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SELF ASSEMBLED CYCLODEXTRIN NANOPARTICLES AS DRUG CARRIER

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ABSTRACT

Modification on cyclodextrin (CD) molecule is the basic requirement for self-aggregation. Different type of aggregates studied previously are micellar, rod shaped, hexagonal, spherical systems. Solubility of cyclodextrins can be understood by the given Highuchi-Cannor phase solubility diagram which explains the different types of CD complexes to design innovative mechanistic approaches. These self-aggregates act as effective nano-carriers in drug delivery for sustained release; targeted drug delivery; gene delivery, ophthalmic delivery, in injectable and many other systems. Different case studies in support of gene delivery, artemisinin nano-carrier system, and nanogel in ocular drug delivery have been discussed to give a better understanding of the various possibilities in CD nano carrier design. Hence this report details the different types of cyclodextrins, their chemistry, physical and chemical properties and the different modifications possible.

KEYWORDS: Cyclodextrin, Aggregates, Nano-carrier, Drug delivery.

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1. INTRODUCTION

Cyclodextrins (CD) are a family of cyclic oligosaccharides with a hydrophilic surface and a lipophilic central. Cyclodextrins are widely used in the pharmaceutical, agrochemical, food and cosmetic industries.In the pharmaceutical industry they are used as complexing agents to increase the aqueous solubility of poorly soluble drugs and to increase their bioavailability and stability.In addition, cyclodextrins can be used to reduce gastrointestinal drug irritation,convert liquid drugs into microcrystalline or amorphous powder, and prevent drug–drug and drug–excipient interactions etc.Recently it has been observed that other types of CD complexes,such as non-inclusion complexes, are also participating in the CD solubilisation of poorly soluble drugs. However, in aqueous solutions CDs are also able to self-assemble to form Nano sized aggregates that can contribute to their solubilizing properties. At low CD concentrations (at about 1%, w/v) the fraction of CD molecules forming aggregates is insignificant but the aggregation increases rapidly with increasing CD concentration. Also, formation of CD complexes can increase the tendency of CDs to form aggregates and can lead to formation of micellar-type CD aggregates capable of solubilizing poorly soluble compounds. That do not readily form the inclusion complex formation of CD aggregates and CD nanoparticles .In its most simple form pure drug nanoparticles are being used to increase apparent aqueous solubility and oral bioavailability of poorly soluble drugs. But more sophisticated nanoparticles can be applied to target drugs delivery to specific cells or tissues, or as vehicles for gene delivery, after parenteral administration. Nanoparticulate drug delivery systems can improve drug bioavailability, modify drug metabolism, reduce drug immunogenicity and drug toxicity, and increase the biological half–life of drugs after systemic administration. In aqueous solutions carbohydrates and oligosaccharides self-associate to form aggregates. Cyclodextrins (CDs) are oligosaccharides that are used as enabling excipients in numerous marketed drug formulations. CDs are known to form nano sized aggregates in aqueous solutions and thus have the potential to develop into sophisticated drug delivery systems. The currently available literature on CD aggregates and CD nanoparticles is being reviewed with emphasis on the physicochemical properties of self-assembled CDs and CD complexes [1].

2. RATIONALE

With the rapidly increasing list of poorly soluble drugs, it is the need of the hour to develop such kind of carrier system which can easily deliver hydrophobic drug molecule having less water solubility. Taking advantage of the well proven capability of cyclodextrins to self-aggregate in nanosize we can develop many specialized drug delivery systems like targeted delivery, gene delivery, insulin delivery, sustained release, and ophthalmic drug delivery systems. Many such self-aggregates like nanoshere, nanoreservoir, micelle, nanogels owing to the ease of manufacture and good drug loading could be employed as excellent nanocarriers.

3. CYCLODEXTRIN

Cyclodextrin consists of (α-1,4)-linked α-D-glucopyranose unit with a lipophilic central and that, under certain conditions, small amounts of cyclic dextrin's or cyclodextrins are also being formed during these degradation processes. Technological advances of the 19th century laid the foundation of carbohydrate chemistry and by the middle of the century a number of relatively pure carbohydrates such as sucrose, cellulose from cotton, starch, glucose, fructose, mannose, and lactose were known to chemists in Europe [2].

3.1 CHEMISTRY

When starch is degraded by cyclomaltodextrin glucanotransferase (EC 2.4.1.19) one of several turns of the amylose helix is hydrolysed and the ends join together to form a cyclic-oligosaccharide called cyclodextrins (CD). The glucose units are linked by-1,4-bonds and the chair formation of the glucopyranose unit shapes the CD molecule into a cone with secondary hydroxyl groups extending from the wider edge and the primary groups from the narrow edge. In aqueous solutions these hydroxyl groups form hydrogen bonds with water making CDs soluble in water. Another feature of the CD structure is the somewhat lipophilic central cavity enabling them to form guest/host type of inclusion complexes. The lipophilicity of their central cavity is comparable to an aqueous ethanolic solution. Most abundant natural CDs consist of six (CD), seven (CD) or eight (CD) glucopyranose units. Although the natural CDs and their Complexes are hydrophilic, their aqueous solubility is rather limited, especially that of a CD. This is thought to be due to relatively strong binding of CD molecules in the crystal state (i.e. relatively high crystal lattice energy), and intramolecular Hydrogen bond within the CD molecule, preventing their hydrogen bond formation with surrounding water molecules. Random substitution of the hydroxyl groups, even by hydrophobic moieties like methoxy functions, will convert the crystalline solids into an amorphous mixture of CD isomers resulting in dramatic improvements in their aqueous solubility. CD derivatives of pharmaceutical interest include the hydroxypropyl derivatives of CD and CD (i.e. HPCD and HPCD), the randomly methylated-CD (RMCD), sulfobutylether-CD (SBECD), and the so-called branched CDs such as glucosyl-CD. Analysis of the crystal structure has shown that the cyclic structure of the CD-molecule is stabilized by intramolecular hydrogen bonds between the secondary OH-groups in position C (2) and C (3) of adjacent glucopyranose units. The secondary OH-groups assume defined orientations, but the primary OH-groups are quite flexible and are able to rotate about the C (5) –C (6) bond. Number of water molecules present in the stable hydrates of the CD lattices ranges from 6.4 for CD to 14.2 for a CD. No covalent bonds are formed or broken during formation of guest/host inclusion complexes in aqueous solutions and guest molecules are in rapid equilibrium with free molecules in the solution. The driving forces for the complex formation include the release of enthalpy-rich water molecules from the cavity (i.e. water molecules that cannot have a full complement of hydrogen bonds), Van Der Waals interactions, hydrophobic interactions, hydrogen bonds, electrostatic interactions, release of conformational and stearic strain as well as charge-transfer interactions. Physicochemical properties of free guest molecules are different from the complexes where the molecules are bind to the host (i.e. the CD) molecules. Likewise, the physicochemical properties of free host molecules are different from those in the complex. Any methodology that can be used to observe changes in additive physicochemical properties can, in theory, be utilized to determine the stoichiometry of the complexes formed and the numerical values of their stability constants. These include changes in solubility, shifts in UV/Vis absorbance, changes in chemical reactivity, as well as in fluorescence, NMR, drug retention (e.g. in liquid chromatography) [3,4].

