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ULTRAVIOLET SPECTROSCOPY AS A PRELIMINARY ANALYTICAL TOOL FOR ASSESSING THE FORMATION OF CYCLODEXTRIN NANOSPONGES

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ABSTRACT

The aim of present study is to evaluate the utility of ultraviolet spectroscopic method for determination of synthesis of β-cyclodextrin based nanosponge. These nanosponges were prepared by melt method, solvent assisted synthesis, ultrasound assisted synthesis and microwave assisted synthesis using β-Cyclodextrin as a polymer and DPC as a cross-linker. FTIR spectra of all the synthesized nanosponges were recorded and the characteristic carbonate peak was obtained at 1740 cm^{-1}. Further spectral analysis was performed using ultraviolet radiations as per the objective. Therefore, ultraviolet spectral copies of all the starting materials, intermediate and end products; nanosponges were recorded and compared in the wavelength region of 190 to 380 nm. β-Cyclodextrin didn't show any peak while DPC showed two peaks at 256 and 217 nm corresponding to phenyl rings and carbonyl groups. However, the hypsochromic shift in λ_{max} value of carbonyl group in synthesized β-CD based nanosponges was observed and prominent peak appeared at 205 $\pm$ 2 nm. This shift can be attributed to the presence of β-Cyclodextrin molecules on either side of carbonyl group in nanosponge rather than phenoxy group present in DPC. Hence, the study could point that ultraviolet spectroscopy can be used as a preliminary tool for confirming the synthesis of β-CD based nanosponges.

Keywords: β-Cyclodextrin, nanosponge, ultraviolet spectroscopy, analytical method

1. INTRODUCTION

Cyclodextrin based nanosponges are the virus sized, solid, three-dimensional scaffold like structures of a long-chain polymer; cyclodextrin and cross-linker. They are biodegradable, nontoxic, non-irritant and highly stable materials which can withstand higher temperatures up to 130ºC and pH in the range of 1 to 11 [1]. In pharmaceutical industry, they represent a relatively novel class of drug carrier for delivery of poorly water-soluble drug molecules, peptides, gas molecules, cosmeceuticals etc. They are also used for controlled drug release purpose as well as for fortification of degradable compounds [2]. Cyclodextrin nanosponges are highly porous nano-vehicles prepared by cross-linking cyclodextrin polymer with different cross-linkers belonging to acid anhydrides, diisocyanates, dicarboxylic acids, dicarboxylic acidchlorides, alkyl dihalides, chlorhydrins, category. Primary hydroxyl group of cyclodextrin is mainly involved in cross-linking process and the carbonate bond is formed during this reaction [3]. Among different types of cyclodextrins, β-cyclodextrin is primarily chosen for nanosponge synthesis because of its larger inner cavity size where it can entrap a wide range of lipophilic moieties. The hydrophilic moieties are also entangled in the canals of nanosponges [4].

The drug can be loaded in nanosponges by a variety of methods like impregnation, freeze drying, spray drying or solvent evaporation. The formed nano-solid structures encapsulate drug in the “sponge” like matrix of nanosponge. These drug loaded nanosponges can further be formulated in the form of oral, parenteral, topical or inhalational dosageforms by mixing with suitable excipients [5]. These medicated nanosponge formulations demonstrated supremely improved solubility, permeability, bioavailability and stability of drugs in several studies.

The synthesis of cyclodextrin based nanosponges is carried out mainly by four methods as melt method, solvent assisted synthesis, ultrasound assisted synthesis and microwave assisted synthesis. The formed nanosponges have a size range of 10 nm to 1000 nm. They are further characterized by different analytical methods like FTIR,Raman spectroscopy or thermal methods to study their foundation [6].

The formation of nanosponge can be confirmed by FTIR spectroscopy where an intense peak is observed in the wavelength region of 1700 to 1750 cm^{-1} which corresponds to formation of carbonate bond [7]. This confirmation can also be achieved by UV-Visible spectroscopy which is a further simple, fast, economic yet reliable

technique. Therefore, the present study aims to use UV-Visible Spectroscopy as a preliminary analytical tool to check the formation of nanosponge. The FTIR spectra of the same samples were also recorded and evaluated with UV-spectra.

