Carbamazepine Microemulsions and Carbamazepine Monolayers used to direct crystal structure to increase bioavailabilty

Ryan Koenes
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Abstract:

Carbamazepine (CBZ) is an anticonvulsant, currently on the market, that has a low absorption rate into the body (bioavailability). Microemulsions and metal crystals have been proposed as ways to increase bioavailability by acting as directors for the packing structure of CBZ when forming crystals. CBZ crystals were formed using both techniques and then were examined using Differential Scanning Calorimetry, Thermogravimetric Analysis, Scanning Electron Microscopy, Optical Light Microscopy, and Scanning Tunneling Microscopy. Using Differential Scanning Calorimetry, Thermogravimetric Analysis, Scanning Electron Microscopy, and Optical Light Microscopy, 3-D crystal structures were made in microemulsions below 30% water-in-oil and 2-D and 1-D crystal structures were made in microemulsions above 30% water-in-oil. Also, these crystals have weaker intermolecular forces. For the metal crystals, the size of the metal center affects the arrangement of the CBZ in the monolayer which resulted in different chiral arrangements of CBZ. Both methods can be used to direct the packing structure of CBZ into crystals in hopes of improving bioavailability.

Introduction:

One problem that continuously keeps arising in the pharmaceutical industry is polymorphism.[1]

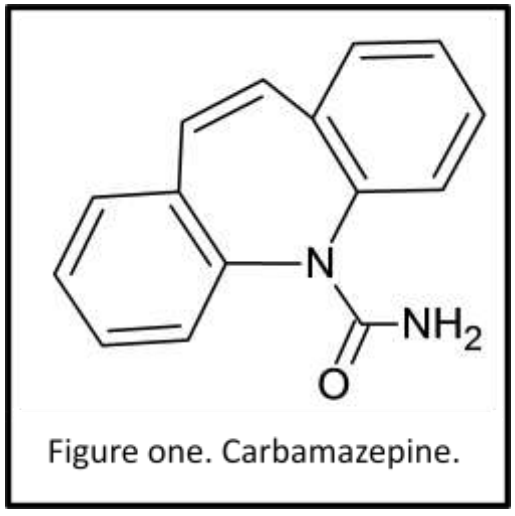

Figure one. Carbamazepine.

Polymorphism is the ability of a drug to appear in multiple crystalline arrangements.[2] Carbamazepine (figure 1) is one particular drug that falls into this category. Carbamazepine, also known as Carbatrol, Epitol, Equetro, and Tegretol,[3] is an anticonvulsant drug that is often used to combat seizures and epilepsy.[3] Carbamazepine has high intestinal permeability but has a low bioavailability. This low bioavailability is due to the limitations posed by Carbamazepine's low water solubility.[4] Carbamazepine has low water solubility

because of the charge stabilization with resonance around the rings and because of the amount of carbon molecules to oxygen or nitrogen molecules. This ratio shows that the molecule has a slightly polar side (the side with the oxygen and nitrogen molecules) but the carbon rings are overbearing and keep the molecule mostly nonpolar. Bioavailability is the amount of drug that is absorbed into the systemic circulatory system and how fast it is absorbed.[5] Bioavailability is important because it helps predict how long it will take for the drug to kick in and how much of that drug is needed in order to feel the desired effect. Microemulsions and metal crystals are two different proposed methods to direct the packing structure of CBZ into crystals in hopes of increasing bioavailability. This would ultimately make orally taken drugs go into effect in the body faster and with a smaller dosage.

Micromulsions are made when two immiscible liquids (e.g., oil and water) are brought together to form a single phase.[6] Macroscopically, these liquids appear to form a single phase, but when examined at the microscopic level the liquids are separate from one another (heterogeneous).[6] A surfactant is often added to the microemulsion in order to overcome the heterogeneous mixture seen microscopically.[6] Surfactants lower the surface tension of a liquid and lower the tension between the two liquids being mixed.[7] What makes microemulsions so useful is that they can have properties such as extremely low interfacial tension and large interfacial area.[6] Interfacial tension is the adhesive forces between 2 liquid phases.[8] Microemulsions also have increased kinetic and thermodynamic stability due to their small size.[6] They are prepared by adding

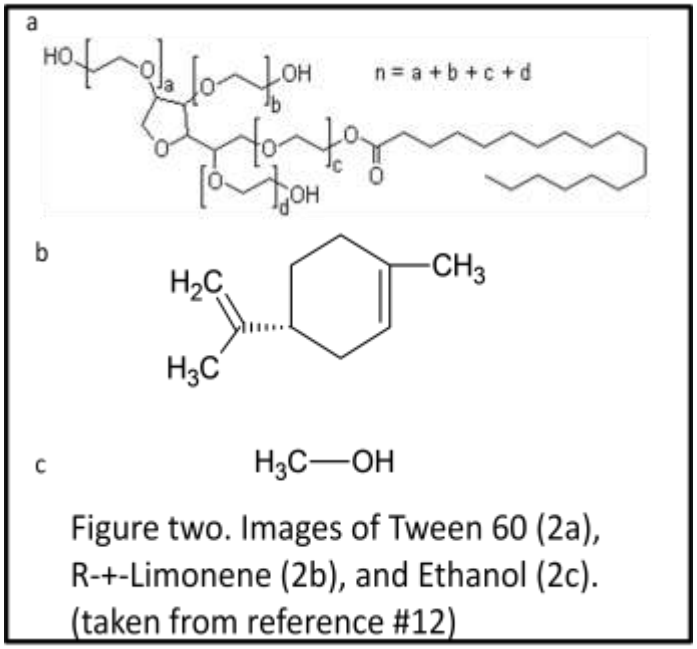

Figure two. Images of Tween 60 (2a), R-+-Limonene (2b), and Ethanol (2c). (taken from reference #12)

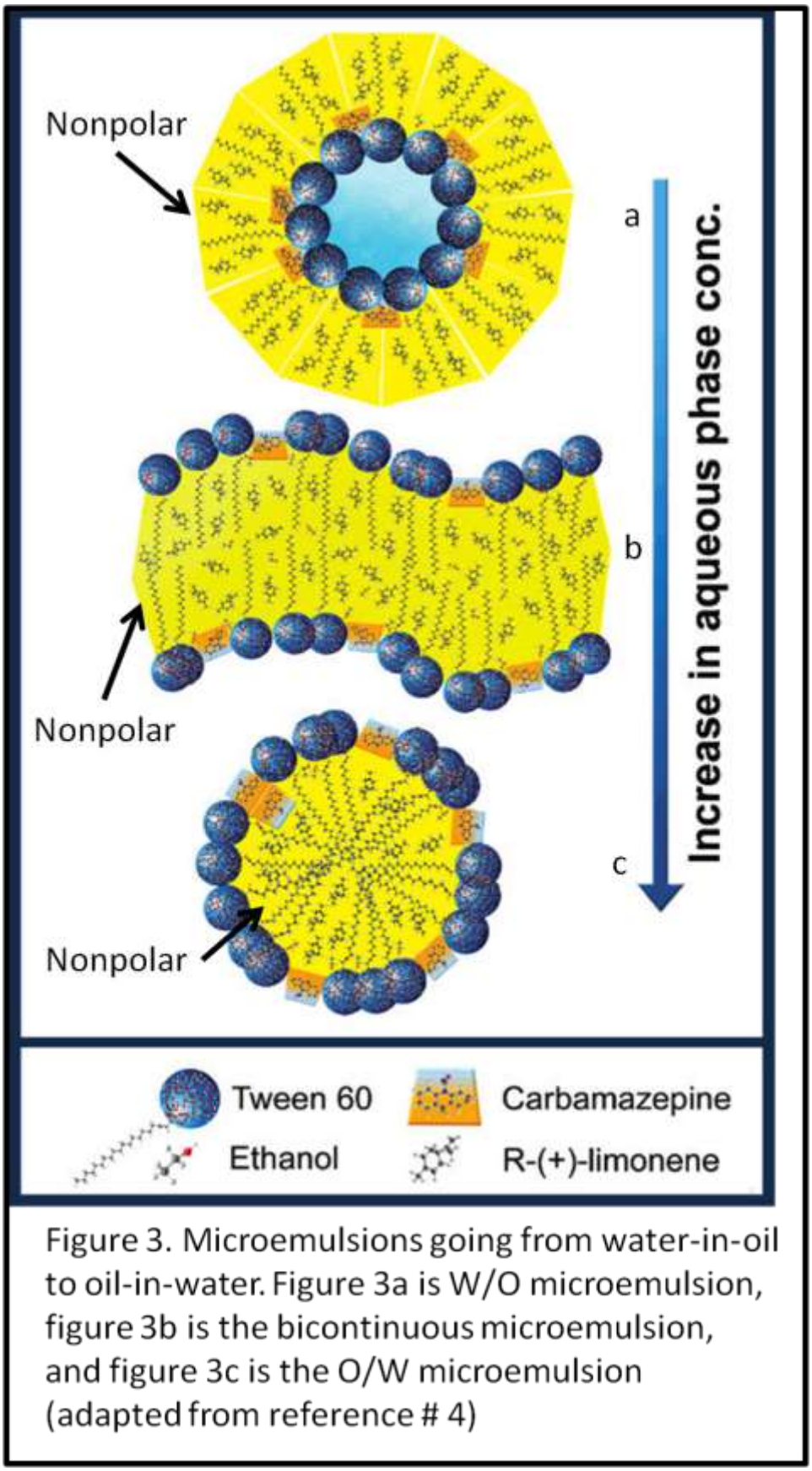

Figure 3. Microemulsions going from water-in-oil to oil-in-water. Figure 3a is W/O microemulsion, figure 3b is the bicontinuous microemulsion, and figure 3c is the O/W microemulsion (adapted from reference # 4)

