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## Cubosomes: A Novel Approach for Delivery of Anticancer Drugs

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### ABSTRACT

Cubosomes are bicontinuous cubic liquid crystalline phases formed by the self-assembly of polar lipids, monolein, in water. They offer unique physicochemical properties than the other common colloidal delivery systems such as liposomes and nanoparticles. The article reviews about definition of cubosomes, advantages and disadvantages, composition and different production and characterization techniques. Moreover, there is an emphasis on the importance of cubosomes for the effective delivery of anticancer drugs.

**Keywords:** Cubosomes; monolein; anticancer; production; characterization.

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## INTRODUCTION

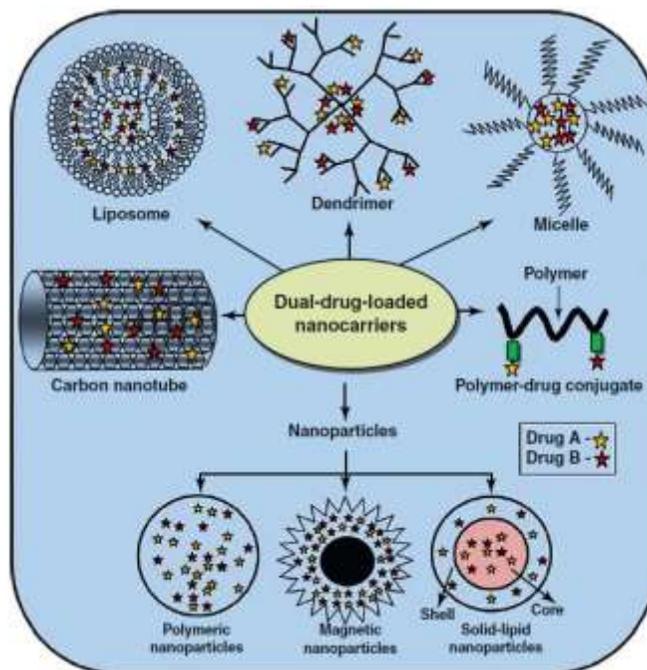
Nanotechnology is the science that focuses on manipulating matter at nanoscale.<sup>1</sup> The use of nanotechnology is widely accepted as a novel approach for cancer treatment. The advantages of using nanoparticles in cancer therapy are to eliminate the possible side effects to the surrounding healthy tissues, to target tumor tissues specifically, to increase antitumor efficacy, to sustain and control release behavior of drugs, to overcome biological barrier difficulties, to prevent the enzymatic degradation and hydrolysis of drugs, and to minimize drug resistance.<sup>2</sup>

The following table describes different types of nanocarriers in addition to their advantages and disadvantages. In addition, Figure 1, illustrates the shape of different nanocarriers.

**Table1: Different Types of Nanocarriers for Delivery of Anticancer Drugs.**

Carrier	Definition	Advantages	Disadvantages
Liposomes	Spherical lipid vesicles composed of amphiphilic phospholipids with a bilayer membrane structure having central aqueous space.	<ul style="list-style-type: none"> <li>✓ Encapsulate both hydrophobic and hydrophilic drugs</li> <li>✓ Lower toxicity</li> <li>✓ Increased drug stability</li> </ul>	<ul style="list-style-type: none"> <li>✓ Poor batch-to-batch reproducibility</li> <li>✓ Difficulties in sterilization</li> <li>✓ low drug loading</li> <li>✓ Low solubility</li> <li>✓ High cost</li> <li>✓ Leakage of encapsulated drugs</li> <li>✓ Degradation by hydrolysis in aqueous solution</li> <li>✓ sedimentation and aggregation on storage</li> <li>✓ Chemical instability due to oxidation of phospholipids<sup>27</sup></li> </ul>
Dendrimers	Synthetic symmetrical macromolecular compounds Consisting of tree-like branches around an inner core	Delivery of a wide variety of hydrophobic and hydrophilic drugs	Tedious preparation Some are toxic <sup>27</sup>
Polymeric micelle systems	Based on block co-polymers with hydrophilic And hydrophobic units that self-assemble in an aqueous Environment into structures composed of a hydrophobic core Stabilized by a hydrophilic shell	<ul style="list-style-type: none"> <li>▪ High accumulation in tumor tissue</li> <li>▪ Efficient for hydrophobic drugs</li> <li>▪ lower toxicity</li> </ul>	<ul style="list-style-type: none"> <li>▪ Low entrapment of hydrophilic drugs<sup>28</sup></li> </ul>
Carbon nanotubes	Carbon cylinders composed of benzene rings	<ul style="list-style-type: none"> <li>▪ Excellent</li> <li>▪ Mechanic strength</li> <li>▪ Ultrahigh surface area</li> <li>▪ Excellent electrical conductivity</li> </ul>	<ul style="list-style-type: none"> <li>▪ Cytotoxicity<sup>29</sup></li> </ul>
Nanoparticles:			
Polymeric	Prepared from a synthetic	<ul style="list-style-type: none"> <li>▪ Increase the circulation</li> </ul>	<ul style="list-style-type: none"> <li>▪ Poor stability</li> </ul>

