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# Estimation of Cholesterol Content in Ghee, Edible Oil, Heated Edible Oils and Its Removal by B Cyclodextrin and Its Nanosponge

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**Abstract**

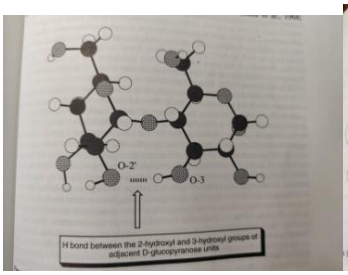
**β cyclodextrin is a cyclic oligosaccharide composed of seven glucose units linked by a 1-4 glucosidic linkage have a truncated cone shaped structure. The inner hydrophobic cavity can trapped many organic compound and form complexes, and the outer hydrophilic surface form complex with polar compound by hydrogen bonding, its nano sponge were synthasised through structural modification using cross linking agent EDTA form highly porous branched matrics nanostructure were assed by IR, FESEM, EDX. used to overcome the problem of cholesterol from human body. We use various brands of ghee like Amul ghee, Sanchi ghee, Purasure ghee and Nova shudh ghee, and different vegetable oil like Soyabean oil, Groundnut oil and Mustard oil, the wrapper of these oil packet mension zero cholesterol but studies shows that they contain cholesterol and these cholesterol content is increases on heating, overeating of these oil and ghee elevate the blood cholesterol level and responsible for cardiovascular disease. In this paper determination of cholesterol in oil and ghee is carried out by Libermann Burchard Spectrophotometric method and its removal is also successively carried out by β cyclodextrin and its nano sponge and it found to be very efficient for removing of 90% of cholesterol. This method is cost effective and ecofriendly.**

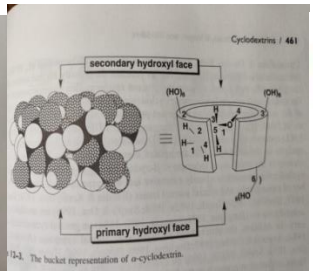
**Keywords: cholesterol, β cyclodextrin, Nanosponge**

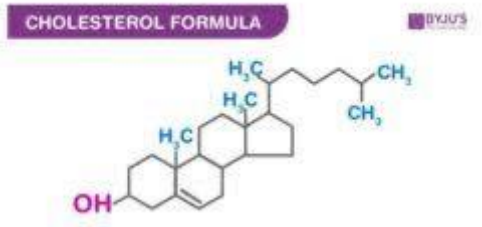
## Introduction:

B cyclodextrin is a non hygroscopic and chemically stable molecule, to improve the efficiency of β cyclodextrin it is modified as nanosponge, free availability of hydroxyl groups of β cyclodextrin makes them suitable for crosslinking with other molecules[1].β cyclodextrin based nanosponge have more interaction sites and higher encapsulation capabilities, higher stability, biocompatibility, encapsulation efficiencies and control over their partical size, solubility and permeability, these are used to reduce cholesterol from food products. Oil is a general term for liquid fat derived for plants while ghee is a clarified butter. Usually oils are full of polysaturated fats which might cause inflammation in the body thus lesser quantities of oil are suggested for the human body and use of heating oil is drastically affects the good cholesterol level of the body and responsible for cardiovascular disease, ghee is a complex lipids of glycerides, free fatty acid, phospholipids, sterol, sterol esters, fat soluble vitamins, carbonyls, hydrocarbons, carotenoids, traces of iron and calcium,98%of ghee is glycerides and rest 2% is cholesterol ,cholesterol is the major component of ghee with at least 95% of total sterols(300 mg per 100 gm of fats).The composition of ghee is not changed up to the 150ºC temperature because of presence of

butyric acid(a short chain fatty acid) but after that deterioration occurs. Due to the cholesterol rich food and its processing the oxidative products are formed which are known as oxysterols undergo further deterioration causes mutagenicity,cytotoxicity,carcinogenicity[2].Almost every adult living in industrialized nation develops some degree of atherosclerosis leads to strokes, heart attacks and other serious health problems, the heart disease is linked to risk factors, the principal risk factors are high cholesterol, high blood pressure, other risk include diabetes, obesity, family history of heart disease and stress. Each animal cell both human and non human contains cholesterol, cholesterol is important in some of the functions of the liver cell and produces all the cholesterol the body needs[3]. There is no need to consume cholesterol in diet.[4] Cholesterol and fats move through the body protein packeges called lipoproteins low density(LDL)carry cholesterol to the organs through arteries, the LDL deposit their load through the inner walls of the arteries and promote atherosclerosis ,they are known as bad cholesterol, the higher the LDL level there is a greater risk of heart problems. Good cholesterol found in high density lipoproteins (HDL) moves back to the liver where it is disposed of. People who exercise regularly ,do not smoke and stay their ideal weight tend to have higher level of HDL's. The higher intake of ghee, oil and heated oil creates a fear of hypercholestermia, heart disease leading to heart cancer, there are number of physical, chemical, biological process have been developed for the reduction of cholesterol content of dairy product and oil but the only method involving the use of β cyclodextrin and its nanosponge synthasised by EDTA have been found success.




