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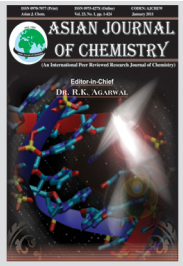

MINI REVIEW

Removal of Cholesterol by β-Cyclodextrin

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This paper reviews cholesterol removal methods by β-cyclodextrin or its derivatives. Cholesterol was complexed by β-cyclodextrin or its derivatives in two major ways *i.e.*, molecular imprinting and direct inclusion. It revealed that β-cyclodextrin dimers linked *via* different bridges exhibited much higher binding capability than β-cyclodextrin itself, of which, a β-cyclodextrin dimer linked by a thioether bridge exhibits the highest binding constant with cholesterol, which reaches up to $(3.30 \pm 0.80) \times 10^6$.

Key Words: Cholesterol, Removal, β-Cyclodextrin, Molecular imprinting.

INTRODUCTION

Cholesterol is a cylclopentapolyhydrophenanthrene derivative, which was found in cholelith as early as 18th century. Cholesterol exists in the body of animals extensively, abound in brains and nerve systems, also in kidney, spleen, skin, liver and bile. Its solubility is similar to fat, insoluble in water and soluble in ether, chloroform, *etc*. Cholesterol is an essential material in animal tissue cells, able to take part in the construction of cell membrane and they are also the raw materials to synthesize bile acid, vitamin D and steroid hormone. Cholesterol has extensive physiological action inside bodies, however, high content of cholesterol will lead to atherosclerosis, venous thrombus, *etc*. As a result, it's crucial to decrease unnecessary high content of cholesterol in order to maintain good health. So far, extensive studies have been carried out in the efficient removal of cholesterol by different means, such as steam distillation, supercritical fluid extraction, extraction with different solvents. Among these, β-cyclodextrin has been widely used in the efficient removal of cholesterol in the dairy industry.

Cyclodextrins are cyclic organic compounds obtained by enzymatic transformation of starch. Among the class of "host" molecules, β-cyclodextrin is one of the most abundant natural oligomers and corresponds to the association of seven glucose units which cavity exhibits a hydrophobic character whereas the exterior is strongly hydrophililic. This peculiar structure allows various substrates to be included in the cavity *via* non-covalent bonds to form what is called supramolecular complexes (also called inclusion complexes). This paper reviews the removal of cholesterol by β-cyclodextrin series of host compounds in the past 20 years, which will provide to the researchers some hints in the design of medicines for the efficient removal cholesterol from human bodies.

Cholesterol removal methods by β-cyclodextrin series of host compounds

Molecular imprinting of β-cyclodextrin/cholesterol: Molecular imprinted polymers (MIPs)[1,2] have been extensively investigated in the field of diagnostics and clinical analysis[3], drug delivery[4], environmental monitoring[5], *etc*. Asanuma *et al.*[6] devised a molecular imprinting technique to choose cyclodextrin to fit appropriated protions of large guest molecules.

Molecular imprinting technique is to utilize the molecular recognition function between a template molecule and the monomers which are employed in the polymerization of a rigid macroporous polymer to create a network of microporous cavities within the polymer matrix. After removal of the matrix, the rigid macroporous polymer provides a rigid microporous environment to bind guest molecules. The range of template is wide-from low molecular compounds[7-9] to macromolecules, *e.g.*, proteins[10-13].

Cleide *et al.*[14], devised a silica matrix, imprinted with the inclusion complex of β-cyclodextrin/cholesterol, in which tetraethoxysilane was used as a pure silica matrix (PSM) and also a precursor for the molecular imprinting of β-cyclodextrin/cholesterol (MIP/β-CD:chol). MIP/β-CD:chol has a high cholesterol adsorption and the adsorption fits the Langmuir

isotherm model. The maximum adsorption capacity is 76.5 mg cholesterol/g-adsorbent. However, if only pure silica matrix was used as the template, the maximum cholesterol adsorption was much higher, 251 mg/g. It's the porous structure that plays a key role in the adsorption. If a more open porous structure was designed, greater cholesterol adsorption capacity would be achieved.

Katarzyna *et al.*[15], devised a photochemical molecular imprinting technique for the removal of cholesterol, in which β-cyclodextrin was modified with cinnamoyl chloride (BCC) and crosslinked with either hexamethylenediisocyanate (HMDI) or toluenediisocyanate (TDI) under UV light, as a result, the molecular imprinted polymers were obtained photochemically. After BCC was photo-crosslinked to form polymeric matrix, its adsorption to cholesterol was twice as much compared to nonphoto-crosslinked material under the same conditions, which could reach 11.2 mg/g-MPI. Hexamethylenediisocyanate was found to be a better crosslinking agent and a higher adsorption capacity compared to toluenediisocyanate. It's probably due to the greater sizes in the matrix of hexamethylenediisocyanate. However, the adsorption capacity of BCC was much lower than MIP/β-CD:chol[14] which could reach up to 251 mg/g-adsorbent.

