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Original Article

Synthesis of Neomycin Inclusion Complex and Improving Its Physicochemical properties.

Hemat M. Dardeer [1], Fatma M. Mohammed[2], Arafat Toghan[1,3], Aleya A. Mohamed[2*]

1Chemistry Department, Faculty of Science, South Valley University, Qena, Egypt

2Chemistry Department, Faculty of Science, Aswan University, Aswan, Egypt

3Chemistry Department, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia

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Abstract:

This study interest with formation of the inclusion complex of Neomycin antibiotic with β-cyclodextrin by the colloidal tectonic approach. The new inclusion complex of Neomycin was characterized by Fourier-transformation infrared (FTIR) to confirm the chemical structure of the prepared materials by the shifting that occur in the frequencies. X-ray diffraction (XRD) was investigated. The results show the crystallinity properties of the Neomycin was increased after loading process. The morphology of the prepared Neomycin inclusion complex was examined by Scanning electron microscope (SEM). Moreover, the UV–visible spectroscopy was studied and indicates the optical properties of the Neomycin enhanced after loading. The results of this study established the insertion of Neomycin into the cavity of β-cyclodextrin has been improved the morphology structure, crystallinity and the optical properties of the Neomycin drug.

Keywords*:* Cyclodextrin, Neomycin, SEM, Inclusion complex, XRD

1. Introduction

Cyclodextrins are natural oligosaccharides composed of six (α-cyclodextrins), seven (β-cyclodextrins), eight (ɣ-cyclodextrins), or more glucopyranose units linked by α-(1,4) linkages. They are also known as cycloamylose and cyclomaltose (Eastburn & Tao, 1994; Villiers, 1891). They are formed by intramolecular transglycosylation by degradation of starch by the enzyme cyclodextrin glucanotransferase (CGTase) (Szejtli, 1998). Cyclodextrins are natural polymer with a hydrophilic outer surface and a hydrophobic central cavity (Fig. 1).

Corresponding author*: E-mail address: a_gad53@yahoo.com

The chair creation of the glucopyranose units gives the cyclodextrin molecule a cone-like shape, with secondary hydroxyl groups covering from the wide end and primary groups from the thin end. This provides the cyclodextrin molecule a hydrophilic outer surface, but the lipophilicity of its central cavity is equivalent to that of aqueous ethanol (Frömming & Szejtli, 1993).

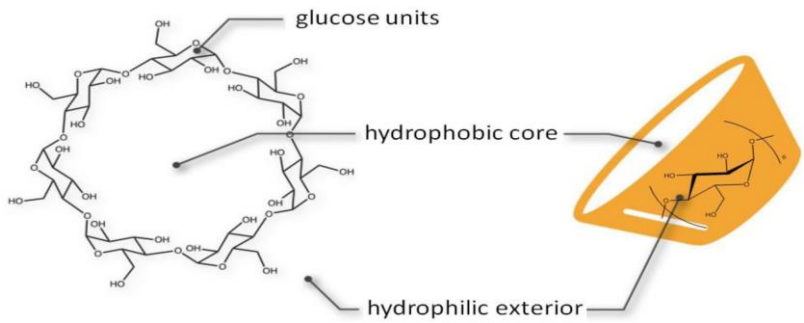


Fig 1. Cyclodextrin: chemical and toroid structures (Ioele et al., 2017).

Small and medium-sized molecules of many different sizes can be present in the holes of CDs. The association, also known as "host-guest inclusion complexation," results from the interaction of the guest molecules with the cavity of the host molecules thus, no covalent link is created in this process. Between unspecific interactions of a solute with the solvent, known as solvation, and incredibly specific receptor contacts, is where this "molecular recognition" gets its specificity (Caira, 2019; Crini et al., 2018). Hydrophobic, van der Waals, electrostatic, as well as the capacity to create hydrogen bonds, are the driving forces for the inclusion complexation (Liu & Guo, 2002).

