An Insight To Pullulan: A Biopolymer in Pharmaceutical Approaches

Deepak Kumar, Nisha Saini, Vinay Pandit, Sajid Ali

School of Pharmaceutical Sciences, Shoolini University, Solan (H.P), India
Email: deepakkaushik354@gmail.com
School of Pharmaceutical Sciences, Shoolini University, Solan (H.P), India
Email: nishasaini10287@gmail.com
School of Pharmaceutical Sciences, Shoolini University, Solan (H.P), India
Email: vinay2121@gmail.com
School of Pharmaceutical Sciences, Shoolini University, Solan (H.P), India
Email: sajidalipharma4u@gmail.com

Abstract

Pullulan is a non-ionic polysaccharide obtained from fermentation of black yeast like Aureobasidium pullulans and is currently exploited in food and pharmaceutical industries due to its unique characteristics. Due to its properties like non-toxic, non-immunogenic, non-carcinogenic, non-mutagenic, pullulan is being explored for various biomedical applications viz., gene delivery, targeted drug therapy, tissue engineering, wound healing, and also being used in diagnostic applications like, perfusion, receptor, and lymph node target specific imaging and vascular compartment imaging. The unique linkage of α (1→4) and α (1→6) in pullulan endows this polymer with distinctive physical traits, including adhesive property and the ability to form fibres. This review article presents an overview of properties, production, derivatives of pullulan, and recent advances of pullulan.

Keywords: Pullulan; Polysaccharide; Biomedical; Diagnostic application; Medical imaging; Linkage.

1 Introduction

Biopolymers are the generally water soluble gums, produced by a variety of micro-organism, which have novel and unique physical properties. Because of these properties, these have found a wide range of applications in food, pharmaceutical and other companies. Some of the applications include their use as
emulsifiers, stabilizers, binders, gelling agents, coagulants, lubricants, film formers, thickening agents and suspending agents. Advances in technology occur due to the exploitation of properties offered by new polymeric materials like blends, composites, etc. Blend of polymers are prepared by physical mixing of two or more polymers. The resulting blending system has the property superior to any one of the individual polymer [1-3]. Pullulan is one of the polymers obtained from the fermentation medium of the black yeast like Aureobasidium pullulans (as it forms a black pigment, melanin so called black yeast) [4]. It shows activity against for enzymes such as invertase, amylases, glucose oxidase, β-glucosidase, fructosyltransferase, and small quantities of proteolytic enzyme [5-7]. Pullulan was first reported by Bernier in 1958 and the structure was elaborated by Bender et al in 1959 [8]. It comprises of maltotrose units connected by α (1→4) glycosidic bond, whereas consecutive maltotriose units are connected to each other by α (1→6) glycosidic linkages. Basic linkages in pullulan and its enzymatic hydrolysis sites are shown in fig. 1.

![Basic linkages in pullulan and enzymatic hydrolysis site](image_url)

Fig. 1: Basic linkages in pullulan and enzymatic hydrolysis site

Structure of pullulan and pullulan acetate are shown in fig. 2 and fig. 3 respectively. The application of pullulan is emerging as a source of polymeric materials, which are economical and competitive with the natural gums produced from marine algae and other plants [9]. Applications of pullulan biopolymer are
based on biodegradability of the polymer, so it is packed in food packaging films, coating of food containers for perishable fruit and vegetables [10].

![Structure of pullulan](image1)

**Fig. 2: Structure of pullulan**

![Structure of pullulan acetate](image2)

**Fig. 3: Structure of pullulan acetate**

### 2 Historical Aspects Related to Pullulan

Origin of pullulan occurred 6-7 decades earlier i.e. in 1950s. *Aureobasidium pullulans* was first described as *Dematium pullulans* by De Bary. Bernier was the first to isolate pullulan form *Aureobasidium pullulans* in 1958. Bender et al studied the novel polysaccharides in 1959 and named it pullulan. In 1960s, the basic structure of pullulan was resolved [11]. They discovered the enzyme pullulanase, which hydrolyses α-(1→6) linkages in pullulan and converts to maltotriose. Thus, pullulan is viewed as α-(1→6) linked polymer of maltotriose.
subunits. Cately and his coworkers established the occurrence of randomly distributed maltotetraose [12].