**Materials and Methods:-**

All the chemicals used in the experiment were analytical grade purchased from merk, β cyclodextrin, the samples of pure ghee, edible oil, cholesterol, Libermann Burchard reagent (acetic anhydride & sulphuric acid),chloroform. ethanol(sigma Aldrich)

**Instrument:**

UV –Visible Apel spectrophotometer

**Standard Cholesterol Solution**

10 mg of standard cholesterol dissolved in 10 m;l chloroform, shaked well.

**Libermann Burchard Reagent:**

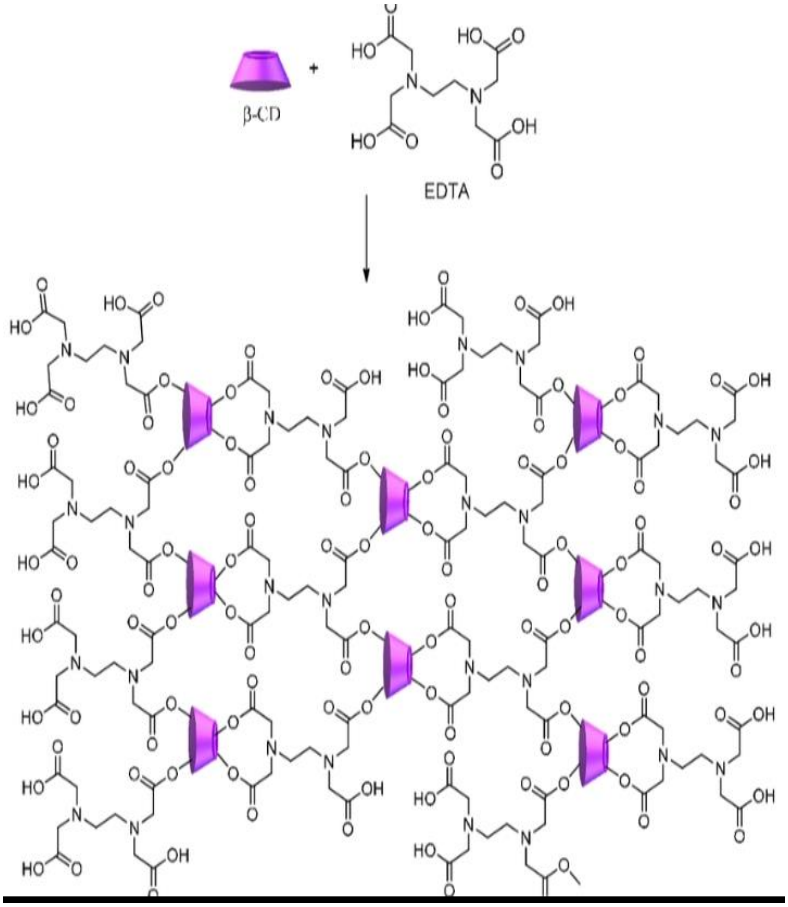
0.5 ml of sulfuric acid dissolved in 10 ml of acetic anhydride, coverd and kept in ice bucket

**Libermann Burchard Method**

The Libermann Burchard Method is a colorimetric method in which cholesterol react with chloroform, acetic anhydride and concentrated sulfuric acid to produce characteristic green colour which is measured spectrophotometrically.

**Preparation method of nanosponge:-**

;- 5 gm of B cyclodextrin mixed with 8 ml of NaOH solution and stirred for 24 hr at room temperature 0.7 ml of EDTA was mixed into the reaction mixture and allowed to stirred for 3 hr at 30 *c* acetone was added in to the reaction mixture and solution was allowed to stand for 24 hr at 50 c ,white precipitate were obtained filtrate and dried it in oven at 60 c ,acetone is added again filtrate them dried it in oven at 60 c and characterized by IR spectroscopy and found to be very efficient for removal of cholesterol[5].