Numerous reviews touch on the value of CD applications in pharmacy. The use of CDs in pharmacies has been reviewed historically (Uekama et al., 1998). A collection of articles (Dardeer & Toghan, 2021; Radwan et al., 2021) provide general summaries. The improvement of weakly water-soluble medications' solubility and the ensuing rise in the bioavailability and effectiveness of the active compounds, as well as the facilitation of their controlled release in multiple trials (Hemat M. Dardeer, Safaa A. Abbas, et al., 2022; Mahgoub et al., 2023). Additionally, numerous cases of special CD inclusion complexation applications are described (Hemat M. Dardeer, Ahmed G. Taha, et al., 2022; Ibrahim et al., 2023). Through molecular encapsulation, CDs were frequently utilized in the pharmaceutical industry to increase the stability and solubility of insoluble pharmaceuticals in water or organic solvent-water mixtures. The ability to encapsulate CDs with medications heavily depends.

The membrane system is impermeable to CDs and CD complexes without compromising the integrity of the membrane structure, but they can interact with the membrane surface

(Hammoud et al., 2019; Leclercq, 2016). By removing cholesterol, they can, for instance, affect the membrane's make-up. Numerous studies have looked into how pharmaceuticals are transported to the lipid bilayer surface. It has recently been demonstrated that different cyclodextrin derivatives interact differently at the membrane surface (El Achkar et al., 2020; Khuntawee et al., 2015) and to some extent allow the medication to pass through the membrane. Native CDs can be administered orally with nearly no limits because they have "GRAS" classification, which indicates that the Food and Drug Administration (FDA) has determined that they are safe for human usage. The sole restriction on -CD consumption is a daily maximum of 5 mg/kg. The reduction of post-meal glycemic reactions was approved for -CD in 2013 and it was added to the European Union Register on nutrition and health claims (Hotarat et al., 2020).

Neomycin (Fig.2) is an aminoglycoside antibiotic that exhibits bactericidal activity against Gram-negative aerobic bacteria and some anaerobic bacteria for which resistance has not emerged (Huidobro et al., 2009). It is generally ineffective against Gram-positive and anaerobic Gram-negative bacteria. Neomycin is presented in oral and topical formulations such as creams, ointments, and eye drops (Hubicka et al., 2015). Neomycin belongs to the aminoglycoside class of antibiotics, comprising two or more amino sugars linked by glycosidic bonds. Neomycin was revealed in 1949 by microbiologist Selman Waksmann and his student Hubert Lechevalier at Rutgers University. It is formed naturally by the bacterium Streptomyces fradiae (Alsamarrai et al., 2019).

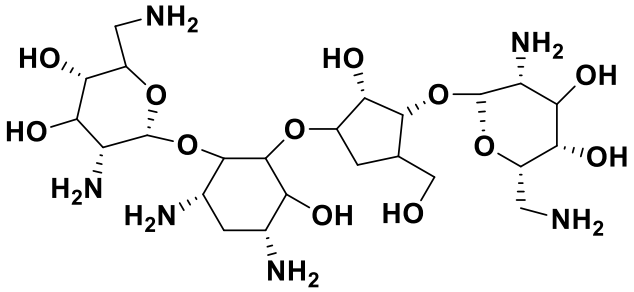


Fig 2. Neomycin structure

From this point the aim of this study is to improve the physical and chemical properties of Neomycin by encapsulation into β-CD to form stable inclusion drug complex.

2. Experimental

2.1 Chemicals and reagents

Neomycin, beta-cyclodextrin and dimethylformamide were purchased from Merk Co., Germany. All chemicals were used as received without additional purification.

2.2 synthesis of Neomycin drug D/ β-CD inclusion complex

Neomycin was loaded into the β-CD by the colloidal tectonic approach in which (4g) of β-CD was dissolved in 40 ml DMF. Then was stirred for 6 hrs till obtain clear solution. After that the Neomycin drug (1g) was dissolved in 10 ml DMF then added to the reaction mixture with stirring at room temperature for 15hrs, then the reaction mixture heated for 2hrs at 70 ºC. The formed solution was poured into a petri dish and the solvent was evaporated at room temperature to give inclusion complex D/β-CD as white powder.

2.3. Characterizations

2.3.1. Fourier-transformation infrared (FTIR)

The structure of the Neomycin drug and the prepared inclusion drug complex was studied by using Fourier-transformation infrared （FTIR) spectroscopy at room temperature (infrared spectrometer: Jasco Model 4100 – Japan) in the wavenumber region of 4000 – 400 cm^{1}.

2.3.2. X-ray diffraction (XRD)

The phase structure and crystallite size of the products were determined by XRD measurements taken at room temperature with a powder diffractometer (Brucker D8 Advance, Germany) equipped with a Cu K radiation source, = 1.5406 and 2 in the range (5–80°).