Pullulan has the safe history of use in Japan as a food ingredient and as pharmaceutical bulking agent. The main use of pullulan has been as a glazing agent having oxygen barrier properties [13]. It has Generally Regarded As Safe (GRAS) status in US for a wide range of applications. Human volunteer studies have only reported the abdominal fullness at doses of 10 g pullulan per day with some mild gastrointestinal symptoms at higher doses. Pullulan is accepted for use as an excipient in pharmaceutical tablets and is listed in the Japanese Standards for Ingredients for drugs [14,15]. The commercial production of pullulan began in 1976 by the Hayashibara Company, in Okayama Japan [16]. Pullulan production was an outgrowth of starch syrup production, noted in 1883. Pullulan films were commercialized by Hayashibara in 1982.

3 Properties of Pullulan

Dry pullulan is white to off-white tasteless, odourless powder which forms a viscous non-hygroscopic solution when dissolved in water at 5-10%. Pullulan starts to decompose at 250 °C and chars at 280 °C. it is highly soluble in water, dilute alkali, insoluble in alcohol and other organic solvents expect dimethylsulphoxide and formamide. As pullulan is highly water soluble so it can be used as a carrier for drug and it helps in controlled release of drug in plasma. It has the molecular weight within the range of 5000 – 9000000 g/mol with straight unbranched chain and is very flexible molecule having the property of “random – coil” (depending on sedimentation coefficient and intrinsic viscosity measurement). Pullulan is non-toxic, non-mutagenic, non-carcinogenic, odorless, tasteless, and edible [17,18]. Furthermore, pullulan has a considerable mechanical strength and other functional properties viz. adhesiveness, film formability, enzymatically – mediated degradability [19]. Pullulan is biodegradable, impermeable to oxygen, and is not attacked by the digestive enzymes of the human gut, hence can be used as carrier for oral delivery of drug. Pullulans solutions have relatively low viscosity, resembling gum arabic [16]. It can be used as low-viscosity filler in beverages and sauces. The viscosity of pullulan solution doesn’t change with heat, change in pH, and most metal ions including sodium chloride.

4 Production of Pullulan

Pullulan is usually produced on industrial scale by the fermentation of liquefied starch under specified parameters using a specific, not genetically modified, non-pathogenic and non-toxigenic strain of Aureobasidium pullulans.
4.1 Pullulan production by fermentation

The production of pullulan depends on the fermentation parameters viz., the morphological state and the fungal strains. In commercial production [16], *Aureobasidium pullulans* is cultivated on medium, comprises of starch hydrolysates of dextran equivalent to 40-50, at 10-15% concentration. The fermentation medium consists of peptone, phosphate and basal salts. The pH of the culture media is initially adjusted to pH 6.5, which falls during the first 24 hours to a pH of 3.5. Maximal growth of culture media occurs within 75 hours. Optimal pullulan yields are obtained within about 100 hours. Cultures are stirred, aerated, and the temperature is maintained at temperature of 30°C. Yield of greater than 70% of initial substrate is claimed. Culture conditions and strain selection are important parameters in obtaining high molecular weight pullulan, which is relatively free of melanin. *Aureobasidium pullulans* cells are removed from media by filtration of diluted culture broth. Melanin is removed by treating it with activated charcoal. Pullulan is recovered and purified by precipitation with organic solvents, particularly alcohol. It may be further purified by the use of ultrafiltration and ion exchange resins.