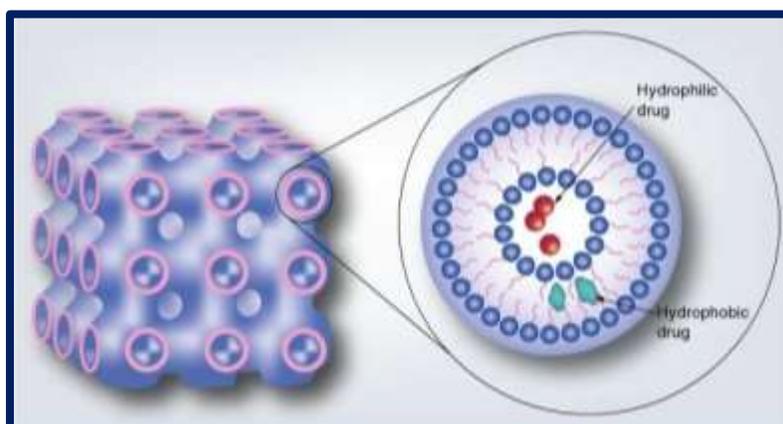
nanoparticles	Polymeric block together with biodegradable Polymers	half-life <ul style="list-style-type: none"> <li>▪ Drug targeting</li> <li>▪ Simplicity</li> </ul>	<ul style="list-style-type: none"> <li>▪ Poor batch to batch reproducibility</li> <li>▪ Sterilization difficulties</li> <li>▪ Low drug loading</li> <li>▪ No well controlled release</li> <li>▪ presence of toxic co-solvents.<sup>30</sup></li> </ul>
Magnetic nanoparticles	Nanoparticles with magnetic surface	Magnetic-mediated drug delivery to the cancer tissues	
Solid-lipid nanoparticles	made from solid lipids and stabilized by surfactants	<ul style="list-style-type: none"> <li>• Biocompatible</li> <li>• Biodegradable</li> <li>• Flexibility of size</li> <li>• Higher efficacy Excellent reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Gelation tendency</li> <li>• Particle size growth<sup>31</sup></li> </ul>



**Figure 1: Different Types of Nanocarriers for Delivery of Anticancer Drugs.<sup>2</sup>**

#### Definition of cubosomes:

Garg, defined cubosomes as nanoparticles with size ranges from 10 -500nm in diameter.<sup>3</sup> They are composed of bicontinuous cubic phase liquid crystals that consist of lipid bilayer separating two continuous but nonintersecting water regions, like “honeycombed”. Because of the interconnectedness of their structure, cubosomes are similar in appearance and rheology to cross-linked polymer hydrogels. X-ray scattering technique identified cubosomes as dots square, slightly spherical shaped in which each dot shows the presence of pore having aqueous cubic phases in lipid water system (Figure 2).<sup>4</sup>



**Figure 2: Cubic Structure of Cubosomes with Different Drug Loading Abilities.<sup>6</sup>**

Cubosomes can be administrated through different routes such as oral, percutaneous and intravenous route.<sup>5</sup>