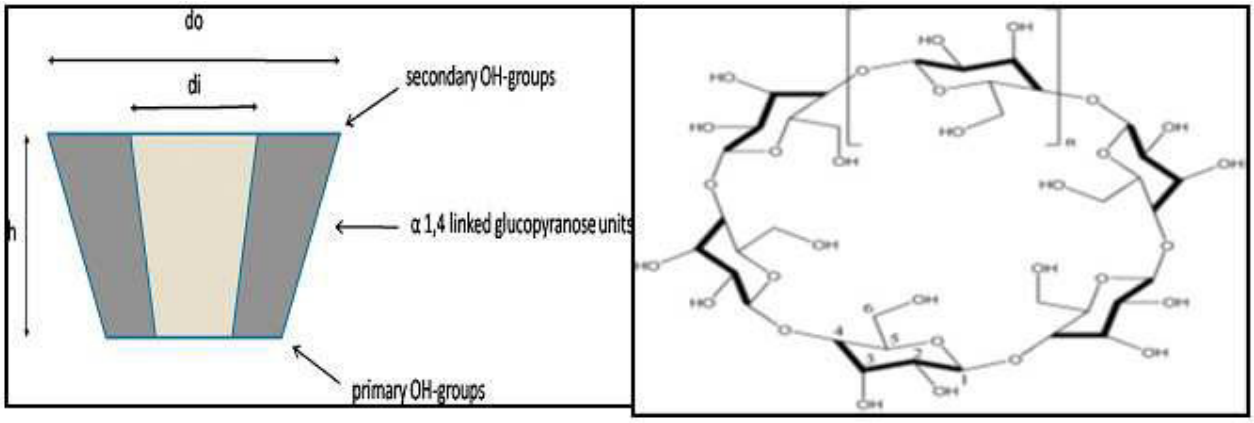

Figure 1
1, 4- linked d- glucopyranose units in cyclodextrins (n=1 CD; n=2 CD n=3 CD), showing the primary hydroxyl group in position C (6) and the two secondary ones in positions C(2) and C(3) [1,22].

3.2 PROPERTIES OF CYCLODEXTRIN

TABLE 1 [1,4].

Property	α-Cyclodextrin	β Cyclodextrin	ϒ Cyclodextrin
Molecular weight of anhydrous compound (Dalton)	973	1135	1297
Number of glucopyranose units	6	7	8
Number of water molecules present in the stable hydrates of the CD lattices			
Total number	6.4	9.6	14.2
Inside the cavity	2	6	8.8
Approximate dimensions (nm)			
Height (H):	0.78	0.78	0.78
Inner diameter (di)	0.50	0.62	0.80
Outer diameter (do)	1.46	1.54	1.75
Solubility in water at 25 ◦C (mg/ml)	129.5±0.7	18.4±0.2	249.2±0.2
K1:1(population mean±standard deviation,25 ◦C)	130±8	490±8	350±9

3.3 PHASES-SOLUBILITY PROFILES AND CLASSIFICATION OF COMPLEXES

The Higuchi–Connor classification of the complexes is based on their phase-solubility diagrams, i.e., how the apparent solubility of a solute molecule (e.g. drug molecule) changes with increasing concentration of dissolved ligand (e.g. CD) due to the enhanced aqueous solubility of the complex formed. In aqueous solutions A-type phase-solubility profiles are obtained when the solubility of the solute increases with increasing ligand concentration through formation of water-soluble complexes. When the complex is first order with respect to solute and first or higher order with respect to ligand then A_L-type phase solubility profile is obtained. If the complex is first order with respect to the solute but second or higher order with respect to ligand then A_P-type phase-solubility profile is obtained. A_N-type phase-solubility profiles can be difficult to interpret but the negative deviation from linearity may be associated with ligand-induced changes in the dielectric constant of the aqueous complexation media, changes in complex solubility or aggregation of the ligand molecules. B-type phase-solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation media. In general, the water-soluble CD derivatives form A-type phase-solubility profiles while the less soluble natural CDs frequently form B-type profiles. The phase-solubility profiles do not verify the formation of inclusion complexes. They only describe how increasing CD concentration influences, drug solubility. To distinguish between inclusion and other types of complexes experimental

results from phase-solubility studies have to be supported by other experimental results from, for example, UV/ Vis, fluorescence and/or NMR studies (The most common type of drug/CD complexes is the 1:1 drug/CD complex(D/CD) where one drug molecule (D) forms a complex with one CD molecule (CD):D + CDK1:1 D/CD The value of K1:1 is most often between 50 and 2000$M^{-1}$with a mean value of 129, 490 and 355$M^{-1}$for CD, CD and CD, respectively. For 1:1 drug/CD complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram. CDs are also able to form non-inclusion complexes where, for example, the hydroxyl groups on the outer surface of the CD molecule form hydrogen bonds with the drug of interest. It has been shown that βCD forms both inclusion and non-inclusion complexes with dicarboxylic acids and that the two types of complexes coexist in aqueous solutions (Likewise acridine/dimethyl-CD 2:1 complex is formed when a 1:1acridine/dimethyl-βCD inclusion complex forms a non-inclusion acridine/dimethyl-βCD inclusion complex forms a non-inclusion complex with a second acridine molecule and some 1:2 and 2:2 drug/CD complexes have been shown to consist of a mixture of inclusion and non-conclusion complexes. This could explain why the values of the equilibrium constants (i.e. The K values) for the complex formation are sometimes concentration dependent and why their numerical values are frequently dependant on the method applied. However, in dilute solutions inclusion-type guest/host CD complexes are probably more common than non-inclusion complexes. In aqueous solutions CDs are not only able to form inclusion and non-inclusion complexes, but also to form hydrogen bonds with neighbouring CD molecules [20,21].

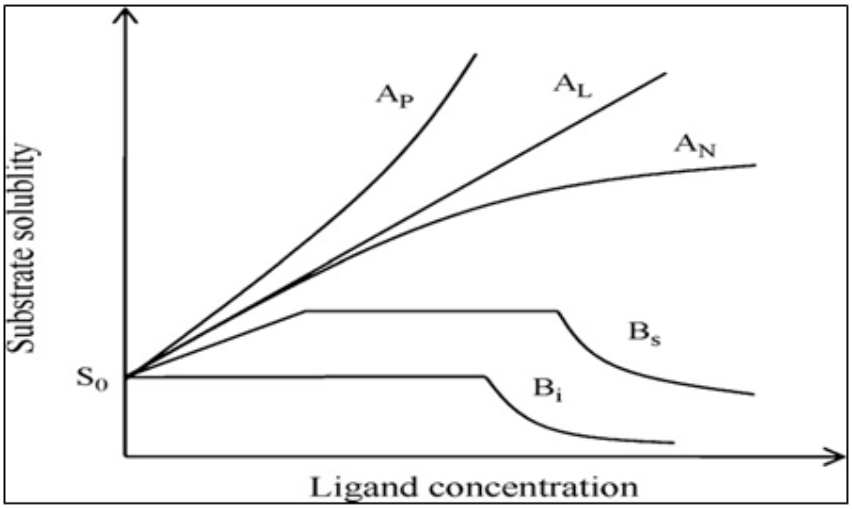

Figure 2
Phase-solubility profiles and classification of complexes [20].

4. NANOPARTICLE

4.1 INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nano spheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

4.2 ADVANTAGES AND DISADVATAGES OF NANOPARTICLES

4.2.1 ADVANTAGES

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration

- They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects
- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc [10,12].

4.2.2 DISADVANTAGES

- In spite of these advantages, nanoparticles do have limitations. For example,
- Their small size and large surface area can lead to particle particle aggregation, making physical handling of Nanoparticles difficult in liquid and dry forms.
- In addition, small particles size and large surface area readily result in limited drug loading and burst release.

4.3APPLICATIONS

4.1. Tumour targeting using nanoparticulate delivery systems

The rationale of using nanoparticles for tumour targeting is based on 1) nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumour targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles; 2) nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ [12].

4.2. Nanoparticles for oral delivery of peptides and proteins

Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose in diabetic rats for up to 14 days following the oral administration [10.12].

4.3. Nanoparticles for gene delivery

Nanoparticles loaded with plasmids DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the derivative endo-lysosomal compartment to the cytoplasmic compartment 74. Hedley et al. 75 reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein [2].