2. MATERIALS AND METHODS

2.1.Materials

Anhydrous β-cyclodextrin (BCD) and Diphenyl carbonate (DPC) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Acetone, Dimethyl formamide (DMF), Ethanol, Methanoland triethylamine (TEA) were obtained from Merck Life Sciences Pvt. Ltd., Mumbai, India. Deionized water was used for all the experiments.

2.2.Methods

2.2.1. Preparation of plain beta cyclodextrin nanosponges

Different beta cyclodextrin nanosponges were prepared by melt method (CDNS-MM), ultrasound assisted synthesis (CDNS-UAS), solvent assisted synthesis (CDNS-SAS) and microwave assisted synthesis (CDNS-MAS) using diphenyl carbonate (DPC) as a cross-linker. The 1:4 molar ratio of anhydrous β-cyclodextrin and DPC was used during all the synthetic procedure. All the synthesis methods are summarized in **Figure 1**. Both the excipients were finely homogenized in a mortar and pestle and weighed accurately.

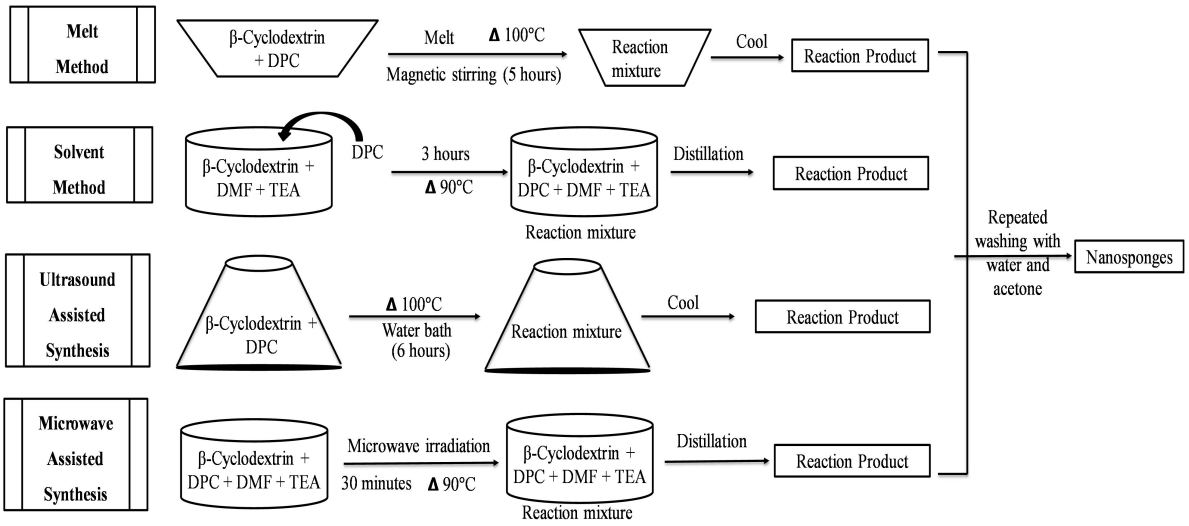

Figure 1: Synthesis of β-cyclodextrin nanosponges

2.2.1.1. Synthesis of β-cyclodextrin nanosponges by melt method (MM)

In melt method, weighed quantity of anhydrous β-cyclodextrin was added to the molten DPC under magnetic stirring. The mixture was heated to 90–100°C for 5 hours under continuous stirring. The phenol crystals formed at the neck of flask during this process were removed properly during regular time intervals. After completion of

reaction, the reaction mixture was allowed to cool to room temperature and the obtained solid product was washed with distilled water repetitively to remove unreacted β-CD. Further washing with ethanol was then carried out to remove phenol and unreacted DPC. The test for phenol was carried out after every washing by using 1% w/v ferric chloride solution. The washed product was then dried at 60°C in hot air oven for 3 hours. The final product so obtained was a white, fine powder which was stored at room temperature in a desiccator for further use [8].

