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Mini Review on Synthesis and Characterization Methods for Ternary Inclusion Complexes of Cyclodextrins with Drugs

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Drug dependence and tolerance has become a major issue related to human health and significantly affects people living in the world today. Cyclodextrins (CDs), a family of cyclic oligosaccharides, have been widely used in the field of pharmaceutical sciences to formulate host-guest inclusion complexes with desired pharmaceutical drugs in order to improve their bioavailability and absorption by the human body. To further enhance the properties of CD-drug binary inclusion complexes, the formulation of ternary CD-drug inclusion complexes with an additive (for example polymers, organic acids, amino acids, and co-solvents) as the ternary component have been vastly explored by researchers worldwide. This review article offers a brief but comprehensive overview on various applicable synthesis and characterization methods employed by researchers for the formulation of ternary inclusion complexes of cyclodextrins with pharmaceutical drugs.

Key words: Cyclodextrin; ternary complex; inclusion complex; pharmaceutical drugs

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Drug dependence is unavoidable when it comes to chronic medical conditions such as high blood pressure and diabetes. As the human body adapts to certain drugs, it will gradually become more tolerant to their effects, which leads to a higher drug intake that may result in overdose or various toxic side effects. One of the key factors in the planning of a safe and effective drug delivery system is its solubility in water. This is because the formulation, absorption and bioavailability of a medicinal drug are inseparably dependent on its solubility in water [1]. Generally, many drugs have relatively low water solubility due to the increasing use of lipophilic molecules in their chemical composition. One of the most effective ways to improve the aqueous solubility of pharmaceutical drugs is to use cyclodextrins (CDs) [2]. CDs are able to modify the physicochemical properties of a drug, leading to the formation of inclusion complexes [3]. Interestingly, inclusion complexes of CDs can be synthesised in the solid, liquid, or gaseous states [4]. Such drug-CD inclusion complexes possess greater dissolution rates and shorter drug release times which increase their absorption efficiency in the human body. This ultimately leads to the higher bioavailability of these drugs, which may allow the drug dosage to be reduced [5]. However, the use of CDs may be limited in some specific cases because the guest drug molecules must be able to fit partially or completely within its cavity. Therefore, the factors affecting the formation of a CD inclusion complex include ease of accommodation of drug molecules within the CD cavity, formation stoichiometry, therapeutic dosage as well as the toxicity level of the chosen CD [6].

To date, several research papers have reported the synthesis of CD-drug inclusion complexes, either in the form of a binary system or a ternary system. A binary CD-drug system consists of two main components, CD and the desired drug, in which CD serves to improve the solubility and bioavailability of the drug. As shown in Figure 1, the drug molecule is incorporated into the CD cavity which contains lipophilic holes in its inner part in order to form the CD-drug binary inclusion complex [7]. In a ternary system (Figure 2), a third component (typically an acid or a polymer) is added to the abovementioned binary system as a catalyst, to further enhance drug solubility and drug-release. For instance, when a water-soluble polymer is mixed into an initially binary CD-drug system, it has been shown to be capable of increasing the amount of the drug delivered in vitro, as well as reducing the concentration of CD required for complexation by up to 80% [8]. Various types of non-covalent bonds may be involved in the binding of guest drug molecules in the CD cavity, for instance dipole-dipole interactions and van der Waals forces [9].

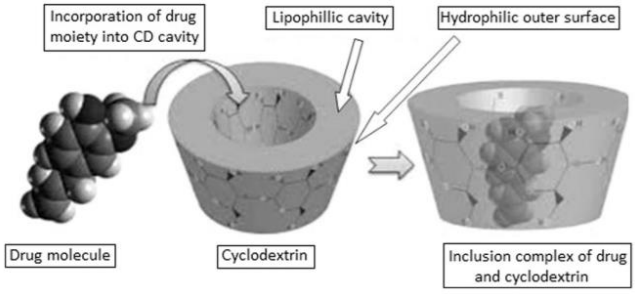

Figure 1. Binary CD-drug inclusion complex [7]

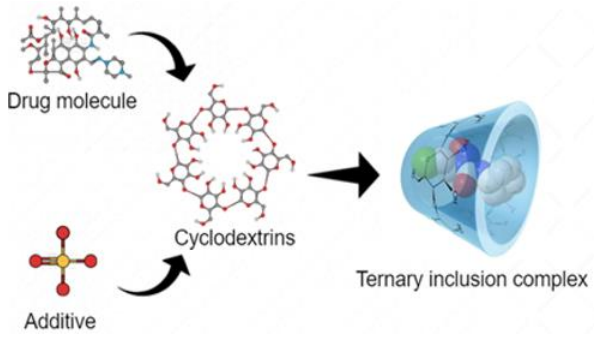

Figure 2. Ternary CD-drug inclusion complex

Table 1. Pharmaceutical products containing CDs [11]

Drug/cyclodextrin	Trade name	Formulation	Company (country)
α-Cyclodextrin (αCD)			
Alprostadil	Caverject Dual	Intravenous solution	Pfizer (Europe)
Cefotiam-hexetil HCl	Pansporin T	Tablet	Takeda (Japan)
Limaprost	Opalmon	Tablet	Ono (Japan)
PGE1	Prostavastin	Parenteral solution	Ono (Japan); Schwarz (Europe)
β-Cyclodextrin (βCD)			
Benexate HCl	Ulgut, Lonmiel	Capsule	Teikoku (Japan); Shionogi (Japan)
Cephalosporin	Meiact	Tablet	Meiji Seika (Japan)
Cetirzine	Cetrizin	Chewable tablet	Losan Pharma (Germany)
Chlordiazepoxide	Transillium	Tablet	Gador (Argentina)
Dexamethasone	Glymesason	Ointment, tablet	Fujinaga (Japan)
Dextromethorphan	Rynathisol	Synthelabo (Europe)	
Diphenhydramine and chlortheophylline	Stada-Travel	Chewable tablet	Stada (Europe)
Ethinylestradiol and drospirenone	Yaz	Tablet	Bayer (Europe, USA)
Iodine	Mena-Gargle	Solution	Kyushin (Japan)
Meloxicam	Mobitil	Tablet and suppository	Medical Union (Egypt)
Nicotine	Nicorette	Sublingual tablet	Pfizer (Europe)
Nimesulide	Nimedex	Tablets	Novartis (Europe)
Nitroglycerin	Nitropen	Sublingual tablet	Nihon Kayaku (Japan)
Omeprazole	Omebeta	Tablet	Betafarm (Europe)
PGE2	Prostarmon E	Sublingual tablet	Ono (Japan)
Piroxicam	Brexin, Flogene, Cicladon	Tablet, suppository	Chiesi (Europe); Aché (Brazil)
Tiaprofenic acid	Surgamyl	Tablet	Roussel-Maestrelli (Europe)
2-Hydroxypropyl-β-cyclodextrin (HPβCD)			
Cisapride	Propulsid	Suppository	Janssen (Europe)
Indometacin	Indocid	Eye drop solution	Chauvin (Europe)
Itraconazole	Sporanox	Oral and intravenous solution	Janssen (Europe, USA)
Mitomycin	MitoExtra, Mitozytrex	Intravenous infusion	Novartis (Europe)
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD)			
Aripiprazole	Abilify	Intramuscular solution	Bristol-Myers Squibb (USA); Otsuka Pharm. (USA)
Maropitant	Cerenia	Parenteral solution	Pfizer Animal Health (USA)
Voriconazole	Vfend	Intravenous solution	Pfizer (USA, Europe, Japan)
Ziprasidone mesylate	Geodon, Zeldox	Intramuscular solution	Pfizer (USA, Europe)
Randomly methylated β-cyclodextrin (RMβCD)			
17β-Estradiol	Aerodiol	Nasal spray	Servier (Europe)
Chloramphenicol	Clorocil	Eye drop solution	Oftalder (Europe)
γ-Cyclodextrin (γCD)			
Tc-99 Teboroxime[a]	CardioTec	Intravenous solution	Squibb Diagnostics (USA)
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)			
Diclofenac sodium salt	Voltaren Ophtha	Eye drop solution	Novartis (Europe)
Tc-99 Teboroxime[a]	CardioTec	Intravenous solution	Bracco (USA)