**Cholesterol detection method**

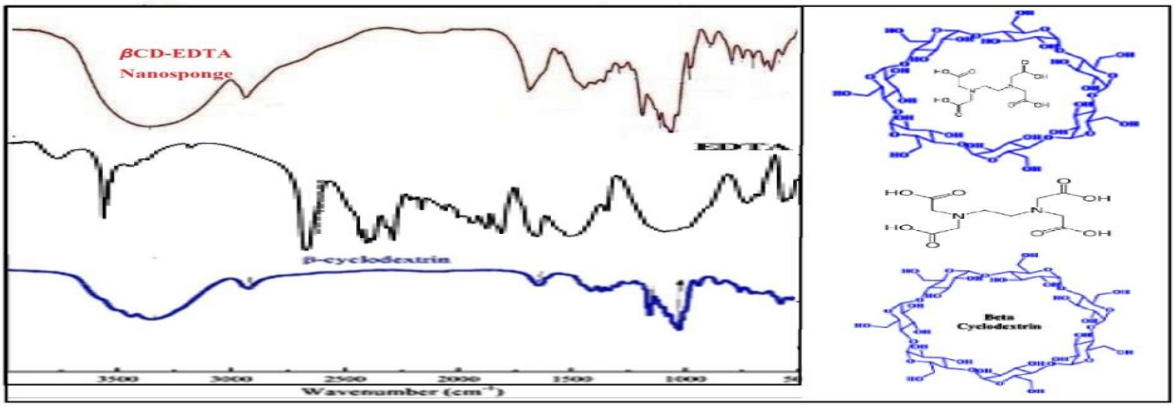
1ml of ghee or oil sample taken in a beaker add 5 ml of chloroform in it add 2 ml of Libermann Burchard reagent, a green colour appears, this indicate the presence of cholesterol. Now take the absorbance of this solution on spectrophotometer, this is the absorbance for cholesterol.[5]

**Removal Method of Cholesterol**

Take 1 ml of ghee or oil sample in a beaker add 5 ml of ethanol in it then allowed to cool at -20$^0$C for 2 hr. After 2 hr solution was precipitated for the separation of two phases and supernatant fraction was collected, allow the supernatant fraction on water bath for removal of ethanol for 5 minutes. This fraction was mixed with 3 gm of β cyclodextrin dispersed in 6 ml of ethyl acetate and 6 ml of distilled water, this sample was stirred on magnetic stirrer for 15 minutes ,then allow to centrifuge for 20 minutes at 2000 rpm white precipitates were obtained and dried it at room temperature Take 1 gm of this precipitate in a beaker add 50 ml of distilled water in to it and add Libermann Burchard reagent, colour changes of the reaction mixture indicate that cholesterol trapped by β cyclodextrin .Now take the absorbance on spectrophotometer[6]

**Results & Discussion**

We have synthesized βCDNS and characterized by IR spectroscopy, β CD & synthesized βCDNS was used for removal of cholesterol in oil and ghee. βCDNS formation was also characterized using IR vibrational spectroscopy, commonly this study focuses on comparing the signals of native βCD and βCD forming nanosponges and recognizing the vibrational signal of the carbonyl group ,which is an indicator of β CD crosslinking. Fig.shows IR spectra of β CD and βCDNS. Characteristic peaks of β CD are observed at 3363cm$^{-1}$ {O-H alcohol streaching}2924 cm$^{-1}${C-H streaching},1417cm$^{-1}$ ,1368cm$^{-1}$ 1157cm$^{-1}${O-H bending}1080 cm$^{-1}$ and1029 cm$^{-1}${C-O streaching}.These data are consistent with literature data{ }For β CDNS the characteristic peaks are located mostly in the same regions observed for β CD, but with shifts or variations in intensity due to changes in the chemical environment .These were observed at 3366 cm$^{-1}$ {O-H alcohol streaching},2928 cm$^{-1}${C-H streaching},1645cm$^{-1}${c=O streaching,1367,1234,and 1155cm$^{-1}${O-H bending}and1079 cm$^{-1}$ and 1030cm-1{C=O streaching},3400-3500 cm$^{-1}${N-H streaching}