2.2.1.2. Synthesis of β-cyclodextrin nanosponges by solvent assisted synthesis (SAS)

In preparation of cyclodextrin based nanosponges by solvent assisted synthesis method, anhydrous β-cyclodextrin was weighed and dissolved in definite amount of DMF. The mixture was then sonicated and for 10 minutes and stirred on a magnetic stirrer for 5 minutes to get a uniform solution. Weighed quantity of DPC was then added to it, followed by the addition of 1 ml of TEA. The solution was stirred further for 3 hours on a magnetic stirrer at a temperature of 90 ºC. After the completion of reaction, the solution was allowed to cool to room temperature and the solvent was distilled out. The obtained crude nanosponges were purified with water and acetone, were dried in hot air oven and stored in a desiccator for consequent use [9].

2.2.1.3. Synthesis ofβ-cyclodextrin nanosponges by ultrasound assisted synthesis (UAS)

In ultrasound assisted synthesis, weighed amounts of anhydrous β-cyclodextrin and DPC (1:4 molar ratio) were mixed in a mortar and pestle and taken in a conical flask. This mixture was heated to 100ºC for 6 hours by means of water bath. During heating process, the crystals of phenol get formed at the neck of flask; which were removed carefully in a regular manner. After reaction completion, the mixture was kept aside to cool to room temperature and was purified by repeated washing with water and acetone as described above. The obtained product was fine, white powder that was stored in a desiccator at room temperature for further use [10].

2.2.1.4. Synthesis of β-cyclodextrin nanosponges by microwave assisted synthesis (MAS)

Microwave assisted synthesis method employed the use of microwave radiations for rapid synthesis of cyclodextrin based nanosponges. Briefly, weighed quantities of anhydrous β-cyclodextrin and DPC were dissolved in 30 ml of DMF and to it 1 ml of TEA was added. The mixture was the

placed in a microwave system, 2450 MHz. The temperature of the system was adjusted to 90 ºC, the power was maintained at 420 W and the reaction was carried out for 30 minutes. After the elapsed time, solvent of reaction mixture was removed by distillation and the obtained product was purified with water and acetone. The obtained white was dried in hot air oven for 3 hours at 60°C and stored in a desiccator for subsequent use [11].

2.2.2. Fourier-transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra were recorded using a Perkin-Elmer Sp.2 spectrometer within the wave number region of 4000-500 cm^{-1}.

2.2.3. Ultraviolet spectroscopy

The ultraviolet analysis of β-cyclodextrin, DPC, physical mixture of β-cyclodextrin and DPC in 1:4 molar ratio and β-cyclodextrin based nanosponges synthesized by all the methods were carried out in the wavelength region of 190 to 380 nm. An incompletely synthesized nanosponge was also analysed by UV spectroscopy. Every sample of concentration 20 µg/ml was prepared using methanol as solvent.

3. RESULT AND DISCUSSION

3.1. Percentage yield of nanosponges

The percentage yield of β-cyclodextrin based nanosponges (1:4 molar ratio) synthesized by all the four methods is shown in **Table 1**. The yield was found to be high when the nanosponges were synthesized by melt method and ultrasound assisted synthesis method.

3.2. Fourier-transform infrared spectroscopy

The FTIR spectra of β-cyclodextrin, DPC, their physical mixture prepared in 1:4 molar ratio is shown in **Figure 2**. The FTIR spectrum of β-cyclodextrin showed broad aliphatic hydroxyl O-H stretch at 3293 cm^{-1} and the corresponding aliphatic C-H stretch at 2926 cm^{-1}. The C-O stretch was also observed at 1080 cm^{-1}. The characteristic peaks of DPC at 1778 cm^{-1} and 1758 cm^{-1} were also seen in the FTIR spectrum of DPC. The physical mixture of β-CD and DPC also showcased the characteristic peaks of the both in its FTIR spectrum. The formation of β-cyclodextrin based nanosponges synthesized by all the four methods, namely, melt method, solvent assisted synthesis, ultrasound assisted synthesis and microwave assisted synthesis were confirmed by FTIR spectroscopy and their FTIR spectra are shown in **Figure 2**. The FTIR spectra of all the nanosponges show a characteristic peak of carbonate bond at 1740cm^{-1}which confirmed its appropriate synthesis [7].