4.4. Nanoparticles for drug delivery into the brain

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB [11].

5. SELF AGGREGATION OF CYCLODEXTRINS

5.1 MECHANISM OF SELF AGGREGATION

It has been known for some time that in aqueous solutions CD molecules have the tendency to self-associate to form aggregates,

but the interest has gained momentum with recent advancements in analytical technologies .The activity coefficients, as well as viscosity and density dependencies on α CD and ϒ CD concentrations, allowed the assume that formation of CD dimmers could possibly be the reason of the observed deviations from ideality. For the next decade physicochemical, pharmacokinetic and toxicological properties of CDs were intensively investigated. During development of CDs as pharmaceutical excipients and drug carriers many of their undesirable physicochemical properties were revealed and investigated, such as their limited aqueous solubility and spontaneous opalescence of aqueous CD solution .The necessity to defy these 'undesirable' CD features arose and series of thoroughly planned studies were carried out by different team assuming a wide variety of methods, assuming self-aggregation as the most probable explanation. Abundant data were obtained with light scattering (LS) methods, both dynamic and static, as they yield precious information on aggregate size and extent of aggregation process in a solution. The general observation is that the aggregates of the parent αCD, βCD and ϒCD tend to grow with increasing CD concentration. The largest aggregates are observed for βCD, which can be up to several micrometres' in diameter. The anomalously low solubility of βCD is explained by the intensity of aggregate formation, which becomes notable at βCD concentrations above 3mM and which is reversely formation, which becomes notable at βCD concentrations above 3m M and which is reversely proportional to hydration extent. It should be remembered that hydrogen bonds play important role in both processes, i.e. solubilisation and aggregate formation. This explanation of low solubility of βCD is furthermore supported by several observations. For example, the substituted βCDs show significantly increased solubility with decreased or even abolished tendency of self-aggregation as in case of 2-methyland3-methyl-βCD. Both RMβCD and HPβCD show negligible aggregation at 12mM while aggregate diameter does not exceed 70 molecules. Also, when the pH of aqueous CD solutions is increased to 12 or above, the OH groups of the CD molecule become ionized resulting in dissociation of the CD aggregates. Finally, chaotropic additives that break hydrogen bonds, such as urea or sodium chloride, cause notable depression of the self-association. These evidences clearly indicate that OH groups of native CDs participate in self-aggregation rather than solvation. Since hydrogen bonds are known to be both saturable and direction dependent it is evident that intermolecular hydrogen binding of βCD molecules limits or prevents hydrogen bond formation with water that leads to solubility depression. The same explanation is valid for peculiarity of aqueous ϒCD solutions, which are well-known to become spontaneously turbid at ϒCD concentrations of 8mM(1%, w/v) or above illustrates well the relationship between ϒCD aggregation and physicochemical properties such as diffusion coefficient, viscosity, activity coefficient and hydrodynamic radius . It should be emphasized that formation of large aggregates does not necessarily indicate extensive aggregate formation. In pure aqueous CD solutions the fraction of molecules participating in aggregate formation is often very low. For example, the mass contribution of the αCD-aggregates in aqueous 12mM αCD solution does not exceed 0.8% (, that of βCD only 0.0011% in 10mM in ϒCD solution and that of ϒCD only 0.02% in 12mM ϒCD solution [1].

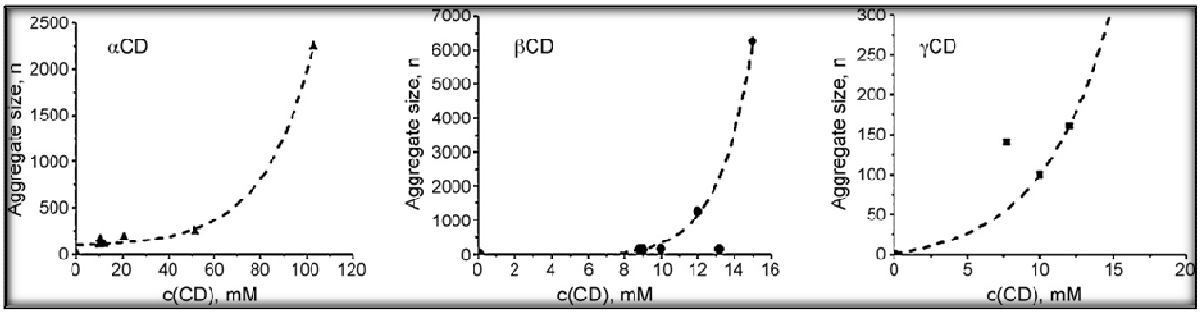

Figure 3
An average size of native CD aggregates (of molecules) versus CD concentration observed by light scattering [1,4].

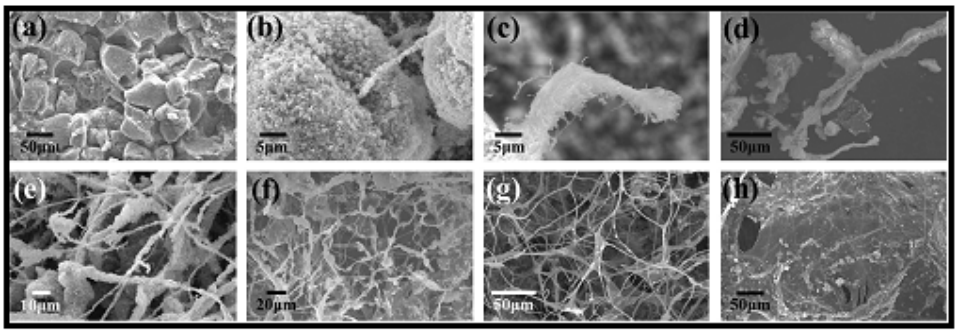

Figure 4
SEM images show progression of the self-assembly in time: (a) 0 days, (b) 2 days, (c) 4 days, (d) 5 days, (e) 6 days, (f)10 days, (g) 20 days, and (h) over 30 day [3].

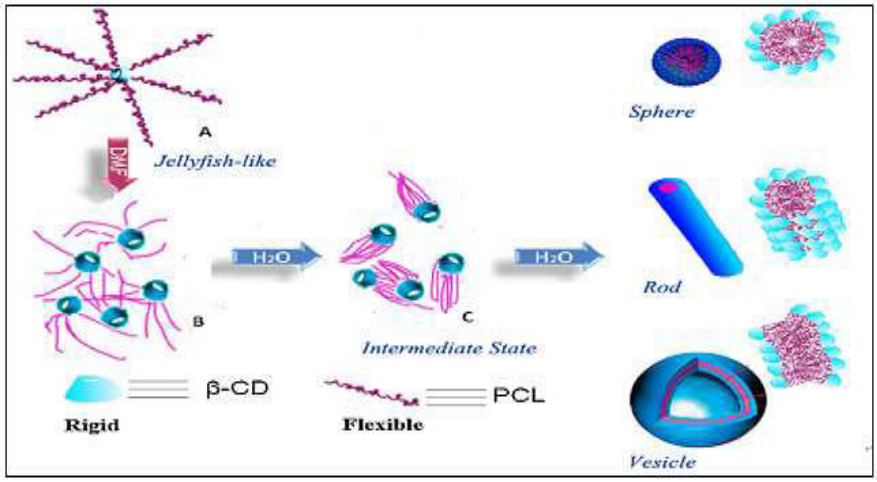

Figure 5
A proposed mechanism leading to the self-assembled morphologies of amphiphilic rigid-coil copolymer CD-PCL [22].

