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REVIEW ARTICLE

Cancer Oriented Cubosomes - A Review

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ABSTRACT

Conventional chemotherapeutic agents often fail, not due to their inability to kill cancer cells, but because of their inability to distinguish cancer cells from normal cells resulting in suboptimal efficacy combined with severe toxic side effects. Nanoparticles (cubosomes) have the potential to improve the biodistribution of chemotherapy drugs by protecting them from degradation, delivering them directly to the tumour site and/or preventing them from affecting healthy tissues. Recently, few anticancer drugs have been successfully encapsulated in cubosomes and characterized physicochemically. Overall, cubosomes have great potential in drug nano formulations for melanoma therapy owing to their potential advantages, including high drug payloads due to high internal surface area and cubic crystalline structures, relatively simple preparation method, biodegradability of lipids, the ability of encapsulating hydrophobic, hydrophilic and amphiphilic substances, targeting and controlled release of bioactive agents like proteins and drugs. The interstitial pressure tends to increase with increasing tumour volume and remain lower in the outermost areas of the tumour. Finally, malignant cells within solid tumours tend to be tightly packed and are heterogeneous in nature. Thus, while the leaky nature of tumour vessels can promote nanoparticle deposition and accumulation, the microenvironment creates a number of barriers that prevent these delivery systems from effectively accessing tumour cells and thus reaching their full potential as the 'silver bullets' of anticancer therapies.

KEYWORDS

Cubosomes, Biodistribution, Tumour, Chemotherapy

INTRODUCTION

Definitions of Cubosomes

Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phase[1].

Cubosomes are nanoparticles which are self assembled liquid crystalline particles of certain surfactants with proper ratio of water with microstructure. Cubosomes are nanoparticles but instead of the solid particles usually encountered, cubosomes are self-assembled liquid crystalline particles with a solid-like rheology that provides unique properties of practical interest.

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History

Despite the early recognization (in 1980) large scale manufacture of cubosomes was difficult due to their complex phase behavior and viscous properties. The cubic phases are unique as possess very high solid like viscosities because of their intriguing bicontinuous structures. Cubic phases can be fractured and dispersed to form particulate dispersions which are colloidally and/or thermodynamically stable for

longer period of time. Certain surfactants spontaneously form cubic phases when mixed with water above a certain concentration. Determination of their honeycomb structure was carried out by Luzzati and Husson, Luzzati *et al.*, Larsson and Hyde *et al* between 1960 and 1985. The term "Cubosomes" were coined by Larsson that reflects the cubic molecular crystallography and similarity to liposomes. Effort to develop scalable processes to produce cubosomes in large scale is under development. A few anticancer drugs have been successfully encapsulated in cubosomes and characterized[1].

Structure

The basic structure of cubosomes includes honeycombed structures separating the two internal aqueous channels along with large interfacial area. Cubosomes are nanoparticles, more accurately nanostructure particles of a liquid crystalline phase with cubic crystallographic symmetry formed by the self assembly of amphiphilic or surfactant like molecules. The cubosomes having high internal surface area (Figure 1) along with cubic crystalline structures.

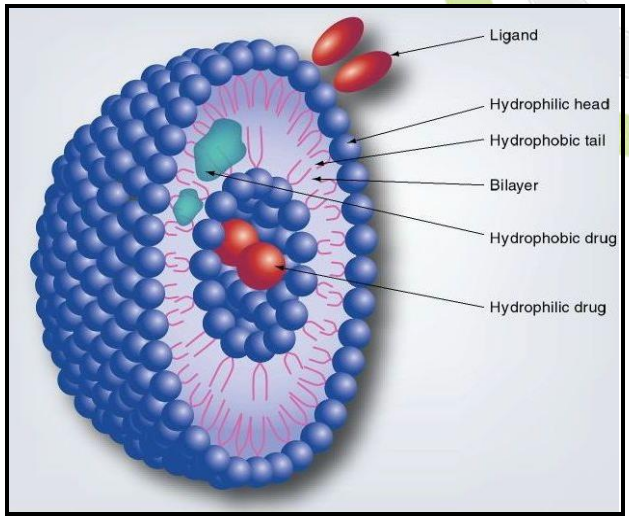

Figure 1: Honeycombed structure separating two internal aqueous channels along with large interfacial area

The cubic phases possess a very high solid like viscosity, which is a unique property because of their intriguing bicontinuous structures which enclose two distinct regions of water separated by a controlled bilayer of surfactant. applications. Amphiphilic molecules form bicontinuous water and oil channels, where "bicontinuous" refers to two distinct (continuous, but non-intersecting) hydrophilic regions separated by the bilayer. The interconnectedness of the structure results in a clear viscous gel similar in appearance and rheology to cross-linked polymer hydrogels. However, monoglyceride-based cubic gels possess significantly more long-range order than hydrogels and, because of their composition (i.e., lipid and water), excellent biocompatibility.

Advantages of Cubosomes[1]

1. High drug payloads due to high internal surface area and cubic crystalline structures.
2. Relatively simple method of preparation.
3. Biodegradability of lipids.
4. Capability of encapsulating hydrophilic, hydrophobic and amphiphilic substances.
5. Targeted release and controlled release of bioactive agents.
6. While most liquid crystalline systems transform into micelles at higher levels of dilution, cubosomes remain stable almost at any dilution level because of the relative insolubility of cubic phase forming lipid in water. So, cubosomes can easily be incorporated into product formulations. Cubosomes are typically produced by high energy dispersion of bulk cubic phase, followed by colloidal stabilization using polymeric surfactants. After formation, the dispersion is formulated into a product and is then applied to a substrate, usually skin or mucosal surface. After that materials are either absorbed or released via diffusion.
7. The cubic phases of cubosomes can be fractured and dispersed to form particulate dispersions that are colloidally and/or thermodynamically stable for longer time.