Generally, CDs and their derivatives are non-toxic to the human body as they can only cross biological membranes to a certain extent. This is one of the reasons why CD-drug inclusion complexes have been widely explored in the last few decades [10]. In the pharmaceutical industry, binary CD inclusion complexes are commonly used to improve the dissolution profile of certain less water-soluble drugs. The first pharmaceutical drug employing CD in its formulation was launched in Japan in 1976, and it was a potent labour inductor – E2/β-CD prostaglandin in tablet form. At present, there are more than 40 binary CD-drug inclusion complex products in the global market (Table 1) [11]. However, many drugs with low aqueous solubility often contain organic solvents or emulsifiers in their formulation, which may cause mild to severe side effects, such as gastrointestinal or ocular irritation [12]. Hence, ternary CD:drug:polymer inclusion complexes have been explored by researchers worldwide as a measure to overcome this issue. As shown in Table 2, there are a handful of studies in which ternary CD:drug:polymer inclusion complexes were used with drugs available in the market, some of which have shown promising results worth further exploration [1].

Table 2. Pharmaceutical products in the form of CDs:drug:polymer [1]

Drug	CD	Water-soluble polymer	Reference
17β-estradiol	HP-β-CD	CMC	Loftsson *et al.*, 1994
	HP-β-CD	PVP	Loftsson, Brewster, 1996
Acetazolamide	β-CD	HPMC, CMC, PVP	Loftsson, Fridrilksdóttir, 1998
	HP-β-CD	CMC, PVP	Loftsson *et al.*, 1994
	HP-β-CD	HPMC, CMC, PVP	Loftsson *et al.*, 2005b
Triamcinolone acetonide	HP-β-CD	CMC	Loftsson *et al.*, 1994
Alprazolam	β-CD	HPMC, CMC, PVP	Loftsson , Fridrilksdóttir, 1998
	HP-β-CD	CMC	Loftsson *et al.*, 1994
Carbamazepine	SBE-β-CD	HPMC, PVP	Smith *et al.*, 2005
	HP-β-CD	CMC, PVP	Loftsson *et al.*, 1994
	β-CD	HPMC, CMC, PVP	Loftsson, Fridrilksdóttir, 1998
	HP-β-CD	HPMC, CMC, PVP	Brewster, Loftsson, 2007
Celecoxib	HP-β-CD	HPMC, PEG, PVP	Chowdary, Srinivas, 2006
Clotrimazol	HP-β-CD	CMC, PVP	Loftsson *et al.*, 1994
Dexamethasone	HP-β-CD	HPMC	Loftsson *et al.*, 1994
	β-CD	HPMC, CMC, PVP	Loftsson, Fridrilksdóttir, 1998
Diazepam	HP-β-CD	CMC	Loftsson *et al.*, 1994
Econazole	HP-β-CD	CMC, PVP	Loftsson *et al.*, 1994
Ethoxzolamide	HP-β-CD	CMC, PVP	Loftsson *et al.*, 1994
	β-CD	HPMC, CMC, PVP	Loftsson, Fridrilksdóttir, 1998
Finasteride	RM-β-CD	HPMC, CMC, PVP	Brewster, Loftsson, 2007
	HP-β-CD	PVP	Asbahr *et al.*, 2009
Gemfibrozil	β-CD	PVP	Sami, Philip, Pathak, 2010
Gefitinib	HP-β-CD	PVP, HPMC	Phillip Lee *et al.*, 2009
Glibenclamide	β-CD, HP-β-CD, SBE-β-CD	HPMC	Savolainen *et al.*, 1998
Glimepiride	β-CD, HP-β-CD, SBE-β-CD	HPMC, PEG, PVP	Ammar *et al.*, 2006
Griseofulvin	α-CD, β-CD and γ-CD	PEG	Wulff, Aldén, 1999
	β-CD	CMC	Dhanaraju *et al.*, 1998
Hydrocortisone	HP-β-CD	CMC	Loftsson *et al.*, 1994
	HP-β-CD	HPMC, PVP	Loftsson, Sigurdardottir, 1994
	HP-β-CD	HPMC, CMC, PVP	Loftsson *et al.*, 2005b
	RM-β-CD	HPMC, CMC, PVP	Loftsson *et al.*, 2005b
Indomethacin	α-CD, β-CD and γ-CD	PEG	Wulff, Aldén, 1999
Irbesartan	β-CD	PEG, PVP	Hirlekar, Sonawane, Kadam, 2009
Lamivudine	β-CD	PVA	Selvam, Geetha, 2008
Lamotrigine	β-CD	PEG, PVP	Shinde *et al.*, 2008
Lovastatin	β-CD, RM-β-CD	PVP	Süle, Csempesz, 2008
Meloxicam	HP-β-CD	PVP	El-Maradny *et al.*, 2008
Methazolamide	β-CD	HPMC, CMC, PVP	Loftsson, Fridrilksdóttir, 1998
	HP-β-CD	HPMC, CMC, PVP	Loftsson *et al.*, 2005b
Miconazol	HP-β-CD	CMC	Loftsson *et al.*, 1994
Midazolam	SBE-β-CD	HPMC	Loftsson *et al.*, 2001
Naproxen	β-CD, HP-β-CD	PVP	Mura *et al.*, 2001
Nicardipine	β-CD	PEG	Quaglia *et al.*, 2001
Oxazepam	HP-β-CD	CMC, PVP	Loftsson *et al.*, 1994
	HP-β-CD	HPMC, CMC, PVP	Brewster, Loftsson, 2007
Prednisolone	HP-β-CD	CMC	Loftsson *et al.*, 1994
	β-CD	HPC	Uekama *et al.*, 1983

In this review article, we focus solely on summarising the synthesis and characterisation methods of ternary CD-drug inclusion complexes, the latest advancement in this field.

1. Synthesis Methods for CD-drug Ternary Inclusion Complexes

1.1. Spray Drying

Spray drying has shown promising results for the preparation of ternary CD inclusion complexes in terms of physicochemical properties of the products [13]. Through this method, CD is dissolved in a solvent of choice along with the drug and supplementary substances. The liquid is then converted into solid particles via an atomization process, whereby droplets of fluids are placed in contact with a high temperature gaseous medium and are converted into a fine dry powder with zero moisture content [14]. The main reason why spray drying has become a priority synthesis technique for most researchers is that it is considered rapid, convenient and reproducible in just a single step. Besides, it is highly practical and relevant to both large manufacturing industries and small research laboratories. There is also no requirement for purification or modification of the product obtained by spray drying, unlike the emulsion or solvent evaporation methods [15]. Moreover, spray drying is less costly and less time consuming when compared to the freeze-drying method which involves long hours of deep cooling that consumes substantial amounts of energy [16]. Furthermore, spray drying is particularly suitable for thermally sensitive chemical substances. Despite the use of a solvent, the evaporation process is relatively fast due to the high surface area-to-volume ratio [17]. With zero moisture content in the fine powdered form, the shelf life of these synthesised products is also expected to increase significantly, which makes this method useful in various areas of industry, from encapsulation of drugs to aromatic oils, pigments, and flavourings [18]. In the field of science, spray drying is often employed to produce microparticles and nanocomposites [19]. Even though the spray drying method has many advantages, it comes with several drawbacks too. The laboratory experimental yields of products obtained using this method are low and usually range between 20% and 70%, due to some of the product sticking or attaching to the surface of the chamber walls, depending on the pressure applied [20]. In addition, the mass production of nanometre-scale particles via spray drying is not feasible in the long run due to the lack of sufficient forces to atomize fluids into large amounts of submicron particles, which causes a problem in the manufacturing industry where size and distribution are the main factors involved [21].