2.3.3. Scanning electron microscope (SEM)

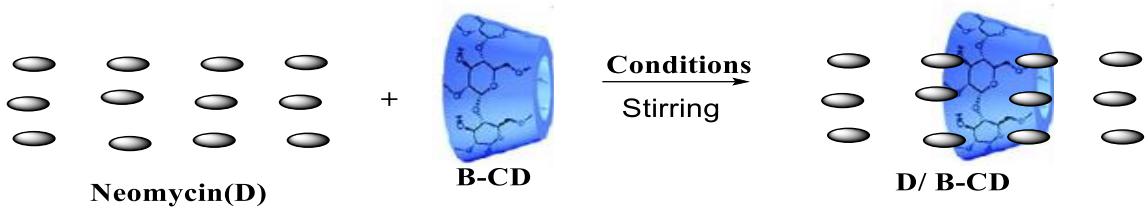
Scanning electron microscope SEM (JEOL SEM model JSM – 5500 – Japan) with accelerated voltage 20 kV was used to study the morphological structures of the produced materials.

2.3.4. UV–visible spectroscopy

The optical characteristics of the produced materials were determined using UV–visible spectroscopy. The UV–visible spectra were obtained by using a UV–visible spectrophotometer (PG Instruments, model T80, UK) and quartz cells with a path length of 1 cm with wavelengths ranging from 200 to 800 nm. To modify the baseline, DMF was utilized as a blank.

3. Results and discussion

The synthesis of Neomycin inclusion complex was conducted using β-CD. Scheme 1 indicate the mechanism has been proposed for the synthesis of this inclusion complex by dissolving the β-CD into DMF then Neomycin (D) is added drop wisely with stirring to form inclusion Neomycin complex.



Conditions: i) DMF, stirring, 70 ^{0}C,2 hrs

Scheme1: Synthetic route for the synthesis of the inclusion complex (D/ β-CD).

3.1 FT-IR

Figure 3. Indicate the FTIR spectrum of the prepared inclusion complex (D/β-CD) in comparing with pure Neomycin drug (D) and pure β-CD. The FTIR spectrum of pure Neomycin drug (D) showed band at 3348 cm^{-1} due to the hydroxyl groups ν [OH] and band at 2903 cm^{-1} correlated to (CH-aliphatic) group. The absorption band at 1133 cm^{-1} which characterized the vibration of (C-O-C) group and band at 1046 cm^{-1} due to (C-O) groups. The FTIR spectrum of pure β-CD and inclusion complex (D/β-CD) show several characteristic bands at 3272 and 3247 cm^{-1} represented for ν [OH] symmetric stretching respectively, characteristic bands due to CH aliphatic at 2922 and 2927 cm^{-1}. In addition to characteristic bands at 1156 and 1161 cm^{-1} due to C-O-C vibration and bands at 1022 and 1027 cm^{-1} due to C-O groups. The absorption band of ν [OH] symmetric stretching in inclusion drug complex (D/β-CD) was shifted to lower frequency compared to those of in bulk β-CD (Dardeer, 2018). In addition, the absorption bands for ν [CH-aliphatic], ν [C-O-C] vibration and ν [C-O] stretching in inclusion complex (D/β-CD) were shifted to higher frequencies compared to those in pure β-CD as shown in Table (1). The shifting that occurs in the absorbance bands could strongly illustrates the formation of an inclusion complex between cyclodextrin and Neomycin drug D. The enhancement in frequencies is due to the insertion of the drug through the cavity of the cyclodextrin rings. In contrast, the decreasing in frequencies is due to the creation of Vander Waals forces, hydrogen bonds between β-CD groups and the active groups in Neomycin drug.