Disadvantages of Cubosomes[1]

1. Large scale production is sometimes difficult because of high viscosity.

Forms[2]

Three macroscopic forms of cubic phase are typically encountered: precursor, bulk gel, and particulate dispersions (cubosomes). The precursor form exists as a solid or liquid material that forms cubic phase in response to a stimulus, such as contact with liquid. Bulk cubic phase gel is an optically isotropic, stiff, solid like material. Cubic gel in equilibrium with water can be dispersed into particles called cubosomes, analogous to the formation of vesicles from lamellar liquid crystalline material. A recent review provides a comprehensive summary of active ingredients delivered by cubic phase. Despite intense interest in cubosome applications, we have found no work examining the practical aspects of large-scale processing and production of cubosomes.

Liquid Cubosome Precursors

Following the difficulty and expense of high-shear dispersion of viscous bulk cubic phase to form cubosomes, it is desirable to seek less aggressive processes of manufacture. High-energy processes being expensive and difficult to scale-up, also proves to be harmful to thermosensitive ingredients like proteins. In some product applications, the in situ formation of cubosomes is desired, such as during hand washing or mouth rinsing. To avoid high-energy processing and produce them in situ a strong driving force exists resulting in the development of a liquid phase precursor to cubosomes. The hydrotrope dilution process is found to consistently produce smaller, more stable cubosomes. In this process the particles are formed by nucleation and growth, as employed in crystallization and precipitation processes. This is achieved by dissolving the monoolein in a hydrotrope (ethanol) which prevents liquid crystalline formation. All this is achieved without the need of high shear, minimizing the risk of degrading the cubic liquid crystalline structure. The liquid precursor process allows for easier scale up of cubosome preparations and avoids bulk solids handling and potentially damaging high energy processes.

Powdered Cubosome Precursors

Powders composed of dehydrated surfactant coated with polymer are termed as powdered cubosome precursors. Hydration of the precursor powders forms cubosomes with a mean particle size of 600 nm, as confirmed by light scattering and Cryo-TEM. A water-soluble non-cohesive starch coating on the waxy lipid prevents agglomeration and allows control of particle size. The lipids used to make cubosomes are waxy, sticky solids, rendering them unable to form small discrete particles. Spray drying technique is an excellent process to produce these particles. Spray drying produces encapsulated particles from an emulsion of liquid droplets or a dispersion of solid particles in a concentrated aqueous polymer solution. Nozzle is used for the continuous and dispersed phases spraying throughout to create suspension droplets that are contacted with a heated, dry air stream flowing in the opposite direction. As a result of this excess water immediately evaporates, leaving dry powder particles composed of the dispersed phase encapsulated by a shell of the formerly dissolved polymer. Spray-drying processes are easily scaled up and are already widely employed for manufacturing consumer products like detergents and foods. Moreover, the process provides an easy route to preload active drug into the cubosomes prior to drying. Finally, the polymer coating on the powder imparts surface properties to the hydrated cubosomes that can be tailored by proper selection of the encapsulating polymer. Such powders offer some process and performance advantages to liquid phase hydrotropic cubosome precursors.

Manufacture of Cubosomes

1. Cubosomes can be manufactured by two distinct methods:
2. Top down technique.
3. Bottom up technique.
4. Preparation of ALA loaded cubosome dispersions.
5. Nucleation.

6. From Pseudo-Binary Systems.
7. In the Presence of Hydrotrope.

Top-Down Technique[3]

It is the most widely used procedure initially reported in 1996 by Ljusberg- Wahren. Bulk cubic phase is first produced and by application of high energy such as high pressure homogenization it is processed into cubosomes nanoparticles. Bulk cubic phase resembles a clear rigid gel formed by water-swollen cross-linked polymer chains. The cubic phases differ in that they are a single thermodynamic phase and have periodic liquid crystalline structure. Cubic phases ruptures in a direction parallel to the shear direction, the energy required is proportional to the number of tubular network branches that rupture. It is the most widely used in research area, where by bulk cubic phase is first produced and then dispersed by high energy processing in to cubosomes nanoparticles. Bulk cubic phase is resembling a clear rigid gel farmed by water swollen crossed linked polymer chains; whereas cubic phases are like liquid crystalline structure. The cubic phase's exhibits yield stress that increases with increasing amount of bilayer forming surfactant and oils. Warr & Chen gave the cubic phases may behave as lamellar phases during dispersion with increasing shear, dispersed liquid crystalline particles are forming at intermediate shear rates, where as defect free bulk phase reforms at higher shear rates.

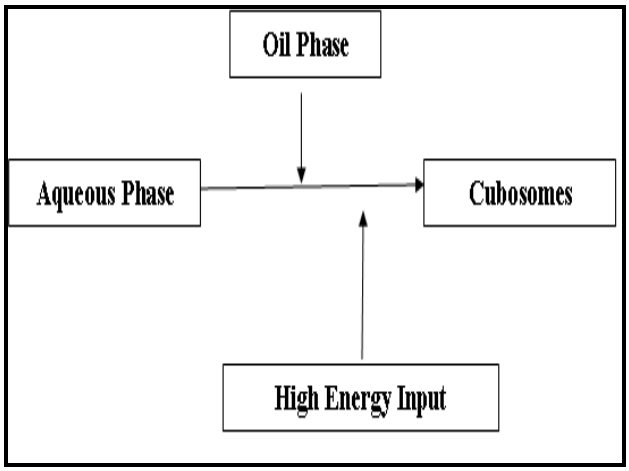

Figure 2: Illustration of the top-down approach[4]

Based on most existing studies comparison of dispersion produced by sonication and high pressure homogenization suggests the formation of complex dispersions containing vesicles and cubosomes with time dependent ratios of each particle type. Coarse cubosomes on micron scale possess the same D-surface structure as their originating bulk cubic phase, but after homogenization, the P-surface dominates because of added polymers.