1.2. Solvent Evaporation

A common alternative to the spray drying method mentioned above is the solvent evaporation method, also known as the solution evaporation method. For the formation of the inclusion complex, both the CD and the drug are dissolved in a common solvent and stirred till homogeneous. This solution is placed in a rotatory evaporator or left in a fume hood until the solvent evaporates, and then filtered to remove undissolved compounds. The residue is dried to obtain the solid complex [22]. The main factors which affect the encapsulation of drugs synthesised using this method are the rate of solvent removal, solubility of drugs, distribution coefficient and molar mass of drugs. The advantages of using the solvent evaporation method include prevention of thermal degradation of drugs due to the low temperatures used in the evaporation of organic solvents. The formation of crystals can also be minimised, just as with the spray drying method, although the latter has a better dissolution profile. A minimal amount of residual solvent is able to mostly preserve the activity of bioactive compounds in the inclusion complex [23]. As for the disadvantages of this method, any unevaporated solvent may affect the chemical properties and stability of the drug, thus reducing the level of drug-loading efficiency. Furthermore, the selection of a common miscible solvent for the desired drugs and components in the inclusion complex may not be easy, due to their varying solubilities in organic solvents [24].

1.3. Kneading

This method is mainly employed in smaller scale experiments, especially in research laboratories, due to the ease of preparation and low cost. Various factors are taken into consideration in this method, including molar ratios, initial water content and mixture composition [25]. To utilise this synthesis method, known amounts of CD and the powdered drug are mixed simultaneously in a mortar, followed by addition of aqueous ethanol to produce a paste-like mixture, which is further kneaded with a pestle until homogeneous before being dried under vacuum or in an oven for at least one day. The kneading method is especially favourable to use for guest compounds with poor water solubility as the mixture is dissolved slowly during the formation of the inclusion complex. Additionally, inclusion complexes formed using this method give a very good yield [26]. As high temperature conditions are not necessary, the kneading method is widely used in the industry for encapsulation of essential oils [27]. However, in actual practice, the drug release level of a CD inclusion complex formed using this method is relatively low when compared to the solvent evaporation method [28]. Furthermore, the formation of crystals tends to be greater than with the spray drying method, which

implies that the spray drying method possesses a better dissolution profile [29]. Lastly, the kneading method is not suitable for large scale production in the manufacturing industry due to speed and quantity requirements.

1.4. Freeze Drying/Lyophilization

An effective way to utilise the freeze drying method is by continuously stirring a known proportion of CD and the guest drug molecules in water, after which the resultant homogeneous solution is freeze-dried, washed with organic solvent, and dried under vacuum conditions [30]. The main factors which play an important role in determining the formation of an inclusion complex via this method include the time span of lyophilization for each product, heat transfer quality, cooling speed, erosion resistance of the components involved, thermal current exchange on the external surface and the volume of lyophilization vacuum space. Due to the use of very low temperatures, this method is highly recommended if the components involved in complex formation are thermo-labile [31]. Additionally, freeze-drying provides a very good yield of the inclusion complex, and it is relatively easy to increase or decrease the amount of the desired product, which makes this method viable for mass production. As such, freeze-drying is widely used in CD-based inclusion complex formation. The CD most commonly used is hydroxypropyl-β-CD, due to its excellent water solubility [32]. Several types of essential oils such as cinnamon, clove, estragole, black pepper, thymol and thyme, as well as their major active ingredients, have been encapsulated in hydroxypropyl-β-CD using the freeze-drying method [33]. Researchers sometimes perform freeze-drying as a substitute for the solvent evaporation method because both methods use a common solvent to dissolve the components involved in the formation of inclusion complex and are also able to form a completely amorphous powdered product with a high percentage of binding between the CD and the drug [30]. However, the freeze-drying method has some disadvantages, one of which is that the process is time-consuming and involves long hours in the freeze-dryer. Freeze-drying costs at least five times as much as conventional drying methods, which may not be feasible or economic for smaller research laboratories in the long run. Further, humidity may cause microbial growth within the synthesised products if improperly stored [34].

1.5. Co-precipitation

The method of co-precipitation is useful for components that are insoluble in water. In this method, a known amount of the desired drug is dissolved in an organic solvent and added to an aqueous CD solution. The mixture is kept under constant agitation with fixed parameters and shielded from external sources of light. Upon cooling, the precipitate formed is vacuum filtered, washed and dried to obtain the inclusion complex [35]. Interestingly, Razak et al. found that co-precipitation may be one of the best methods to synthesise the inclusion complex of β-CD and isoniazid, as the complex formed using this method displayed optimum stability when exposed to different conditions, compared to other methods such as freeze-drying and kneading [36]. The benefits of using this method include reduced energy consumption and inhibition of thermal degradation of the synthesised inclusion complex due to the shorter time required for drying, as the water content is almost zero. Additionally, the synthesised inclusion complex forms new solid crystalline phases which significantly improves the physicochemical properties of the guest drug molecules, such as reduced volatility, reduced oxidation rate and greater thermal stability, thus reinforcing the potential application of these complexes in the pharmaceutical and food industries [37]. Although co-precipitation provides promising results in the synthesis of inclusion complexes, it has not attracted the attention of the manufacturing sector due to its low percentage yield of products and the fact that large scale preparation consumes too much time and is thus uneconomic for commercialization [38].

1.6. Milling

Milling is a well-known method in the field of mechanochemistry, and can be used for the preparation of a solid phase ternary inclusion complex. This method makes use of a powerful tool which is supported by the input of mechanical energy to induce mechanochemical transformations. Among all the mechanical grinding methods, ball-milling is one of the most used laboratory methods to prepare inclusion complexes. Through this method, both drug and CD in solid form are physically mixed together, and the mixture is introduced into an oscillatory ball mill where it is ground for a set period of time. The fine powdered product is then sieved through a 60-mesh sieve [39]. Ball milling is especially useful when the desired drug component has very low solubility, because only the solid phase is involved. Besides, this method greatly enhances the dissolution rate and bioavailability of drugs by amorphization of the crystalline drug, although a little crystallinity may still be observed [40]. Unlike other methods, milling is considered to be an approach which supports green-chemistry principles as the procedure does not require the use of toxic organic solvents. On the surface, this method may be similar to kneading as both involve grinding, however it is different in that kneading only requires simple blending, while this method requires binding and impact to a much greater extent through very powerful blending forces. Despite the usefulness of milling, it is less efficient when it comes to mass production because heat elimination and cooling of the milling chamber is necessary at set intervals, which may

consume a lot of time [41]. In addition, solid-state characterization methods for the synthesised complex may be limited, especially in cases where a combination of free and bound drug components are present, causing multiple amorphous and crystalline phases to co-exist due to the unoptimised drug to CD ratios used during the reaction process. The complexity of such characterizations requires great expertise and costly equipment, which limits the use of this technique [42].

1.7. Overall thoughts on Synthesis Methods for Ternary CD-drug Inclusion Complexes

Although there are many options available for the preparation of CD-drug ternary inclusion complexes, each method has its benefits and flaws, which should be carefully considered. For instance, in small laboratories which have limited instruments, the kneading, co-evaporation and solvent evaporation methods are wise choices as the procedures are simple and do not require very advanced apparatus. Whereas for the spray-drying, freeze-drying, and milling methods, the use of specific or high-tech instrument is required, which may not always be available for smaller scale research. All in all, the products that are synthesized by different methods may have slight to obvious differences in terms of physical and chemical properties, while the physical states of the starting materials play an important role in choosing the most suitable method.