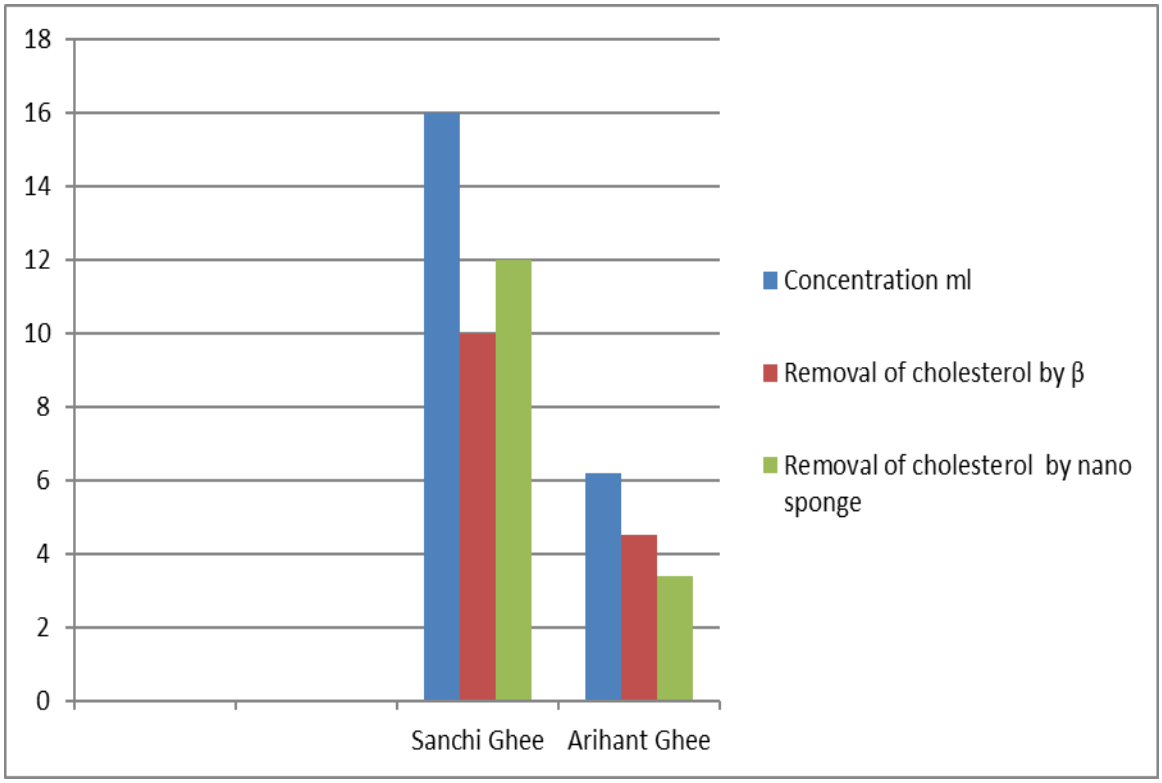


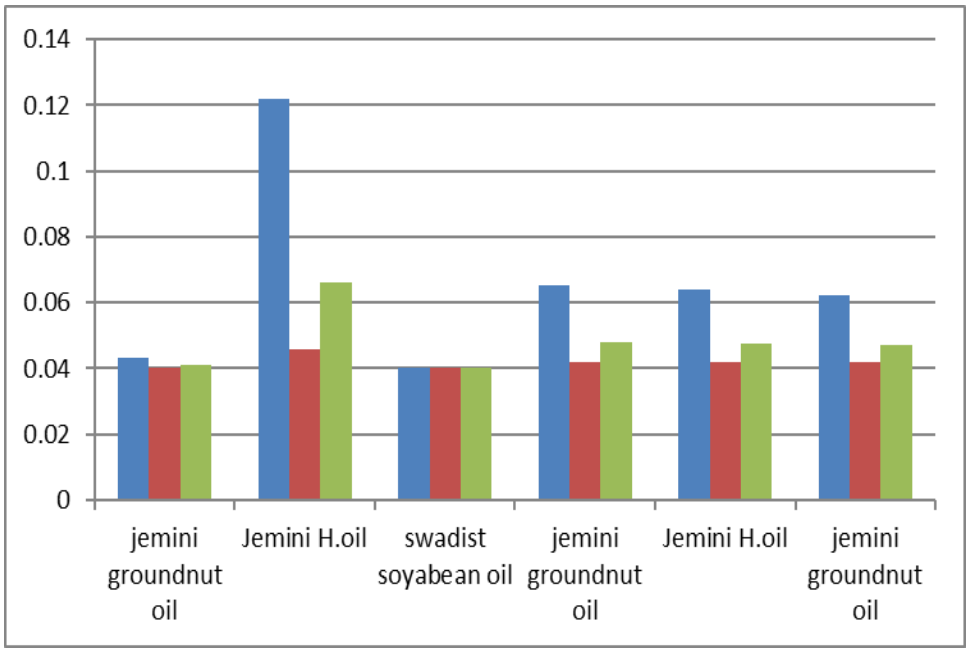

**Characteristics of β CdNs;-**

The presence of EDTA in the modified β-CD material was verified by FTIR spectroscopy. The characteristic peaks of the carbonyl groups of esters and carboxylic groups were observed at 1738 and