3.3. Ultraviolet spectroscopy

The recorded ultraviolet spectrum of the starting materials namely, β-CD, DPC, their physical mixture, the synthesized nanosponges and an incompletely synthesized nanosponge are shown in the **Figure 3**. The corresponding peaks, i.e. the λ_{max} values and their absorbance readings are shown in **Table 2**.

The ultraviolet spectrum of β-Cyclodextrin does not show any peak in the wavelength region of 190 to 380 nm while the UV spectrum of DPC show two prominent peaks at 256 nm and 217 nm. Presence of two phenyl rings in DPC were responsible to show peak at 256 nm while the peak corresponding to 217 nm can be attributed to the presence of carbonyl group flanked by two phenoxy groups. The physical mixture of β-CD and DPC also show the same peaks as that of DPC with lowered absorbance values; as the concentration of DPC has reduced in the mixture. The ultraviolet spectral copies of β-CD based nanosponges synthesized by all the methods show similar spectral pattern with λ_{max}values in the region of 205 $\pm$2 nm and 271 $\pm$2 nm. The carbonyl group of DPC is responsible for cross-linking of DPC with β-Cyclodextrin liberating the phenol. This carbonyl group now skirted by β-Cyclodextrin molecule is responsible to show prominent peak at 205 $\pm$ 2 nm. While the very low absorbance value peak at 271 $\pm$ 2 nm can be due to the trace of phenolic group in the nanosponge. The reaction intermediate does not show any peak in the ultraviolet region.

Table 1: Percentage yield of β-cyclodextrin based nanosponges

Type of nanosponge	Percentage yield (%) ± SD, n = 3
β-CDNS by melt method	**32.65 $\pm$0.019**
β-CDNS by solvent assisted synthesis	**20.07 $\pm$0.036**
β-CDNS by ultrasound assisted synthesis	**29.16 $\pm$0.024**
β-CDNS by microwave assisted synthesis	**17.12 $\pm$0.103**