2. Characterization Methods for Ternary CD-drug Inclusion Complexes

2.1. Phase Solubility Studies

One of the most significant techniques for characterization employed by many researchers is the phase solubility study [43]. The existence of a synthesised inclusion complex in aqueous solution is not considered a confirmation for the existence of the same complex in the solid state. Hence, the synthesised product in powder form has to be tested to determine whether it is the desired inclusion complex or merely a simple physical mixture of the guest drug molecules and CD [44]. In a famous publication by Higuchi and Connors, complexes were classified based on the phase solubility profiles derived from the molecular interactions between the host and the guest molecules (Figure 3). Type A curves indicate the formation of inclusion complexes that were soluble, whereby A_P shows positively deviating isotherms, A_L shows linearity while A_N shows negatively deviating isotherms; type B curves indicate the formation of inclusion complexes that were poorly soluble, whereby B_I shows limited solubility and B_S shows insolubility [45]. As in the case of ternary inclusion complexes, there were no studies found on the use of the phase solubility technique to determine the molar ratio of the three components involved in the formulation. However, Jadhav and Pore have applied this technique to investigate the solubilities and possible stoichiometries of binary and ternary systems of bosentan (BOS) and hydroxypropyl-β-CD (HPβCD) with or without the presence of L-arginine (ARG) as the ternary component. Using this method, the molar ratio of BOS:HPβCD was found, and the stability constant (K_S) was calculated using the gradient, while ARG was added as the third molecule to determine whether it had a positive effect towards the solubility of BOS and how it affected the phase solubility profile [46]. Generally, the molar ratio of host to guest molecules in an ordinary inclusion complex is 1:1, with several exceptions for complexes formed with multifunctional or long-chain guest molecules [47]. Through intensive study, it was observed that a complex with a stability constant K_S around 100 – 1000 mol/L was deemed optimal for the formation of an ideally stable complex, while $K_S < 100$ indicated an unstable complex, and Ks > 1000 indicated overly high stability whereby the guest molecules were tightly bonded in the CD cavity [48].

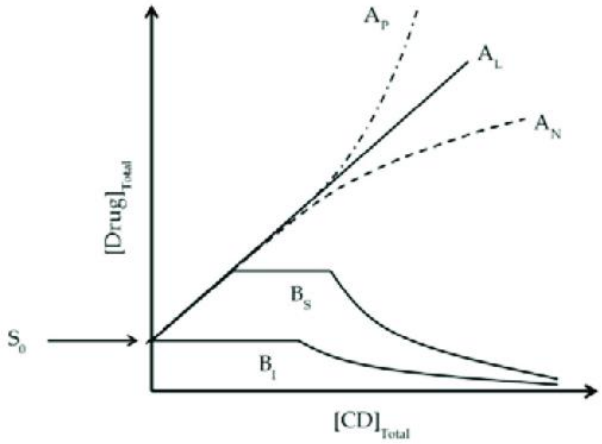

Figure 3. Phase solubility profiles by Higuchi and Connors [45]

2.2. Continuous Variation Method/Job's Plot

In the continuous variation method, identical concentrations of reactants are mixed in such a way that the total volume is kept constant, but the mole ratio of the reactants varies methodically [49]. Despite the lack of research on the determination of the stoichiometry of all three components involved in the formation of ternary CD-drug inclusion complexes using a spectroscopic method, an interesting study by Ahmad and Narayanaswamy reported the use of the continuous variation method or Job's plot to obtain molar ratios of the three individual components involved in the formation of ternary complexes of aluminium (Al) with

eriochrome cyanine R (ECR) and cetylpyridinium chloride (CP). For the ternary complex of Al:ECR:CP, the Al:ECR ratio was held constant and mixed beforehand. A fixed concentration of this solution was then mixed with CP of the same concentration but varied in terms of mole fraction, while the total volume in each mixture was held constant. The absorbance for each mixture was taken and the molar ratio which corresponded to the maximum absorbance indicated the stoichiometry of the components involved. As shown in Table 3, Al: ECR was held at a constant 1:3 ratio before mixing with CP to obtain solutions of various mole fractions for further analysis (not all mole ratios were used). In Figure 4, the plot of absorbance versus mole fraction for two sets of solutions (with total volumes of 3 mL and 6 mL) showed that the maximum absorbance was achieved when the mole ratio was about 0.25, and so (Al: ECR):CP was 1:3, which gave the ratio of 1:3:3 for this ternary complex [50]. Similarly, El-Didamony [51] also applied the continuous variation method to determine the stoichiometric ratio of the SPFX: Pd(II):eosin ternary complex. The difference in this method was the use of excess eosin to find Pd(II):SPFX, the use of excess Pd(II) to find eosin: SPFX, and the use of excess SPFX to find eosin: Pd(II). Figure 5 shows the summary of the results, where (a) Pd(II):SPFX was 1:1 in excess eosin, (b) eosin: SPFX was 1:1 in excess Pd(II), and (c) eosin: Pd(II) was 1:1 in excess SPFX, all of which are used to deduce a mole ratio of about 0.50, which led to the final ratio of 1:1:1 for the overall ternary complex On a side note, most researchers do not attempt the calculation of the stoichiometry ratio for all three components in a CD-drug ternary inclusion complex because the ternary component (for instance a surfactant, polymer, or emulsifier) is often not directly involved in the binding reaction and is only added to enhance the solubility, bioavailability, and pharmacological activity of the desired drug molecule [52]. Regardless, the two experimental methods mentioned above may be useful in obtaining the stoichiometry ratios for every component involved in the formation of a CD-drug ternary inclusion complex, especially in cases where the ternary component plays an important role in affecting drug release, so that suitable adjustments can be made for optimum synthesis.

Table 3. Solutions prepared for Job's plot in determination of Al: ECR: CP ratio [50]

Mole ratio	Al: ECR (2.0 moldm^{-3})/mL	CP (2.0 moldm^{-3})/mL	Total volume/mL
0.0	0.0	6.0	6.0
0.1	0.6	5.4	6.0
0.2	1.2	4.8	6.0
0.3	1.8	4.2	6.0
0.4	2.4	3.6	6.0
0.5	3.0	3.0	6.0
0.6	3.6	2.4	6.0
0.7	4.2	1.8	6.0
0.8	4.8	1.2	6.0
0.9	5.4	0.6	6.0
1.0	6.0	0.0	6.0

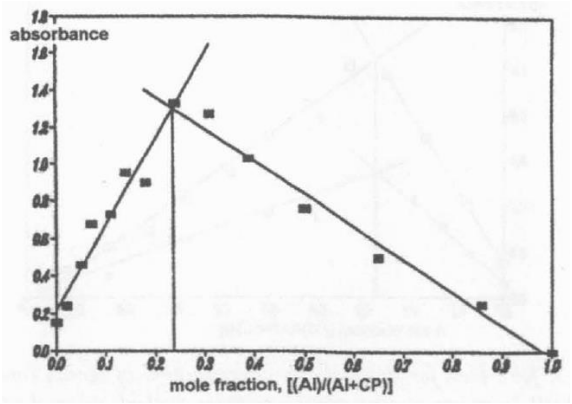

Figure 4. Job's plot for determination of Al: CP in the ternary complex when Al: ECR = 1:3 [50]

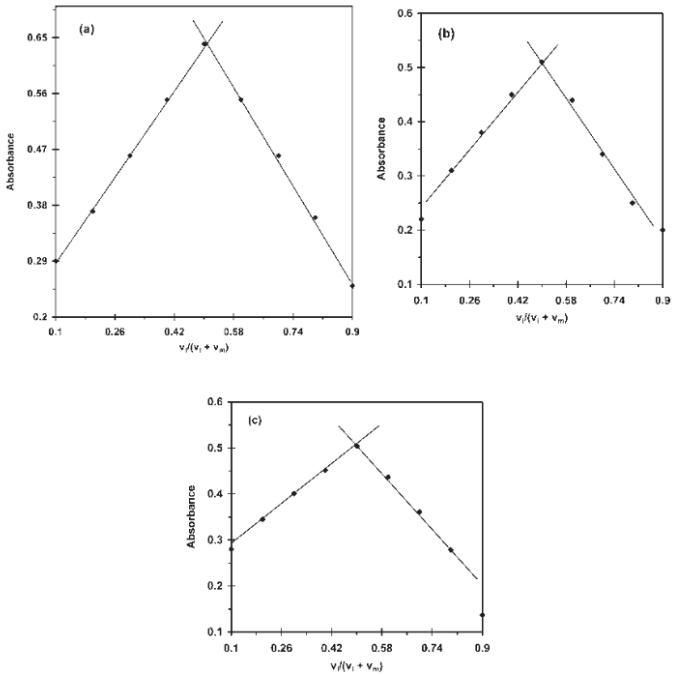

Figure 5. Job's plots for (a) Pd(II)-SPFX in excess eosin, (b) eosin-SPFX in excess Pd(II), and (c) eosin-Pd(II) in excess SPFX (c) [51]