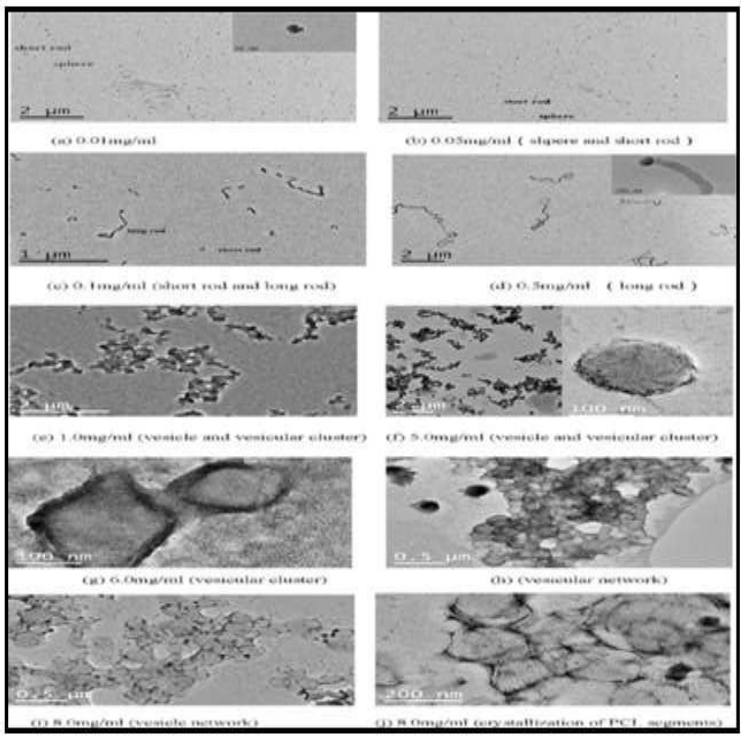

Figure 6
TEM images of CD-PCL18 self-assembled micelles in water with different initial concentrations in DMF. (a) 0.01 mg/ml; (b) 0.05 mg/ml;c) 0.1 mg/ml; (d) 0.5 mg/ml ; (e) 1.0 mg/ml; (f) 5.0 mg/ml; (g) 6.0 mg/ml; (h) 6.0 mg/ml; (i)8.0 mg/ml; (j) 8.0 mg/ml [11].

5.2 MODIFICATIONS ON CYCLODEXTRINS

TABLE 2
Hydrophobically modified β-cyclodextrins [1]

β-CD derivative	Abbreviation	Degree of substitution (DS)
Acetyl-β-cyclodextrin	Ac-β-CD	7
Carboxymethyl-ethylhexylglycidyl-β-cyclodextrin	CMEHG-β-CD	4.2/2.8
Hydroxypropyl-β-cyclodextrin	HP-β-CD	6.3
Hydroxypropyl-hydroxyhexyl-β-cyclodextrin	HPHH-β-CD	2.8/2.8
Hydroxypropyl-phenylglycidyl-β-cyclodextrin	HPPG-β-CD	3.5/2.8
Methyl-β-cyclodextrin	M-β-CD	12.6

TABLE 3
CD-conjugated polymers for biomedical applications [4]

Type of polymer	CD type	Synthesis method	Biomedical applications
Dextran and its derivatives	α-CD, β-CD	Conjugating to the polymers or oxidized Products	Drug delivery
Hyper branched PEI	β-CD	Coupling to the preformed polymer via “click chemistry	Biomimetic biological membranes or solubilisation
Hyper branched polyglycerol	β-CD	Conjugating to the preformed polymer via nucleophilic reaction	Drug delivery
Copolymer of oligo-(ethylene glycol) methyl ether acrylate,N-	β-CD	Conjugating to the preformed copolymers via “click chemistry	Drug delivery

isopropylacrylamide, and acrylate			
Polyaspartamide	β-CD	Conjugating β-CD to the preformed polymers via nucleophilic reaction or "click chemistry	Drug delivery
8-Arm star PEG	β-CD	Conjugating to the preformed polymer	Drug delivery
Chitosan and its derivatives	β-CD or its derivatives	Coupling or nucleophilic reaction between CD and chitosan	Drug delivery, gene delivery
olyamidoamine (PAMAM) Dendrimer	α-, β-, and γ-CD	Conjugating CDs to the preformed polymers	Drug delivery, gene delivery
Linear and branched PEI	β-CD	Conjugating CDs to the preformed polymers	Drug delivery, drug/gene co-delivery
Poly(ε-lysine)	β-CD	Conjugating CDs to the preformed polymers	Gene delivery
Hyper branched poly(amido amine)	β-CD	Michael addition copolymerization	Gene delivery

5.3 PREPARATION METHODS OF CYCLODEXTRIN SELF AGGREGATES

5.3.1 Preparation of CD-PCL Micelles

The obtained CD-PCL18 copolymer was initially dissolved in common solvent DMF to form a series of copolymer solutions with different initial concentrations (0.01–8.0 mg/ml) at room temperature.5 ml Deionized water was added dropwise to 2 ml CD-PCL18 solutions. The solution was stirred for another 2 h unless otherwise noted, and then a twofold excess of water (5 ml) was added to quench the resulting micelles before dialysis against water to remove the remaining DMF solvent. After 48 h, the volume of the solution increased to about 12 ml. The final concentration of the obtained copolymer solution in water varied from 0.002 mg/ml to1.3 mg/ml. Similarly, a 10 ml solution of 2.0 mg/ml CD-PCL18 in DMF was slowly dropped into 10 ml of FcA aqueous media (2.0 mg/ml) under sonication, followed by dialysis against deionized water. After 48 h, the volume of the solution increased to 40 ml with the addition of deionized water to obtain an aggregate solution with a concentration of 1.0 mg/ml for further experiments. The critical aggregate concentration (CMC) of CD-PCL18 in water was measured by the fluorescent probe method. 5 l of6×10−3 mg/ml pyrene solution in acetone was added to CD-PCL18 aqueous solutions with different concentrations and the solutions were sonicated for 10 min before fluorescence emission measurements [14].

5.3.2 Nanoassemblies preparation – Nanogel

Nanoassemblies were obtained by mixing at room temperature equal volumes of aqueous solutions of pβ-CD and of MD, at concentrations ranging from 1 to 10 mg/mL. Their mean diameter and the size distribution were determined at different time intervals by quasi-elastic light scattering (QELS) using a Coulter Nanosizer (Model N4MD, Coultronic, France). According to the need, samples were diluted with milliQ water in order to maintain the count per second between 5×104 and 1×106. Each sample was measured three times for 1min at 20°C and at an angle of 90°. Both unimodal and size distribution processor analysis were performed. The experiments were made in triplicate. Nanoassemblies' suspensions were centrifuged (20,000 ×g, 30min). Supernatants were collected and freeze-dried, in order to determine the weight of the polymers which did not form nanogels. The production yields were calculated from the mass ratio of polymers forming nanoassemblies and the polymers initially introduced in the fabrication procedure .Aqueous solutions of the two polymers were mixed at room temperature. Typically, the system separated in two phases, a highly viscous liquid phase and a supernatant. However, a very narrow domain was found where the system behaved in a completely different way: immediately after mixing the two polymers, suspensions of stable spherically shaped nanogels were obtained in a dispersing medium devoid of soluble polymer [6].

5.3.3 Nanoreservoir systems

Reservoir-type nano systems were prepared by injecting an organic phase containing acetone (10 mL),γ-CD-C10, benzyl benzoate,and a lipophilic surfactant, Montanex-80, into aqueous phase containing distilled water (20mL) added with a hydrophilic surfactant, Montanox-80, under magnetic stirring (500 rpm) at 25 °C. The organic solvent was removed under reduced pressure and the suspension was concentrated until a final aqueous volume corresponding to 50–60% of initial distilled water volume. ART-loaded

systems were prepared using an initial drug amount of 20 mg in the organic solution. All preparations (sphere and reservoir colloidal suspensions) were finally rendered isotonic with glucose solution [8].