Bottom-Up Technique[3]

In this cubosomes are allowed to form or crystallize from precursors. The formation of cubosomes by dispersing L2 or inverse micellar phase droplets in water at 80°C, and allow them to slowly cool, gradually droplets get crystallizes to cubosomes. This is more robust in large scale production of cubosomes. The cubosomes at room temperature is by diluting monoolein-ethanol solution with aqueous poloxamer 407 solution. The cubosomes are spontaneously formed by emulsification. Another process is also developed to produce the cubosomes from powdered precursors by spray drying technique. Spray dried powders comprising monoolein coated with starch or dextran form cubosomes on simple hydration. Colloidal stabilization of cubosomes is immediately provided by the polymers. In this cubosomes are allowed to form or crystallize from precursors.

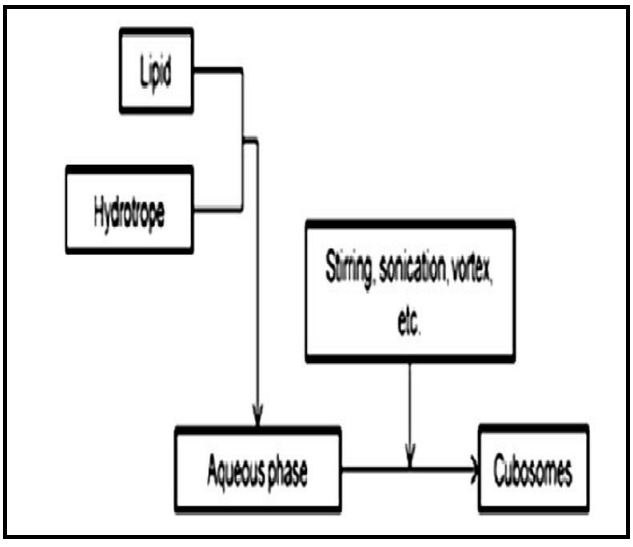

Figure 3: Illustration of the bottom-up approach[4]

The bottom-up approach first forms the nanostructure building blocks and then assembles them into the final material. It is more recently developed technique of cubosome formation, allowing cubosomes to form and crystallize from precursors on the molecular length scale. The key factor of this technique is hydrotrope that can dissolve water insoluble lipids into liquid precursors. This is a dilution based approach that produces cubosomes with less energy input when compared top down approach.

Preparation of ALA Loaded Cubosome Dispersions[4]

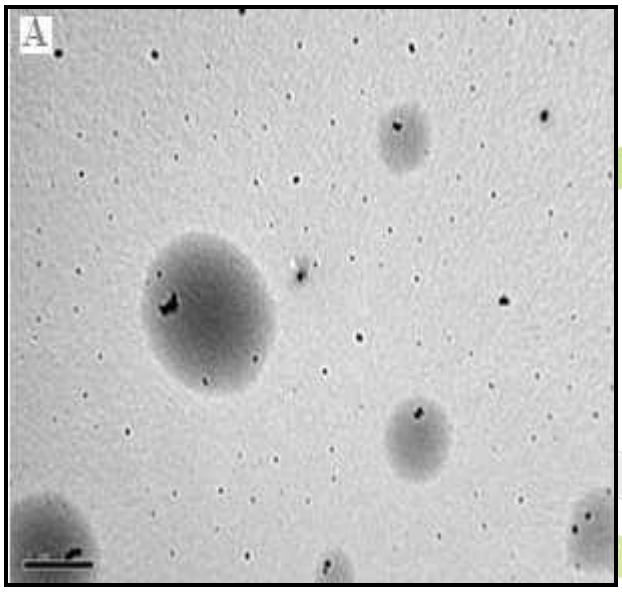

Figure 4: TEM photograph of ALA loaded cubosomes prepared by emulsification of GMO/P407 in water using different GMO concentrations[4]

Cubosome dispersions were fabricated using two different methods. The first method was through fragmentation of GMO/P407 bulk cubic gel. GMO (5.0%) and P407 (1.0%) were firstly melted at 60^0C in a hot water bath, after which ALA (25, 50 or 100 mg) was added and stirred continuously to dissolve. Deionized water was gradually added and vortex mixed to achieve a homogenous state. After equilibration for 48 hrs at room temperature, an optically isotropic cubic gel phase was formed. After addition of 10 ml of deionized water, the cubic gel was first disrupted by mechanical stirring. The crude dispersion was subsequently fragmented by intermittent probe sonication at 200 W energy input under cooling in a 20 ^{0}C water bath for 20 min. The second method was achieved through the emulsification of GMO and P407 in water followed by ultrasonication. Dispersion is composed of 5% GMO (with 1% P407 and 5% ethanol) in 89% water. GMO and P407 were gently melted at 60^0C and mixed; ALA ethanolic solution was then added to the melt. The resultant mixture was then added drop wise to deionized water preheated at 70^0C and ultrasonicated at maximum power of 130 kW for 15 min at the same temperature. All dispersions were stored in glass vials at ambient temperature (23 ^{0}C) protected from light.

Table 1: Composition of ALA cubosome dispersion

Dispersion	GMO %w/w	P407 %w/w	Ethanol %w/w	Water %w/w
D1[a]	05.0	1.0	-	94.0
D2[b]	05.0	1.0	5.0	89.0
D3[b]	10.0	1.0	5.0	84.0
D4[b]	15.0	1.0	5.0	79.0
D5[b]	15.0	2.5	5.0	77.5
D6[b]	15.0	5.0	5.0	75.0

a :Prepared by top-down approach.

b :Prepared by bottom-up approach.

Making Cubosomes by Nucleation[5]

The dilution (nucleation) process provides the ability to produce cubosomes without laborious fragmentation. The best way to anticipate appropriate dilution pathways is by charting trajectories on the ternary diagram. Dilution with water is essentially equivalent to drawing a line from some composition to the water apex (Figure 5). Of course, phase diagrams speak to thermodynamic properties; dilution has a large kinetic component so that this is an approximation.