controlled amounts of water-to-oil or oil-to-water in nanometer dispersions.[6] In these microemulsions Tween 60, R-+-limonene, and ethanol (figure 2(a), 2(b), and 2(c) respectively)[4] were mixed. Tween 60 is a molecule that has a polar head and a nonpolar tail and serves as the surfactant in the microemulsion.[4] R-+-limonene is also a nonpolar molecule and was added to the microemulsion with ethanol (which is polar).[4] R-+-limonene and ethanol served as the oil and water phases in the microemulsion (respectively).[4] As the amount of water is increased from 0% to 90% (ultimately going from water-in-oil, to bicontinuous, to oil-in-water) the microemulsion turns from a reverse single layer membrane, to a bicontinuous membrane, and finally to a single layer membrane (figure 3).[4] From these microemulsions, crystals were formed. These crystals were then examined with a Differential Scanning Calorimetry, Thermogravimetric analysis, scanning electron microscopy, and optical light microscopy.

Differential Scanning Calorimetry (DSC) (figure 4) is a timely technique used to measure heat flow versus temperature.[9] There are three types of DSC; Power compensated, heat flux, and modulated.[9] Heat Flux is a common technique and is when observing the crystals produced from the microemulsions.[9] Heat flux DSC measures the differences in the heat flow between a sample crystal and a reference crystal.[9] A reference crystal is used to see what is expected and what is obtained from the new sample. The crystals are set in the pans, which are on the raised platforms, on top of the thermoelectric disks.[9] Heat is sent

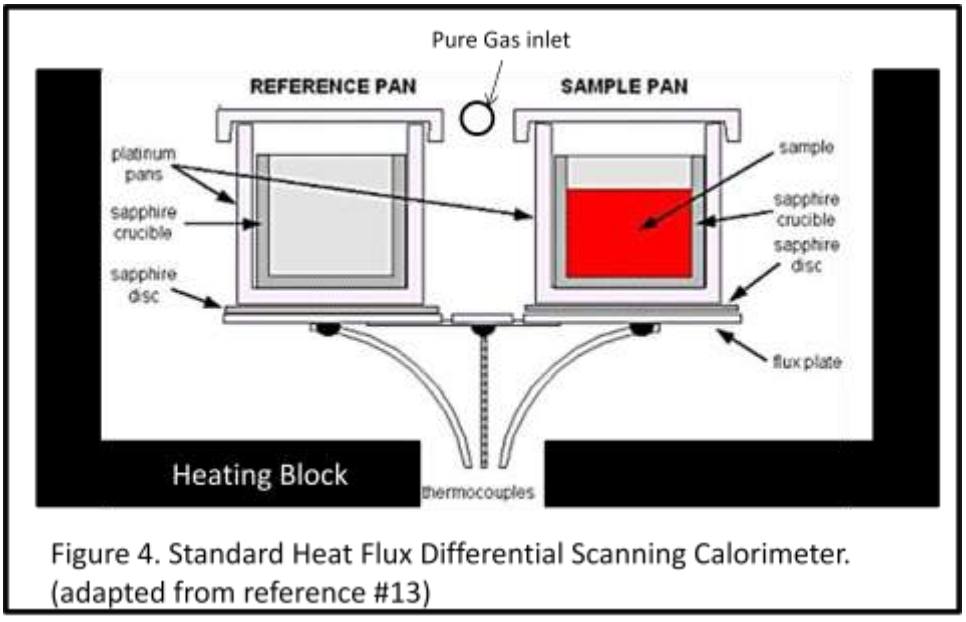

Figure 4. Standard Heat Flux Differential Scanning Calorimeter. (adapted from reference #13)

into the pans via the thermoelectric disk.[9] The difference in heat flow is monitored by the Chromel-constantan thermocouple.[9] A Chromel-constantans thermocouple is an industrial thermometer that consists of two dissimilar metals joined together at one end.[10] These thermocouples act as the sensor and detect the changes in energy given off or put in to the crystal. In a DSC thermogram, upward peaks symbolize exothermic activity (heat released from the crystal) and downward peaks symbolize endothermic activity (heat put into the crystal).[9] Thermogravimetric analysis was used in tandem with DSC.

Thermogravimetric analysis (TGA) (figure 5) is a technique used to measure the change in the mass of a sample over a temperature gradient.[9] TGA has four main components; the furnace, a pure gas system, a thermobalance, and a computer for data processing, acquiring, and instrumental control.[9] A typical furnace has a temperature range of 25°C to 1000°C. [9] TGA furnaces also have an accuracy of 1°C and a precision of ± 0.1°C.[9] The furnace has to be insulated in order to avoid heat transfer to the balance.[9] The furnace is purged with argon or nitrogen gas in order to prevent any oxidation of the sample being analyzed.[9] In

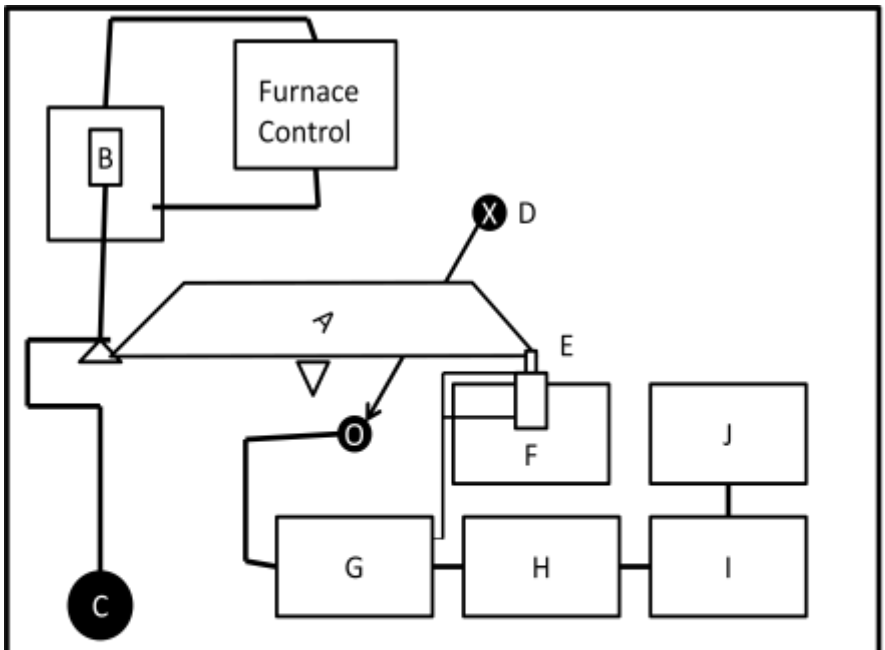

Figure _5. Thermobalance componenets. The balance is shown here as A, the sample cup and holder are B; the counterweight is C; The lamp and photodiodes are D; the magnetic coil is E, the permanent magnet is F; G,H, and I are the data processing components; J is the display and printer.

order to get more defined peaks, the purge gas can be switched mid-analysis.[9] This would increase definition because it would allow for molecules that are similar to be separated based on the type of gas used and their interaction with that gas. Thermobalaces have ranges from 1 milligram to 100 milligrams and they can detect changes as small as one microgram.[9] When a change in mass occurs, a deflection of the balance beam occurs.[9] This forms a shutter between one of two photodiodes and the lamp.[9] This causes an imbalance in the phototube. The signal from the phototube gets amplified and fed into the coil.[9] This coil is located between two poles of a permanent magnet.[9] These magnets return the balance beam to the original position.[9] Each time this imbalance occurs the computer records the photodiode current.[9] It then transfers the signal into a mass loss versus temperature graph.[9] This graph is often represented as the percent mass loss on the Y axis and the temperature gradient on the X axis.

