* (zone inhibition in mm).

CONCLUSION

The Schiff base of chitosan was synthesized by the reaction of chitosan with 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde.The optimized reaction condition obtained in the preparation of biopolymeric Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde is Chitosan **:** 0.5 g (dry basis), [Acetic acid] **:** 0.15 mol/L[mol] **:** 6.00:1.00, Time **:** 18 h,Temperature **:** 45^{0}C. The value of maximum percentage yield, in the case of biopolymeric Schiff base of chitosan and 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxaldehyde is found to be 69.71%. The antifungal activities of chitosan and Schiff base of chitosan were investigated against *Penicillium and A.niger* and the result indicates that the Schiff base of chitosan has better antifungal activites than chitosan. The antifungal acitivity of Schiff base increases with an increase in concentration. As a novel chitosan derivative, the Schiff base of chitosan improves the antifungal activity of chitosan and expands the antifungal spectrum compared with chitosan itself.

Acknowledgement

We are very thankful to the Mahtani chitosan Pvt. Ltd., Veraval, Gujarat to provide chitosan for this work.

REFERENCES

[1] E. Khor and L.Y. Lim, *Biomaterials.*, **2003**, 24, 2339-2349.
[2] C. Shuiping, U.Guozhong, Z. Hongyan, *Carbohydrate Polym.*, **2005**, 60(1), 33-38.
[3] L.Mui, D.Yumin, Y. Jiamhong, Z. Huayue, *Carbohydrate Polym.*, **2004**, 55(3), 291-297.
[4] R. Patale, B. P.Vandoma, *Carbohydrate Polym.*, **2011**, 85(1),105-110.
[5] R.A.A. Muzzarelli, *Cell Mol. Biol. Life Sci*, **1997**, 53, 131-136.
[6] H. Serag and G. Edress, *European J. of Experimental Bio.*, **2011,** 1(4), 87-92.
[7] K.D.Yao, Peng T., M.F.A. Goosen, J.M. Min, Y.Y. He, *J. of Applied Polym. Sci.*, **1993**, 48(2), 343-354.
[8] A. Kundawala, V. Patel,H. Patel and D. Choudhary, *J. of Der Pharmacia Sinica*, **2011**, 2 (5), 88-97.
[9] S. Senthil, A. Saha, K. Kavitha and S. Basu, *J. of Der Pharmacia Sinica*, **2012**, 3 (6), 616-623.

[10] Prerna Dhawade and R. Jagtap, *J. of Der Chemica Sinica*, **2012**, 3(3), 589-601.
[11] S. Kalyan, P. Sharma, V. Garg, N. Kumar and J. Varshney, J. of Der Pharmacia Sinica, 2010, 1(3), 195-210.
[12] K.D. Yao, Y.J. Yin, M.X. Xu, F. Wang, *Polymer Int.*, **1995**, 38(1), 77-82.
[13] K. Saitu, S. Nagaoka, T. Shirosaki, M. Horikawa, S. Mastanda, J. Hirotaka, *Carbohydrate Res.*, **2012**, 349, 52-58.
[14] M. C. Rodignez-Anguelles, C. Sieiro, C. Roberto, *J. of colloid and interface sci.*, **2011**, 364, 80-86.
[15] K.S.V. Rao, R. Reddy, L.Yong-ILL, C. Kim, *Carbohydrate Polym*, **2012**, 87(1), 920-925.
[16] J. Zhishen, S. Dongfeng, X. Weilliang, *Carbohydrate Res.*, **2001**, 333, 1-6.
[17] S. Hirano, K. Nagamura, M. Zhang, S.K. Kim, B.K. Chung, M. Yoshikawa, *Carbohydrate Polym.*, **1999**, 38(4), 293-298.
[18] K. Kurita, S. Mori, Y. Nishiyama, M. Harata, *Polymer Bull.*, **2002**, 48(2), 159-166.
[19] G.K. Moore, G.A.F. Roberts, *Int. J. of Biomacromolecules*, **1981**, 3,337-349.
[20] H. Sashiwa, Y. Shigemasa, *Carbohydrate Polym.*, **1999**, 39(2), 127-138.
[21] J. Santos, E. R. Dockal, E. Cavalheiro, *Carbohydrate Polym.*, **2005,** 60(3), 277-282.
[22] J. Xiaoxiao, W. Jiangtao, J. Bai, *Carbohydrate Res.*, **2009**, 344(6), 825-829.