CHAPTER 3

SYNTHESIS, CHARACTERIZATION AND CHOLESTEROL UPTAKE STUDIES OF ETHERS AND ESTERS OF DEXTRAN OR CELLULOSE
3.1. Introduction

Cholesterol is the steroid with formula C_{27}H_{46}O. Cholesterol is an essential component of cell membrane and is essential for the production of many steroid hormones, bile acids and Vitamin D.

Scheme 3.1: Structure of Cholesterol

Excessive cholesterol leads to many central nervous system diseases like Alzheimer’s disease, Parkinson’s diseases, brain stroke, cardiovascular diseases, including arteriosclerosis and hypertension.\textsuperscript{1,2} It is well documented in literature that excessive cholesterol can lead to loss of cell membrane fluidity and activation of inflammatory mediators.\textsuperscript{1} Hypercholesterolemia is one of the several risk factors for coronary heart disease.\textsuperscript{3}

Neil S. Cameron \textit{et al.}\textsuperscript{4} studied the serum cholesterol reduction by using poly(\(N,N,N\)-trimethylammoniumalkylacrylamide chloride) – based hydrogels. It has been well established that dietary fiber has many health benefits including decrease in the serum low density lipoproteins (LDL)-cholesterol, improving triglycerides, and body weight management.\textsuperscript{5} Chitosan is also known example of a biopolymer that inhibits the absorption and enterohepatic circulation of bile acids, leading to a decrease
of plasma cholesterol levels accompanied by an increase in the compensatory oxidative synthesis of the bile acids from the hepatic cholesterol.\textsuperscript{6} Jing \textit{et al.}\textsuperscript{7} investigated the effect of chitosan on patients with renal failure and found that chitosan effectively reduced total serum cholesterol levels and increased serum haemoglobin levels. Gallahar \textit{et al.}\textsuperscript{8} determined the hypocholesterolemic effect of a supplement containing equal amount of chitosan and glucomannan on blood lipid concentrations and fecal excretion of fat, neutral sterols and bile acids. Bokura \textit{et al.}\textsuperscript{9} investigated the effectiveness of chitosan in reducing the serum cholesterol without concomitant diet therapy. Apple skin or pulp is a rich source of dietary fibers and can be used to reduce the risk of cholesterol gallstone formation. Minekus \textit{et al.}\textsuperscript{11} determined the effect of partially hydrolyzed guar gum on the bioaccessibility of dietary fat and cholesterol.

The hydrophobically modified dextran are reported for use in the lipid delivery system for release of hirudin a 65- amino acid peptide (MW 7000), the most potent and specific known inhibitor of thrombin.\textsuperscript{12} The ether-type anion exchangers based on hydroxy group-containing polysaccharides and polymerized hydroxy group-containing polysaccharides, including dextran and cellulose can be advantageously used in pharmaceutical compositions and processes for lowering hypercholesteremia and reducing serum bile acids.\textsuperscript{13} Zhong \textit{et al.}\textsuperscript{14} prepared novel hydrophilic molecularly imprinted polymers using acryloyl-containing hydrophilic monomers (including cholesteryl acrylate and acryloyl-6-amino-6-deoxy-β (or γ)-cyclodextrin), and analyzed their steroid-binding properties in 2-PrOH by HPLC. Turley \textit{et al.}\textsuperscript{15} compared the metabolic effects of \textit{Psyllium mucilloid} and two other non-absorbable polymers (cholestyramine and surformor) on sterol metabolism in the hamster and found that all
these polymers significantly lowered plasma total cholesterol concentration and the level of cholesterol carried in low-density lipoproteins.

Polysaccharide contains high density of hydroxyl groups and hence can be easily functionalized by chemical and enzymatic method. This chapter includes the synthesis, characterization of the modified dextran other than by etherification. Cellulose was also modified to make a comparison and their applicability also evaluated in cholesterol uptake. The alkoxide dextranate and alkoxide cellulose was prepared from dextran and cellulose by treating with alkali metal hydroxide.\(^{16}\) Dextran ethers and cellulose ethers were then synthesized from the corresponding alkoxide by chemical etherification with alkyl halide as discussed earlier in Chapter 2. Dextran esters and cellulose esters were synthesized enzymatically from the dextran and cellulose, respectively. The characterization of all the synthesized polymers was carried out by FTIR, TGA, SEM and XRD. The synthesized polymers were studied for cholesterol uptake from the body fluid.