5.3.4 Preparation of IND-loaded micelles

IND was loaded into PCEC–b-CD co-polymer micelles by a modified dialysis method. Briefly, IND and PCEC–b-CD co-polymers at ratios of 3/100, 5/100 and 10/100 were dissolved in dimethyl formamide (DMF) and placed in a dialysis bag (molecular weight cut-off (MWCO) 3500). As DMF was gradually removed by dialysis in deionized water for 24 h at 80 ℃ and the IND-loaded co-polymer micelle (IND-M) solution formed. This solution was lyophilized to obtain dry IND-loaded micelle powder. After redissolving in DMF the drug loading capacity (LC) and encapsulation efficiency (EE) of the drug-loaded micelles were determined from the absorbance at a wavelength of 319 nm using an ultraviolet spectrophotometer (TU-1800PC, Beijing Purkinje General Instrument Co., Beijing, China) and calculated using the following equations. PCEC–b-CD did not interfere with ultraviolet absorption by IND in the wavelength range 200–400 nm [9].

5.4. SHAPES AND SIZES OF SELF AGGREGATES OF CYCLODEXTRINS

Another interesting feature of CD aggregates is their shape, which can be studied by a variety of microscopy techniques. Long with spherical particles which can be observed in aqueous βCD and ϒCD solutions a wide spectrum of 'exotic' forms can be found. Especially wide variety of βCD aggregates have been reported by using Cryo-TEM technique. In addition to spherical structures they found disks and large sheets consisting of welded fibers. Perhaps, the most natural arrangement of CDs in aggregates are long fibers (or rod, worm-like structures), that are organized similar to the channel type of CD crystal structure, i.e. stacks of molecules oriented in all possible ways: head-to-head, head-to-tail and tail-to-tail. In addition, due to dynamic properties of a liquid solution medium and non-covalent binding abilities of CDs, such fibers can form higher-order congregations as mentioned previously. Alternatively self-aggregation can be studied by monitoring the colligative properties of CD solutions. This is based on the fact that formation oligomeric structures inevitably affect physicochemical properties such as vapour pressure, osmotic pressure, freezing point and boiling point. A classical way of studying solute aggregation is measurement solution's osmolality, either by monitoring changes in its vapour pressure or the freeze-point depression using pure solvent as a reference. The formation of aggregates decreases the number of particles in comparison to ideal solution, where the number of particles is equal to the number of nonelectrolyte molecules, and aggregation is manifested as negative deviation of solution osmolality from its expected value [1.4].

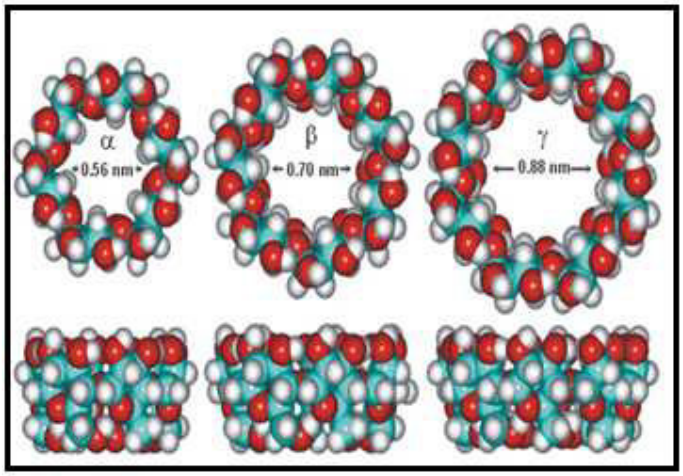

Figure 7
Moleculecular arrangement of Cyclodextrin [18]

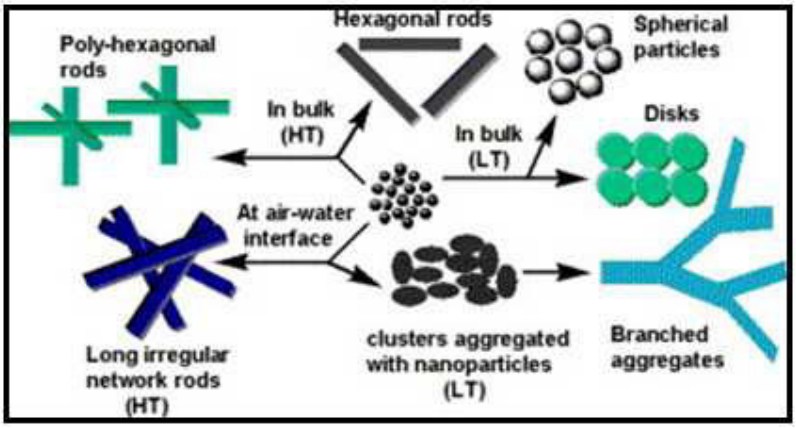

Figure 8
Different shapes of cyclodextrin aggregates [15]

Table 4
Self-assembly of the natural αCD, βCD and ϒCD [1]

Cyclodextrin type	Aggregate shape	Aggregate diameter at given concentration	Analytical method	Weight fraction of CD forming aggregates
α-CD	Spherical coils (elongated Nanostructures	68±20nm (12mM)	DLS	0.8% in 12mM
βCD	Spherical disks, sheets, welded fibres, rod or worm-like structures	194±10nm (10mM) DLS 6 nm (3Mm)	DLS,TEM	0.0011% in 10mM
ϒCD	Spherical	90nm (3mM) DLS 174±38nm (12mM) no aggregates l 112±37nm (12mM)	DLS DLS NMR DLS	

5.5. DETECTION METHODS

Now-a-days different advanced analytical methods are used to detect the self-assembled cyclodextrin aggregates. These methods are based on physical properties of molecules. The basic properties they are measured is like chemical environment in NMR, hydrodynamic radius in LS, and direct scanning in TEM, mechanical scanning of particles surfaces in AFM, and determination changes in freezing point that is osmolality are measured. In follow different analytical techniques are given with their principle and advantages and disadvantages.

5.5.1 NMR (nuclear magnetic resonance)

In this technique, investigation of the electronic environment of an atom and its interaction with adjacent atoms is done. Very sensitive to changes in the molecular environment (e.g. forming of inclusion complexes.) .Results can be difficult to interpret, especially when numerous atomic interactions are being investigated [19].

5.5.2 LS (light scattering)

In this technique determination of the hydrodynamic radius (Rh) of a particle by the light scattered from it. Direct observation of the particle size Only Rh is obtained not the particle size and shape, difficult to find acceptable laser intensity that produces reliable results. Concentrated solutions produce signal noise; filtration or centrifugation of these solutions changes their composition. The aggregate size increases linearly with time.

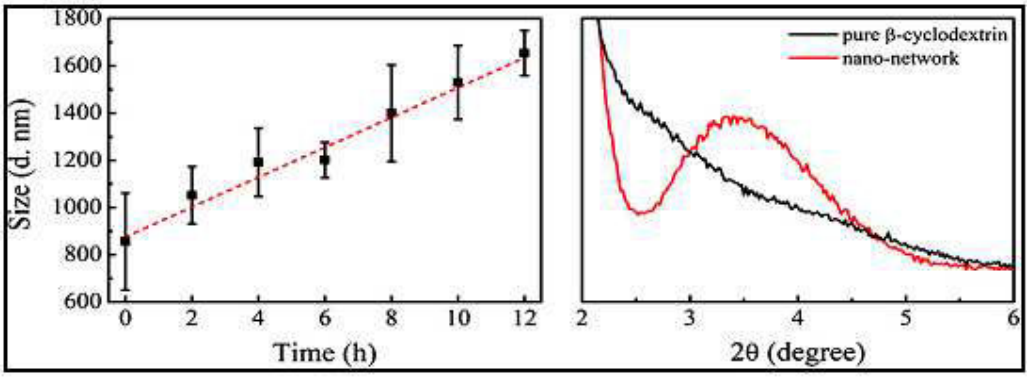

Figure.9

(a) DLS data of the peak aggregate size in the growth solution during exposure. (b) SAXRD data for the pure CD hydrate powder (black line) and mature TiO2–CD network (red line) [2].