2.3. Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) has always been the most popular method for characterization of the molecular interactions between host and guest drug molecules of ternary inclusion complexes in the solid state. FT-IR can identify the functional groups present in inclusion complexes because their vibrations are associated with characteristic infrared absorption bands, and the absorption bands of individual components before complexation are retained despite the formation of complexes [53]. Upon complexation, the absorption bands or intensities of certain functional groups may change after the guest drug molecules are encapsulated in the CD cavity due to the loss of certain bending or vibrating functional groups during molecular interactions [54]. However, the absorption spectrum of a synthesised ternary drug-CD inclusion complex is usually dominated by CD bands as it contains many repeated D-glucopyranose units, hence the spectra of the synthesised inclusion complex may look similar to that of the CD [55]. According to the literature, the β-CD spectrum shows characteristic intense bands around 3300-3500 cm^{-1} (O-H stretching), 2800-3000 cm^{-1} (C-H stretching) and 1000-1300 cm^{-1} (C-O-C stretching) [56]. Comparison is usually made between FT-IR spectra of the CD, the pure drug components, and the synthesised ternary inclusion complex, where extra attention is given to the characteristic absorption regions of CD and the drugs used in the formulation to confirm the successful formation of a ternary inclusion complex. For example, in Figure 6, carvedilol displays characteristic peaks at ~3340 cm^{-1} (O-H and N-H stretching peaks which are merged due to very close wavenumber range), ~2920 cm^{-1} (C-H stretching), ~1590 cm^{-1} (N-H bending) and ~1250 cm^{-1} (O-H bending and C-O stretching). The spectrum of the ternary inclusion complex of carvedilol, β-CD and citric acid retained most of the characteristic peaks of pure carvedilol and β-CD, except for a few observable changes. For instance, the O-H and N-H stretching peaks merged and broadened dramatically at approximately 3340 cm^{-1}. Several slight differences include the 1480-1600 cm^{-1} region which may be caused by skeletal vibrations of the aromatic C=C bonds. Meanwhile, the peaks in the low wavenumber region (1000-400 cm^{-1}) were mostly unchanged, which indicates that the overall molecular symmetry was not affected much during the formation of the ternary inclusion complex, despite certain restrictions in molecular vibration and bending [57]. These findings provide solid evidence that the synthesized ternary inclusion complex possessed most of the characteristic functional groups of pure carvedilol and β-CD, thus confirming the synthesis was successful.

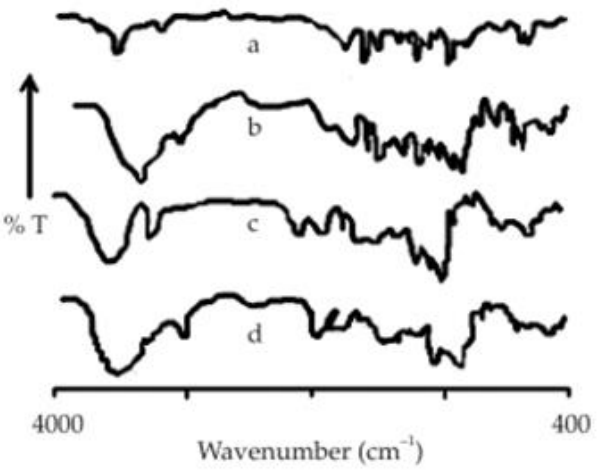

Figure 6. FT-IR spectra of (a) pure carvedilol, (b) physical mixture, (c) complex by kneading and (d) complex by spray drying. Ternary complex: carvedilol, β-CD and citric acid [57].

2.4. Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy (NMR) is often one of the techniques employed to confirm the formation of an inclusion complex. NMR provides very strong evidence for the formation of inclusion complexes, whereby the chemical shift changes in inclusion complexes during complexation are used to provide explanations for the host-guest interactions between CD and the guest drug molecules, even if the interactions mainly rely on intermolecular forces and not chemical bonds [58]. As shown in Figure 7, it is a well-known fact that β-CD has a hollow cone-shaped geometry, with H3 and H5 being the inner protons [59]. Generally, the inclusion of drug molecules in the β-CD cavity changes the chemical shift values of H3 and H5 in β-CD. These values are calculated using the equation, $\Delta\delta = \delta$ (free) - δ (complex), in which a positive value shows upfield shifts and a negative value shows downfield shifts. Occasionally, the H6 proton of β-CD may also be affected due to its location at the rim of the β-CD cavity [60]. In relation to this, Patel and Hirlekar presented the ^{1}H-NMR characterization of the cinnarizine: β-CD: hydroxy acid ternary inclusion complex to show the host-guest interactions between the involved components. Interestingly, they performed the characterization based on a stepwise method, from pure components to the binary system and finally the ternary system with the addition of either citric acid or tartaric acid as a co-complexing agent (Figure 8). From Table 4, it can be observed that the H3 and H5 protons of HPβCD experienced significant upfield chemical shifts in the binary complex A1, as well as in ternary complexes A3 and A6, thus providing evidence for the inclusion of cinnarizine in the HPβCD cavity [61]. Other NMR techniques such as nuclear Overhauser effect spectroscopy (2D-NOESY) and rotating-frame Overhauser effect spectroscopy (2D-ROESY) are frequently used to determine the atomic configuration of a molecule, even those that are not closely connected by chemical bonds [62]. These can be used to support the results obtained from ^{1}H-NMR by showing the cross-correlation between neighbouring atoms to gain more conformational information about the synthesised ternary inclusion complex. Delrivo et. al. [63] carried out several 2D-ROESY experiments to investigate the formation of three different types of sulfadiazine: β-CD: amino acids ternary inclusion complexes. As displayed in Figure 9, the 2D-ROESY spectrum of the sulfadiazine: β-CD: L-arginine ternary inclusion complex shows that Ha and Hb of the drug are correlated with the inner H5 and H6 of β-CD, and with H3 of L-arginine. From this, it can be predicted that the aromatic ring of sulfadiazine was inserted into the β-CD cavity during complexation. Additionally, the drug had certain interactions with L-arginine which was added as an auxiliary substance to improve complexation efficiency.