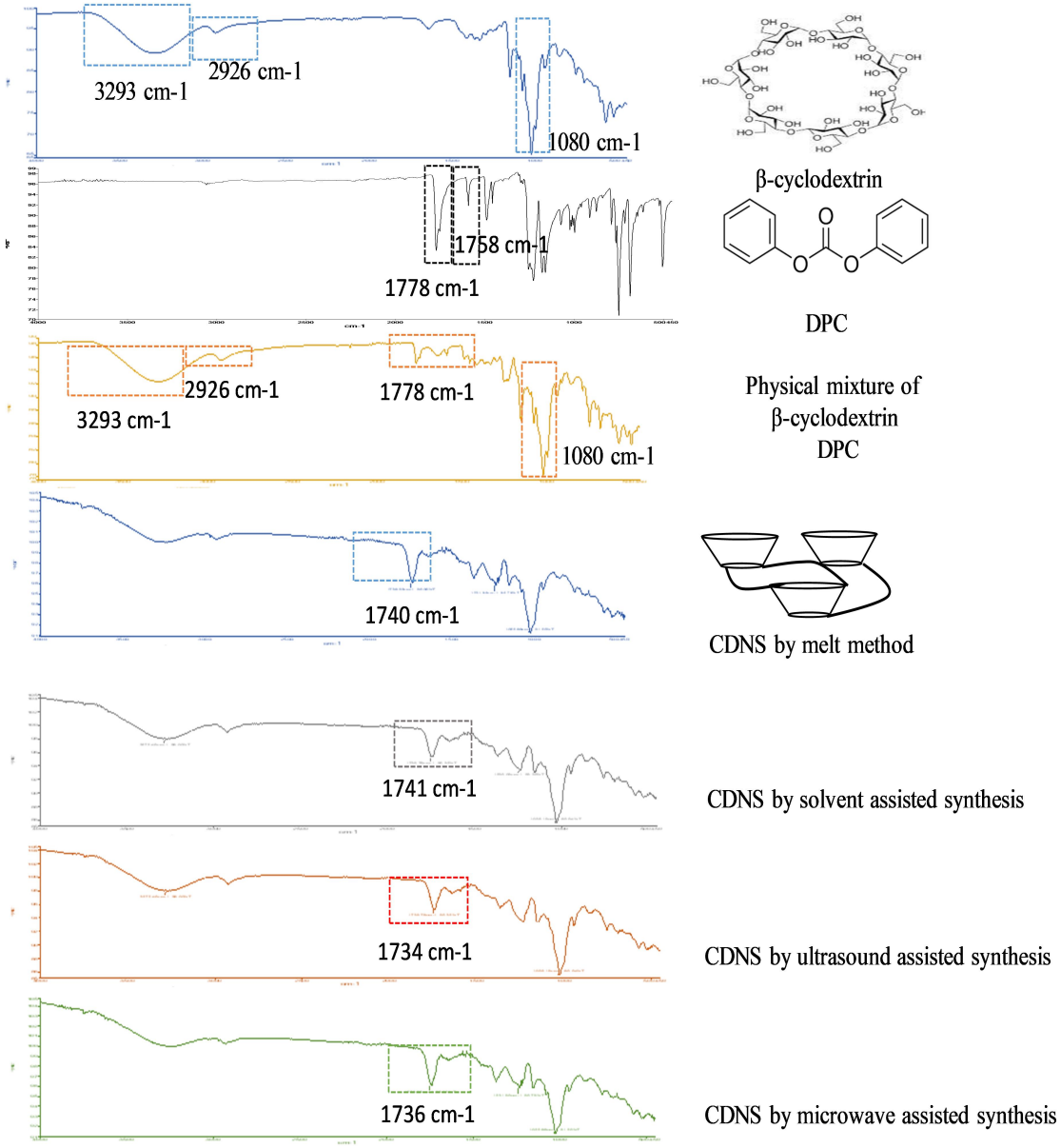

Figure 2: FTIR spectra of β-CD, DPC, physical mixture of β-CD and DPC (1:4 molar ratio), plain β-CD based nanosponges prepared by different synthetic methods