5.5.3 TEM (transmission electron microscopy)

In this technique direct imagining of a sample by an electron beam. Image of the particles showing their sizes and shapes. Samples have to be solid and coated with gold or carbon. Mechanical scanning of particle surfaces were done and direct observation of the particle size and shape Cantilever must be able to scan the sample without scratching, particles must be fixed for scanning [17].

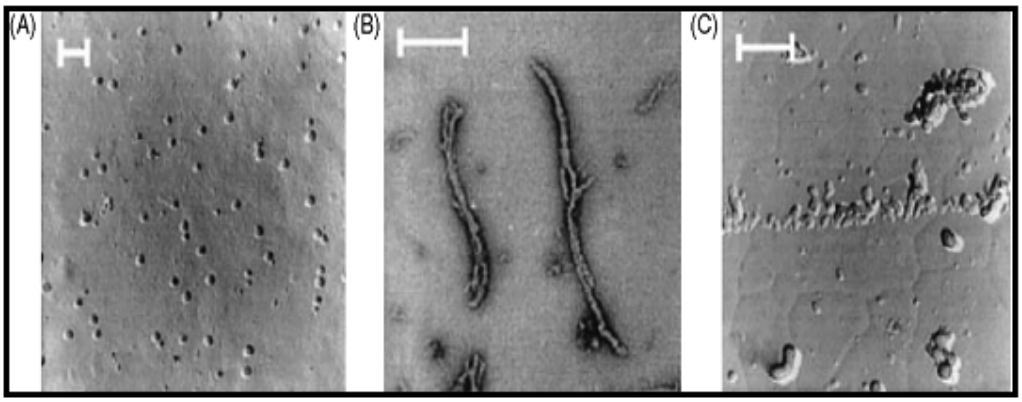

Figure10

Micrographs made by transmission electron microscopy. Each bar represents 200 nm[17].

5.5.4 Osmolality

Osmolality is the colligative property and it is solely depends upon particle numbers .In this technique determination of changes in the freezing point depression or vapour pressure lowering due to formation of particles. Indirect observations of particles formation were done. This technique could not give information about the particle shapes and sizes [1].

5.5.5 SEM (Scanning electron microscopy)

The microstructural evolution of the networks was determined by arresting the growth after various growth times. Growth is effectively terminated by washing sample in copious amounts of de-ionized water. The network is first frozen in pure water and then loaded into a freeze dryer overnight under vacuum at a temperature of −50◦C. The resulting dry networks are self-supporting 3D porous monoliths. The microstructure of the networks is analysed with scanning electron microscopy (SEM, JEOL JCM 5700). SEM specimen are cut from the freeze dried samples and coated with a thin layer of high purity gold to reduce charge-up effects during SEM imagining [5].

5.5.6 AFM (atomic force microscopy)

In this technique mechanical scanning of particle surfaces was done. Direct observation

of the particle size and shape. Cantilever must be able to scan the sample without scratching; particles must be fixed for scanning.

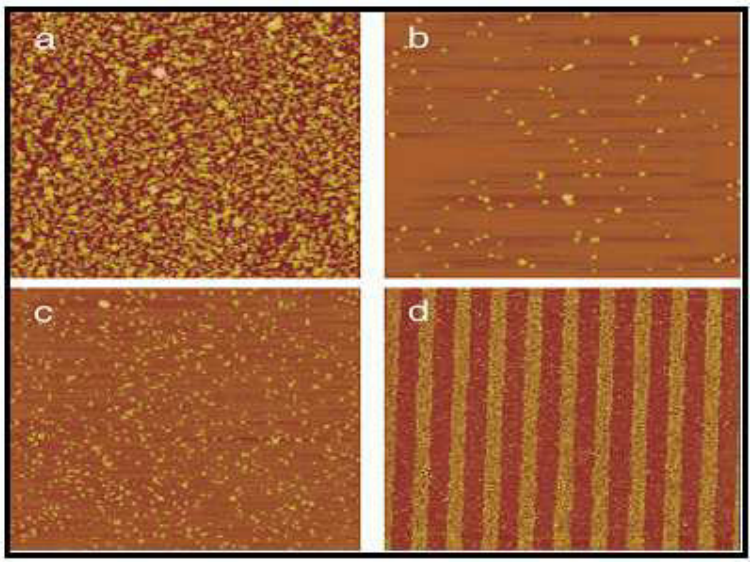

Figure 11
AFM height images (z scale: a, b: 250 nm; c, d: 300 nm) obtained after the deposition (2 min at pH 9.2) of β-cyclodextrin-(a, b, d) [3].

5.6.ADVANTAGES AND DISADVANTAGES OF CYCLODEXTRIN SELF AGGREGATES

5.6.1 ADVANTAGE

- It is use in many formulations and no compatibility issue.
- Aggregation size is nanometre hence it can be inject.
- It encapsulating property used to reduce adverse effect of many drugs.
- It can use in many drug delivery systems like sustained release, targeted drug delivery system, parenteral, ophthalmic drug delivery.
- It is safe and nontoxic.
- It is generally regarded as safe hence advantages.
- Drug loading is higher.

5.6.2 DISADVANTAGES

- Natural cyclodextrins are not used due to its solubility problem.
- Modification is required for self-aggregation.
- Scale up is problem.
- Process parameter controlled is difficult.
- For analytical propose require advance analysis techniques then cost is problem.

5.7. APPLICATIONS

5.7.1 TARGETED DELIVERY OF siRNA IN HUMANS

The development of new therapeutics that attempt to engage RNAi pathway has opened a new area of drug discovery and development. Traditional drug discovery and development involve large scale screenings of small molecule libraries with initial screening hits Subjected to subsequent medicinal chemistry approaches to create enhanced versions. Translation of small molecule experimental therapeutics into the clinic has led to high failure rates for a variety of reasons that include lack of efficacy, poor pharmacokinetics (PK), and so on. Drug discovery and development utilizing RNAi based mechanisms allow for significantly different strategies than those utilized in traditional drug discovery and development. They have the potential to provide therapies for many targets that are currently undruggable and to do so with higher success rates in the clinic. While there are many ways to engage RNAi pathways, the majority of the clinical trials currently being conducted involve small interfering RNA (siRNA). The initial clinical trials employ

naked siRNA that is administered locally (to the eye, lung, or skin) [2].

5.7.2 CONTROLLED RELEASE DRUG DELIVERY

Aqueous nano and microgels are outstanding polymer materials because of their interesting properties such as small size, high porosity, large surface area, high chemical functionality, ability to swell in different solvents (e.g. Water), and stimuli-sensitivity. Nano- and micro-gels can be used in colloidal form as micro reactors, drug carriers, and antimicrobial agents but also as building blocks for design of colloidosomes, hydrogels, and films targeting such applications as controlled release, tissue engineering and optics. Are outstanding polymer materials because of their interesting of polymer nano and micro-gels is extremely important for their application in different areas of science and technology. So far colloidal polymer networks have been modified by incorporation of ionisable or reactive groups, integration of biomacromolecules or nanoparticles [15].

5.7.3. INTRAVENOUS DRUG ADMINISTRATION

This is very much important route of administration. Many drug which having high presystemic metabolism or not absorbable through GIT which is given by this route. Self-aggregates of cyclodextrin means modified cyclodextrin having higher water solubility and it can used by this route, and aggregate is in nano size it is easily injectable [23].