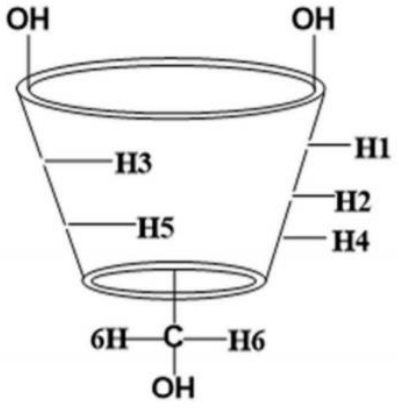

Figure 7. Structure of β-cyclodextrin with protons labelled

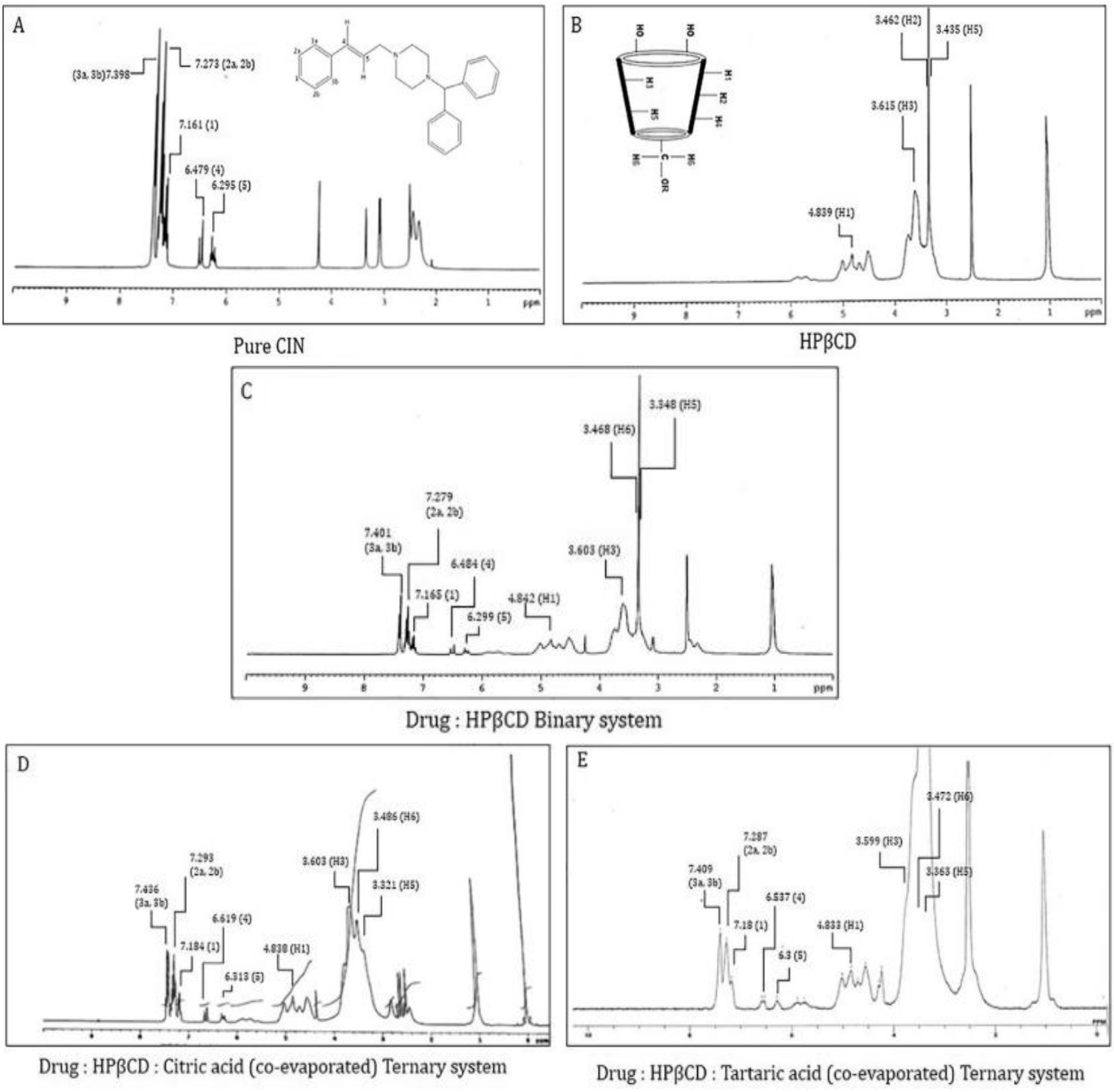

Figure 8. ^{1}H-NMR spectrum for (A) pure CIN, (B) pure HPβCD, (C) CIN:HPβCD binary system, (D) CIN:HPβCD:CA ternary system and (E) CIN:HPβCD:TA ternary system with peak representations of CIN and HPβCD protons [61].

Table 4. Chemical shifts of HPβCD protons in free and complexed forms [61]

HPβCD proton no.	δ_{free}	A1 (CE) $\delta_{complexed}$	$\Delta\delta$ (ppm)	A3 (CE) $\delta_{complexed}$	$\Delta\delta$ (ppm)	A6 (CE) $\delta_{complexed}$	$\Delta\delta$ (ppm)
H1	4.839	4.842	−0.003	4.838	0.001	4.833	0.006
H3	3.615	3.603	0.012	3.603	0.012	3.599	0.016
H5	3.435	3.348	0.087	3.321	0.114	3.363	0.072
H6	3.462	3.468	−0.006	3.486	−0.024	3.472	−0.01

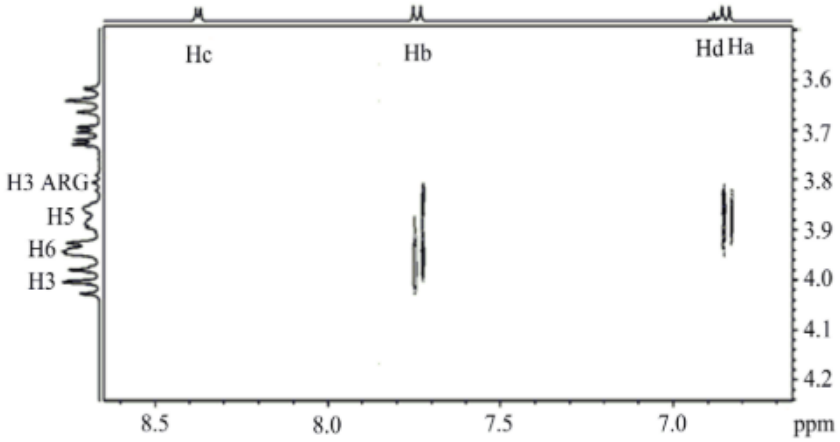

Figure 9. 2D-ROESY spectrum of the sulfadiazine:β-CD: L-arginine ternary inclusion complex [63]

2.5. Thermal Studies

Two viable techniques that can be used for thermal characterization of a ternary inclusion complex in the solid-state are differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC can be used for the confirmation of inclusion complexes because the melting point, boiling point and sublimation point of the guest drug molecules that are encapsulated in the CD cavity will either be shifted or disappear completely in the thermograms [64]. From the literature, the DSC thermogram of β-CD displays a very broad endothermic peak between 60 – 110 °C which indicates a loss of water molecules in the dehydration process, followed by a solid-solid phase transition at about 214 °C [65]. For the application of DSC in the characterization of binary and ternary inclusion complexes, Li and Yang analysed thermograms of ST-246, HPβCD and meglumine (MEG) individually, as well as of their ternary systems. For the ternary inclusion complex synthesized through physical mixing, the thermogram showed peaks at ~125 °C and ~180 °C which correspond to the characteristic melting points of MEG and ST-246, although these had shifted slightly compared to the pure components. This result is explained by the presence of unreacted molecules in the physical mixture. Whereas for the ternary inclusion complex synthesized by freeze-drying, no characteristic melting peaks of any pure components were observed. This was most likely due to the conversion of crystalline molecules into an amorphous structure, indicating the formation of the inclusion complex [66].