Takayuki *et al.*[16], reported the adsorption of cholesterol by imprinted β-cyclodextrin polymers which was crosslinked by toluene 2,4-diisocyanate, cholesterol as the template. The binding activity of imprinted β-cyclodextrin polymers was twice as much of non-imprinted polymers prepared by crosslinking β-cyclodextrin with toluenediisocyanate in the absence of templates. The adsorption was reversible and carried out in water/THF mixtures. Under the experimental conditions (25 ºC), 70 % of the cholesterol was bound by the imprinted β-cyclodextrin polymer and more than 85 % of the equilibrium amount was adsorbed within 1 min, which shows that the adsorption was efficient and prompt.

Removal of cholesterol by β-cyclodextrin derivatives: β-Cyclodextrin can be modified in order to increase its solubility, decrease its toxicity and increase its capacity to include guest molecules. As a result, β-cyclodextrin derivatives have a wide application in pharmaceutical industry, food industry and environmental industry, *etc*. Due to the variety of modification, β-cyclodextrin and its derivatives have been extensively studied in cholesterol complexation and removal[17-26]. After appropriate modification, β-cyclodextrin can efficiently extract cholesterol, *e.g.*, hydroxypropyl-β-cyclodextrin could efficiently extract membrane cholesterol from red blood cells[27,28]. β-Cyclodextrin or its 2-hydroxypropyl or methyl derivatives could extract cholesterol from cultured cell membranes[29,30] or to change cell membrane cholesterol levels at will (both depletion and repletion[30,31].

Methyl substituted β-cyclodextrin (MβCD) is widely applied in cholesterol depletion in mammalian cells[32]. Due to the damage of intramolecular hydrogen bonding caused by the existence of methyl groups, methyl substituted β-cyclodextrin has a better solubility in aqueous media and lower toxicity to mammalian cells than β-cyclodextrin, which will have a better application prospect. Igor *et al.*[33] reported that 1 or 2 mM concentration of methyl substituted β-cyclodextrin can reduce cholesterol inside T lymphoma Jurkat cells up to 63 ± 5 or 75 ± 4 %, respectively. Appropriate methyl substituted β-cyclodextrin concentration is crucial to cholesterol removal, since methyl substituted β-cyclodextrin can cause cell damage[34] and disruption of lipid bilayers[35].

β-Cyclodextrin dimers linked *via* different linkage are also widely used in cholesterol removal, since the cholesterol length is roughly twice the length of β-cyclodextrin cavity. Many cyclodextrin oligomers have been synthesized and investigated recently[36-38]. The research focused on the design of the linkers and their physiological effects.

Alcalde *et al.*[39] synthesized a series of β-cyclodextrin dimers linked by succinic acid (**1**) or ethylene diamine tetraacetic acid, EDTA (**2**), a β-cyclodextrin monomer modified by amino acid followed by succinic acid (**3**) (Fig. 1). Their ability to extract cholesterol was investigated. Their results showed that the above appropriately modified dimers or monomer had a great efficiency in cholesterol removal. The stoichiometry of the complexes between β-cyclodextrin dimers (**2**, **3**) and cholesterol is 1:1, while the monomer (**1**) forms two complexes with molar ratios of 1:1 and 1:2 (cholesterol/monomer). The binding constants are listed in Table-1, which clearly verifies that appropriate modified β-cyclodextrin dimers have much stronger binding effects with cholesterol than monomers.