3.2. Experimental

3.2.1. Materials

Dextran (MW 5,00,000), cellulose, lipase, cholesterol (HiMedia, Mumbai, India), sodium hydroxide, potassium periodate, bromooctane, stearic acid, lauric acid and hexane (SD Fine Chemicals, Mumbai, India), were used as received.

3.2.2. Chemical Modification of Dextran and Cellulose

3.2.2.1. Synthesis of Dextran Derivatives

3.2.2.1a. Synthesis of Dextran Ethers

Dextran ethers were prepared as described in Chapter 2.
3.2.2.1b. Synthesis of Dextran Esters

The dextran stearate was prepared enzymatically. Dextran (1.5 g) and stearic acid (1.5 g) was suspended in hexane and a specific amount of lipase (300 mg) was added. The mixture thus obtained was stirred in Chemical Reactor (M/s Autochem, US) at 60 °C for 8 h. The product obtained was washed thoroughly with methanol. The dextran laurate was also prepared by following the similar scheme.

3.2.2.2. Synthesis of Cellulose Derivatives

3.2.2.2a. Synthesis of Cellulose Ethers

Cellulose ethers, octylcellulose and hexadecylcellulose were prepared by following the similar scheme as discussed earlier for the dextran ethers (Chapter 2). The amount of cellulose, octyl bromide and hexadecyl bromide taken were 1 g, 1.1 g and 1.7 g, respectively, on the basis of the molar ratio.

3.2.2.2b. Synthesis of Cellulose Esters

Cellulose esters, cellulose stearate and cellulose laurate, were synthesized in a similar way as discussed for dextran stearate and dextran laurate under sub-section 3.2.2.1b above. The cellulose and the organic acid were taken in 1:1 molar ratio with that of the anhydroglucose unit of cellulose.

3.2.3. Characterization of Dextran Derivatives

The evidence of modification was obtained from the characterization by various physical methods. Candidate functionalized polymers were characterized by TGA, FTIR SEM and XRD to provide evidence of modification on the polymer backbones. Different biopolymer derivatives were characterized by FTIR on Nicollet 5700 Spectrophotometer in KBr. Scanning electron micrographs (SEM) for all the
synthesized polymers were recorded on Jeol JSM-6100 scanning microscope. Thermogravimetric analysis of dextran and its derivatives was carried out in double pan SHIMADZU DTG-60H (simultaneous TGA/DTA module) thermal analyzer. The thermal investigation was carried out by heating samples in a platinum crucible under nitrogen atmosphere at a heating rate of 20 °C/min. The XRD spectrum was obtained by using powdered polymer samples. The XRD patterns of samples were recorded on Philips PANANALYTICAL XPERT-PRO X-ray diffractometer using a typical wavelength of 1.54060 Å (Cu-Kα radiation).

Different cellulose derivatives were characterized by same techniques but for the TGA.

3.2.4. Cholesterol Uptake by Dextran and Cellulose Derivatives

A desirable level of cholesterol in human beings should be less than 200 mg/dL (5.17 mmol/L). The level between 200 mg/dL and 239 mg/dL (5.17-6.18 mmol/L) are considered borderline. The levels at or above 240 mg/dL (6.21 mmol/L) are considered as high total cholesterol level. Hence the concentration of stock solution prepared for cholesterol uptake studies was 240 mg/dL. The cholesterol stock solution was prepared in the body serum. The different synthesized modified dextran polymers and modified cellulose polymers were separately immersed in a specified volume of the cholesterol stock solution prepared as above at 37 °C. The specific amount of cholesterol stock solution was separated at different time intervals and cholesterol amount was estimated colorimetrically using UV-Vis spectrophotometer. The coloring agent for cholesterol determination was prepared as follows. The stock reagent was prepared by dissolving 10 g of FeCl₃·6H₂O in the deionized water using a 100 mL volumetric flask. Prior to use 1.0 mL of stock reagent was transferred into 100 mL flask and concentrated H₂SO₄ (3N) was added to make volume. To 3.0 mL of the test solution was added 2.0 mL of
FeCl₃ coloring solution and the optical density values were observed from the UV-Vis spectrophotometer at 560 nm. The concentration of cholesterol was assessed from the standard curve.