Reflected Light Microscopy was used to view the surface of the microemulsion crystals (figure 6).[11] In Reflected Light Microscopy, the object observed cannot have light pass through it, thus the light must reflect off of the surface back to the microscope.[11] Reflected Light Microscopes typically have two eyepieces and a trinocular tube head where a digital camera is often mounted in order to take pictures of the crystals surface.[11] The stage of the microscope is controlled electronically with a specimen holder which allows for precise movements and better focusing.[11] Light starts in the lamp

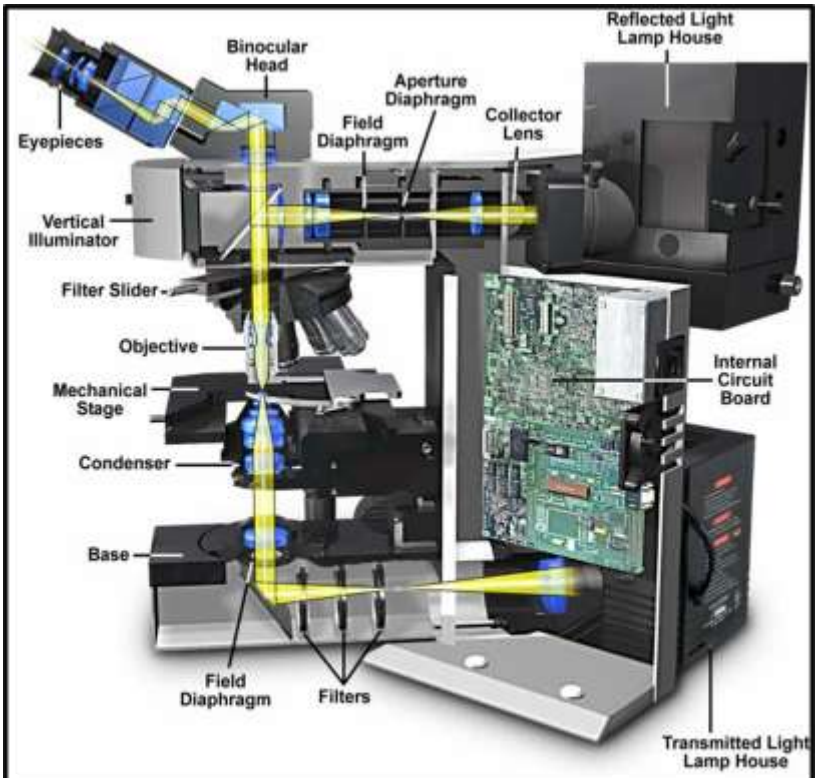

Figure 6.Cutaway diagram that shows the components of a microscope fitted for transmitted and reflected light. (image adapted from reference 11)

house and passes through the vertical illuminator.[11] The top surface of the crystal is in the upright position on the stage.[11] The illuminator is positioned at a 90 degree angle to the surface of the crystal and once the surface of the crystal is in focus, a digital image is taken.[11]

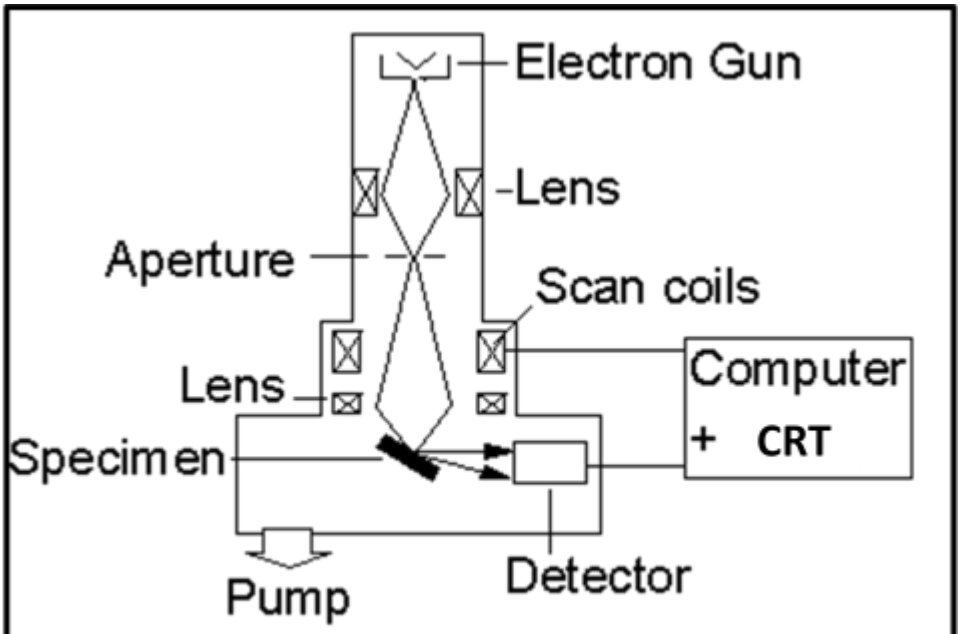

Figure 7. Scanning Electron Microscope with Cathode Ray Tube display and recorder. (taken from reference 13)

Scanning Electron Microscopy (SEM) (figure 7) was the final technique used to closely examine the crystals obtained from the microemulsion.[9] Scanning Electron Microscopy was used to obtain more refined images of the crystals being studied.[9] When obtaining images by SEM, a finely focused beam of electrons is directed at the surface of the crystal.[9] The beam is scanned across the crystals surface in a *raster scan*.[9] *Raster scans* are scans where the electron beam is swept across the crystal surface horizontally.[9] Once the beam hits the edge of the crystal, the beam moves down the y axis and goes back to the point of origin.[9] This process is repeated until the total desired surface area is scanned.[9]

Metal centers were also used in an attempt direct CBZ crystal packing structure and ultimately improve bioavailability. Metal crystals were coated with a monolayer (single layer of CBZ molecules) of carbamazepine and then examined with Scanning Tunneling Microscopy (STM) (figure 8).[9] STM is capable of resolving features at the atomic level on the surface of a solid.[9] STM is often performed

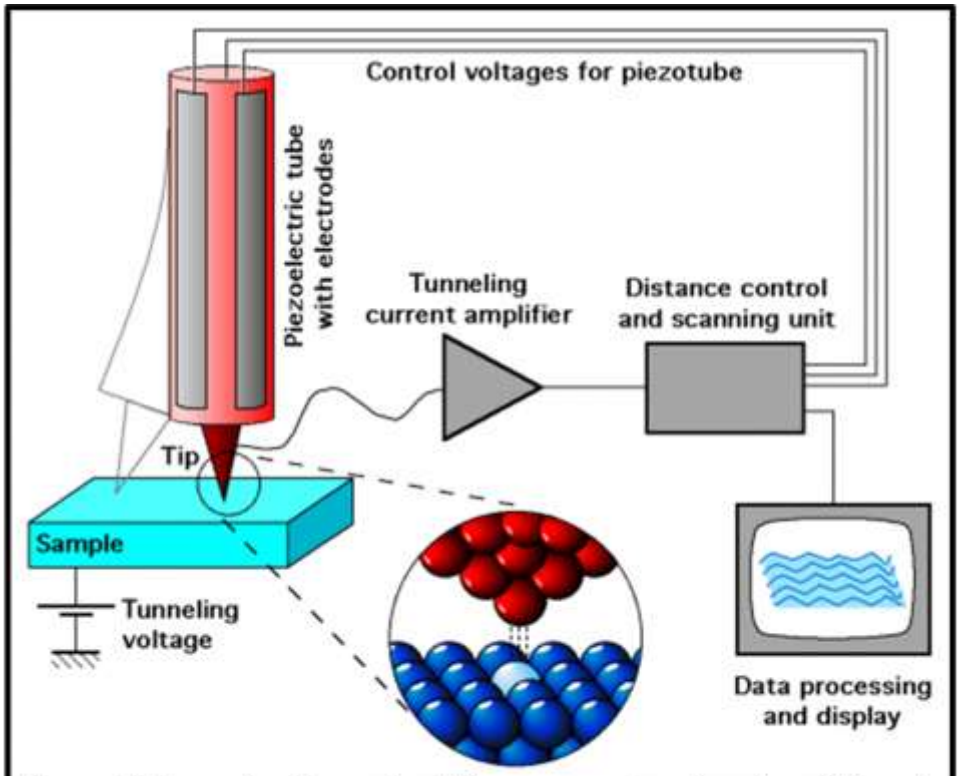

Figure 8. Scanning Tunneling Microscope showing the different parts of the instrument. (taken from reference 14)

inside of a vacuum in order to avoid contamination.[9] STM scans the surfaces of a conducting solid in a raster pattern using a very fine metallic tip.[9] The computer sets the position of the tip in relation to surface being studied, and it determines the raster scan coordinates to follow (in terms of X and Y axis).[9] Once this is determined, the computer adjusts the height of the tip (Z) and records the voltage for the X,Y, and Z coordinates.[9] Once these voltages have been recorded the tip continues down the X axis until it reaches the end of the surface.[9] Then the tip resets at the original X location but moves down the Y axis and continues the raster scan.[9] Once the surface is scanned completely, the voltages of the recorded X, Y, and Z locations are converted into contour maps.[9] The tip is very sensitive to the distance (Z) between the tip and surface of the substance which allows for atomic resolution.[9] When the final image is produced from the data, darker spots indicate lower areas on the surface of the monolayer and lighter areas indicate higher areas on the surface of the monolayer.[9]

With microemulsions, carbamazepine crystals were produced and examined with differential scanning Calorimetry, Thermogravimetric analysis, optical light microscopy, and scanning electron microscopy. Metal crystals will be coated with a monolayer of CBZ and will be examined with STM.