**Advantages of cubosomes:** <sup>7, 8</sup>

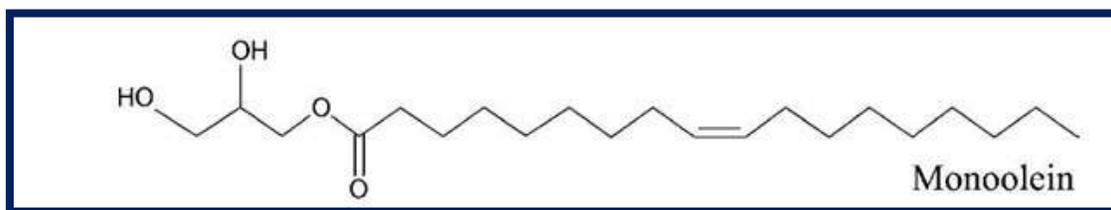
- Sustained and targeted release profiles for drugs
- Entrap both lipophilic and hydrophilic drugs
- Thermodynamically stable for longer time
- Low cost
- High drug loading due to high internal surface area and cubic crystalline structures
- Simple preparation method
- Biocompatible and non- toxic
- Excellent bioadhesive properties
- Easily incorporated into product formulations
- Excellent solubilizers
- Skin permeation enhancement

**Disadvantages of cubosomes:**

- Large scale production is sometimes difficult because of high viscosity<sup>9</sup>
- Low entrapment of water soluble drugs due to presence of large amounts of water inside cubosomes<sup>10</sup>

**COMPOSITION OF CUBOSOMES:**

Monoolein, amphiphilic lipid, is unsaturated C18 monoglyceride which is similar to non-ionic surfactants (Figure 3). It is yellow waxy paste with a characteristic odor, relatively insoluble in water and stable against temperature changes. It has many advantages as it is nontoxic, biodegradable and biocompatible material.<sup>3</sup>

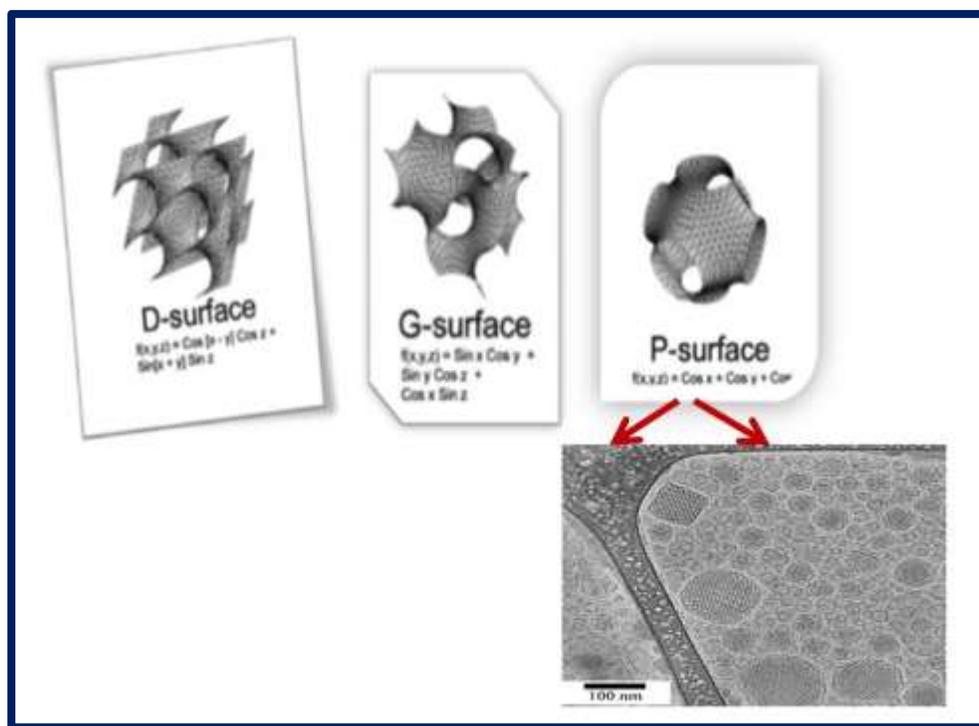


**Figure 3: Chemical Structure of Monoolein.**<sup>11</sup>

Larsson, 1983, claimed that monoolein is the most commonly used monoglycerides that constructs bicontinuous cubic phases upon the addition of water.<sup>12</sup> Copolymers like poloxamer407 are usually added to monoolein-water system to provide system stability and prevent aggregation of cubosomes particles.<sup>13</sup> Poloxamer407 is an amphiphilic nonionic agent that provides steric shielding and stabilization of colloidal particles. Other agents can be used as stabilizing agents for cubosome dispersion such as tween 80, polyvinyl alcohol and polyethylene glycol lipids.<sup>7</sup> In

addition; hydrotrope such as ethanol, polyethylene glycol 200 or propylene glycol is also included in the cubosome formation to improve the solubility of organic solutes in water such as: hydrocarbons, lipids and other water insoluble agents.<sup>14</sup>