3.3. Results and Discussion

These dextran ethers and cellulose ethers were synthesized by chemical method and dextran esters and cellulose esters were synthesized by an enzymatic method. The hydroxyl groups on the dextran and cellulose backbone are the reaction site for modification of dextran and cellulose, and formation of its ethers and esters. The incorporation of long alkyl chain on the backbone rendered the dextran and cellulose derivatives less hydrophilic than the parent backbone.

3.3.1. Mechanism of Modification of Dextran and Cellulose

The dextran and cellulose were derivatized to their ethers and esters and the mechanism of etherification has been presented in Chapter 2 (Scheme 2.3) and the mechanism of esterification is proposed as under. As stated in Chapter 2 the sites available for modification of dextran are C₂, C₃ and C₄ due to α-1,6 linkage in dextran. However, in cellulose the glucopyranose units are joined by β-1,4-glycosidic linkage. Therefore the sites available for modification in cellulose are C₂, C₃ and C₆. Both the natural polysaccharides (dextran and cellulose) were first modified to their sodium salt, which easily reacts with alkyl halides to form the corresponding ethers. Preferable site for modification is 2-hydroxyl group due to its proximity to the anomeric centre of dextran repeat unit. While in cellulose it is 2- and 6-hydroxyl group that are more reactive than the OH- group at position 3. The yield of dextran stearate and dextran laurate obtained was 1.81 g and 2.70 g, respectively, when 1.5 g of dextran and 1.5 g of acid (stearic/lauric acid) was taken. Octylcellulose 1.95 g and hexadecylcellulose 2.3 g was obtained when the cellulose undergo etherification, while 1.9 g of cellulose stearate and 2.5 g of cellulose laurate were obtained when 1.5 g of cellulose underwent
esterification with 1.5 g of the acid (stearic / lauric). The esterification of both the polysaccharides was carried out by using lipase as catalyst in the non-aqueous media. The proposed mechanism of etherification is discussed in Chapter 2 (Scheme 2.3) and the proposed mechanism for esterification is presented as Scheme 3.2:

![Scheme 3.2: Mechanism of Esterification of Dextran/Cellulose](image)

3.3.2. Characterization of Dextran Modified Polymers by FTIR, TGA, and SEM

3.3.2.1. Characterization of Dextran Ethers

Details of the characterization studies of the dextran ethers have been explained earlier in Chapter 2.

3.3.2.2. Characterization of Dextran Esters

3.3.2.2a. FTIR Spectroscopy

The evidence of modification of ester formation was obtained by noting change in the intensity of peaks, shifts in their position in addition to the presence of the
additional peaks. The characteristic peaks are in the range of 3500 – 3200 cm\(^{-1}\) (for O-H stretching, due to the polymeric association) and 1200 – 800 cm\(^{-1}\) (C-O and C-C stretching vibrations of the hexopyranosyl moiety). The intensity due to O–H stretching was very much decreased in the modified dextran esters due to the consumption of the hydroxyl groups in the substitution process (Figures 3.1). In case of dextran laurate the intensity of –OH stretching gets appreciably decreased because of the higher substitution by the attachment of the lauryl group. In both the dextran esters an additional peak appears near 1701 cm\(^{-1}\) and that is ascribed to the C=O stretching.

3.3.2.2b. Characterization of Dextran Esters by TGA

The results of TGA of dextran and its derivatives are presented in Table 3.1. The thermal decomposition of dextran stearate is exhibited in Figure 3.2.1. It degraded in three steps and it has high FDT (655.8 °C). The maximum weight loss, i.e., 59.935% occurred in the second stage (188 °C – 399.5 °C). In the last stage the rate of degradation was slow which is attributed to the char formation. Dextran laurate (Figure 3.2.2) also degraded in three steps. Its FDT is 327.2 °C. The maximum weight loss, i.e., 33.81% occurred in the third stage (301.11 – 327.2) and that is attributed to degradation due to depolymerization. Prior to that anhydride formation takes place due to the loss of water by the reaction of hydroxyl groups present in the close proximity. Dextran esters showed low initial thermal stability as compared to the dextran as is evident from the IDT values. Dextran laurate had the lowest FDT value than dextran and dextran stearate indicating its faster degradation at high DT.