5.7.4 SOLUBILITY ENHANCER

Certain pharmaceutical excipients are known to enhance CD solubilization of drugs. The formation of complex aggregates and non-inclusion interactions between these aggregates and other excipients present in the complexation media. Molecular modelling studies have suggested that malic acid acts as go-between econazole/βCD inclusion complex aggregates reinforcing nanostructures via specific (hydrogen bond and salt bridging) interactions with both components. Similarly significant improvement of the complexation efficiency is observed when small amount of water-soluble polymers is present in the aqueous complexation media [20,21].

5.7.5 OPTHALMIC DRUG DELIVERY SYSTEM

In ophthalmic drug delivery system, self-aggregation of cyclodextrin is used to prepare the nanogels for delivery of many drugs which are used in ophthalmic diseases like uveitis, glaucoma. Nanogels are help to improve the ocular bioavailability and longer retention of drug in eyes. Drug like indomethacin, dexamethasone are delivered by this way [7].

6. CASE STUDIES

6.1 Self-assembled biotransesterified cyclodextrins as Artemisinin nanocarriers.

Recently reported a one-step trans esterification of cyclodextrins (CDs) by vinyl-acyl fatty esters catalysed by thermolysin. By using the solvent displacement method and depending on the experimental conditions, the CD derivatives grafted with decanoic alkyl chains (CD-C10) yielded either nanosphere or nanoreservoir-type systems with a size ranging from 70 to 220 nm. Both types of nanostructures were able to associate artemisinin (ART), a well-known antimalarial lipophilic drug. The formulation parameters were optimized to reach stable and high ART dosage corresponding to drug levels of 0.3 and1.6 mg/ mL in the colloidal suspension, for the spherical and reservoir-type nanosystems, respectively .PEG surface-decorated nanoparticles were also prepared by co-nanoprecipitation of PEG fatty acid estersand CD-C10 molecules. The integration of the PEGylated amphiphiles within the CD-C10 nanostructures did not influence the ART lyoavailability. Both types of ART-loaded nanosystems showed a sustained in vitro release profile over 96 (nanoreservoirs) and 240 h (nanospheres). Finally, the in vitro antimalarial activity was evaluated using the lactate dehydrogenase assay. ART-containing colloidal suspensions inhibited the growth of cultured Plasmodium falciparum, both multi-resistant K1 and susceptible 3D7strains with IC50 values (2.8 and 7.0 ng/ mL) close to those of reference ART solution. These colloidal nano systems

based on CD derivatives and containing ART may provide a promising alternative formulation for injectable use of ART [8].

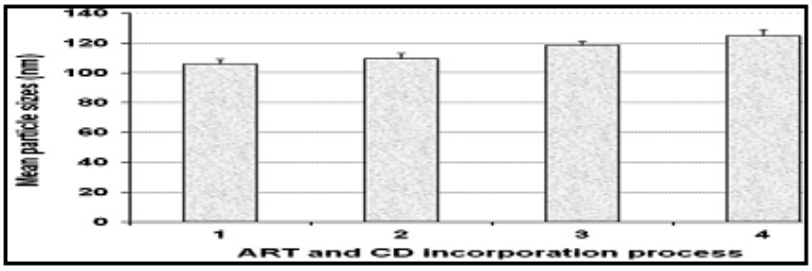

Figure 11
ϒ-CD-C10 particle size (mean ± SD) as a function of ART and CD incorporation process [8].

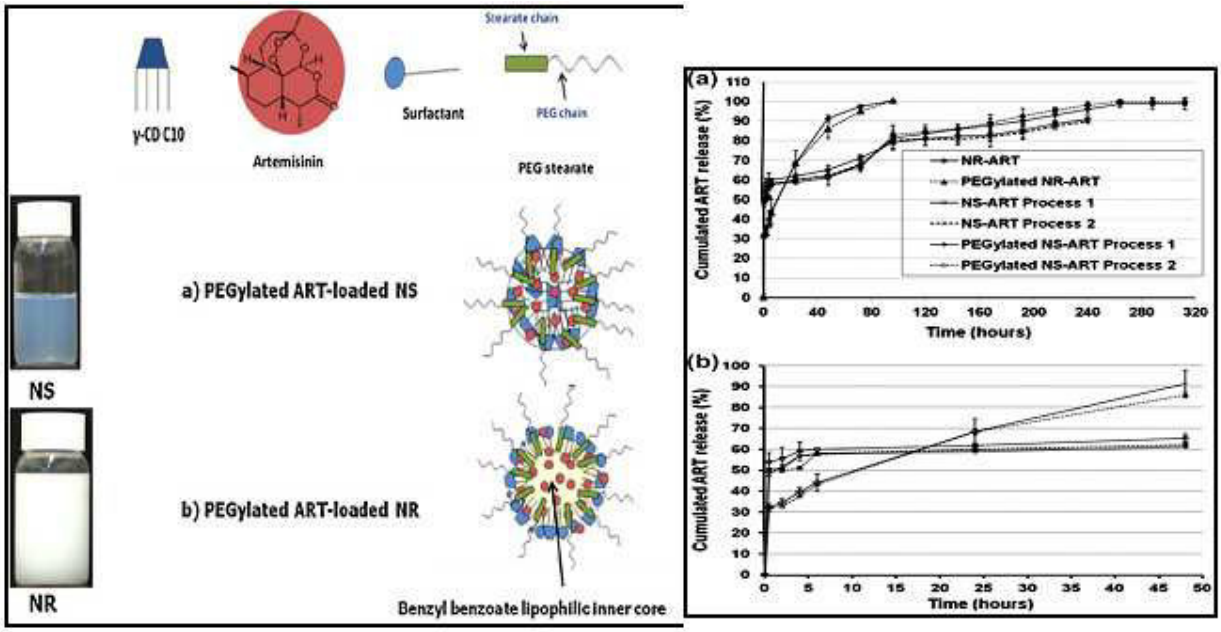

Figure 12, 13
In vitro release profiles of ART from ϒ-CD-C10-based sphere and reservoir type nano systems (n = 3, SD) (a) and in vitro release profiles during the first 48 h (b)PEGylated NR-ART = PNR1 [8] .

Conclusion

Biosynthesized β- and ϒ-CD-C10 are able to form supramolecular assemblies such as nanospheres and reservoir-type nanosystems in the conditions of solvent displacement. PEG surface decorated nanostructures were developed via the interaction of fatty acid esters of different chain length and CD-C10 derivatives. The two types of developed nanostructures were able to associate artemisinin. Stable formulations associated with high drug content were obtained with ϒ-CD-C10. In vitro release characteristics showed an extended release from 4 to 12 days, which was not influenced by the presence of the hydrophilic corona. In vitro antimalarial activity data indicate that both ART-loaded nanosystems are potentially interesting for clinical applications. These colloidal nano carriers may provide a promising alternative for injectable use of ART. In vitro cytotoxicity, complement activation, and macrophage uptake as well as bio distribution studies are in progress in order to assess the safety and stealthiness of these colloidal drug delivery systems.

6.2 Thermosensitive β-cyclodextrin modified poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone) micelles prolong the anti-inflammatory effect of indomethacin following local injection.

A novel biodegradable and injectable in situ gel-forming controlled drug delivery system based on thermosensitive β-cyclodextrin-modified poly (ε-caprolactone)–poly (ethylene glycol)–poly (ε-caprolactone) co-polymer (PCEC–β-CD) was studied in this work. The drug encapsulating capacity has been improved by introducing b-CD bound to the PCEC co-polymer. The prepared PCEC–β-CD co-polymers self-assembled in water to form micelles, and underwent a temperature-dependent gel–sol transition, which was in the form of a flowing injectable solution at low temperatures but became a non-flowing gel at around physiological body temperature. Furthermore, a small hydrophobic drug molecule indomethacin (IND) was successfully encapsulated in PCEC–b-CD micelles by dialysis at a high encapsulation efficiency and drug loading capacity. The IND-loaded micelles (IND-M) exhibited controlled release in vitro. Additionally, a pharmacodynamics study in vivo based on both the carrageen an-induced acute and complete Freund's adjuvant-induced adjuvant arthritis models indicated that sustained therapeutic efficacy could be achieved through subcutaneous injection of IND-loaded micelles. A significant improvement in the anti-inflammatory effect of IND in rat's in PCEC–β-CD micelle [9] .