Surface x-ray diffraction (Figure 4) determines the atomic structure of different monoolein-water systems. The monoolein-water system forms the D-surface at high water levels, the G-surface at lower levels and the P-surface is formed in the monoolein-water system, when a third component, such as caseins or amphiphilic block copolymers e.g. Poloxamer 407 is added.<sup>15</sup>



**Figure 4: Surface X-Ray Diffraction of the Prepared Cubosomes: D-surface (diamond surface), G-surface (gyroid surface), P-surface (primitive surface).<sup>11</sup>**

#### PRODUCTION OF CUBOSOMES:

##### Top down technique

This approach is the most widely used one in research areas to produce cubosome nanoparticles.

Cubosomes preparation is achieved in two steps:<sup>11</sup>

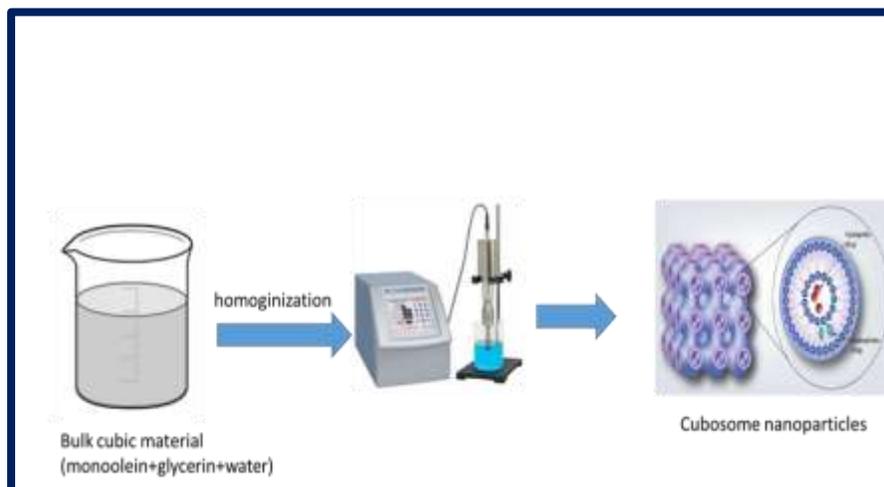
Step 1: formation of viscous bulk cubic phase

The process starts by mixing lipids with stabilizers which yields the bulk cubic phase. The bulk cubic phase is a clear rigid gel formed by water swollen cross linked polymer chain.

Step 2: dispersion of the resultant from step 1 into aqueous medium

The produced bulk cubic phase will require high energy to be dispersed into the aqueous medium which is achieved by either high energy homogenization or sonication, to form the lyotropic liquid crystal nanoparticle.

This method results in producing stable cubosomes with no aggregation for up to 1 year. But its disadvantage is that it requires high energy which is inappropriate when dealing with thermosensitive ingredients such as proteins.