Decomposition temperature (DT) from thermogram for respective 10% weight losses had been calculated (Table 3.2). It is clear there from that 50% weight loss occurred at the same DT (330 °C) for dextran and dextran stearate, however at around
375 °C weight loss from dextran and dextran stearate was 80% and 70%, respectively. In dextran, an endotherm was observed at 293.8 °C and sharp exotherms were observed at 384.6 °C and 543.7 °C. In dextran stearate two endotherms were observed at 79.86 °C and 273 °C and an exotherm at 545.4 °C. In dextran laurate two endotherms were observed at 85.3 °C and 276.4 °C and exotherm at 370.9 °C. Like dextran, the exotherm in all the derivatives of dextran corresponds to the breakdown of the volatile products after maximum degradation. The maximum degradation for dextran, dextran laurate and dextran stearate occurred at 384.6 °C, 370.9 °C and 545.4 °C, respectively.

As the hydroxyl groups were consumed in the derivatization processes, the derivatives therefore exhibited different thermal decomposition behaviour than the dextran backbone. On derivatization to ester a decrease in the initial decomposition temperature was observed than dextran which can be ascribed to the oxygen absorption. The modification opens up the polymer chain of backbone and hence the modified dextran polymers showed lower IDT than dextran. The dehydration and depolymerization are considered as the two main processes associated with the degradation mechanism of polysaccharides.

3.3.2.2c. Characterization of Dextran Esters by SEM

SEM of dextran esters are presented as Figure 3.3. It is evident from the SEM of these polymers that they have different surface morphology. SEM provides evidence of changes affected in the surface morphology after derivatization. The globule like surface of the dextran was changed into porous network like structure in the case of dextran stearate (Figure 3.3.1).
3.3.2.2d. Characterization of Dextran Esters by XRD

The XRD spectra of dextran esters are plotted as the intensity of the diffracted X-rays vs. angle (2θ) and are presented as Figures 3.4. A peak of intensity 439, peak position (2θ) 21.77, \(d\) spacing (4.07 Å), % relative intensity (100) and peak area (91.28) was observed for dextran stearate. Dextran laurate has a peak of intensity 183, peak position (2θ) 20.65, \(d\) spacing (4.29Å), % relative intensity (100) and peak area (24.58). The crystallinity is reduced in the modified dextran esters. It is evident from the XRD (Figures 3.4) that dextran esters are crystalline and dextran stearate is more crystalline than dextran itself.

3.3.3. Characterization of Modified Cellulose

3.3.3.1. FTIR Spectroscopy

The spectra of the peaks within the particular polymers are presented in Figure 3.5. The FTIR spectra of the cellulose shows characteristic absorption peaks near 3400 cm\(^{-1}\) to 3200 cm\(^{-1}\) for -OH stretching, 2901 cm\(^{-1}\) for C-H stretching, 1059 cm\(^{-1}\) and 1163 cm\(^{-1}\) for C-O-C stretching (Figure 3.5.1). In addition to the additional peaks in the spectrum of the modified cellulose basic peaks of cellulose are present with a slight shift in the position of peaks. Apart from the change in the intensity of peaks, the peak due to the O-H stretching gets narrower due to the breaking of polymeric associations. The intensity of peak due to O-H stretching gets remarkably reduced in the case of hexadecyl cellulose and cellulose stearate. That indicates the higher modification in these cases. A high intensity peak at 1440 cm\(^{-1}\) and 1460.6 cm\(^{-1}\) due to the -CH\(_3\) asymmetric bending was observed in the spectrum of octylcellulose ether and hexadecylcellulose ether. In cellulose stearate and cellulose laurate a peak appears at 1717 cm\(^{-1}\) and 1779 cm\(^{-1}\), respectively, due to the C=O stretching of the ester group.
3.3.3.2. SEM Studies

SEM of cellulose and its modified forms are presented in Figures 3.6.1-3.6.3. The surface morphology of cellulose is comparably changed after modification. The round needle like structure appeared in the SEM of the cellulose ethers. Such nano-forest is due to the assembly of the alkyl chains those self assemble due to the hydrophobic interactions.