Youssef et al reported the production of pullulan by using various strains of *A. pullulans* using sucrose and glucose in shaking flask and stirred tank fermenters. They reported maximum concentration of pullulan at 31.3 g/L with pullulan productivity of 4.5 g/L per day [20]. In another study Shabtai and his co-workers produced pullulan in a two stage fermentation process with increased productivity. In the first step, fermentation was carried out using soyabean oil as a carbon source and glutamate as nitrogen source, at pH of 4.5, which resulted in the concentration of pullulan at 15 g/L. In the second stage, the cells were shifted to production, which was carried out using sucrose as a carbon source with nitrogen limitation. They reported the concentration of pullulan about 35 g/L in 50 hours [21]. Recently, Roukas with his co-workers observed maximum concentration of pullulan of 30 g/L in an air-lift fermentor at an aeration rate of 2 vvm (vol/vol/min). West T. P., et al, 1991 produced the pullulan in two cycle process of 165 hours, using either agar or calcium alginate for immobilized cell system. The cells which were immobilized in alginate gave a higher production of pullulan with 4.2 mg pullulan per gram cells per hour during the first cycle and 4.6 mg per gram cells per hour during the next cycle [22].

4.1.1 By using coconut by-products

Thirumavalavan et al synthesized pullulan from coconut by-products using *Aureobasidium pullulans*. The strain was maintained on agar slants at 4°C and subcultured every fortnight time interval. The seed medium comprises of sucrose, potassium dihydrogen phosphate, yeast extract, ammonium sulphate, sodium chloride and distilled water. The medium was autoclaved for 15 min at 121°C, cooled and the pH was adjusted to 7. Then the culture was incubated at 30°C for
36 hours in a rotary shaker incubator at 200 rpm. The highest concentration of pullulan was 54 g/L in coconut milk [23].

4.1.2 By beet molasses:

Goksungur and his co-workers produced pullulan by beet molasses using A. pullulan. The media comprised of sucrose, ammonium sulphate, yeast, potassium dihydrogen phosphate, magnesium sulphate, and sodium chloride. They reported the highest pullulan concentration 35 g/L obtained in molasses treated with sulphuric acid and activated carbon [24].

4.1.3 From agro-industrial waste:

Pullulan can be synthesized from a various carbohydrate substrates incorporated into defined (synthetic) or non-defined media. The latter covers the agro-industrial wastes, which have been shown to be suitable for pullulan production [25,26]. The utilization of these substrates for the production of pullulan seems to be economically advantageous and economically sound.

Different fermentation parameters for the production of pullulan have been studied with defined substrates viz. glucose and sucrose, but the results from the agro-industrial wastes have shown that higher or similar yield of pullulan can be obtained as compared to conventional substrate [27]. Also, pullulan produced by such fermentations is characterized by heterogeneity of both composition and molecular weight [28]. Following are the agro-industrial wastes which have been used for the production of pullulans:

- Grape skin pulp [28]
- Molasses [28]
- Starch waste [29]
- Olive oil wastes [28,30]
- Carob pod [22]

4.2 Biosynthesis of pullulan

Significant work has been done in recent years to determine the pathways for biosynthesis of polysaccharides like pullulan. Taguchi et al noted the biosynthesis of pullulan cell free preparations and acetone dried cells. Frozen cells were disrupted by grinding by alumina. The debris was removed by centrifugation. The precipitation of supernatant was done with 80% saturated ammonium sulphate solution. This protein precipitate catalyzed biosynthesis of pullulan from Uridine-5-diphosphogluucose (UDPG) and ATP gave the yield of 54% of pullulan with respect to the amount of UDPG added [31].
5 Semi Synthetic Derivatives of Pullulan

Pullulan can be easily derivatized in order to enhance its activity and widen the window of its applications. Pullulan can be derivatized in various ways, which are given below:

5.1 Chemical modification

Pullulan can be derivatized to enhance its applications by grafting different chemical structures on the backbone. Pullulan consists of nine hydroxyl groups for the substitution reactions on the repeating unit. The relativities of these hydroxyl groups also depend on the polarity of the solvent and the reagents. The hydroxyl groups of pullulan were subjected to various chemical reactions, leading to the formation of a large number of derivatives, which are given in table 1 [32].