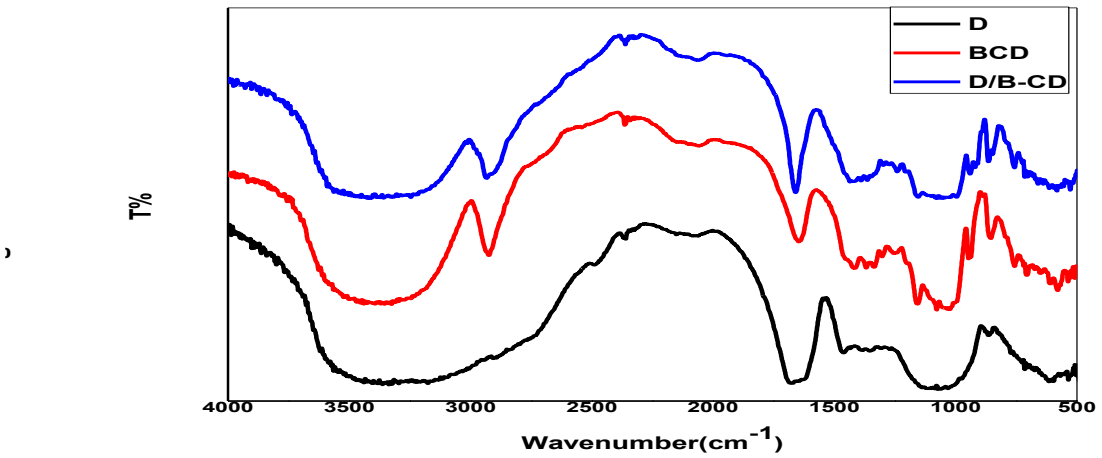


Fig 3. FT-IR spectrum of D, β-CD and D/ β-CD.

Table 1. The change in the absorption bands between β-CD and D/β-CD and pure drug D.

Functional group	Wavenumber, cm^{-1}		Δδ	Wavenumber, cm^{-1}		Δδ
	β-CD	β-CD /D		D	β-CD /D	
v [O-H] stretching	3272	3247	-25	3348	3247	-101
v [CH-aliphatic]	2922	2927	+5	2903	2927	+24
v [C-O-C] vibration	1156	1161	+5	1133	1161	+28
v [C-O] stretching	1022	1027	+5	1046	1027	-19

3.3 XRD analysis

XRD is a useful technique for identifying crystal transformation of drug in pharmaceutical preparation. X-ray diffraction analysis of Neomycin drug (D) and inclusion complex D/ β-CD are presented in Fig. 4. XRD spectrums were recorded at room temperature in the range 5°-80°. The crystal nature for pure Neomycin drug (D) is completely different than the crystal nature for inclusion drug complex. The change in XRD spectrum and crystallinity value between pure Neomycin drug and inclusion complex is due to the cross linker destroying the regularity of the chain of the β-CD during the cross-linking process, also this change supported the synthesis of prepared inclusion complex (Dardeer, 2019; Dardeer & Ebnalwaled, 2019).

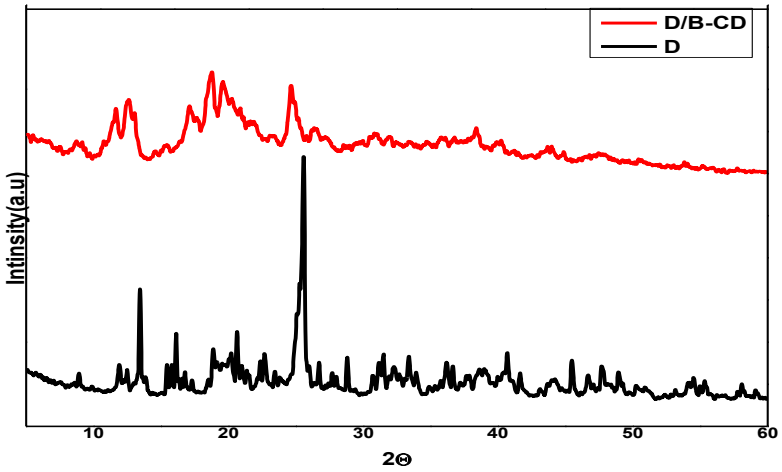


Fig 4. XRD spectrum of Neomycin drug (**D**) and D/ β-CD.

3.3 UV analysis

As shown in Fig.5 the characteristic absorption bands of Neomycin D (dissolved in DMF) were create in 295 nm, 335 nm, 360 nm and 380 nm. While the characteristic absorption peaks of D/ β-CD was appeared at 267nm. When the inclusion complexes were removed with DMF, we detected that its UV absorption of the inclusion complexe was like to that of the β-CD, but it was different from the UV absorption of D, which designated that the UV absorption of D was protected by the external β-CD, thereby it displayed the UV absorption similar to that of the external β-CD. These results showed the construction of the inclusion complexes (Wang et al., 2019) between D and β-CD.