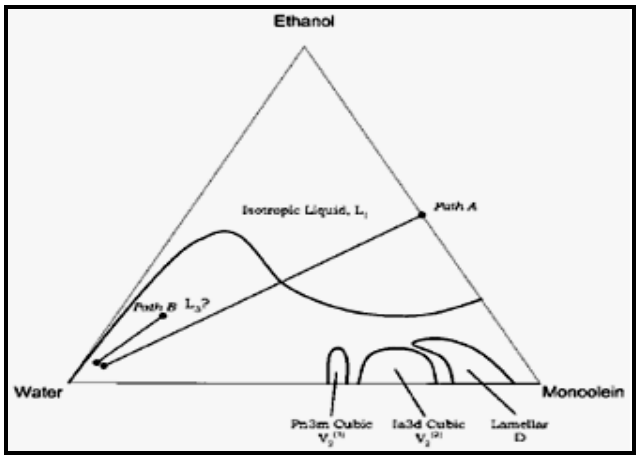

Figure 5: Ternary phase diagram for the monoolein-ethanol water system[5]

The phase diagram also offers a means of determining the yield of cubic phase obtained by a dilution process using the tie lines between the isotropic liquid and the cubic phase in conjunction with the Lever Rule. One of the most logical dilution paths is from the large isotropic L1 region because the isotropic liquid is low viscosity and conveniently mixed with water. Consider that path A (Fig. 5) represents the dilution of an isotropic liquid (50% monoolein, 50% ethanol) with a polymer-water solution to form a colloidal dispersion of cubosomes in water (89% water, 5% monoolein, 5% ethanol, and 1% Poloxamer 407). Cubosomes form spontaneously with minimal energy input other than that required to contact the two liquids and, literally, gentle mixing by hand inversion of the container. The dispersions are estimated to be10%cubosomes dispersed in90%liquid (mostly water). A small amount of polymer is necessary to stabilize the particles against flocculation; the presence of this small amount of polymer does not alter the phase behavior of the system. Without the polymer, the cubosomes will flocculate quite rapidly, on the order of seconds. Cubosomes made by our process (with added polymer) show excellent long-term stability despite the relatively low polymer content. Cubosome dispersions prepared by dilution of isotropic liquid were stable against flocculation for at least 6 months with only 1% polymer present. This is in agreement with the work of Friberg et al., who found that vesicles formed via dilution were more stable than those formed by energy-intensive methods. In contrast, required much higher polymer concentrations (i.e., 4-12%) to stabilize cubosomes for several months as prepared by high-pressure homogenization.

Although speculative, the mechanism for the superior stability of dilution-produced cubosomes is likely the more homogeneous distribution of the stabilizing polymer to the cubosome surfaces during nucleation than during energetic dispersion. Cryo-TEM images of the dispersion made by dilution pathAare shown in Fig.5. Fig.5 shows a cubosome about 300nmin diameter with a lamellar vesicular surface coating. These cubosomes are similar in appearance and structure to those previously noted. It is worth emphasizing that this process creates nanoparticles of cubic liquid crystalline gel without any significant mechanical energy input. It is likely that a phase inversion process occurs as the dilution path crosses the isotropic liquid phase boundary (Fig.5). As a result, interfacial energy is applied instead of mechanical energy toward the dispersion of the cubic gel that forms. On a practical scale, some adjustment of the PSD will likely be needed, but the total energy input will be much less than that needed to disperse bulk cubic gel "from scratch". Finally, in all of the cryo- TEM images shown thus far, some vesicles are always present with the cubosomes. Recalling that trajectories do not reflect kinetic phenomenon, Fig. 5 shows transitional vesicle structures (indicated by the arrows) formed during the dilution. It is believed that these vesicles are precursors for cubosomes and that the remaining vesicles will transform into cubosomes rapidly. More conclusive research into this question is warranted, but the structures are reminiscent of the structures formed by membrane fusion of phospholipid vesicles during their phase transition to the hexagonal phase.

Dilution from the emulsion region provides an interesting contrast to dilution of an isotropic liquid. Emulsions are excellent precursors for cubosome dispersions because they can be easily dispersed and stabilized prior to cubosome formation to prevent liquid crystal

degradation and agglomeration, respectively. In general, the low-viscosity emulsions formed in this region are promising cubosome precursors because their PSD is easily tailored by low shear, stabilized, and finally diluted into the cubic liquid equilibrium region to form cubosomes. A macroemulsion was first prepared (70% water, 20% ethanol, and 10% monoolein) and then diluted with Poloxamer 407 solution to form a cubic liquid dispersion (90% water, 6% ethanol, 3% monoolein, and 1% polymer) using only mild hand agitation.

Cubosome nanoparticles (100-300 nm in diameter) were again formed spontaneously during the emulsion dilution process, as verified by cryo-TEM imaging. Because the cubosome particles were formed from a macroemulsion without any application of shear beyond hand mixing, there appears to be a broader PSD than that produced by dilution from the L1 region (direct nucleation process). In addition to nanoparticles, particles on the order of micrometers were also observed. The longest dimension is about 7 *ím* along the edge. Surprisingly, the particles possess a distinct cubic shape despite their nonsolid state and their large scale relative to the cubic unit cell dimensions, reminiscent of previously reported cubic phase-containing emulsions. These particles are clearly formed with edges that terminate along the principal directions of the unit cell.

Cubosomes from Pseudo-Binary Systems[5]

Cubosomes were first made in a pseudo-binary system of monoolein-water (including polymer at low levels) using the conventional technique of energetic dispersion of bulk cubic gel. Melted Poloxamer 407 (8% w/w) and monoolein (92% w/w) were combined to form a homogeneous solution. The monoolein-polymer solution was then added to deionized water to form a 1.8% mixture of monoolein containing 98% water and 0.2% Poloxamer 407. The mixture was sonicated for 60 min in a controlled temperature ultrasonic bath, maintained at 25°C, to disperse the cubic liquid crystalline gel. Cryo-TEM (Fig.6) revealed mostly square cubosomes that were about 100-300 nm along an edge. The unit cell structure is evident from alternating water (light gray dots) and oil channels (dark matrix). Fourier analysis of the periodicity results in 150 Å, which is consistent with SAXS for monoolein water cubic phases. The three-dimensional shape of the aggregates, however, is elusive. Stereographic images taken at 0 and 15° from the normal (Figure 6) show some blurring in the well-defined matrix of water channels from visualizing successive layers below the top layer. However, the length of the particle edge does not change upon tilting. This is peculiar because rotating a cube by 15° should result in an increase of 22% in size along the direction of rotation. This suggests that the aggregate might be more sphere like or relatively flat, although more distinctly cubic cubosome aggregates have been documented.