Both the monolayer and the crystals were examined in order to determine if they direct the packing structure of CBZ when forming crystals in hopes of finding a way to increase the bioavailability of CBZ.

Results and Discussion:

Carbamazepine is an antiepileptic drug that has high intestinal permeability but due to the low water solubility, it has a low bioavailability. In an effort to correct this problem, carbamazepine microemulsions and metal crystals with a single coated layer of Carbamazepine were examined with a wide variety of instruments in order to see if a new packing structure of the molecules is more efficient and would give the drug better bioavailability.

First, crystals were obtained from the different microemulsions of Carbamazepine in order to compare them to commercial carbamazepine. The microemulsions examined here were 0%, 10%, and 20%, weight percent water-in-oil microemulsions (W/O), 30%, 40%, 50%, and 60% weight percent bicontinuous microemulsions, and 70%, 80%, and 90% weight percent oil-in-water microemulsions (O/W). The bicontinuous microemulsions just have the percent ratio of water to oil (as the percents increase more water is being added). Crystals were made from these microemulsions and the crystals were examined with Differential Scanning Calorimetry, Thermogravimetric Analysis, Optical Light Microscopy, Scanning Electron Mircroscopy, and Scanning Tunneling Microscopy.

Commercial Carbamazepine (CBZ) and the crystals formed from the various microemulsions of carbamazepine were first analyzed with Differential Scanning Calorimetry (DSC) in order to determine their crystalline structure. In DSC, downward peaks indicate heat being absorbed (endothermic) and an upward peaks indicate heat

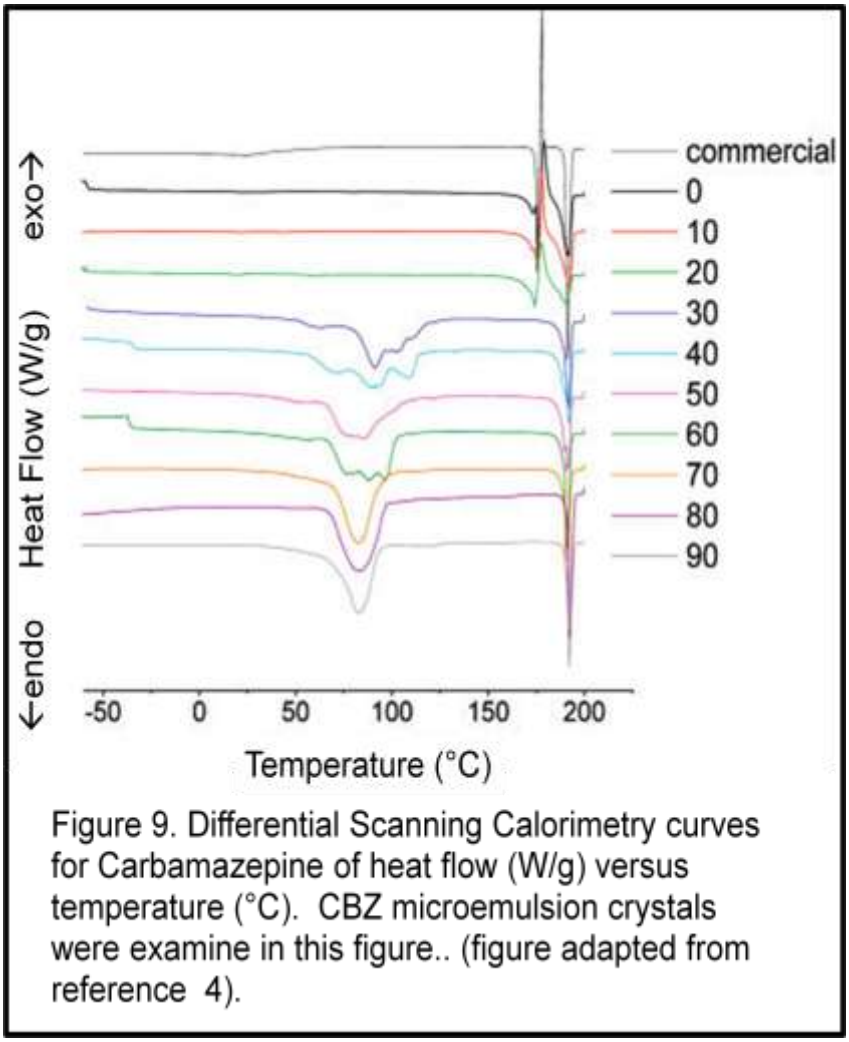

Figure 9. Differential Scanning Calorimetry curves for Carbamazepine of heat flow (W/g) versus temperature (°C). CBZ microemulsion crystals were examine in this figure.. (figure adapted from reference 4).

being given off (exothermic). In figure 9, the top line represents the commercial Carbamazepine. The first peak seen was at 175°C. This downward peak represented a taking in of heat (endothermic) and was the melting of the commercial P-monoclinic form (β CBZ) (figure 10). The next peak seen in figure 1 was at 178°C and was an upward peak representing a release of heat (exothermic). This peak represented the triclinic form (ά CBZ) of CBZ. This form of CBZ is very similar to the commercial form of CBZ but is classified as a slightly altered form because of the slight

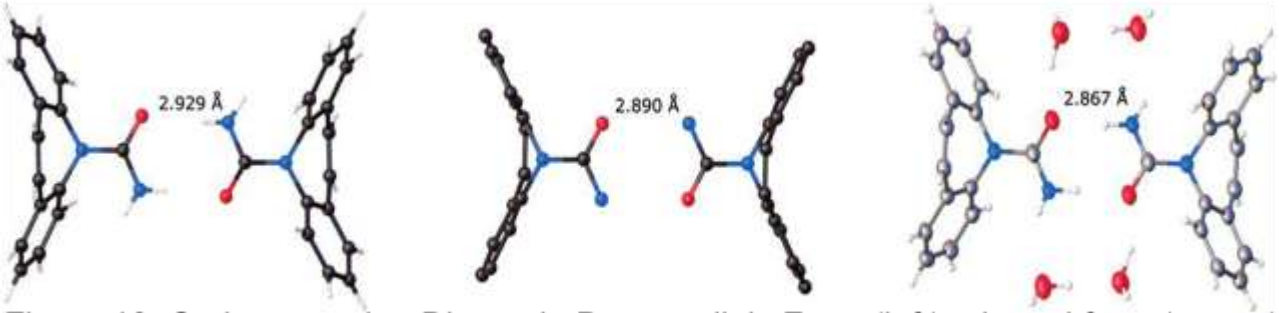

Figure 10. Carbamazepine Dimers in P-monoclinic Form (left), trigonal form (center), and dihydrate (right). (take from reference 15)

shift seen in the exothermic and endothermic peaks. The final peak seen for these microemulsions in figure 9 was located at 191°C and was another downward peak representing the taking in of heat (endothermic). This peak represented the melting of the polymorph or specific crystalline structure. The three peaks represented a "solid-melt-solid" phase transitions that is very similar to the known behavior of commercial CBZ. This means that the CBZ started off as the solid crystal. Then at 175°C heat was absorbed by the crystal and a liquid formed. After this occurred, the liquid recrystalized at 178°C, giving off heat. After all of this is done, the liquid rereleased heat and re-melted to form a liquid. Even though this follows a known standard behavior for CBZ, it does not follow the discoveries of Katzhendler *et al.* who found a solid-solid phase transition (two simultaneous upward, exothermic peaks).

Thermogravimetric analysis (figure 11) was consulted in parallel to DSC. It was noted that the top most line on the graph (which represented commercial CBZ) went straight across the graph. This showed no weight loss for the commercial CBZ while it was heated from 40°C to 200°C. If weight loss had occurred then it could indicate a less stable crystal form and would indicate that something else must be given off from the crystal to have weight loss.