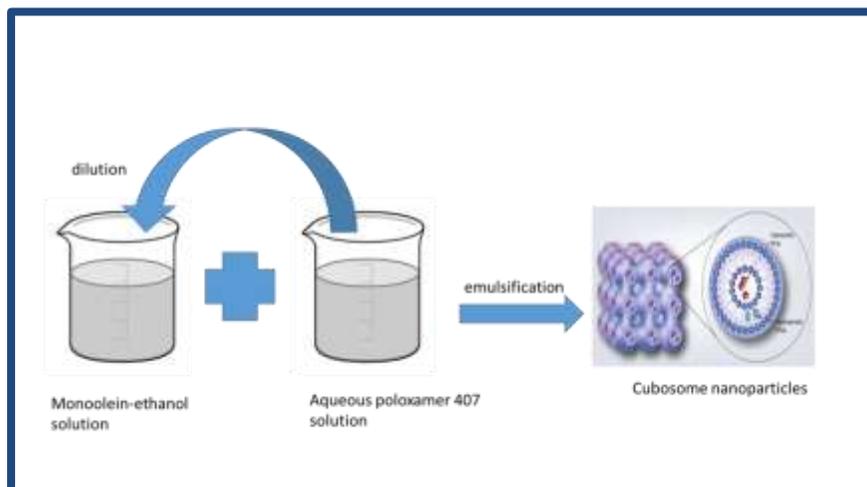


**Figure 5 : Top- Down Technique for Cubosomes Production.**

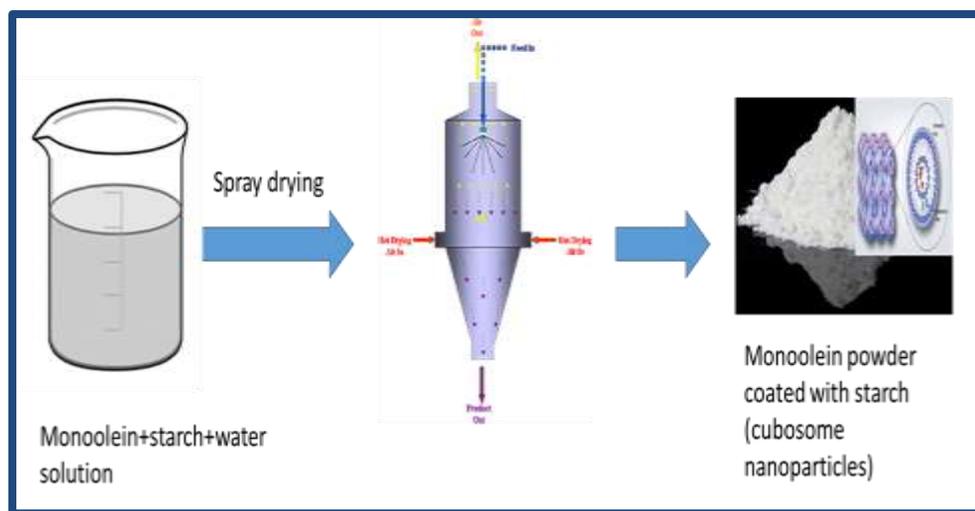
### **Bottom up technique**

As the name suggests, the nanoparticles building blocks are first formed then assembled to form the final material. The bottom up technique has the advantage of being less aggressive than the top down one. In addition, it is easier to scale up the bottom up technique than the top down one. The bottom up technique is less expensive and good for thermosensitive materials such as protein.<sup>7</sup> There are two types of precursors from which cubosomes are crystallized. These precursors are liquid precursor and powder one. The liquid precursor is composed of monoolein and ethanol solution.<sup>11</sup> It is formed at room temperature by the addition of hydrotrope (ethanol) to the molten monoolein. After that, a solution of poloxamer 407 is added to emulsify the monoolein-ethanol solution results in a viscous cubic liquid gel. By diluting the formed gel using water and sonicating it for five minutes, cubosomes nanoparticles are formed.<sup>7</sup>

On the other hand, the powder precursor is composed of monoolein powder coated with either starch or dextran. This precursor which is composed of dehydrated surfactant coated with polymer is then hydrated to form emulsion of liquid droplets. Using spray drying technique, the nanoparticle cubosomes are formed from this powder precursors.<sup>7</sup>



**Figure 6: Bottom-up Technique for Cubosome Production (Liquid Precursor).**



**Figure 7: Bottom-up Technique for Cubosome Production (Powder Precursor).**

#### CHARACTERIZATION OF CUBOSOMES:

##### Differential scanning calorimetry:

It is used to evaluate the physical status of the drug within the cubosomes. Both ingredients of the cubosomes seem to melt together at temperature of around 37°C to 56°C. The thermal events related to the drug's melting point are different from those of the native drug in which no sharp drug melting peak appears at around 200°C, while the thermal events observed between 200°C and 300°C may be related to glyceryl monooleate degradation process.<sup>16</sup>

##### POLARIZED LIGHT MICROSCOPY:

Polarized light microscopy is used to identify the morphology of liquid crystalline depending on the optical birefringence phenomena. It is also useful to differentiate between anisotropic and isotropic material.<sup>17</sup>