Table 1: Schematic chemical structures of the most common pullulan derivatives:

<table>
<thead>
<tr>
<th>Type of reaction</th>
<th>Schematic chemical structure of substituted pullulan (P-OH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etherification</td>
<td>P-O-CH₃ (Permethylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-(CH₂)₂,₃-CH₃ (Alkylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-CH₂-COOH (Carboxymethylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-(CH₂)₂,₃-CH₂-NH₃⁺ (Cationization)</td>
</tr>
<tr>
<td></td>
<td>P-O-CH₂-CH₂-CN (Cyanoethylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-(CH₂)₁,₄-Cl (Chloroalkylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-CH₂-CH₂-(S=O)-CH₃ (Sulfinylethylolation)</td>
</tr>
<tr>
<td></td>
<td>P-O-CH₂-CH₂-CH₂-SO₃Na</td>
</tr>
<tr>
<td></td>
<td>P-O-CH₂-CH₂-N(CH₂CH₃)₂</td>
</tr>
<tr>
<td></td>
<td>P-O-CH₂-CH₂-N⁺(CH₂CH₃)₂-CH₂-CH₂-N(CH₂CH₃)₂</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-CH₃ (Acetylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-(CH₂)₂,₁₄-CH₃ (Alkoylation)</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-CH₃-Cl (Chloroacetylation)</td>
</tr>
<tr>
<td>Reaction</td>
<td>Chemical Structure</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Esterification</strong></td>
<td>P-O-CO-CH(_2)-CH(_2)-COOH (Succinoylation)</td>
</tr>
<tr>
<td></td>
<td>PA-O-CO-CH(_2)-CH(_2)-CO-Sulfodimethoxine (^a)</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-CH(_2)-CH(_2)-CO-Cholesterol (^b)</td>
</tr>
<tr>
<td></td>
<td>P-abietate</td>
</tr>
<tr>
<td></td>
<td>P-stearate</td>
</tr>
<tr>
<td></td>
<td>PA-folate (^a)</td>
</tr>
<tr>
<td></td>
<td>P-cinnamate</td>
</tr>
<tr>
<td></td>
<td>P-biotin</td>
</tr>
<tr>
<td></td>
<td>P-O-SO(_2)-CH(_3)</td>
</tr>
<tr>
<td><strong>Urethane Derivatives</strong></td>
<td>P-O-CO-NH-CH(_2)-CH(OH)-CH(_3)</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-NH-CH(_2)-CH(_2)-NH(_3^+)</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-NH-R (R=Phenyl or hexyl)</td>
</tr>
<tr>
<td></td>
<td>P-O-CO-NH-phenyl</td>
</tr>
<tr>
<td><strong>Urethane derivative/amidification</strong></td>
<td>P-O-CO-NH-(CH(_2))(_n)-NH-CO-chlorestrol</td>
</tr>
<tr>
<td><strong>Chlorination</strong></td>
<td>P-CH(_2)-Cl (C(_6) Substitution)</td>
</tr>
<tr>
<td><strong>Sulfation</strong></td>
<td>P-O-SO(_3)Na</td>
</tr>
<tr>
<td><strong>Azido-pullulan</strong></td>
<td>P-CH(_2)-N(_2)</td>
</tr>
<tr>
<td><strong>Oxidation</strong></td>
<td>P-COOH(C(_6) Oxidation)</td>
</tr>
<tr>
<td></td>
<td>Glycosidic ring opening (periodate oxidation)</td>
</tr>
<tr>
<td><strong>CMP/hydrazone derivatives(^c)</strong></td>
<td>P-O-CH(_2)-CO-NH-doxorubicin</td>
</tr>
<tr>
<td></td>
<td>P-O-CH(_2)-CO-NH-antibody</td>
</tr>
<tr>
<td><strong>CMP/amidification(^c)</strong></td>
<td>P-O-CH(_2)-CO-NH-CH(_2)-(CH(<em>2))(</em>{14})-CH(_3)</td>
</tr>
<tr>
<td></td>
<td>P-O-CH(_2)-O-C(CH(_3))(_2)-R</td>
</tr>
<tr>
<td></td>
<td>with R= poly(methacrylate), poly(methylmethacrylate)</td>
</tr>
</tbody>
</table>
Copolymerization