The next three lines just below the commercial CBZ line in figure 9 represented the crystals generated from the 0%, 10%, and 20% W/O microemulsions. In these microemulsions, the CBZ molecules are located in the interface of the nanodroplet "membrane". In figure 9, there are peaks for the 0%, 10%, and 20% microemulsion crystals at 173°C, 174°C, and 169°C respectively. These peaks are thought to be a β' form of CBZ because of their slight shift to the left of the endothermic peak seen in

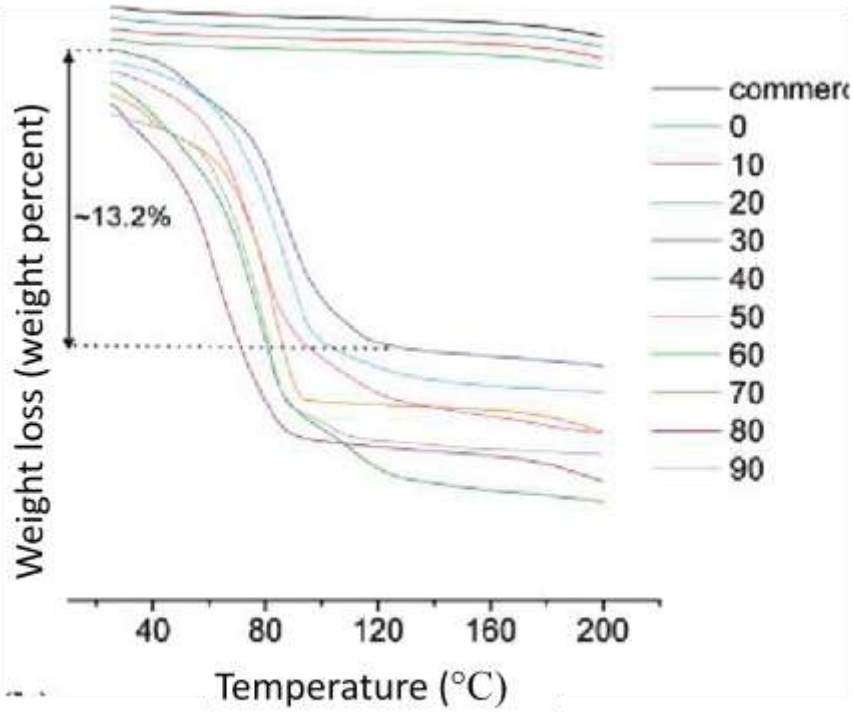

Figure 11. Thermogravimetric Analysis curves showing weight Loss (in weight percent) vs temperature (°C) for the different Microemulsion crystals. (adapted from reference 9).

commercial CBZ at 175°C (melts at a lower temperature). This indicates a lower melting point and a weaker structure. This form of CBZ is thought to have slight structural defects although the specific defects where never examined. This form also seems to be more prevalent than the standard β-CBZ form that is observed. When Thermogravimetric analysis was correlated to these results (figure 11), it was seen that the lines representing these three microemulsions went straight across the graph and showed no weight loss. These crystals also underwent a solid-melt-solid phase transition but they did so at slightly different temperatures (hence calling the crystals formed the β form of CBZ). Also, with no weight loss seen, it was noted that the water did not evaporate out of the crystal.

In figure 9, the bicontinuous microemulsion crystals (30%, 40%, 50%, and 60%) were examined. In these microemulsions the CBZ molecules are located in the membrane of the bilayer that forms. In figure one, broad endothermic peaks are seen from 61°C to 118°C with no exothermic or endothermic activity occurring around the

175°C or 178°C mark. Thermogravimetric analysis was then consulted in search of an answer for this activity (figure 2) and it was noted that there was a sharp drop in the lines representing the bicontinuous microemulsions (30%-60%), all occurring around the same temperature. This corresponds to a 13.2 percent loss in weight. Due to these microemulsions containing a large percent of water, the dihyrdate form of CBZ is formed (figure 10). Thus, this weight loss is the loss of water that is commonly seen when water is evaporated from emulsions. When the weight of water is divided by the weight of the dihydrate CBZ the same percent weight loss is the thermogram is seen:

$$36\ grams\ H_2O \Big/ 272\ grams\ Dihydrate\ CBZ * 100\% = 13.2\%$$

Also, as the amount of water in each microemulsion increases, the endothermic peak shifts further left in figure 9 and it becomes more distinguished. This is due to better containment of the water molecules in the microemulsions with a lower percent of water and a higher percent of oil (figure 3a). With the amount of water being the lowest in the W/O microemulsions, the limited amount of water is better contained. As the amount of water is increased, this containment efficiency lessens. This is seen in the multiple, smaller exothermic peaks showing the water evaporating at slightly different temperatures based on how well it was contained within the crystal. When the amount of water gets too large (as in the O/W microemulsion crystals), there is such an abundance of water that it isn't contained as well and all evaporates at one temperature forming one distinct peak. This suggests that the structure crystal formed by the microemulsion is influenced by the structure of the hydrated CBZ. The peak seen at 191°C, as in the commercial CBZ line, was also an endothermic peak (melt) that

showed the formation of triclinic CBZ. This whole process demonstrated a melt-melt transition (as seen in the two endothermic peaks).

The last three lines in figure 9 represent the microemulsion crystals ranging from 70%-90% O/W. In these microemulsions, the CBZ molecules are, again, in the membrane of the nanodroplet (nonpolar phase) (figure 3c). In the lower three lines in figure 9, an endothermic peak (that is more distinguished) is seen between 81°C and 84°C. Thermogravimetric analysis was then consulted (figure 11) in order to determine the reason for these peaks. The lower three lines in figure 11 represent the O/W microemulsion crystals. As the temperature was increased from 0°C to 200°C, a 13.2 percent loss in weight was noted. This loss was from the evaporation of water from the dihydrate CBZ microemulsion crystal. From this data it was noted that with a CBZ microemulsion above 20 percent, the dihydrate forms. Also in these lines, a peak is observed at 191°C which, again, is for the addition of heat to the crystal in the formation of the triclinic form of CBZ.

Based on what was seen in DSC and TGA, certain predictions can be made for the arrangement of the crystals and their structures. From DSC and TGA, it can be predicted that the 0%-20% W/O microemulsion crystals will have a 3-D structure because no weight loss was seen in TGA and the peaks in DSC most closely resembled those of commercial CBZ. The bicontinuous and O/W microemulsion crystals will have a slightly altered structure (in regards to commercial CBZ) because of the weight loss seen in TGA and because of the other peaks seen in DSC that do not match the commercial CBZ peaks. No definitive crystal structure can be determined from this data. Due to this, optical light microscopy was used to take a closer look at the crystals.

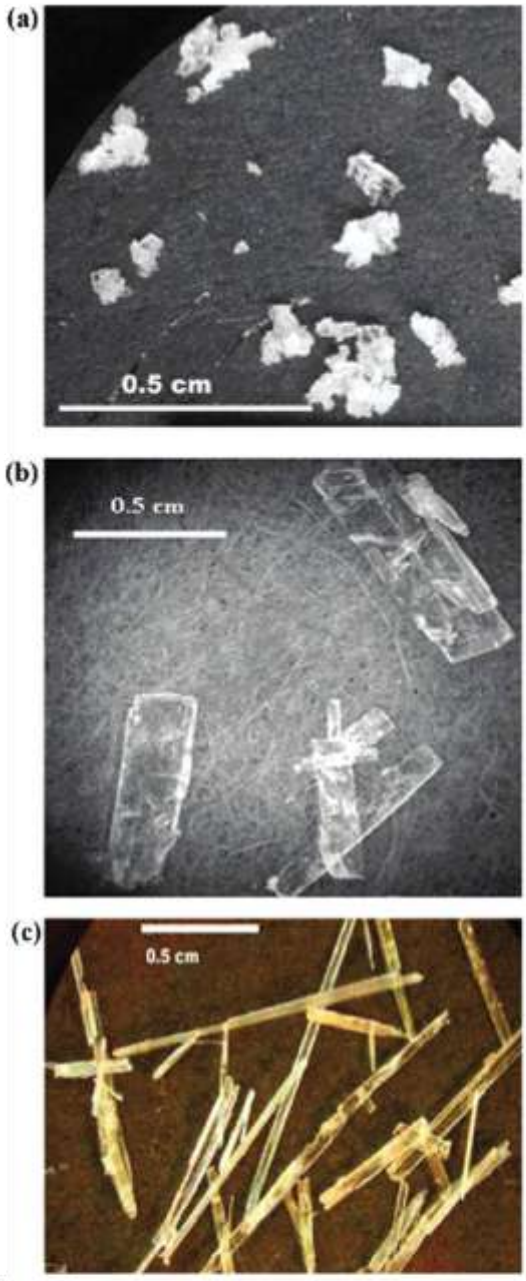

Figure 12. Optical light microscopy images of Carbamazepine microemulsion crystals from microemulsions of 10% water-in-oil (a) 40% bicontinuous (b), and 90% oil-in-water (c). (adapted from reference 9).

Optical light microscopy was used to get a closer look at the surface of the microemulsion crystals in order to more closely observe the crystals surface structure (figure 12; a-c). Figure 12a represents the 10% W/O microemulsion crystal. This microemulsion crystal had prism-like structures that measured 2 to 4 millimeters in width and 3-5 millimeters in length. These prism-like microemulsion crystals were formed in a test tube at room temperature (25°C) after 3 weeks without agitation.

The 40% bicontinuous microemulsion crystals were observed in figure 12b. These crystals measured 0.5-1.5 millimeters in width and 2-10 millimeters in length. These crystals were obtained after 2 weeks of precipitation at 25°C with no agitation. These crystals had a broad size distribution unlike W/O microemulsions.

Needle like structures were seen in figure 12c, which corresponded to the crystals obtained from the 90% O/W microemulsions. These crystals were 0.5 millimeters wide and 10-15 millimeters long. These crystals were obtained in 3-7 days at room temperature (25°C) without agitation.