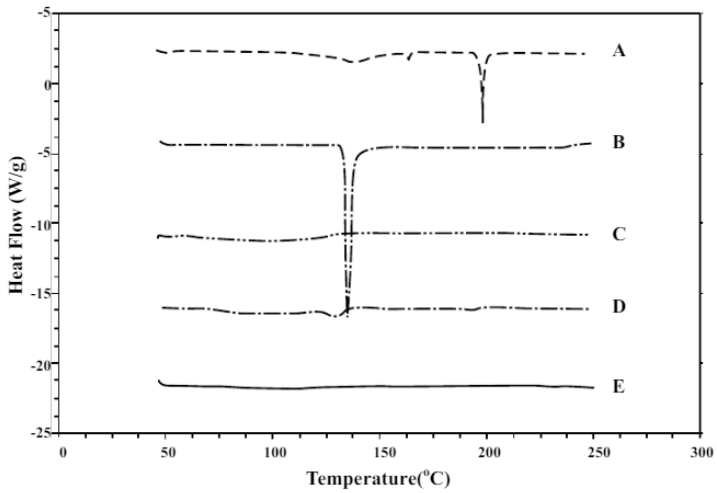

Figure 10. DSC thermograms of (A) ST-246, (B) MEG, (C) HPβCD, (E) ternary inclusion complex by physical mixing, (F) ternary inclusion complex by freeze-drying [66]

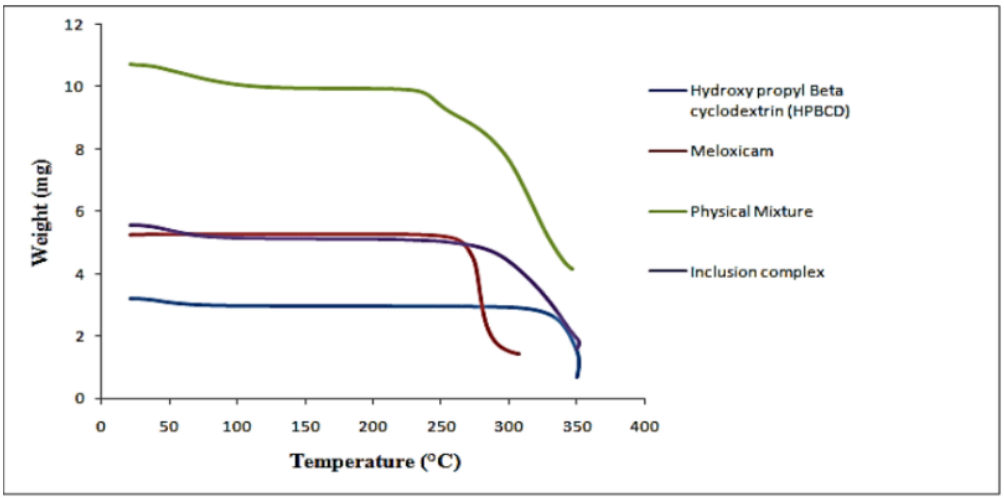

Figure 11. TGA curves of HPβCD, meloxicam, MLX: HPβCD:DEA by physical mixing, and MLX: HPβCD:DEA by solvent evaporation [68]

TGA is used to measure the thermal stability of a compound and identify the changes in weight percent of the compound with respect to temperature. According to the literature, β-CD will suffer a loss of water molecules at temperatures of 25 – 100 °C, and decomposes at temperatures above 300 °C [67]. In Figure 11, the blue curve at the bottom indicates that the ternary inclusion complex of meloxicam (MLX): HPβCD: diethanolamine (DEA) synthesized through the solvent evaporation method showed thermal degradation at a significantly higher temperature and with the lowest relative weight loss compared to its pure components. This provides strong evidence for the successful formation of a ternary inclusion complex, as the drug is encapsulated and gains higher stability in the HPβCD cavity [68].

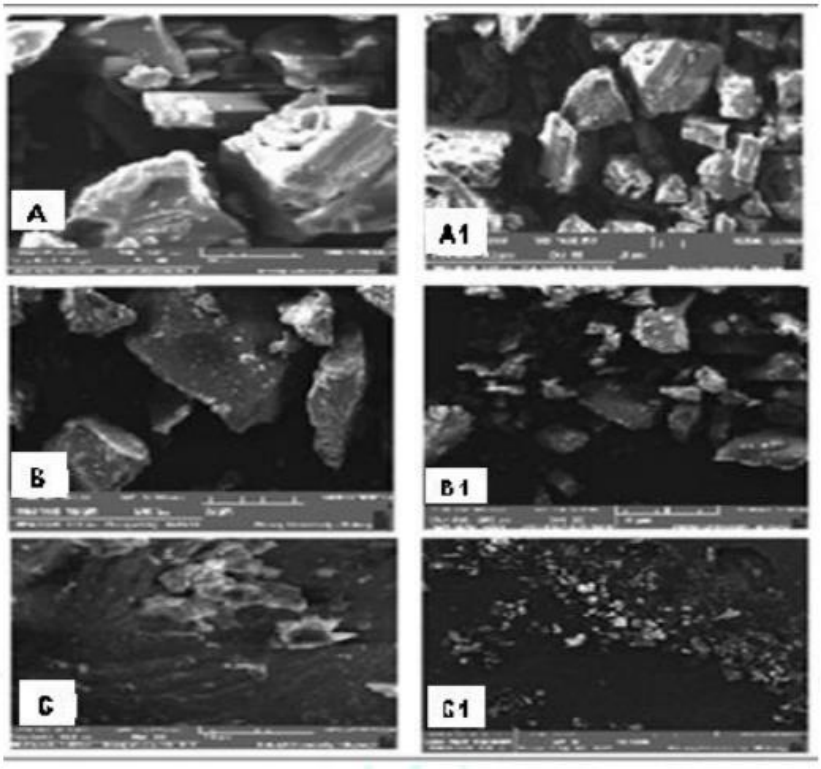

Figure 12. SEM images of (A) EPL, (B) EPL: β-CD binary inclusion complex, and (C) EPL:β-CD:L-arginine ternary inclusion complex [70]

2.6. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is widely used to characterize the surface morphology of synthesised ternary inclusion complexes. Although this technique may be insufficient to prove the formation of a real inclusion complex, the images obtained can be used as supporting evidence. The altered particle shapes and sizes, changes in amorphous or crystalline aggregates as well as structural differences compared to the pure components are key elements in predicting the formation of inclusion complexes and may serve as good reasons for changes in complex solubility [69]. Figure 12(A) shows the surface morphology of the drug epalrestat (EPL), which consisted of well-separated crystalline particles, while Figure 12(B) shows particles which are slightly altered in shape and dimension, most likely due to the natural amorphous structure of β-CD. Lastly, it can be seen from Figure 12(C) that the ternary inclusion complex of EPL: β-CD: L-arginine exhibits vastly different particle shapes and sizes, especially in terms of particle agglomeration, compared to the pure drug and the binary system, and this serves as an indication of the formation of a new solid-state component [70].

2.7. X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis is frequently used to confirm the formation of new compounds from their original components; in the case of inclusion complexes, it is used to determine the crystallinity of the drug in the synthesised complex. X-ray diffractograms of the drug and β-CD will usually display sharp, thin and strong signals which represent their characteristic crystalline peaks. Upon the formation of the inclusion complex, the sharpness and intensities of the characteristic peaks of the individual components will be significantly lower, and some peaks may be fused and broadened, indicating a reduction in the degree of crystallinity, thus confirming the formation of new compound [71]. Meanwhile, the conversion of crystalline components into an amorphous form is closely related to the improved dissolution rate for the synthesised inclusion complex [72]. Based on Figure 13, a sample of pure finasteride (FIN) had intense characteristic peaks in the range of 10-20°, which indicates its natural crystalline character, whereas for HPβCD and the natural polymer chitosan, the XRD patterns showed no sharp peaks, which indicate their amorphous structure. Some characteristic diffraction peaks which belong to FIN were still detected in the physical mixture for FIN: HPβCD: chitosan, indicating incomplete formation of the inclusion complex. However, all the characteristic peaks of FIN disappeared in the FIN: HPβCD: chitosan complex synthesized via the co-evaporation and lyophilization methods, which confirmed the formation of the ternary inclusion complexes. This result can be explained by the higher degree of complexation using the co-evaporation and lyophilization methods, which resulted in greater transformation of the structure from crystalline to amorphous compared to the physical mixture method [73].