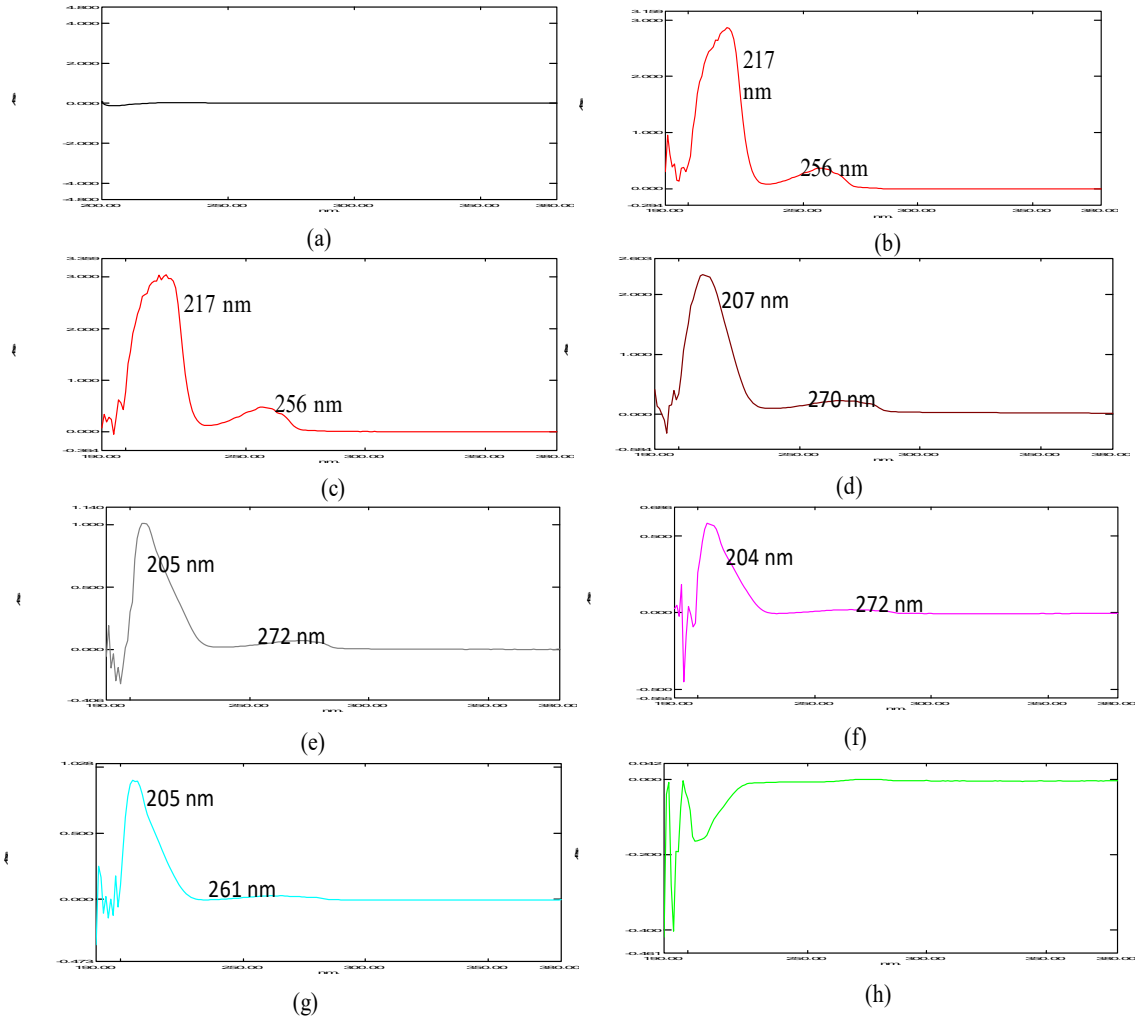

Figure 3: Ultraviolet spectrum of (a) β-CD (b) DPC (c) physical mixture of β-CD and DPC (d) β-CD NS by melt method (e) β-CD NS by SAS (f) β-CD NS by UAS (g) β-CD NS by MAS (h) Incompletely synthesized nanosponge

Table 2: Peak table of Ultraviolet spectroscopy

Sr. No.	Sample	λ_{max}(nm)	Absorbance (units)
1	β-Cyclodextrin	No peak	-
2	Diphenyl carbonate	256	0.365
		217	2.871
3	Physical mixture of β-CD and DPC	256	0.279
		217	2.091
4	β-CDNS by melt method	270	0.186
		207	2.337
5	β-CDNS by solvent assisted synthesis	272	0.011
		205	0.072
6	β-CDNS by ultrasound assisted synthesis	272	0.019
		204	0.053
7	β-CDNS by microwave assisted synthesis	270	0.903
		205	0.87
8	Reaction intermediate	No peak	-

4. CONCLUSION

The basic research carried out in this work was with the intension to assess the use of ultraviolet spectroscopy as a preliminary analytical method for confirming the synthesis of β-CD based nanosponges. So, the nanosponges were synthesized by using β-CD and DPC in 1:4 molar ratio by all the methods namely, melt method, solvent assisted synthesis, ultrasound assisted synthesis and microwave assisted synthesis. All the formulated nanosponges and the starting materials were analysed by FTIR and ultraviolet spectroscopy. The FTIR spectrum of synthesized nanosponges showcased characteristic carbonate peak at 1740 cm^{-1} confirming the cross-linking of β-CD and DPC. The ultraviolet spectra of these nanosponges presented prominent peak at 205 $\pm$2 nm corresponding to carbonate bond formation. Earlier, in ultraviolet spectrum of DPC, the peak corresponding to carbonyl group was obtained at 217 nm which was due to the presence of two phenoxy groups on either side of it. This hypsochromic shift observed in the λ_{max} value of nanosponge as compared with DPC can confirm the synthesis of nanosponge and thus, it can be stated that ultraviolet spectroscopy can accurately depict the synthesis of β-CD based nanosponges and can be used as an introductory tool for characterization of nanosponge.

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