Based on these three results the data supports the predictions made from the Differential Scanning Calorimeter data and Thermogravimetric Analysis. Anhydrous carbamazepine crystals (which most similarly represent the W/O microemulsions) form a 3-D, multisided prism (figure 12a) that has a small amount of size distribution and is large in quantity. This was expected to occur because no weight loss occurred when heating the crystals and the peaks in DSC (for the microemulsion crystals) closely resembled those of the commercial CBZ crystals. Hydrated carbamazepine crystals (which are most similar to the bicontinuous microemulsion and the O/W microemulsion) had a 2D, plate-like structure or a 1D needlelike structure which was seen in figure 12b and 12c. This is most likely due to the evaporation of water from the crystal when heated. While this structure could be predicted from the DSC and TGA data (this among others), optical light microscopy gave a definitive crystal structure.

Crystallization of a bicontinuous or O/W emulsion differ from that of a W/O emulsion in a variety of ways. In W/O microemulsions, the molecules experience a change of phase from liquid to solid through two processes; crystal growth and nucleation. Nucleation is where the start of a crystal is formed within a liquid.[12] From this starting point, the rest of the crystal can then continue to grow and form[12]. In order to form the growth unit cell of the crystal in W/O microemulsions, solute molecules (Tween

60, R-(+)-limonene, and ethanol) and water molecules have to be incorporated into each other.

In the bicontinuous microemulsion, the oils dissolve the crystallized CBZ instead of dissipating the heat of crystallization. The heat of crystallization is the amount of heat required to remove one gram of liquid, in order to form a solid with no change in temperature.[17] This then causes the growth of the crystal in the microemulsions to be orientated towards the water molecules because the water molecules dissipate the heat of crystallization. In addition to this, the orientation of water and oil in the microemulsion has an effect on the crystallization of the bicontinuous microemulsions. From the images obtained from the light microscope (figure 3b) it is noted that the plate like crystals have a large dispersion of size. This is caused by the disorder of the water and oil in the microemulsion.

Finally, the O/W microemulsions have a better dispersion of the heat of crystallization because there is a larger amount of water surrounding the hydrophobic nanodroplet. It is more efficient than the bicontinuous microemulsion because the bicontinuous microemulsion creates a bilayer that isn't as secluded as the O/W nanodroplet (figure3b versus 3c). This is shown in the larger crystals that form that are more linear. Due to the increase in water, the crystal formation process is favored because the water better dissipates the heat needed to form the crystal.

In order to further confirm the crystal structure of the microemulsions, scanning electron microscopy was used to examine the same crystals that were examined with optical light microscopy (figure 13). These images reveal the microscopic features of

crystal structures which then allow bridging between microscopic crystal results and macroscopic observations of carbamazepine.

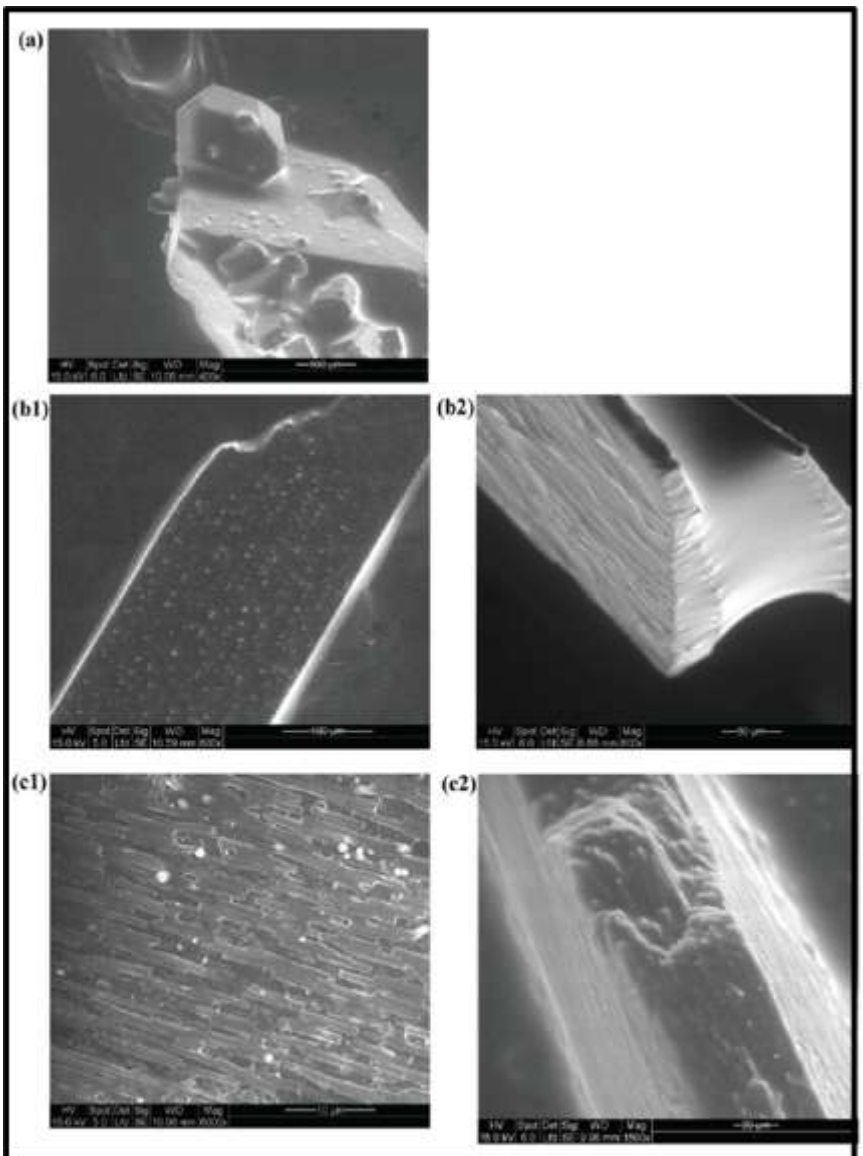

Figure 13. Scanning Electron Microscopy images of the Carbamazepine crystals formed from 10% (a), 40% (b), and 90% (c) microemulsions. Images b1 and c1 were obtained from the center of the crystals and b2 and c2 were obtained from the edge of the crystals. (adapted from reference 4)

In Figure 13a, it is noted that at a microscale level, the surface looks smooth. This structure most resembles commercial CBZ because the crystal structure of both W/O microemulsions and commercial CBZ have smooth surfaces (figure 13a).

In figure 13b1 and 13c1 the bicontinuous and O/W (respectively) microemulsions contain microplatelets on the surface of the crystal. This shows that the surfaces of these microemulsions are not a smooth surface like that of the anhydrous or commercial CBZ.

These platelets run the length of the crystal and range from 2-15 micrometers long with a width of 0.03-0.05 micrometers. The platelets are formed from different sized channels of water and oil that are connected with each other. In the cross sectional view of both crystals, the platelets are seen permeating through the entire width of the crystal. This lead to the conclusion that these platelets are the building blocks for the O/W microemulsion crystals and for the bicontinuous microemulsion crystals. The packing of these crystal structures shows that they have an attachment mechanism that is similar to other common crystallization techniques; *e.g.* biomineralization crystallization.

In the O/W nanodroplets that form, the droplets are 8 nm in diameter and are large enough to form a hydrophilic outer layer with a hydrophobic core (Figure 14a). These nanodroplets also allow a CBZ dihyrdate cluster to precipitate. These nanodroplets can be expanded to micrometer size (figure 14b) through liquid-liquid interactions. In these interactions, the droplets get close enough that their internal contents fuse and the hydrophilic layers combine and expand the droplets size. Once the droplet's are expanded, the CBZ (which composes part of the hydrophilic layer – figure 14b) can come into contact with one another and form a bridge. This bridge is formed in a way resembling sintering; a process where the solution is heated to a temperature just below the melting point until the molecules adhere to each other. Once the CBZ is fused and one of the droplets moves away, another CBZ from another droplet fuses to the growing chain (figure 14c). This continues until a crystal is formed (figure 14d).