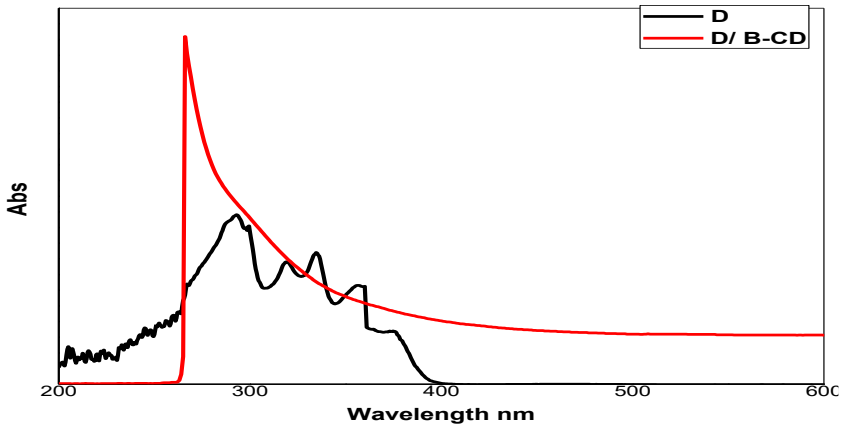


Fig 5. UV spectrum of D and D/ β-CD.

3.4 Surface Morphology

The morphological structure of Neomycin drug (D), β-CD and inclusion complex D/ β-CD were clarifying by SEM analysis. In Fig. 6. which appeared as the following describe, the difference of morphological structure of these compounds powerful indication of our compound already synthesizes and give the following Neomycin drug (D) have the regular shape and smooth with spherical structure, and rough and wrinkled polymeric network with crooked pores. While the SEM image for pure β-CD was appeared as rod like morphology with irregular aggregation. While the SEM image of inclusion complex (D/ β-CD) was appeared as regular and smooth gray clouds with small pores. the difference in the SEM photos between D, β-CD and D/β-CD was supported the synthesis of these materials (Hemat Mohamed Dardeer et al., 2022).

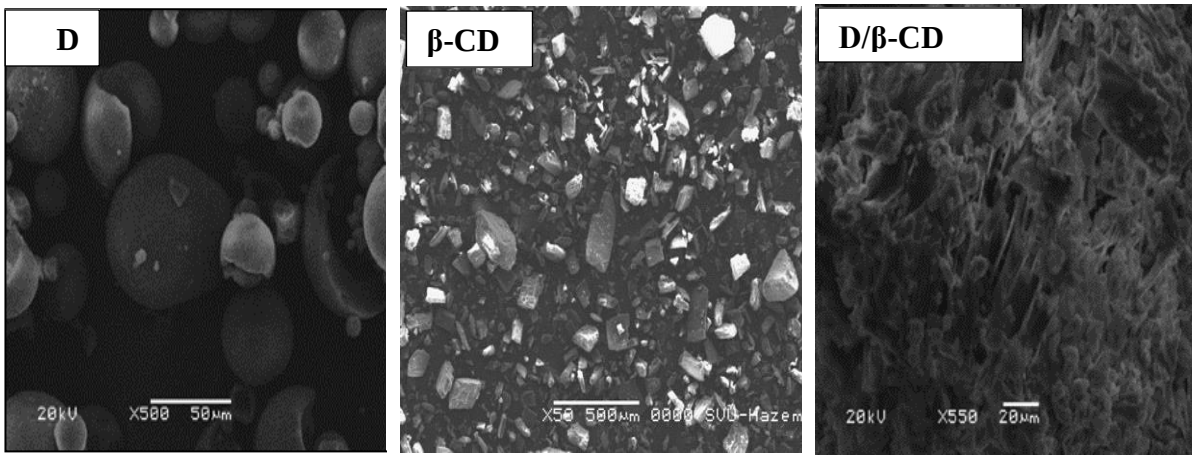


Fig 6. SEM images of Neomycin drug (D), β-CD and D/ β-CD at magnification of 500X.

4. Conclusion

In this study, the inclusion complex of Neomycin drug with β-CD was successfully prepared by the colloidal tectonic approach. The new inclusion complex was examined by the Fourier-transformation infrared (FTIR), X-ray diffraction (XRD), Scanning electron microscope (SEM) and UV–visible spectroscopy which confirmed clearly the difference between the drug before and after loaded β-CD. The results showed that improving the physicochemical properties of drug after insertion into the cavity of the β-CD.

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