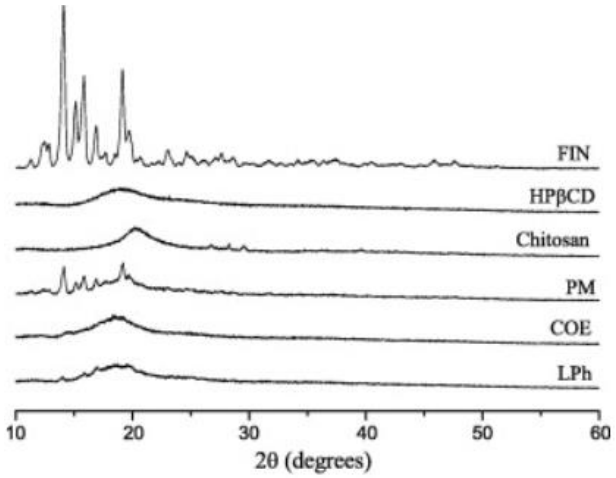

Figure 13. XRD images of pure FIN, HPβCD, chitosan, FIN: HPβCD: chitosan (physical mixture), FIN:HPβCD: chitosan (co-evaporation), and FIN: HPβCD: chitosan (lyophilization) [73]

2.8. Molecular Docking Studies

Molecular docking studies allow a better understanding of the molecular structure of the synthesised ternary inclusion complex and the molecular interactions between the chosen drug and CD during complexation [74]. The molecular modelling process is usually done using computer software such as Hyperchem and Gaussian, whereby the host-guest system is built and designed based on a coordinate system [75]. Therefore, the shape of the complex, its preferred binding orientation and corresponding binding energy can be predicted, along with the orientation, penetration, and rotational angles of the drug molecules inside the CD cavity. As it is possible to observe almost every aspect of the bonding between the drug molecules and CD, predictions of the thermal stability of the synthesized complex can also be made based on the theoretical values of its stabilization energy for different molecular geometries in the equilibrium state. Thus the conformation or molecular arrangement having the most energy-minimized structure with the most negative value can be determined [76]. For example, Figure 14 illustrates the theoretically most ideal and energy-efficient molecular geometry for the binary cinnarizine (CIN): HPβCD inclusion complex and the ternary CIN: HPβCD: tartaric acid inclusion complex. For the binary inclusion complex, it was observed that the 3-phenyl-2-propenyl side chain of CIN was responsible for forming aromatic hydrogen bonds with the two hydroxide (-OH) groups located in the cavity of HPβCD. Additionally, the diphenyl ring of CIN formed an aromatic hydrogen bond with the ethereal oxygen of HPβCD. Whereas for the ternary inclusion complex, tartaric acid (TA) acts as the stabilizer for the complex through the formation of a salt bridge, along with hydrogen bonds between the carbonyl and hydroxyl groups of TA and the piperazine ring of CIN. All theoretical bond lengths were calculated in Angstrom units. The theoretical Gibbs free energy for the binary inclusion complex was -23.48 kcal mol^{-1} while that for the ternary inclusion complex was -52.57 kcal mol^{-1}, which indicates that the ternary inclusion complex has greater stability among the two due to its more negative value. Interestingly, due to electrostatic forces of attraction between TA and CIN, the theoretical electrostatic energy changed drastically from -7.59 kJ for the binary complex to -658.66 kJ for the ternary inclusion complex [61]. Alshehri et al. performed intensive studies on the ternary inclusion complex of piperine (PPR):β-CD:hydroxy propyl methyl cellulose (HPMC) using molecular docking as a reliable method to understand the molecular geometry of the inclusion complex. As shown in Figure 15, the appropriate binding modes and conformation of the molecules involved were identified, where the terminal piperidine ring of PPR protruded out of the β-CD cavity, while the aromatic benzodioxolyl ring of PPR and the (1,4)-beta-D-glucan part of HPMC both occupied the hydrophobic central cavity of β-CD. As such, the binding energy of PPR in β-CD was predicted to be -5.2 kcal mol^{-1}, whereas for HPMC it was predicted to be -4.7 kcal mol^{-1}, both of which can be considered thermodynamically stable due to their negative energy values. Theoretically, this molecular arrangement has minimized steric hindrance as the hydroxypropyl side chain of HPMC is outside the β-CD cavity [77].

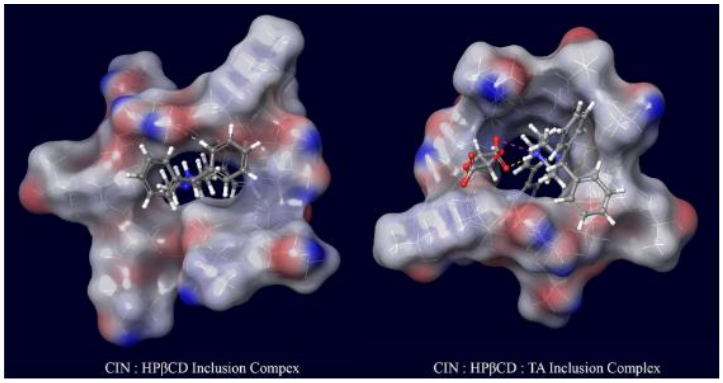

Figure 14. Molecular docking images for CIN: HPβCD binary and CIN: HPβCD: TA ternary inclusion complex. CIN: cinnarizine; HPβCD: hydroxypropyl β-cyclodextrin; TA: tartaric acid [61]

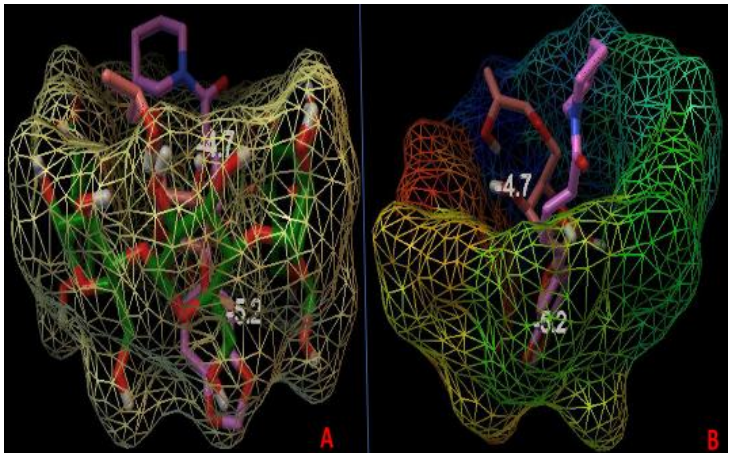

Figure 15. Molecular docking images for PPR: β-CD: HPMC ternary inclusion complex, (A) side view, (B) top view. PPR: grey ; β-CD: wire and mesh; HPMC: orange [77]

2.9. Overall thoughts on Characterization Methods for CD-drug Ternary Inclusion Complexes

For characterization, the results obtained from one method alone is not sufficient to prove the formation of ternary CD-drug inclusion complexes. Therefore, the results obtained from various characterization methods, including FT-IR, NMR, XRD, SEM, and TGA should all be analysed collectively as evidence of the formation of a ternary CD-drug inclusion complex. Lastly, it is important to note that certain characterization methods are only suitable for solid phase samples, while others are used for liquid phase samples. Hence, extra care must be taken to ensure that the product of the synthesis method suits the characterization methods used.

CONCLUSION AND OUTLOOK

This review article has summarized various synthesis methods used by researchers for ternary inclusion complexes of CDs and pharmaceutical drugs. The characterization methods commonly used by researchers to confirm the successful formation of ternary inclusion complexes were also clearly illustrated, with examples of results and case studies obtained from several published research papers. Even though ternary CD-drug inclusion complexes are not something new, they are biomaterials which require thorough consideration of their toxicity and biocompatibility. Exploring useful ways to overcome these issues will encourage further research in this field. There are also possibilities for the improvement of the permeability and stability of synthesized ternary CD-drug inclusion complexes before moving into practical applications. Furthermore, there has been little to no research done on the possible formation of ternary CD-drug inclusion complexes with more than one type of drug simultaneously, and this may require further exploration to verify whether such a synthesis is feasible. All in all, drug delivery systems formulated using CDs in the form of inclusion complexes provide numerous possibilities in the field of pharmaceutical sciences. With the advancement of technology, it is strongly believed that future formulations of ternary CD-drug inclusion complexes will be significantly improved in terms of physical and chemical properties, as well as providing greater drug-release efficiency.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the review article.

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