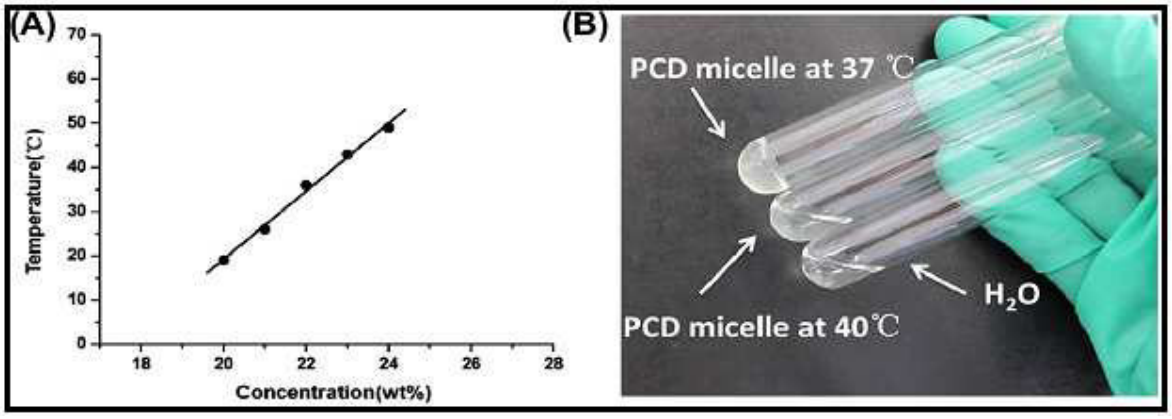

Figure 14

Gelation properties of the PCD micelles. (A) Gel–sol transition diagram of the PCD micelles. (B) Photograph of a PCD micelle (22 wt.%) at 37(gel state) and 40ºC (sol state) in H_2O [9]

Conclusion

In this study a novel amphiphilic co-polymer PCEC1000-6000-1000– β-CD was synthesized and its thermo sensitivity studied.With the help of the solubilisation effect of β-CD for hydrophobic drugs IND loaded micelles with a high EE were successfully prepared by a dialysis method. IND-M was able to release the drug over an extended period in vitro and exhibited a significant therapeutic effect in pharmacodynamics studies in vivo. These results suggest that the PCEC–β-CD micelles might have great potential for sustained release of hydrophobic drugs via local administration in clinical situations.

6.3 Dexamethasone eye drops containing ϒ-cyclodextrin-based nanogels

Sustained release aqueous eye drops of dexamethasone, based on cyclodextrin (CD) nanogels, were designed and tested in vivo. ϒCD units were cross-linked in the form of nanogels by means of an emulsification/solvent evaporation process. The composition of the nanogels was optimized with regard to drug loading and release rate. The eye drops consisted of an aqueous solution of dexamethasone in2-hydroxypropyl-ϒ-cyclodextrin (HPϒCD) medium containing ϒCD nanogels. The nanogels eye drops (containing 25 mg dexamethasone per ml) were tested in rabbits and compared to the commercially available product Maxidex® (suspension with 1

mg dexamethasone per ml). One drop administration of the nanogel eye drops resulted in nearly constant dexamethasone concentration for at least 6 h in the tear fluid (mean concentration±SD = 295±59 _g/ml) whereas the concentration after administration of Maxidex® fell rapidly from 9.72±3.45 µg/ml 1 h after application to 3.76±3.26 µg/ml 3 h after application. We found that the maximum dexamethasone concentration in the aqueous humor (2 h after application) was136±24 mg/ml after application of the nanogel eye drops, and only 44.4±7.8 µg/ml after application of Maxidex®. The dexamethasone nanogel eye drops were well tolerated with no macroscopic signs of irritation, redness or other toxicity [7].

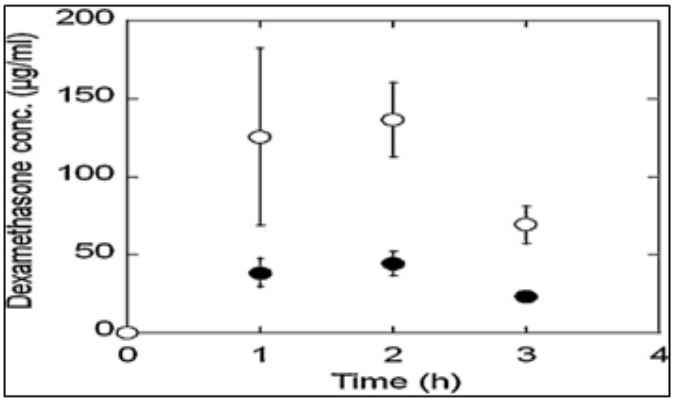

Figure 15
Dexamethasone concentration in aqueous humor after topical administration of one drop of Maxidex® () or of one drop of aqueous dexamethasone nanogel Formulation (o) to rabbit's eyes.

The concentration of dexamethasone reached was significantly higher after administration of nanogels eye drops ($P < 0.04$, paired test) [7].

Conclusions

Dexamethasone/HPϒCD/nanogel eye drop formulation, with pH 7.4 and viscosity of approx. 30 cps, was successfully formulated and compared to Maxidex® suspension. The nanogel formulation contained 25-times more drug than the commercial formulation .where the whole dose was kept dissolved in a colloidal system .No irritation or other side effects were observed after topical application of the CD-based nanogel eye drops to rabbits. Nanogel containing eye drops showed a higher, longer, and more constant concentration dexamethasone on the eye surface. The nanogels may adhere to the ocular surface resulting in sustained drug delivery to the eye. The nanogel-containing eye drops increased the ocular bioavailability and gave high dexamethasone concentrations in the aqueous humor for at least 3 h after administration of the eye drops.

7. CONCLUSION

In aqueous solutions CDs self-assemble to form nano sized aggregates. The aggregate formation is concentration depend to increasing with increasing CD concentration. In the case of the natural αCD, βCD and ϒCD the fraction of CD molecules forming aggregates is in most cases less than 1%, and frequently less than0.1%, in pure aqueous 1% (w/v) CD solutions while the hydrophilic ϒCD derivatives, e.g. the hydroxypropylated and sulfobutylether derivatives, appear to have even less tendency to self-assemble to form aggregates at these low concentrations.

CD derivatives of pharmaceutical interest include the hydroxypropyl derivatives of CD and CD (i.e. HPCD and HPCD), the randomly methylated-CD (RMCD), sulfobutylether-CD (SBECD), and the so-called branched CDs such as glucosyl-CD.

8. FUTURE PROSPECT

The advent of Nanotechnology has unfolded a host of possible techniques to reformulate

various poorly soluble drugs to our advantage. Equipped with the detailed understanding of the cyclodextrin molecule and the various modifications to its basic chemistry it can be effectively used as a nanocarrier for difficult molecules. Also a potential challenge would be to match pace with advancements in analytical techniques and come up with innovative techniques to characterize such aggregates. Thus, with the simplest application of aggregate formation micellar systems of self-assembled drug-CD nanoparticles could be designed to enhance efficacy at the same time reduce drug related side effects. These encapsulated systems could be utilized for sustained release, targeted drug delivery, gene delivery and many others.

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