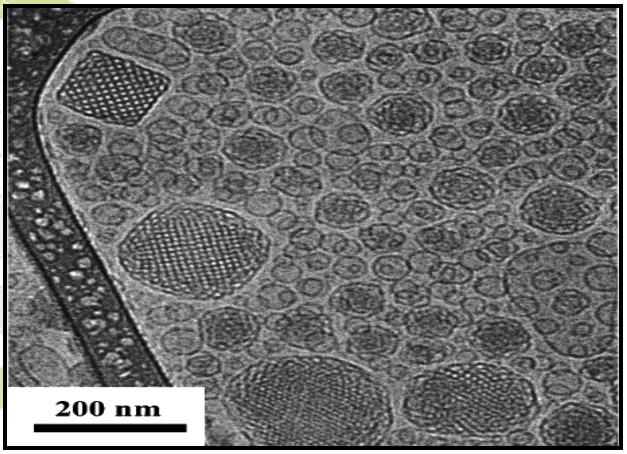

Figure 6: Cryo-transmission electron micrograph of dispersed particles of cubic liquid crystalline material or cubosomes[5]

Cubosomes in the Presence of Hydrotrope[5]

Cubosomes were also formed in the presence of significant levels of hydrotrope by sonication based methods. Bulk cubic gel was fabricated by the combination of molten monoolein (93% w/w) and ethanol (7% w/w) to form a low-viscosity isotropic liquid. A 1.2% Poloxamer 407 solution was added to the liquid, forming a viscous, cubic liquid crystalline gel in the presence of excess water (final composition: 68% monoolein, 26.7% water,5%ethanol, and 0.3% Poloxamer 407). The mixture was sonicated for 5 min.

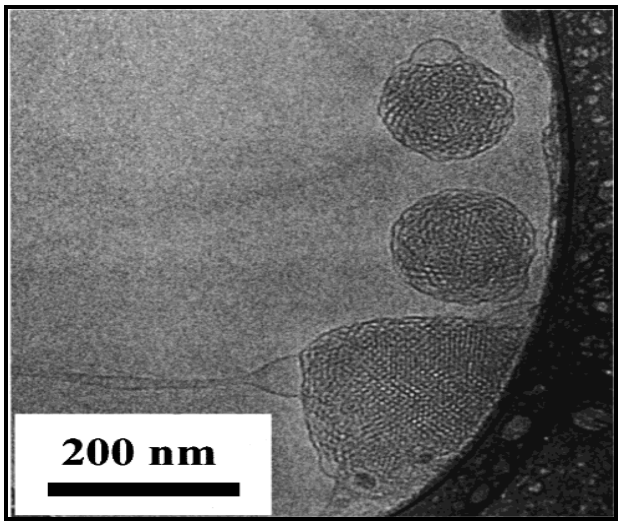

Figure 7: Cryo-TEM image of cubosomes formed by sonicating bulk cubic gel containing ethanol hydrotrope

Figure 7 shows cryo-TEM photographs of two cubosomes about 200nm in diameter. These cubosomes are similar in size and shape to those formed without ethanol, although more circular than square. Also visible is a larger region of cubic liquid crystal attached to the support and displaying a well-defined cubic lattice. The larger pieces of cubic gel form as a result of incomplete dispersion by the short application of ultrasonic energy; this dispersion was macroscopically more opaque (dispersion formed after 60 min of sonication). Large amounts of energy per unit volume are clearly necessary to completely disperse the cubic gel into cubosome nanoparticles when starting from bulk cubic gel. Finally, note that both cubosomes in have a hemispherical-shaped vesicle extending from an edge. The formation of a vesicular coating on cubosomes has been suggested as a thermodynamic means of avoiding exposure of lipid hydrocarbon chains as the cubic liquid crystalline gel is fragmented during dispersion. Formation of cubic liquid crystals in the presence of hydrotrope was confirmed by SAXS measurements on ethanol-containing cubic phase gels. SAXS measurements were made on cubic phase gels of 2% ethanol (i.e., 50% monoolein, 48% water, and 2% ethanol) and compared to those without ethanol (i.e.50% monoolein and50%water).

Drug Loading Capacity of Cubosomes[1]

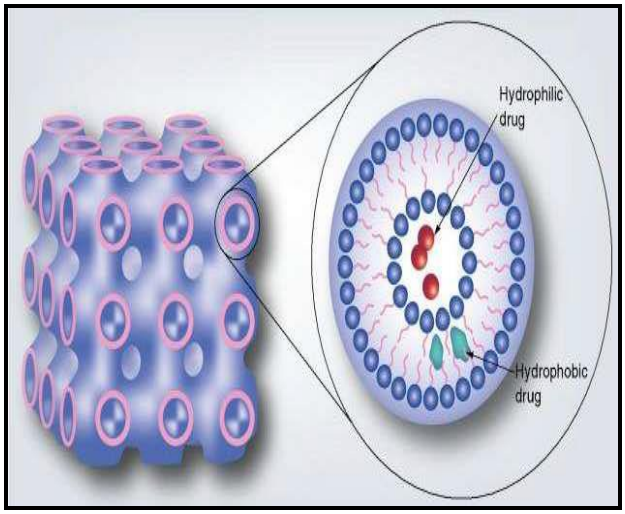

Figure 8: Cubosomes exhibiting its cavernous internal and cubic structure and its membrane composition with different drug loading modalities

The cubosomes generally have different internal cubic structure along with variant composition related to the drug loading modalities. The cubosomes have huge potential in drug nano formulations for melanoma therapy due to their potential advantages consisting high drug payloads.

Cancer Treatment[9,10,11]

Your cancer treatment depends on many factors, including:

1. The type of cancer you have
2. Stage of your cancer
3. Your health
4. Your preferences

The goal of treatment is to kill or remove cancer cells to bring your cancer into remission.

