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Release study of Naproxen, A Modern drug from pH Sensitive Pullulan Acetate Microsphere

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Abstract

The current study represents the latest and new advances in the investigations and applications of the pullulan the wonderful biomaterial which is produced from microbes (generally *Aureobasidium sps.*) and its derivatives for its applications in the development of various therapeutic formulations. Basically pullulan is an exo-polysacharides which is non-carcinogenic, non-immunogenic and non-mutagenic without having any types of toxicological activities. Similar to dextran and other biopolymer it can be used as a plasma expander. The model drug for this studies i.e Naproxen was used which is one of the most potent non-steroid and anti-inflammatory drug for the treatment of various bone related diseases .In our study we have prepared pH sensitive naproxen-pullulan delivery system in which Pullulan acetate was synthesized by treating 2.5 gm of crude pullulan with 25 ml of formamide at 50°C. For the loading of drug 50 mg of Pullulan acetate was dissolved 5 ml of dichloromethane and to it 80 mg of Naproxen was added. The drug loading efficiency of the prepared microparticle was found to be 80%. The microsphere was also showing a pH sensitive swelling behaviour in Phosphate Buffer Saline (PBS) buffer. The release profile of the drug loaded microsphere reveals that pH of the medium was influencing it at *in vitro* condition. Moreover the released amount of drug from the pullulan based microsphere at pH 7.2 was 75 times more than that at the pH 1.2. Therefore Pullulan acetate loaded with Naproxen is a useful polymerised materials for the development and formulation of pH sensitive drug.

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Pullulan, *Aureobasidium sps.*, Naproxen, Phosphate Buffer Saline, pH

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1. INTRODUCTION

Controlled drug delivery systems, which are used to deliver drugs at predetermined rates at predefined time periods, have been used to overcome the shortcomings of various conventional drug formulations [1,2] . There is a significant progress which has been made in the controlled drug delivery system, more advances are yet to be made for the

treatment of many clinical disorders like rheumatoid arthritis, diabetes and various rhythmic heart disorders. In these cases, the drug has to be delivered in response to a particular environmental condition i.e pH in the body [3,4,5].

Pullulan, which is made up of linear α-D-glucan maltotriose and maltotetrose repeating units interconnected by α(1→6) and α(1→4) linkages, is a water-soluble homopolysaccharide produced extra-cellularly by *Aureobasidium pullulans* [6,7]. This polysaccharide is of having so many economic importances with its application in the food, pharmaceutical, agricultural and chemical industries. Now a days material scientist are paying more and more attention to process the inorganic crystallization studies within a largely formulated organic matrix of biologically synthesised compounds like Pullulan. They are generally used for various types of *in-vitro* and *in-vivo drug* delivery studies along with different types of therapeutic formulations etc [8,9,10] .

Naproxen is a no-steroidal and anti-inflammatory drug (NSAIDs). In the living system it acts by reducing the level of hormones that causes inflammation and pain in the body. Naproxen is generally used for the treatment of pain, inflammation caused by different types of diseased conditions and complications such as arthritis, tendinitis, spondylitis, gout etc. [11,12,13,14].

In this study, we have synthesised the hydrophobic pullulan acetate from the hydrophilic pullulan gum by chemical modification with substitution reaction. After that the microsphere using the pullulan acetate was prepared and its swelling capacity was measured at the different pH. The released behaviour of drug loaded pullulan acetate microspheres with respect to pH was also studied.

2. MATERIALS AND METHOD

2.1 Chemicals

The Naproxen (NPX) was purchased from Square Pharmaceuticals Ltd. and Dichloromethane was taken from Sigma Chemical Co. (USA) . Formamide, Pyridine (Chemical grade) and acetic anhydride were purchased from Hi media,India. Pullulan was purchased from TCI Chemicals,India.