##### Photon correlation spectroscopy:

Particle size distributions of cubosomes are mainly determined by using zeta sizer (photon correlation spectroscopy). The sample is diluted with a suitable solvent, adjusted to light scattering intensity of about 300 Hz and measured at 25 °C in triplicate as an average volume weight size. The zeta potential and polydispersity index can also be recorded.<sup>18</sup>

#### **Determination of drug content:**

The drug content of dispersions is evaluated by diluting the filtered dispersion sample in methanol (1:9 v/v) and then analysing for drug content by high performance liquid chromatography (HPLC).<sup>19</sup>

#### **Determination of entrapment efficiency:**

Entrapment efficiency and drug loading of cubosomes can be determined using ultra filtration technique in which untrapped drug concentration is determined by subtraction from the total drug added.<sup>9</sup>

#### **In vitro drug release studies:**

In vitro release of drug from cubosomes is evaluated using a dynamic dialysis method. The samples of various formulations were placed in dialysis bags (cellulose membrane), then immersed in release medium at 37±1 °C under specific paddle rotation speed. At predetermined time intervals, a 5 ml sample was withdrawn and immediately replaced with an equal volume of release medium and then the concentration of released drug is measured.<sup>20</sup>

#### **Stability studies:**

The physical stability of cubosomes can be studied by investigation of organoleptic and morphological properties as a function of time. Particle size distribution and drug content can be measured at different time intervals.<sup>19</sup>

### **APPLICATIONS OF CUBOSOMES AS A CARRIER FOR ANTICANCER DRUGS:**

Tilekar reported that loading of anticancer drugs into cubosomes is superior to the other colloidal delivery systems such as liposomes and nanoparticles.<sup>7</sup> They improve stability of anticancer drugs against enzymatic degradation, increase drug target to the tumour site and decrease the adverse effects of anticancer drugs.

#### **Dacarbazine:**

Dacarbazine is the first choice medication for management of melanoma. However, its available formulation (intravenous injection) suffers from many disadvantages. These include: pain and hence patient incompliance, incomplete absorption and photosensitivity. Bei and coworkers

suggested cubosomes as a potential nanocarrier to overcome these problems, in addition to its bioadhesion and skin penetration enhancement properties which are required in melanoma.<sup>16</sup>

### **20(s)-protopanaxadiol:**

It is a ginsenoside that exhibits potent anticancer effects.<sup>21</sup> It shows low bioavailability in rats and dogs due to poor oral absorption and extensive metabolism by cytochrome p450.<sup>22</sup> Jin<sup>23</sup> and coworkers prepared 20(s)-protopanaxadiol as cubosome loaded with piperine as an approach to improve the drug bioavailability. They found that the drug bioavailability was increased after its incorporation in cubosome loaded with piperine due to an increase in absorption and inhibition of metabolism rather than improved drug release. Thus, cubosome loaded with piperine may be considered as a promising oral carrier for anticancer drugs.

### **Amphotericin B:**

Amphotericin b is considered as a golden standard and a life-saving drug in the treatment of many severe fungal infections. However, its clinical use is limited by its toxicity and poor solubility, necessitating hospitalization and parenteral administration.<sup>24</sup> Yang and coworkers, first developed amphotericin b-loaded cubosomes for oral administration in an attempt to increase patient compliance and reduce the drug nephrotoxicity. They found that amphotericin b-loaded cubosomal formulation orally administrated in rats did not show nephrotoxicity and its relative bioavailability was approximately 285% as compared to the commercially available product, fungizone®.<sup>25</sup>

### **Doxorubicin:**

Doxorubicin is a potent cytotoxic agent, which is used to treat wide range of cancers such as hematological malignancies, carcinoma and soft tissue sarcomas.<sup>26</sup> It is preferable to be administered in combination with coenzyme q10 in order to improve anticancer activity of doxorubicin with reduced cardiotoxicity.<sup>27</sup> The oral administration of this combination is challenged by extensive first pass metabolism of doxorubicin and the highly lipophilic with large molecular weight of coenzyme q10 leading to poor bioavailability.<sup>28</sup> Swarnakar and coworkers reported that the formulation of a combination of doxorubicin & coenzyme q10 as phytantriol cubosomes results in improving the therapeutic efficacy of doxorubicin after oral administration.

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