Remission happens when the cancer is under control or is responding to treatment. There are 3 major types of cancer treatments. These treatments are available as pills that can be given by mouth or they have to be infused through the vein. Your doctor may choose to combine these treatments.

1. Chemotherapy uses medicines to kill cancer cells

2. Radiation therapy uses energy beams to kill cancer cells
3. Surgery removes as much of the cancer as possible

Cancer Treatment Side Effects

Cancer treatment may make you sick. Everyone experiences side effects differently. The side effects you have depend on your treatment type. Many side effects can be managed or treated. Some side effects can disappear over time.

Chemotherapy

1. Anemia (low blood count that can make you tired and short of breath)
2. Fatigue (feeling tired and weak)
3. Hair loss
4. Increased chance of bruising, bleeding, and infection
5. Nausea and vomiting

Radiation Therapy

1. Tiredness
2. Nausea with or without vomiting (most common when
3. the stomach or brain is treated)
4. Diarrhoea and bleeding in bowels
5. Memory loss
6. Skin changes including dryness, itching, peeling or
7. blistering
8. Infertility that may be temporary or permanent

Surgery

1. Pain
2. Tiredness
3. Bleeding
4. Infection
5. Reactions around the surgery area such as:
    - Swelling
    - Tenderness
    - Stiffness
    - Draining

Anti-Cancer Drugs Enclosed in Cubosomes over Chemotherapy, Radiation Therapy and Surgery[6]

Recently few anticancer drugs have been successfully encapsulated in cubosomes and characterized physicochemically. The unique structure of this promising nanocarrier suggests its application in melanoma therapy. In order to specifically target nanomedicines to tumours, different approaches have been envisaged, with passive and active targeting of cancer cells having been shown to be valid approaches in preclinical and clinical studies. Passive targeting exploits the pathophysiological properties of the tumour vasculature which is generally highly disorganised with enlarged gap junctions between endothelial cells and compromised lymphatic drainage allowing for the extravasation of nanocarriers with sizes up to several hundred nanometres. Objects of this size cannot pass through the tight junctions that exist within the endothelial cell lining of the vessels of healthy tissues (Figure. 9 & 10). Passive targeting is largely dependent on the ability of a drug nanocarrier to exhibit an increased circulation lifetime resulting in enhanced accumulation at the target site. Circulation time is dictated by the nanoparticle physicochemical properties (size, charge, biodegradability, solubility, shape, rigidity), which can be easily manipulated in the majority of the delivery systems described. The most common modification used to evade macrophage capture and increase circulation time is accomplished by making the nanoparticle surface hydrophilic through the addition of a polyethylene glycol (PEG) coating on the surface. The majority of the nanoparticle-drug formulations used clinically and in development rely mainly on passive targeting. As a means of increasing recognition of target cells by nanoparticles, active targeting has been implemented. Active targeting utilises specific ligands such as peptides or antibodies that bind to molecules

specifically expressed or overexpressed on target cells. Thus, active targeting does not actually improve overall accumulation at the tumour site, but rather enhances cellular uptake of the particles following their passive extravasation due to the leaky vasculature. Transferrin and folate ligands are two examples of commonly used active targeting moieties in nanomedicine formulations targeting tumours. The only clinically approved actively targeted nanomedicines are antibody–drug conjugates used in the treatment of leukemias and lymphomas. Currently, no actively targeted nanoparticle formulations are approved for clinical use, with only a small number in clinical trials. Despite the ample evidence and extensive research effort supporting the benefits of both passively and actively targeted nanomedicines in the treatment of cancer, clinically, both strategies have met with only moderate success. This is likely due to the fact that the complexity of the tumour microenvironment (tumour heterogeneity, vascularity, location) is commonly overlooked and will have a major effect on nanoparticle extravasation, accumulation, and penetration into the tumour. The tumour microenvironment is highly heterogeneous in composition with as much as half of its volume occupied by noncancerous cells and dense extracellular matrix. Furthermore, the hyperpermeable nature of the tumour vasculature, while being ideal for allowing nanoparticles to enter into tumour tissue, also allows fluid to leak from the vessel into the tumour microenvironment, thereby causing extraordinarily high interstitial pressure throughout the tumour interior. The interstitial pressure tends to increase with increasing tumour volume and remain lower in the outermost areas of the tumour. Finally, malignant cells within solid tumours tend to be tightly packed and are heterogeneous in nature. Thus, while the leaky nature of tumour vessels can promote nanoparticle deposition and accumulation, the microenvironment creates a number of barriers that prevent these delivery systems from effectively accessing tumour cells and thus reaching their full potential as the 'silver bullets' of anticancer therapies.