1679 $cm^{-1}$.The peak were observed at 1025 $cm^{-1}$,representing the OH bending vibration and OH symmetric, while asymmetric streaching was noted at 3300-3400 $cm^{-1}$ [1,8] .The C-O-C vibration was noted at 1153 $cm^{-1}$ and C-H vibration was also found at 1153 and 1029 $cm^{-1}$ [1,9]

It is clear from the result that cholesterol in ghee and oil is present in higher amount and it is effectively removed by β cyclodextrin and its nano spomge.In case of Sanchi ghee 16ml of cholesterol is present and 10ml is effectively removed by β cyclodextrin and 12ml is removed by β cyclodextrin. In case of Arihant ghee 6.2ml of cholesterol is present and 4.5ml is removed by β cyclodextrin and 3.4ml is removed by β cyclodextrin nano sponge. In case of purasure ghee 4.7ml cholesterol is present and 3.4ml is removed by β cyclodextrin and 3.2ml is removed by β cyclodextrin nano sponge. In case of Gopi Shree Pure Ghee 6.8 ml of cholesterol is present and 2.6ml is removed by β cyclodextrin and 3.4ml is removed by β cyclodextrin nano sponge. In case of Nova Shudh Ghee 3.7ml of cholesterol is present and 2.5ml is removed by β cyclodextrin and 3.2ml is removed by β cyclodextrin nano sponge. In case of oil it is formed that mustard oil have lowest cholesterol its absorbance is 0.004 and after removal by and groundnut oil have highest cholesterol

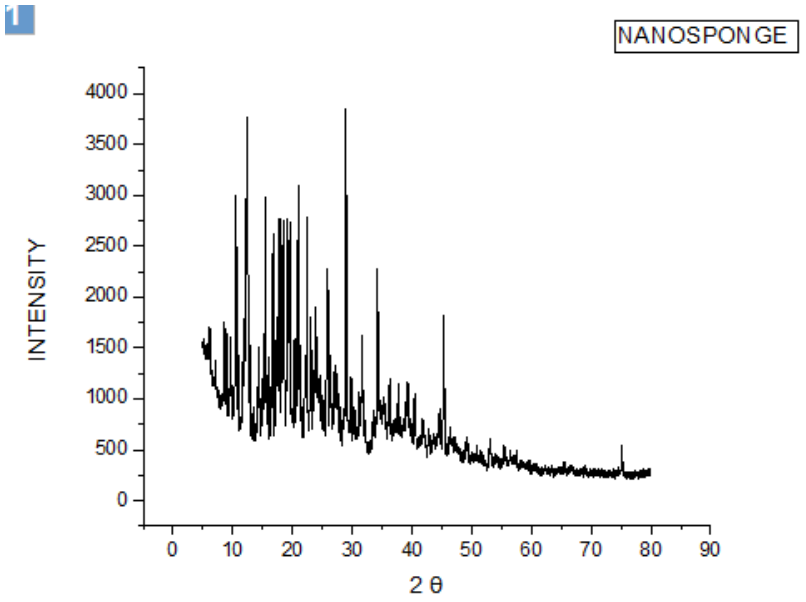





Concentration removal by B cyclodextrine removal by nano sponge

It is clear from bar diagram that cholesterol is present up to certain amount shown by blue bar and it is effectively removed by nanosponge shown by green bar as compared to β cyclodextrine shown by red coloured bar.

**XRD Analysis**




X-ray powder diffraction pattern analysed and compared with literature for the β cyclodextrin nanosponge. The sharp peaks in the graph for intensity vs 2θ values indicate the amorphous nature of the sample.X ray powder diffraction pattern shows numerous reflection in low to high angle region indicative of amorphous phase. Careful observation of the pattern in this figure reveals the fact that this

pattern are different from the starting material. The 2 θ value for β cyclodextrin is found to be 14º and 23º,this value of 2θ change when interaction occurs with

**Conclusion**

It is clear from the result that cholesterol is already present in edible oil & ghee and it is increases on heating. About 99% of Indian kitchen heated oil is used, so by the use of β cyclodextrin & its nanosponge cholesterol is successively controlled It is a very safe, easy, ecofriendly &cost effective method to control the amount of cholesterol.

**Acknowledgement**

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