<table>
<thead>
<tr>
<th></th>
<th>poly(hydroxyethylmethacrylate) and poly(sulfopropylmethacrylate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-O-CH₂-O-C(CH₃)₂-R</td>
<td>with R = poly (butylmethacrylate)</td>
</tr>
<tr>
<td>P-O-CH₂-O-C(CH₃)₂-R</td>
<td>with R = poly (N- isopropylacrylamide)</td>
</tr>
<tr>
<td>P-O-[CO-CH(CH₃)-O]ₙ-H</td>
<td>(polylactide)</td>
</tr>
<tr>
<td>P-O-poly(2-isopropyl-2-oxazoline)</td>
<td></td>
</tr>
</tbody>
</table>

P - Pullulan

- Derivatives prepared from pullulan acetate (PA). Substitution occurred on some of the remaining free hydroxyl groups
- Derivatives prepared from succinoylated pullulan. Substitution occurred on carboxylic acid
- Derivatives prepared from carboxymethylpullulan (CMP). Substitution occurred on carboxylic groups.

Chemical modification includes:

### 5.1.1 Carboxymethyl pullulan

Carboxymethylation is the most widely used reaction done on the neutral polysaccharides in order to allow further chemical modification or to favor the solubility in the aqueous solutions. The hydroxyl groups of the pullulan are activated as alcoholate in the alkaline aqueous solution to allow the nucleophilic substitution of chloride from monochloroacetic acid. Several works has been done to understand the behavior of self assembled or cross-like carboxymethyl pullulan [33-36].

### 5.1.2 Sulphation

With the aim of developing a new alternative to heparin, pullulan was derivatized by reacting with sulphur. The final property of sulphated pullulan depends on the temperature, solvent and duration of action and the reagent used for the sulphation. Mahner et al reported a homogenous distribution of the sulphate along both polysaccharidic backbones [37]. Later, Alban et al confirmed these results by determining the degree of the sulphation of the hydroxy group occurred in the order C₆ > C₂ > C₃ > C₄ irrespective of the weight of the pullulan [38]. The sulphated pullulans were obtained by stepwise sulphation of pullulans with SO₃ – pyridinde complex in dimethyl formamide (DMF) at 75 °C and 95 °C for 3 – 8 hours, in which the pullulans having the molecular weight 50 kDa (soluble in DMF) and 200 kDa (insoluble in DMF) were used.
6 Applications of Pullulan

6.1 Biomedical and Pharmaceutical applications:
The use of pullulan in biomedical field is emerging contemporarily due to its properties viz., non-toxic, non-immunogenic, biocompatible, and inert nature.

6.1.1 Tissue engineering and grafting

The surface and the bulk property of any biomaterial is important as it influences the dynamic reactions that take place at tissue implant interface. These properties or the change in the property which can take place over time in-vivo, should be known for designing the biomaterial for specific applications and this can be easily done with pullulan. Na and his co-workers conjugated the pullulan with an interferon-water-soluble low molecular weight recombinant protein which had both anti-viral and immunoregulatory activity. This allowed the user to preserve the biological activity of the drug while enhancing its liver accumulation [39]. Surface modification is an utmost tool for tissue engineering purpose. The surface modification can be easily done with pullulan as it comprises nine hydroxyl groups for substitution reactions on the repeating unit.

Applications of pullulan can be enhanced by grafting different chemical groups on it as it contains nine hydroxyl groups, which can be easily substituted. The weight of pullulan increases when grafting is done. The increase in weight of pullulan after grafting clearly indicates the grafting of concerned group on to pullulan. Gao et al characterized the graft yield, which is shown below [40]:

\[
\text{Grafting ratio } \% G = \frac{\text{Weight of the grafted chain}}{\text{Weight of pullulan}} \times 100
\]

\[
\text{Grafting Efficiency } \% E = \frac{\text{Weight of the grafted chain}}{\text{Weight of polymer formed}} \times 100
\]

\[
\text{Grafting ratio } \% G = \frac{\text{Weight of the polymer used}}{\text{Weight of the monomer used}} \times 100
\]

6.1.2 Pullulan as a carrier for drug delivery

Stimuli-sensitive polymer systems have been used as materials for the delivery of drug [41,42]. Gheorge et al grafted poly(N-isopropylacrylamide-co-acrylamide) onto the pullulan in order to confer their temperature sensitivity. Then the remaining hydroxyl groups of the pullulan were reacted with succinic anhydride.
to introduce the pH-sensitive units (-COOH), resulting into the more hydrophilic nature than the native pullulan [43]. Thus, pH-sensitive pullulan microspheres can be prepared for controlled delivery of the drug.