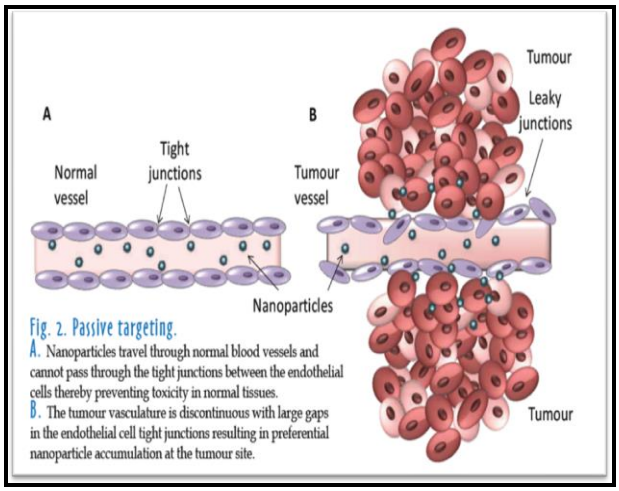

Figure 9: Action of cubosome incorporated drugs on tumours

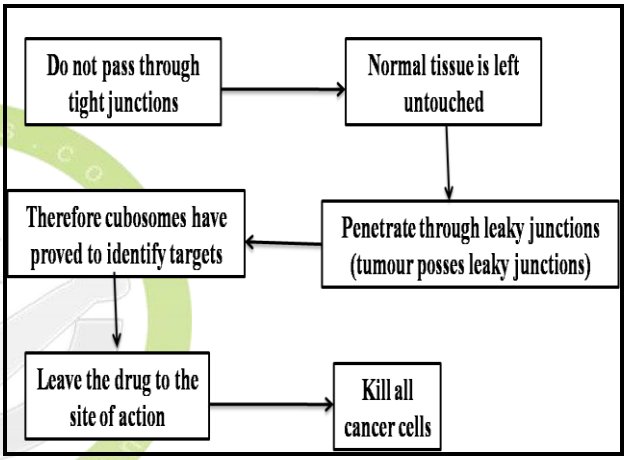

Figure 10: Action of cubosome incorporated drugs on tumours

Table 3: Comparison of drugs with and without cubosomes

Drug alone	Drug enclosed in cubosomes
Fail to distinguish normal cells from cancer cells	Distinguishes normal cells from cancer cells.
Low efficacy	More efficacies.
Less biodistribution	More biodistribution.
Severe toxic side-effects	Reduced side-effects.

Affect healthy tissues	Prevent affecting healthy tissues.
Eg: Cisplatin	Eg: Dacarbazine, Camptothecin

The optimized formulation based on formulation parameters resulted from 100 mg of monoolein, 107 mg of polymer and 2 mg with diameter of 104.7 nm and encapsulation efficiency of 6.9%. Optimal formulation based on process parameters was obtained from 24,000 rpm of homogenization speed, 5.5 min of duration and 76°C of temperature with cubosome formulation of 85.6 nm in size and 16.7% in encapsulation efficiency. Dacarbazine inside cubosomes was in crystal form. These dacarbazine-loaded cubosomes had great potential in melanoma treatment[12].

Dacarbazine (DTIC), a water soluble drug, is currently used as a first line chemotherapy medication against melanoma. However, current therapies are not ideal. For example, the reference drug in melanoma therapy, dacarbazine, is potent, but it has some serious side effects. Firstly, it is normally administered intravenously, which is painful and usually not patient compliant. Secondly, the absorption of dacarbazine is generally erratic, slow, and incomplete. Thirdly, the drug is light sensitive and unstable. One promising strategy to overcome these limitations is to encapsulate this drug using nanocarriers or nanoparticulate systems intended for controlled drug delivery. In recent years, cubosomes (cubosome dispersions) entered the drug nanocarrier library as a novel member due to their great potential as an alternative drug delivery system relative to liposome. Cubosomes, especially made of binary systems, monoolein–water, are one of the most studied binary systems. These are aqueous surfactant systems that can self-assemble into thermodynamically stable bicontinuous cubic liquid crystalline phases. They are viscous isotropic and have a large internal surface area (~400 m^2/g). Cubosomes are capable of loading lipophilic, hydrophilic, and amphiphilic drugs. Because of the three-dimensional nanostructure with hydrophobic and hydrophilic domains, cubic liquid crystalline phases have been applied in pharmaceutical drug delivery. The large interfacial area can provide a complex diffusion pathway for sustained release of entrapped drug molecules, whereas lipid constituents are biocompatible, bio-adhesive, and digestible. Previous research on drug encapsulation within cubosomes concerned the study of somatostatin, insulin, indomethacin, and rifampicin. Cubosomes have also been investigated for different pharmaceutical applications (peptides, enzymes, antimuscarinic drugs, antibiotics, and analgesic delivery) and extensively reviewed .Although the properties of bioadhesion and penetration enhancement of cubosomes suggest their potential utility in skin cancer (e.g., melanoma) treatment, there is currently no formulation addressing this need. Moreover, there is emerging interest in using statistical methods to optimize pharmaceutical formulations. However, to our knowledge, such methods have seldom been used specifically for drug-loaded cubosome formulation. The present study is concerned with the first production and the characterization of cubosomes (using such methods as a novel nanomedicine) for dacarbazine that could eventually be used by a transdermal route to improve drug stability, efficacy, and safety.

Preparation of Dacarbazine-Loaded Cubosomes

Drug-loaded cubosomes were prepared through an adapted coarse method .Briefly, for each sample, a volume of 15 ml of chloroform was used to completely dissolve GMO and Pluronic F127. The chloroform was allowed to evaporate under reduced pressure at 60 rpm and at a temperature of 60 ± 2°C, leading to the formation of a thin film at the bottom of the flask. A volume of 50 ml of PBS buffer saline (pH = 7.4) was used to dissolve the drug. This solution was added to the dry lipid film to form coarse dispersions. A sonicator was used to briefly mix the lipid film and water phase together, and the mixture was used to keep the coarse dispersions under hot water (80 ± 2°C) for 15 min in a water bath. The hot mixture was

transferred swiftly to a beaker in which a homogenizer (IKA ULTRA-TURRAX T-25, Staufen, Germany) was used for 1 min at the speed of 13,500 rpm to prepare uniform dispersion. Cubosomes were formed when the dispersion cooled down to room temperature gradually. Aluminum coils were used to cover the sample vials in order to protect samples from direct light. The dispersions were then used for future tests and evaluation[13].

Several studies have been carried out on the physical and chemical properties of cubosomes. The preparation of cubosomes based on different materials has been reported[14-17]. It has also been reported that cubosomes transform into hexasomes, exhibiting a time-resolved behavior due to pH-induced lipid hydrolysis[18]. The internal and structural changes of cubosomes could be controlled by adjustment in lipid composition[19]. The specific type of cubosomes was researched to identify their detailed structure[20,21]. The cubic symmetry and ionexchange properties[22], the bilayer phase transition[23], the effect of vitamin E and polymer on cubosome structure[17], the instability of cubosomes in plasma due to interactions with lipoproteins (high-density lipoprotein and low-density lipoprotein) and albumin[24] were also reported.