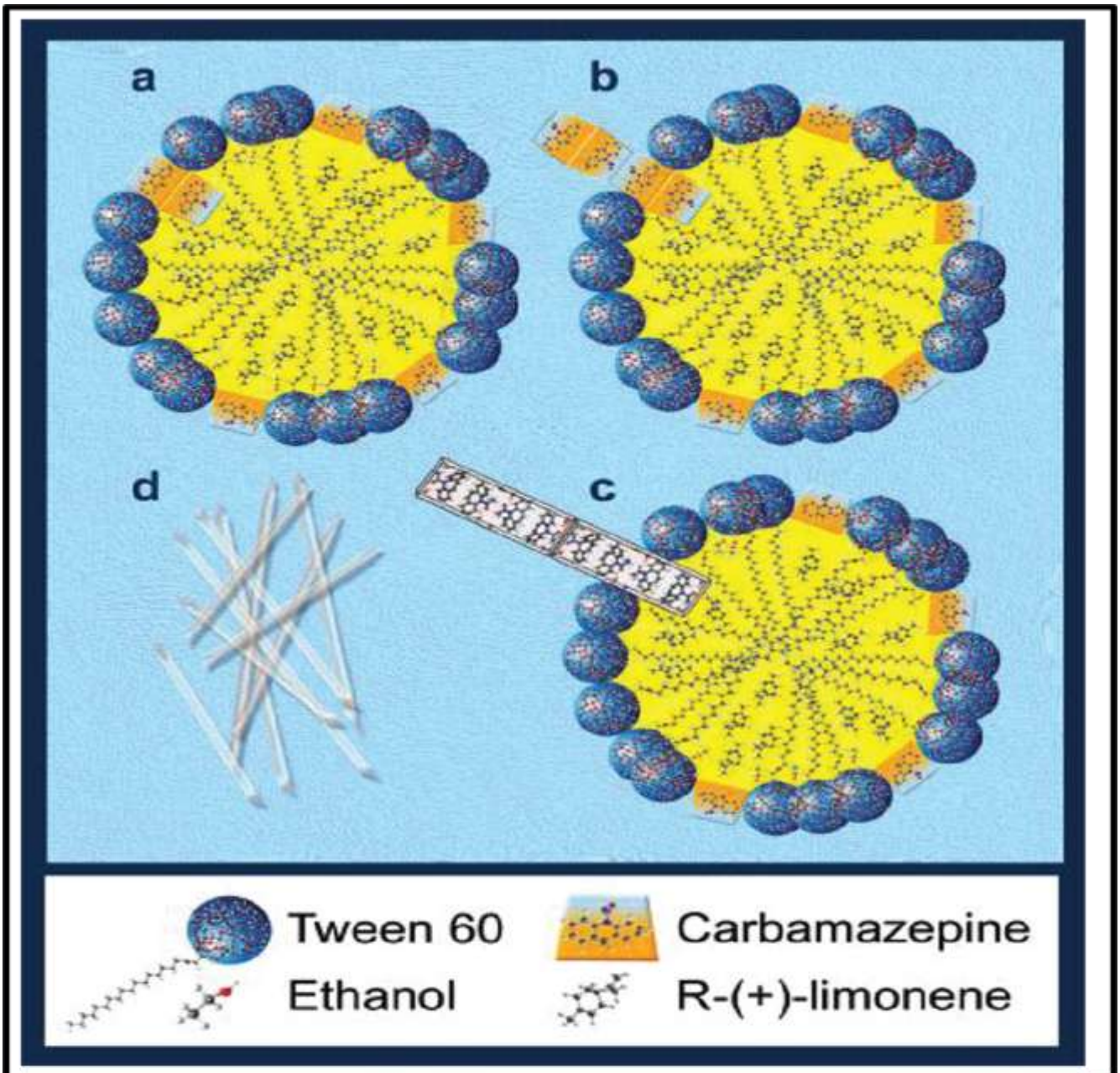

Figure 14. Crystallization of Carbamazepine in an oil-in-water microemulsion. Figure 14(a) shows the microemulsion droplet with Carbamazepine situated in the outer membrane of the nanodroplet. Figure 14(b) shows the carbamazepine molecules coming together on the droplet's surface (crystal growth). Figure 14(c) shows the formation of the continued growth of the oil-in-water microemulsion crystal. Figure 14(d) is the finalized crystal. (taken from reference 1)

After the crystals were formed and extracted from the microemulsion, they were ground up. In standard molecule-molecule growth of crystals, the crystals are continuous in structure and are strong (i.e. hard to grind). The obtained crystals were much weaker and required little effort in order to grind them into powder. This supported the above method of crystallization because the method above leads to weaker attachment-type bonding which would then make the crystals easier to grind (unlike the standard crystal structure formation which leads to tougher bonds and stronger crystals)

Scanning Tunneling Microscopy (STM) is a newer technique that has been used to get an even more distinct view of a crystal structure. STM was used to test the packing efficiency of a newer crystal structure involving CBZ and metal ions. First, 2 mm^2 * $2mm^2$ crystals of Gold (111) (Au) and Copper (111) (Cu) were mounted onto a single STM plate (for consistency purposes). These crystals were then coated with a monolayer (ML) of carbamazepine. A monolayer (ML) is a single layer of molecules that is closely packed.

One panel was plated with both metal crystals, coated with the monolayer of CBZ and then examined with STM. When examined, a three lobed structure involving both Au and Cu centers was seen on the surface of the panels. These three lobed structures were linked to two different trimers that formed; the CBZ trimer involves the rings above the plane of the molecule with the amide groups in the center of the trimer (figure 15b) and the Dibenzazepine trimer involves the rings above the plane of the molecule with nothing in the center or the trimer (figure 15b) The CBZ trimer forms an equilateral triangle with the metal ion in the center and each side measuring 8 angstroms. The dibenzazepine trimer has sides measuring 6 angstroms and forms with the backside of

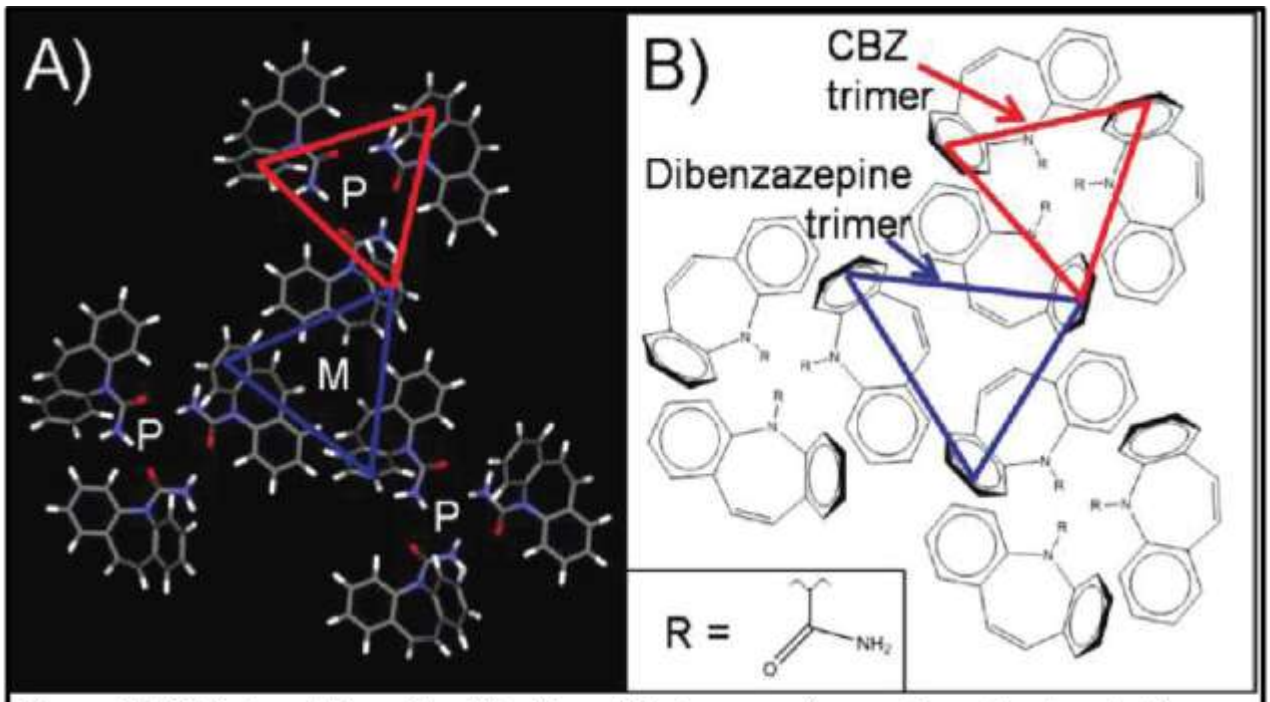

Figure 15. Chiral and trimer identification of Carbamazepine packing structure. In figure 15(a) the chiral trimers are seen are those on a gold molecule (P chirality is right handed chirality and M is left handed chirality). In figure 15(b) the same concept is shown but each trimer is labeled and this shows how the trimers are arranged in terms of the CBZ molecules. (taken from reference 1)

the CBZ molecules (opposite the amide group). As see in figure 15 in the CBZ trimer, the amide group and the aromatic ring sticking out of the plane of the paper at a 53° angle are both in close proximity to the metal center. This configuration allows for hydrogen bonding interactions between adjacent amide groups. Also, with part of the molecule being out of the plane, there is less steric hinderance, which then allows adjacent molecules to get much closer to the amide groups. This results in organizational chirality with the centers.

The molecular structure obtained here is unlike anything previously observed for CBZ (as cross referenced in Cambridge Structural Database)[1]. In figure 6 each trimer demonstrates specific chirality because of the ring that is above the plane of the page. As seen here, the CBZ trimers are associated with a P chiral center and a M

chiral center. Chiral molecules are molecules which are not superimposable on their mirror images. “P” chirality is equivalent to right handed chirality or R and “M” chirality has left handed chirality of S. P and M chirality are commonly used for helical complexes.