2.2 Synthesis of Pullulan Acetate from Pullulan

Pullulan is a hydrophilic polysaccharide by nature, so it is very essential to change the hydrophilic nature into a hydrophobic for its applications in the controlled drug delivery system. We had prepared hydrophobic pullulan acetate by substituting a proton ion at glucose into acetyl group (Fig. 1).The synthesis of Pullulan acetates was done as follows; in which 2.5 g of pullulan was suspended in 25 ml of formamide solution and dissolved by vigorous stirring at 400 rpm. After that 60 ml of pyridine with 150 ml of acetic anhydride were added to this suspension which was stirred at 500 rpm for 2 days. The synthesized Pullulan acetate was extracted after reprecipitation from 250 ml of water. The pullulan acetate got by this process was confirmed by the use of FT-IR spectroscopic analysis [15,16,17].

2.3 Pullulan Acetate Microspheres preparation and Drug Loading Procedure

50 mg of pullulan acetate was made to dissolve in 5 ml of dichloromethane and to this solution 80 mg of Naproxen was added. Then, the solution was homogenised at gentle rpm in the room temperature until it was dissolved completely. In order to prepare the microspheres, this homogenised solution was dropped very slowly into 50 ml of double distilled water with stirred conditions in the homogeniser again to evaporate the organic solvent for 2 hr at room temperature. NPX-loaded microspheres were obtained by centrifugation at 10,000 rpm, in 4°C for 15 min and then subjected to be dried at 70°C for 1hr to get the final microsphere samples. To get the

amount of drug that had been loaded, the freeze-dried pullulan acetate microspheres were suspended in methanol, vigorously stirred for 30 min and then sonicated for 10 min. The resulting solution was centrifuged at 12,000 rpm for 20 min and the supernatant was taken for the measurement of drug concentration using UV spectrophotometer.

2.4 Swelling Measurement

The swelling is defined as the weight of water absorbed per unit weight of dried pellets which has been calculated by measuring the weight of swollen pellets. Swelling of the NPX-loaded microsphere was measured as a function of pH. But the resulted microspheres are not useful to measure the swelling behaviour because of their very small size. For this reason, the pullulan acetate pellets were used . For this process, 500 mg of pullulan acetate was dissolved in 50 ml of dichloromethane solution. The solution was transferred to a petri dish (polyethylene), and then dried in a vacuum oven at a constant temperature (37°C) until there is no detectable changes in weight had been observed. The pellets (0.7 cm, 23 mg) that has been obtained was subjected to place in aqueous media with two different pH values (1.2 and 7.4) until equilibrium was achieved. The degree of swelling (α) was then calculated from the following formula.

$$\alpha = \frac{W_g - W_o}{W_o}$$

Where W_o is the initial weight of the micro particles and W_g is the weight of the micro particles at equilibrium swelling in the medium [18].

2.5 *In vitro* Release Studies of NPX-loaded Microspheres

The release study of the drug loaded microspheres was carried out as follows; 50 mg of NPX-loaded microspheres in 1 ml of 0.15M phosphate buffered saline (PBS; pH 7.4) or HCI in PBS (pH 1.2) were put into two different dialysis tube which was introduced into 100 ml of respective buffer and was kept in a stirrer at gentle rpm at room temperature. At specific time intervals, the released sample was taken out to find the concentration of the released drug and replaced respectively with freshly prepared buffer [20,21,22].The concentration of the released NPX was estimated by using UV spectrophotometer at 319 nm.

2.6 FT-IR Analysis

Fourier transform infrared (FTIR) spectra were recorded in a Perkin Elmer-Spectrum RX1 spectrometer over KBr pellet. Total 32 scans were performed in which 2.5mg of pullulan sample was well blended manually with 63 mg of KBr powder. These mixtures were then desiccated at 50°C for 12 hours under low pressure before starting the FTIR measurement [23,24].

3. RESULTS AND DISCUSSION

3.1 Synthesis of Pullulan Acetate from Pullulan

Pullulan is a hydrophilic polysaccharide by nature. It was modified to hydrophobic by replacing the hydroxyl groups with acetate groups to produce a hydrophobically modified pullulan that is 'Pullulan Acetate' after reacting with formamide, pyridine and acidic anhydride as shown in the Fig.1 with the acetylation substitution reactions.