3.3.3.3. XRD Patterns

The XRD spectra of cellulose and cellulose derivatives are presented in Figure 3.7. It is evident from the XRD spectrum that the crystallinity of cellulose is affected after modification (Figure 3.7.1). Hexadecyl cellulose shows a peak of intensity 148, peak position (2θ) (24.42), d spacing (3.64 Å), % relative intensity (100) and peak area (32.0) (Figure 3.7.2). A peak of intensity 322, peak position (2θ) (24.03), d spacing (3.7 Å), %relative intensity (100) and peak area (318.71) was observed for cellulose stearate (Figure 3.7.2). Cellulose laurate (Figure 3.7.2) has a peak of intensity 319 at (2θ) (22.69), d spacing (3.91 Å), % relative intensity (100) and peak area (16.06).

3.3.4. Cholesterol Uptake Studies

3.3.4.1. Cholesterol Uptake by Dextran Modified Polymers

The concentration of cholesterol was determined by colorimetric method. The uptake was studied with stock solution of 2400 ppm at 37 °C. The dextran exhibited the least uptake of cholesterol than its modified polymers (Figures 3.8). The uptake of cholesterol in the case of dextran remains constant after 6 h. After 12 h it showed a maximum uptake of 45.20% of cholesterol. Cholesterol uptake by all the polymers
initially increased with time, and remained almost constant after 6 h. Octyldextran ether and hexadecyldextran ether were found to be more efficient with uptake efficiency of 85.48 % and 84.5 % after 4 h, respectively (Figure 3.8.1). Thereafter it decreased and even after 12 h the uptake efficiency of dextran ethers was found to be high than dextran. However the dextran esters like dextran had maximum uptake of cholesterol after 2 h, and after 6 h exhibited almost a constant uptake (Figure 3.8.2). It is clear from the graphs that the dextran ethers are more efficient adsorbent for cholesterol uptake or removal from the body fluid than the dextran and dextran esters. The octyldextran had cholesterol uptake of 82.1% even after 12 h, i.e., it is the most efficient among all the dextran-based polymers for the cholesterol removal. It is proposed that dextran have entangled chains because of interactions between the hydroxyl groups present on it, therefore, had less cholesterol uptake. Like dextran, the dextran esters also have intermolecular interactions between the hydroxyl groups and carbonyl groups that reduce the efficiency of these polymers for cholesterol uptake. But the dextran ethers has lower number of hydroxyl groups i.e. the lesser intermolecular interactions and lesser network formation, and the bulky cholesterol molecule can be easily taken up by these polymers. The hexadecyldextran exhibited slightly lower uptake of cholesterol than octyldextran that might be because the alkyl chain was long enough to curl up and hence lesser cholesterol uptake. The cholesterol is possibly held on the polymer materials by the hydrophilic-hydrophilic and hydrophobic and hydrophobic interactions. Since longer chains while promote the later type of interactions do not allow the former to happen as these curl up and inhibit the close proximity of cholesterol with the polymer backbone.
3.3.4.2. Cholesterol Uptake by Modified Cellulose

The cholesterol concentration was determined by the similar method as discussed above. Cellulose esters exhibit lower cholesterol uptake than cellulose and cellulose ethers (Figures 3.9). All the forms of modified cellulose esters exhibited first increase, then a decrease in the %uptake value upto 6 h and thereafter these remained almost the same. Cellulose exhibits an increase in the cholesterol uptake upto 4 h and thereafter remained constant. After 12 h it showed a maximum of 72.04% uptake of cholesterol. Octyl cellulose and hexadecyl cellulose exhibited an increasing trend in the cholesterol uptake efficiency upto 4 h and thereafter remained almost constant (Figure 3.9.1). The maximum uptake efficiency of octylcellulose and hexadecylcellulose is 87.91% and 86.84%, respectively. Cellulose esters had maximum uptake of cholesterol after 2 h, thereafter a decrease was observed and after 6 h exhibited almost constant uptake (Figure 3.9.2). Like the dextran ethers, cellulose ethers were also found to be more efficient comparative to cellulose and cellulose esters. Even after 12 h octyl cellulose had cholesterol uptake value 87.91%. It is more efficient in cholesterol removal among
all the polymers. Cellulose forms a strong inter-transient network by interaction between the hydroxyl groups and that result into lower uptake of cholesterol. But in cellulose ethers the crystalline structure of the cellulose is broken and result is lesser intermolecular interaction of the hydroxyl groups because modification results in the consumption of good number of hydroxyl groups (Scheme 3.4). The result is manifested in higher cholesterol uptake.