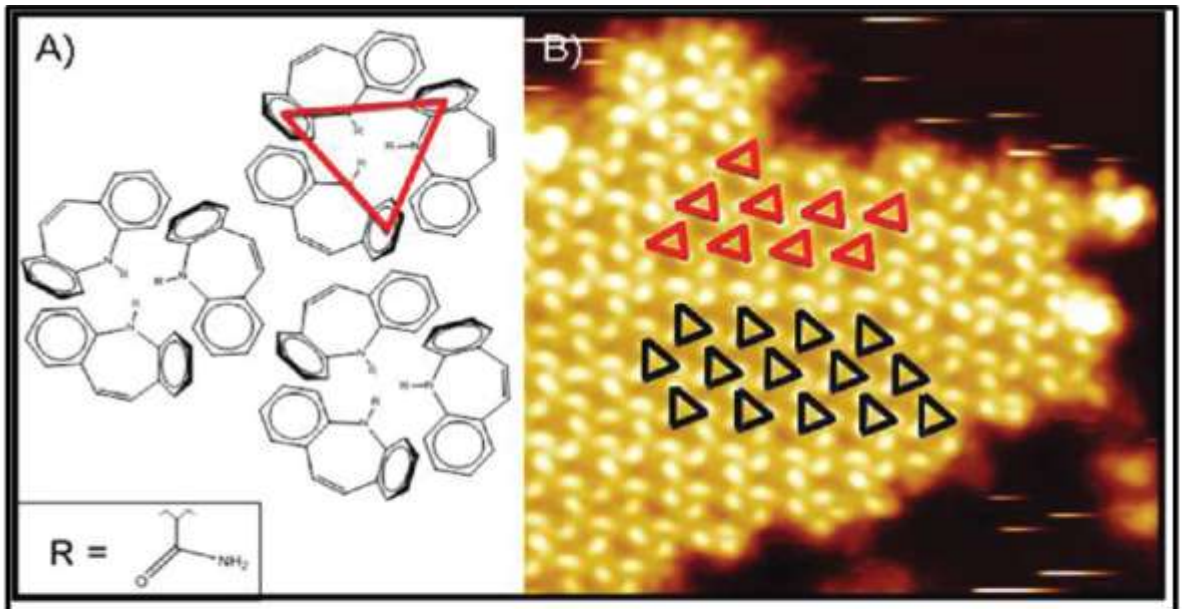

Figure 16. Homochiral Carbamazepine on Gold 111. Figure 16(a) shows how the carbamazepine arranges to form an equilateral triangle. Figure 16(b) shows the dispersion of the homochiral trimers. (P chiral arrangement is red and M chiral arrangement is black). (figure taken from reference 1).

On Au, the CBZ trimers produce different specific chiral configurations (figure 16). These chiral configurations are homochiral in their vicinity. Homochiral means that all of the neighboring CBZ trimers have one chiral configuration and all of the neighboring dibenzazepine trimers have the same chiral configuration. In figure 16b, it is seen that the percentages of P and M chiral configurations can be calculated and it is seen that there is a 50:50 split of P to M chiral configurations on the crystal (as expected).

On the Cu crystal surface, a large mixture of M and P trimers were seen, instead of the three-fold symmetry observed on Au crystal. In Cu, 2.7 CBZ molecules per nm^2 were seen where Au only had 1.7 CBZ molecules per nanometer squared. The three-fold trimer seen in Au cannot be seen in Cu because Cu is too small (*i.e.* 5*5*5 angstrom in Cu compared to 8*8*8 angstrom in Au). Due to this decrease in size, the three-lobed structure is attributed to the dibenzazepine (figure 17). In this figure, the gold triangles are where the dibenzazepine forms the trimer (this is what the blue triangle is over and symbolizes) and the black holes are where the CBZ trimer forms (this is what the red triangle is over and symbolizes). This dibenzazepine packing is much closer on the Cu surface than on the Au surface. With this geometry, the dibenzazepine trimers appear to

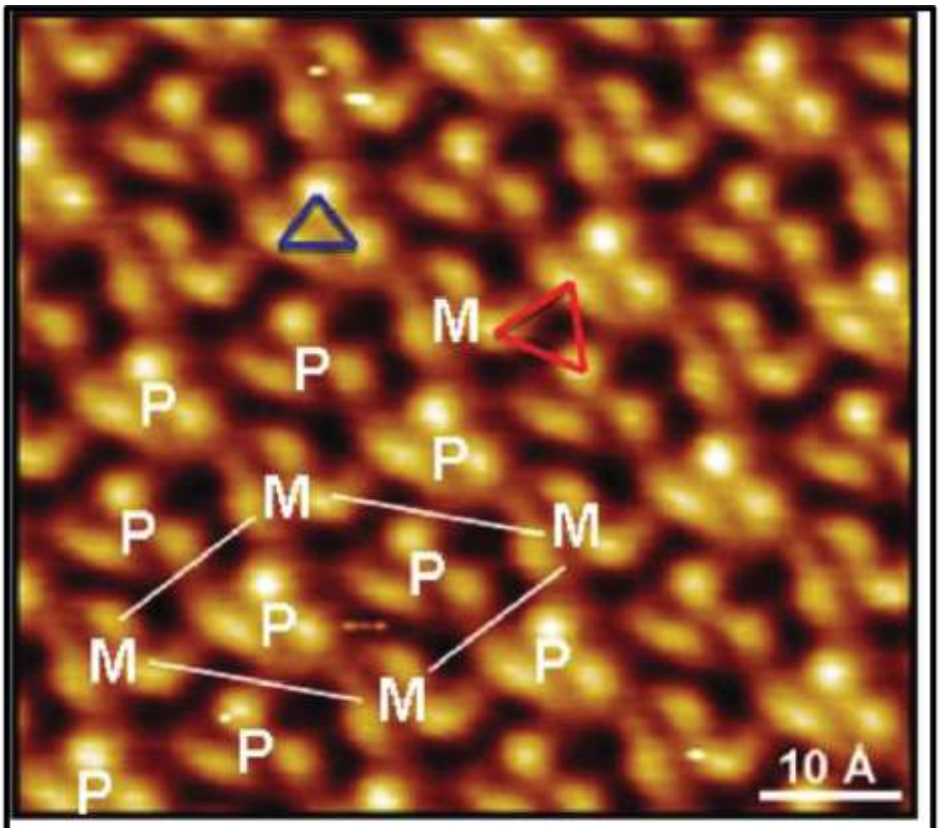

Figure 17. Heterochiral Carbamazepine domains on copper 111. The two types of trimers shown are the dibenzazepine trimer (blue) and Carbamazepine trimer (red). The white letters denote the chiral Arrangements with the P denoting the dibenzazepine Trimer and the M denoting the CBZ trimer. (figure taken from reference 1)

be closest while the CBZ trimers appear to be more distant. This is seen in the gold triangles in figure 8 that correlate to the dibenzazepine trimers and the black holes in that same figure correlate to the CBZ trimers. Each dibenzazepine trimer posses a six fold chiral symmetry with six “P” trimers surrounding a central “M” trimer which arrange hexagonally. This rhombus configuration denoted by the M’s with lines between them is the unit cell and it shows that the packing structure for Cu is a heterochiral instead of homochiral. Heterochiral means that there is a mixture of the different types of chiral configurations near each other (mixtures of P and M chiral configurations together). This is seen because the homochiral packing arrangement has all the same packing types in the vicinity where here it does not. Due to the fact that the packing is much more efficient on Cu, this leads to the conclusion that heterochiral packing is much tighter than homochiral packing structure in this case. The more efficient packing structure indicates that the size of the metal ion will affect on the packing structure and, ultimately, the strength of the crystal structure. This is so because the tighter the packing structure, the closer the two bonded molecules are. With this, the bonds are under less strain and they are stronger which leads to a more stable structure.

From all of this it is seen that metal surfaces can act as chiral directors in polymorph formation. Due to the chiral selectivity of metal surfaces, it can be noted that the metal has a strong influence on the initial stages of crystal growth. This also shows that metal surfaces aid in the complex 2-dimensional molecular structure formed by the packing of the CBZ trimers.

Conclusion

In conclusion, microemulsions and metal crystals can both be used to direct the crystal structure of carbamazepine. With microemulsions, the weight loss observed in Thermogravimetric Analysis and the extra peaks seen in Differential Scanning Calorimetry are two examples of this. Also in the optical light microscopy images, 3D crystal structures for the 0-20% W/O microemulsions and 2D and 1D crystals for the bicontinuous and O/W microemulsions were observed. Finally, in Scanning electron microscopy, the smooth 3D structure of the W/e microemulsion crystals and the platelet building blocks of the 2D and 1D crystals are also examples of this. With the metal crystals, the chiral trimers that associate with the metal crystals is an example of the crystal packing structure designation. With these crystal packing structure directors, there is hope to find a more efficient way to organize CBZ into crystals in order to improve their bioavailability.

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