CONCLUSION

The use of nanomedicines in localised drug delivery has received a lot of attention over the past couple of decades and resulted in several clinically approved formulations. These systems have been shown to have a number of advantages over conventional chemo-therapeutics; however, they have not yet reached their full potential as anticancer agents. This is likely due to the fact that until more recently, features of the tumour microenvironment that can create barriers to effective nanoparticle delivery have been largely overlooked. With improved understanding of how the tumour micro-environment affects nanoparticle delivery and distribution within tumours, strategies can be developed to better address and overcome the shortcomings of current delivery systems. Thus, future anticancer therapies using nanomedicine can be envisioned to specifically kill all cancer cells within the tumour while leaving normal tissue in the body virtually untouched.

REFERENCES

1. Bhosale, R. R., Osmani, R. A., Harkare, B. R., & Ghodake, P. P. (2013). Cubosomes: The Inimitable Nanoparticulate Drug Carriers. *Scholars Academic Journal of Pharmacy*, *2*(6), 481-486
2. Prashar, D., Sharma, D. (2011). Cubosomes: A Sustained Drug Delivery Carrier. *Asian Journal of Research in Pharmaceutical Sciences, 1*(3), 59-62.
3. Urvi, S., Dhiren D, Bhavin, P., Patel, U., Shah, R. (2013). Overview of Cubosomes: A Nano Particle. *International Journal of Pharmacy and Integrated Life Sciences, 1*(5), 36-47
4. Saly, S., Ehab, R. B., Sabry, B. (2013). The Design and Evaluation of Novel Encapsulation Technique for Topical Application of Alpha Lipoic Acid. *Journal of Advanced Pharmaceutical Research, 4*(1), 13-22.
5. Spicer, P. T., Hayden, K. L. (2001). Novel Process for Producing Cubic Liquid Crystalline Nanoparticles (Cubosomes). *Langmui, 17*, 5748-5756.
6. Sagnella, S., and Drummond, C. (2012). Drug Delivery: A Nanomedicine Approach. *Australian Biochemistry, (43)*, 5-7.
7. Patrick, T., Spicer, Matthew L., Lynch; Bicontinuous Cubic Liquid Crystalline Phase and Cubosome Personal Care Delivery Systems. Available from http://www.nonequilibrium.com/CubicLiquidC
8. Thadanki, M., Srivalli, P., & Prabha, K. (2011). Overview of cubosomes: a nano particle. *International Journal of Research Pharmaceutical Chemistry*, *1*, 535-41.

9. American Cancer Society Web site. http://www.cancer.org/. Accessed May 10, 2011.
10. National Cancer Institute Web site. http://www.cancer.gov/. Accessed May 10, 2011.
11. Managing Side Effects. The Leukemia & Lymphoma Society.http://www.lls.org//attachments/National/br_1171992654.pdf#/diseaseinformation/managingyourcancer/treatmentnextsteps/sideeffects/. Accessed May 10, 2011.
12. Bei, D. (2009). *Preparation and characterization of dacarbazine-loaded cubosomes as alternative nanoformulation for melanoma treatment*. University of Missouri-Kansas City.
13. Bei, D., Marszalek, J., & Youan, B. B. C. (2009). Formulation of Dacarbazine-Loaded Cubosomes - Part I: Influence of Formulation Variables. *AAPS PharmSciTech*, *10*(3), 1032-1039.
14. Garg, G., Saraf, S., & Saraf, S. (2007). Cubosomes: an overview. *Biological and Pharmaceutical Bulletin*, *30*(2), 350-353.
15. Uyama, M., Nakano, M., Yamashita, J., Handa T. (2009). Useful modified cellulose polymers as ne emulsifiers of cubosomes. *Langmuir*, *25*, 4336-4338.
16. Yaghmur, A., Laggner, P., Almgren, M., & Rappolt, M. (2008). Self-assembly in monoelaidin aqueous dispersions: direct vesicles to cubosomes transition. *PloS one*, *3*(11), e3747.
17. Dong, Y. D., Larson, I., Hanley, T., & Boyd, B. J. (2006). Bulk and dispersed aqueous phase behavior of phytantriol: effect of vitamin E acetate and F127 polymer on liquid crystal nanostructure. *Langmuir*, *22*(23), 9512-9518.
18. Salonen, A., Muller, F., & Glatter, O. (2008). Dispersions of internally liquid crystalline systems stabilized by charged disklike particles as pickering emulsions: basic properties and time-resolved behavior. *Langmuir*, *24*(10), 5306-5314.
19. Yaghmur, A., de Campo, L., Sagalowicz, L., Leser, M. E., & Glatter, O. (2006). Control of the internal structure of MLO-based isasomes by the addition of diglycerol monooleate and soybean phosphatidylcholine. *Langmuir*, *22*(24), 9919-9927.
20. Angelov, B., Angelova, A., Papahadjopoulos-Sternberg, B., Lesieur, S., Sadoc, J. F., Ollivon, M., & Couvreur, P. (2006). Detailed structure of diamond-type lipid cubic nanoparticles. *Journal of the American Chemical Society*, *128*(17), 5813-5817.
21. Yaghmur, A., de Campo, L., Sagalowicz, L., Leser, M. E., & Glatter, O. (2005). Emulsified microemulsions and oil-containing liquid crystalline phases. *Langmuir*, *21*(2), 569-577.
22. Trikalitis, P. N., Rangan, K. K., Bakas, T., & Kanatzidis, M. G. (2002). Single-crystal mesostructured semiconductors with cubic Ia 3 d symmetry and ion-exchange properties. *Journal of the American Chemical Society*, *124*(41), 12255-12260.
23. Nakano, M., Kamo, T., Sugita, A., & Handa, T. (2005). Detection of bilayer packing stress and its release in lamellar-cubic phase transition by time-resolved fluorescence anisotropy. *The Journal of Physical Chemistry B*, *109*(10), 4754-4760.
24. Leesajakul, W., Nakano, M., Taniguchi, A., & Handa, T. (2004). Interaction of cubosomes with plasma components resulting in the destabilization of cubosomes in plasma. *Colloids and Surfaces B: Biointerfaces*, *34*(4), 253-258.