- **Liver targeting** study focuses on the blood compatibility of the cationic pullulan, physico-chemical characterization, uptake of nanocomplex by hepatocytes and *in-vitro* transfection. Liver targeting can be achieved by using drug loaded pullulan. Xi and his co-workers studied the liver binding affinity of the modified pullulan *in-vitro* in hepatocytes and *in-vivo* in mice [44].

- Pullulan can be used for **tumour cell targeting**. Scomparin et al designed two new anti-cancer polymers for tumour cell targeting. Pullulan was derivatized with either doxorubicin or doxorubicin and folic acid. Then, pullulan was activated by periodate oxidation and functionalized by reductive conjugation cysteamine and 1.9 kDa ammonium polyethyleneglycol [PEG (NH$_2$)] [45]. This study suggests that doxorubicin-pullulan bioconjugates possess suitable properties for passive tumour targeting while folic acid conjugate of pullulan has a limited effect on selective cell uptake.

### 6.1.3 Pullulan as a carrier for gene delivery:

Application of pullulan to the gene delivery is being explored. Gene therapy using viruses have been performed, but the major drawback of using viruses is that they are immunogenic, disease causing and can be hazardous to health. Pullulan being non-toxic and biocompatible is investigated for gene delivery application. Hosseinkhani et al mixed the pullulan derivative with a plasmid DNA in aqueous solution containing zinc ions to obtain the conjugate of pullulan derivative with Zn$^{2+}$ coordination [46].

### 6.1.4 Medical imaging:

Recently nanotechnology has been used for earlier detection of cancerous cell in the body. Quantum dots, which are the nano-size semiconductor particles has attracted many scientists in biological field. They are used as fluorescent probes for cell tracking. Hasegawa et al developed cholesterol pullulan and amino group modified cholesterol pullulan nanaogels for the delivery of quantum dots into cells in comparison to conventional cationic liposome which has the disadvantage of forming aggregates ones gets into the cells. Nanoparticles were prepared by mixing nanaogels of derivatized pullulan with quantum dots. They reported the intensity of fluorescence per cell and compared with liposomal-quantum dots complex. The particles with higher number of amino group showed fluorescence
up to 3 – 4 times more than that of control. They concluded that cellular uptake of cholesterol pullulan was enhanced by introducing cationic groups and simultaneously the quantum dot’s better than the conventional cationic liposomes and these nanoparticles could be a fluorescent probe for medical imaging [47].

6.2 Plasma expander

Pullulan was also explored as a potential blood-plasma substitute like that of dextrans. Polymers which are highly water soluble in nature can be used as plasma expanders and pullulan is water soluble polymer. It has been reported that pullulan to be used as plasma expander with molecular weight of about 60 kDa [16]. They observed that pullulan having high molecular weight increased the venous pressure whereas low molecular weight pullulans were rapidly excluded from the organism leaving the stage of secondary hemorrhagic shock. Thus, pullulan to be used as plasma expander, should have an effective therapeutic range of molecular weight. Shingel and his co-worker developed an anionically modified pullulan via gamma irradiation which was used as a base for blood – plasma substitute [48].

6.3 Molecular chaperons

Molecules having the chaperon like activity are able to catch and release proteins. Molecular chaperons bind to denatured proteins in order to prevent irreversible aggregation. Then chaperon molecules release the proteins. Water soluble polymers such as polyethylene oxide (PEO) have been tried to increase the recovery yield of parent protein during refolding [49]. These polymers prevent the aggregation of proteins by blocking their hydrophobic surface. Nomura et al developed hydrophobized pullulan nanogels having the properties of molecular chaperons [50]. They reported the release of complexed proteins from the nanogels in their refolded forms in the presence of cyclodextrins. They concluded that these amphiphilic nanogels trap the denatured proteins and cyclodextrin acts as an effector molecule to control the binding ability of chaperon molecule to proteins.