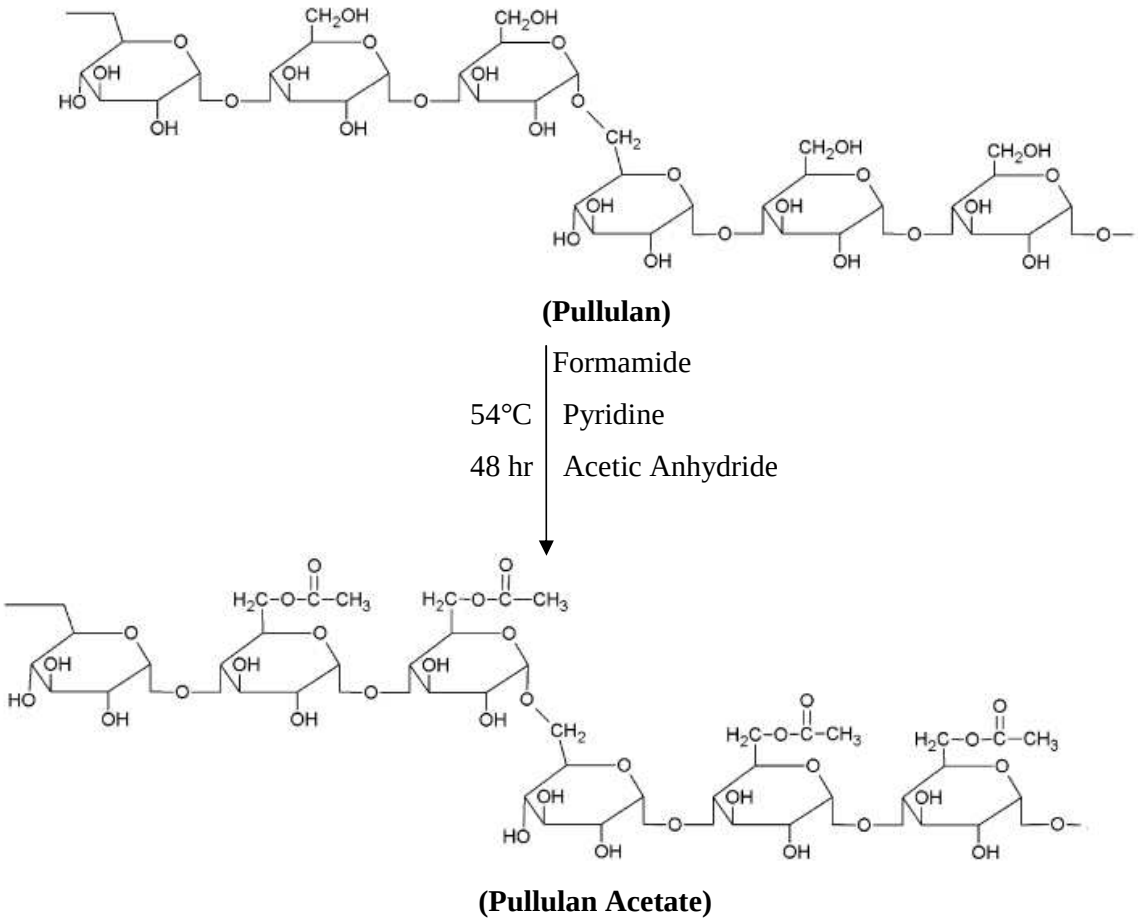

Fig.1: Synthesis of Pullulan Acetate from Pullulan by Acetylation

3.2 FT-IR Analysis of Pullulan, Pullulan Acetate and Drug loaded Pullulan Acetate

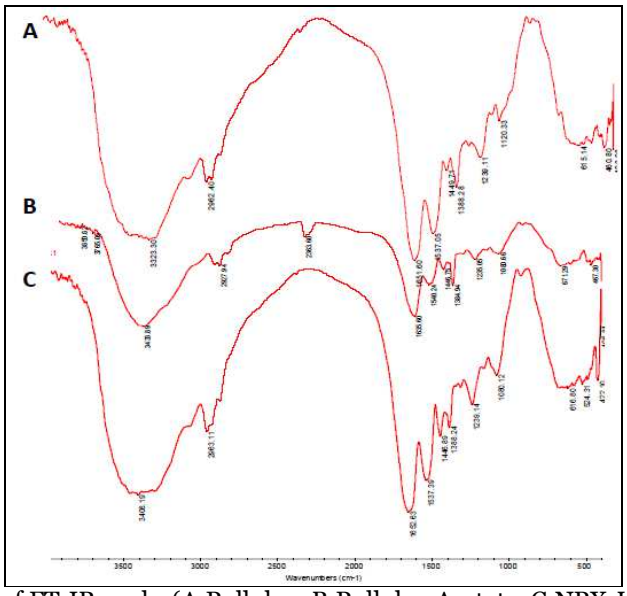

Fig.2: Combined plots of FT-IR peaks (A:Pullulan, B:Pullulan Acetate, C:NPX-Loaded Pullulan Acetate microsphere)

The Pullulan and Pullulan Acetate were analyzed with FT-IR (Fig. 2).The spectra showed the formation of extra acetate group, indicated by C=O stretching (wave number = 1652 cm^{-1}), deformation of CH- (wave number =1388 cm^{-1}) and O-C=O bonds (wave number = 604 cm^{-1}). The bands observed in the micro particles spectrum did not show any shift, suggesting that no new chemical bond was formed after preparing the formulation and the results confirmed that the drug is physically dispersed in the polymer.

3.3 Swelling Capacity Measurement

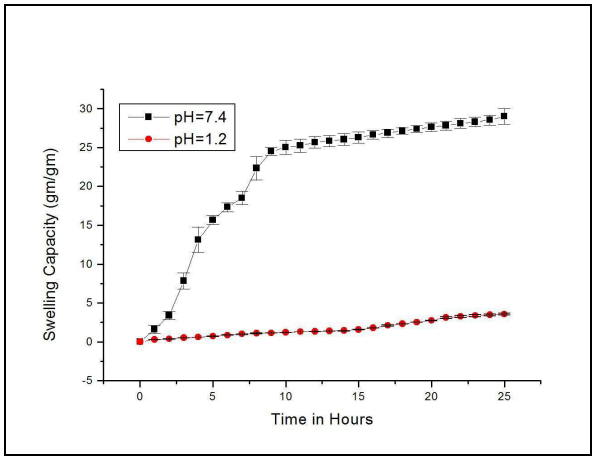

Fig.3: Swelling Profile of NPX-Loaded Pullulan Acetate

Swelling properties of microparticles was measured as a function of pH 1.2 and pH 7.4. One of the key factors for controlling the sustainable controlled release pattern of the drug is the swelling properties of microspheres which has been affected by the surrounding pH of the plasma [25] . The swelling of pullulan acetate pellets at pH 7.4 was approximately 9 times greater than that at pH 1.2. In the acidic condition (pH, 1.2) the carboxylic groups is generally protonated due to