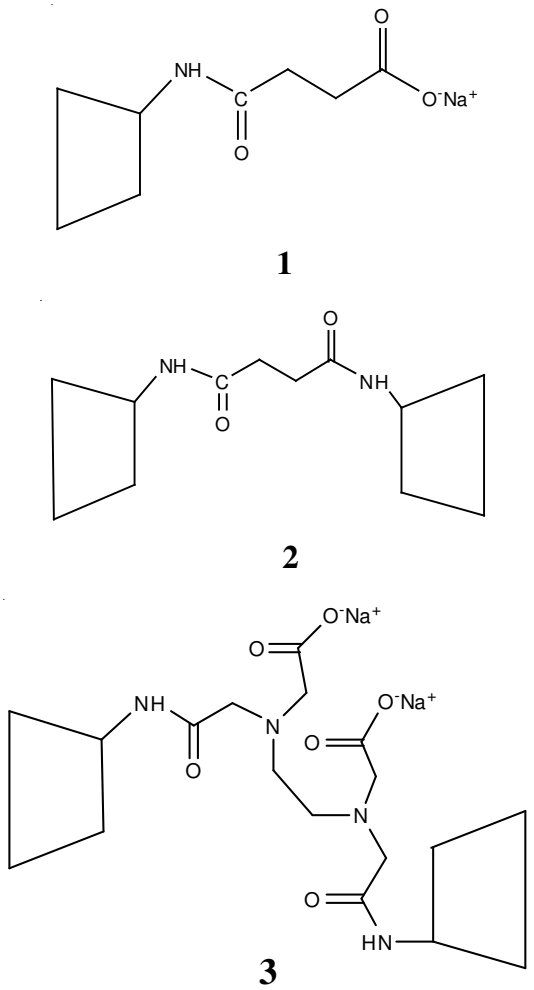


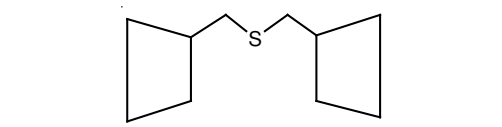
Fig. 1. β-cyclodextrin dimers synthesized by Alcalde *et al.*[39]

TABLE-1
BINDING CONSTANTS OF β-CYCLODEXTRIN DIMER **1-3** AND CHOLESTEROL IN WATER AT 25 ºC

Host	Guest	K_a (M^{-1})
2	Cholesterol	$(5.9 \pm 0.3) \times 10^4$
3	Cholesterol	$(8.8 \pm 0.2) \times 10^4$
1	Cholesterol	73 ± 19 ($K_{1:1}$)
1	Cholesterol	204 ± 65 ($K_{1:2}$)

Table-1 lists the binding constants of dimer **1-3** with cholesterol.

Breslow and Zhang[20] synthesized a series of β-cyclodextrin dimers linked by a thioether bridge or hydroxypropyl linked polymer (Fig. 2) and their inclusion process with cholesterol and its enhancement of cholesterol solubility in water were studied.



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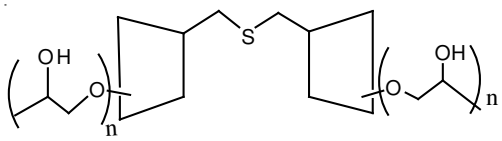

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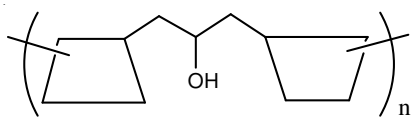

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Fig. 2. β-Cyclodextrin dimers synthesized by Breslow and Zhang[20]

The following table (Table-2) lists the binding constants of Host-Guest complexes in water at 25 ºC.

TABLE-2
BINDING CONSTANTS BETWEEN HOST AND GUEST IN WATER AT 25 ºC

Host	Guest	K_a(M^{-1})
4	Cholesterol	$(3.30 \pm 0.80) \times 10^6$
5	Cholesterol	$(1.47 \pm 0.62) \times 10^5$
6	Cholesterol	$(5.07 \pm 0.58) \times 10^4$
β-Cyclodextrin	Cholesterol	1.7×10^4
Hydroxypropylated β-CD	Cholesterol	1.9×10^4

From Table-2, it is clear that the dimer linked *via* thioether has the strongest binding capability to cholesterol whose binding constant reaches up to $(3.30 \pm 0.80) \times 10^6$.

During the inclusion of cholesterol by β-cyclodextrin, water was also involved. Claudy *et al.*[40], investigated the inclusion phenomenon of cholesterol by β-cyclodextrin by means of DSC, TG, X-ray diffraction and ^{13}C NMR spectroscopy and suggested that three molecules of host molecules were necessary to include cholesterol completely due to the large size of cholesterol, meanwhile, 17 % weight of water corresponding approximately to 42 molecules of water were included in the host-guest complexes in the forms of hydrogen bonding. Only under low temperature, anhydrous inclusion can be made. Thermal degradation of the complex begins at 200 ºC which reveals that cholesterol and β-cyclodextrin form a stable inclusion complex.

Conclusion

This paper summarizes the inclusion of cholesterol by β-cyclodextrin and its derivatives. β-Cyclodextrin can form stable inclusion complex with cholesterol. After appropriate modification, β-cyclodextrin dimers or polymers will have more recognition sites and larger cavities to include cholesterol, meanwhile, water will involve the inclusion process. As a result, the binding constant of β-cyclodextrin dimers or polymers will be increased in a remarkable degree in comparison to β-cyclodextrin. Among the published results, the β-cyclodextrin dimer linked by a thioether bridge exhibits the highest binding constant with cholesterol, which reaches up to $(3.30 \pm 0.80) \times 10^6$. Due to the multiple diseases caused by unnecessary high content of cholesterol, in order to decrease the high content of cholesterol in human organs, *e.g.*, blood, as for the design of appropriate β-cyclodextrin dimers or polymers, low toxicity and minimal side effects must be taken into serious consideration. After undergoing clinical diagnosis, β-cyclodextrin derivatives can be made in the form of peroral medicine or direct hyperdermic injection. Effective removal of cholesterol will be no longer an obstacle for human beings.

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