![Cellulose and Modified Cellulose](image)

**Scheme 3.4: Graphical Presentation of Cellulose and Modified Cellulose**

### 3.4. Conclusions

The dextran and cellulose were modified to their ethers and esters and their modification was confirmed by different techniques (FTIR, SEM, TGA and XRD). The cholesterol uptake efficiency of dextran and cellulose, and their modified ethers and esters were studied by colorimetric method.

Among all the synthesized polymers dextran ethers as well as cellulose ethers were found to have higher cholesterol uptake efficiency than the corresponding esters or pristine forms of the two biopolymers studied. Cellulose and its modified polymers exhibited better results than dextran and its modified polymers, the reason can be the
basic structural difference between dextran and cellulose. As dextran has 1,6-
polyglucose linkages instead of 1,4-linkage in cellulose modification/derivatization
occurs at the other hydroxyl groups than 6-hydroxyl as in cellulose and there is no
substantial change in the morphology of dextran while cellulose structure opens up
considerably to allow more interactions with the cholesterol molecules. The cholesterol
is possibly held on the polymer materials by the hydrophilic-hydrophilic and
hydrophobic and hydrophobic interactions and on derivatization with longer chains the
former are weakened. For the similar reasons ethers with moderate alkyl chain length
(C_8) shows better result than the other derivative.
Figure 3.1.1: FTIR Spectrum of Dextran Stearate
Figure 3.1.2: FTIR Spectrum of Dextran Laurate
Figure 3.2.1: TGA and DTA of Dextran Stearate
Figure 3.2.2: TGA and DTA of Dextran Laurate
Figure 3.3.1: SEM of Dextran Stearate
Figure 3.3.2: SEM of Dextran Laurate
Figure 3.4: XRD Diffraction Pattern of (a) Dextran (b) Dextran Stearate and (c) Dextran Laurate
Figure 3.5.1: FTIR Spectrum of Cellulose
Figure 3.5.2: FTIR Spectrum of Sodium Cellulose
Figure 3.5.3: FTIR Spectrum of Octylcellulose
Figure 3.5.4: FTIR Spectrum of Hexadecylcellulose
Figure 3.5.5: FTIR Spectrum of Cellulose Stearate
Figure 3.5.6: FTIR Spectrum of Cellulose Laurate
Figure 3.6.1: SEM of Cellulose (a) 50000 X and (b) 100000 X
Figure 3.6.2: SEM of Octylcellulose (a) 50000 X and (b) 100000 X
Figure 3.6.3: SEM of Hexadecylcellulose (a) 50000 X and (b) 100000 X
Figure 3.7.1: XRD Diffraction Pattern of (a) Cellulose, (b) Sodium Cellulose and (c) Octylcellulose Ether
Figure 3.7.2: XRD Diffraction Pattern of (a) Hexadecylcellulose, (b) Cellulose Stearate and (c) Cellulose Laurate
Figure 3.8.1: $P_u$ of Cholesterol by Dextran Ethers as a Function of Time
Figure 3.8.2: $P_a$ of Cholesterol by Dextran Esters as a Function of Time
Figure 3.9.1: $P_u$ of Cholesterol by Cellulose Ethers as a Function of time
Figure 3.9.2: $P_u$ of Cholesterol by Cellulose Esters as a Function of Time
Table 3.1: TGA and DTA Data for Dextran and Its Esters

<table>
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<tr>
<th>Polymer</th>
<th>Decomposition Stages</th>
<th>Decomposition Range (°C)</th>
<th>Initial D.Temp. (°C)</th>
<th>Final D.Temp. (°C)</th>
<th>DTA</th>
<th>% wt. Loss</th>
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<td>327.2</td>
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Table 3.2: Decomposition Temperature for Dextran and Its Esters

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<th>Decomposition Temperature (DT) at Every 10% Weight Loss</th>
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<th>Dextran Laurate</th>
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<td>90%</td>
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<td>331.37</td>
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<td>-</td>
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