substitution reaction, may be due to which pullulan acetate microspheres is not swell in a extent but on the other hand at the neutral condition (pH, 7.4) the degree of ionization of carboxylic group in pullulan acetate microspheres is greater than that at pH 1.2. Due to which there is a increase in the swelling of microspheres.

3.4 *In vitro* Release Studies

Fig. 4 shows the release pattern for NPX-loaded microspheres with drug content of 50 (Wt%). NPX released from the microsphere was much faster at pH 7.4 than that at pH 1.2.The possibility is that the solubility of NPX is dependent upon the pH of the PBS buffer. There also may be other possibility that the swelling of pullulan acetate depends upon the pH-sensitive factor. At pH 1.2, the carboxylic groups of pullulan acetate were protonated due to which the pullulan acetate microspheres did not swell [26,27] . Thus NPX was not released from the pullulan acetate microspheres. In contrast, at pH 7.4 the amount of NPX released increased generally because the microsphere swelling enhanced effectively due to the ionization of carboxylic groups at a neutral pH. Therefore, the initial release of NPX was started by the pH-sensitive diffusion of drug contained at the microsphere surface, then this release phase was followed by the pH-sensitive swelling behaviour of microspheres. The *in-vitro* release studies were performed at room temperature for the period of 14 hours with two different pH in the triplicate form.