6.4 Hydrophobized pullulan conjugates for drug delivery: A recent development

Pullulan hydrogels as drug delivery systems in the form of microgels and nanogels have been studied. Slow release of drug into the plasma helps in attaining the therapeutic benefits [51]. Gupta and his co-worker prepared hydrogel nanoparticles of cross-linked pullulans with glutaraldehyde in order to develop a DNA carrier system, improving the gene loading efficacy, controlled release
properties, biocompatibility and enhanced stability. Also, hydrophobized pullulan-based nanogels interact with molecular assemblies such as liposomes and oil-water emulsion. As a result, hydrophobised pullulan conjugates were used for targeting of drugs viz., metronidazole, nicotinic acid, sulfathilzole, mitoxantrone and epirubicin [52].

Most of the cited paper in the field of hydrophobized pullulan reports the self assembly of cholesterol-bearing pullulan as stable hydrogel nanoparticles in which pullulan is non-covalently bind by associating cholesteryl moieties is shown in fig. 4.

![Diagram showing the formation of cholesterol-pullulan conjugate-based nanoparticles by self aggregation in aqueous solution](image)

**Fig. 4: Formation of cholesterol-pullulan conjugate-based nanoparticles by self aggregation in aqueous solution**

### 7 Recent Advances with Pullulan

Recent advances done in pullulan are described in table 2.
Table 2: Recent advances of Pullulan:

<table>
<thead>
<tr>
<th>Recent research</th>
<th>Advantages of pullulan</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast disintegrating tablet using pullulan as diluent</td>
<td>Tablet hardness was found to increase without increasing the disintegrating time with high concentration of pullulan.</td>
<td>[53]</td>
</tr>
<tr>
<td>Pullulan /Silver Nanoparticles composite nanospheres using electrospray techniques for antibacterial application.</td>
<td>Controlled spherical structure by controlling the concentration of pullulan, enhanced antibacterial activity</td>
<td>[54]</td>
</tr>
<tr>
<td>Self assembled nanogels of hydrophobized pullulan</td>
<td>Size stability, micelles showed long term colloidal stability with nearly negative neutral charge</td>
<td>[55]</td>
</tr>
<tr>
<td>Pullulan acetate coated magnetic nanoparticles for hyperthermia</td>
<td>Nanoparticles have high magnetite content, good biocompatibility, good heating property in magnetic field, and have evident cellular uptake by tumor cells.</td>
<td>[56]</td>
</tr>
<tr>
<td>Rapid dissolving films of cetirizine hydrochloride using pullulan as a film forming agent</td>
<td>Pullulan acted as rapid film forming agent.</td>
<td>[57]</td>
</tr>
</tbody>
</table>

8 Conclusion

Pullulan has gained a lot of attention in the past few decades due to its unique properties. Pullulan is an edible and bio-polysaccharide with numerous applications in the field of food and pharmaceutical industries. The unique property of pullulan is due to its glycosidic linkage. Pullulan is synthesized by fermentation of coconut by-products, beet molasses, agro-industrial waste. Pullulan can be easily derivatized by means of chemical reaction. Pullulan has important application in the field of biomedical and pharmaceutical field viz., tissue engineering & grafting. Pullulan has been used for liver and tumour target delivery of drug. Pullulan has the application in the field of targeting of drug to liver and cancer cells. Pullulan has occupied a niche area in food and pharmaceutical field.

References


[29] Shabtai Y., Mukmenev J., Enhanced production of pigment-free pullulan by a morphogenetically arrested Aureobasidium pullulans (ATCC 42033) in a two stage fermentation with shift from soybean oil to sucrose, Applied Microbiology and Biotechnology, 43, 1995; 595-603.


[37] Mahner C., Lechner M. D., Nordmeier E., Carbohydrate Research, 331, 2, 2001; 203.