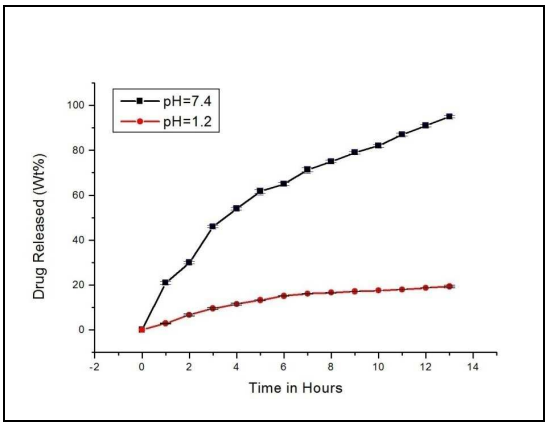

Fig.4: *In-vitro* Release study of NPX-Loaded Pullulan Acetate microsphere (80mg of drug was loaded with polymers)

3.5 Effect of pH on Drug Release

We have tested release of NPX from pullulan acetate under the condition of two different values of pH (changes from 1.2 to 7.4) in the intervals of 4 hours .The release of the drug from microspheres with alterations of the pH (the pH was set at 1.2 for first 4 hr, then set to 7.4 for next 4 hr successively). At the initial pH of 1.2 for a period of 4 hours, no drug released was observed. When the pH of the aqueous environment has changed to 7.4 the drug was released from microspheres quickly. Moreover at a pH 1.2 , the release of drug was stopped, and once the pH 7.4 medium was reinstalled, the release of drug started again. The main cause for this types of alternation with respect to the release is due to the fact that, at pH 7.4, the microspheres disintegrated where as at pH 1.2 there is not any disintegration of microsphere and only disintegration of the microspheres facilitate the rapid sustainable release of the NPX.

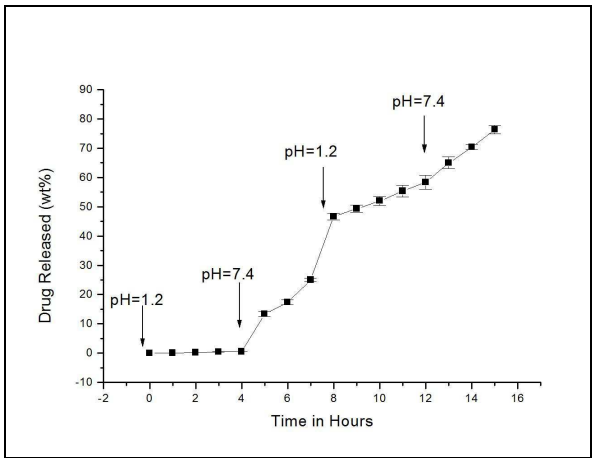

Fig.5: Effect of pH on the release of drug from the Pullulan acetate microsphere

4. CONCLUSION

In this study, Pullulan Acetate was synthesized by changing the degree of acetylation successfully. Pullulan Acetate was synthesized by substitution reaction method which can be a good carrier of the NPX *in vitro*. The pH-sensitive release of NPX from the microspheres and to the release of the drug from the surface after swelling, due to the chemical composition of the microspheres with disintegration and the pH of the surrounding environment. Therefore, pullulan acetate can be a useful for pH sensitive drug delivery system. Due to the good biocompatibility of pullulan, Pullulan Acetate may be useful in the delivery of antitumor drugs, and the further investigations in SEM and TEM analysis with *in vivo* release studies with model organisms are in progress.

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