Natural Turmeric Encapsulated Layered Double Hydroxides as Anti-microbial Nanohybrid

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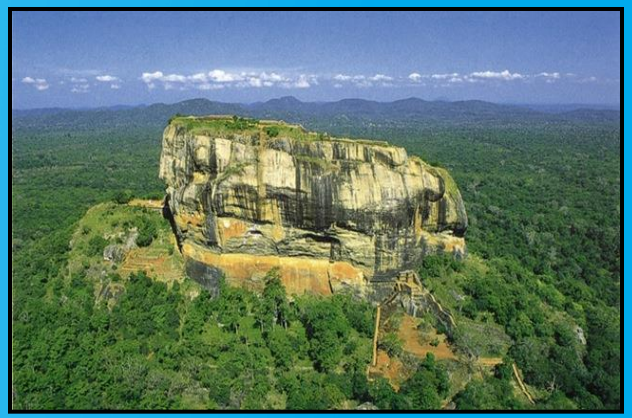
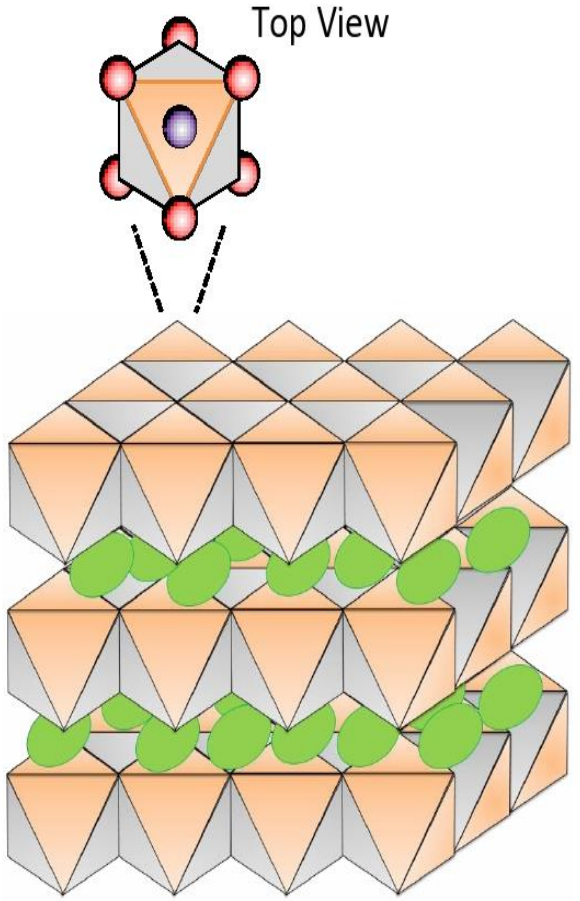


- In nature there are so many natural compounds with therapeutic potential, but due to their low bio availability, solubility and stability the application of those natural compounds in the pharmaceutical industries are limited.

- There are number of approaches which researchers are researching on to increase the stability these natural therapeutic compounds, and one of them is the combination of natural products and nano technology.

Turmeric

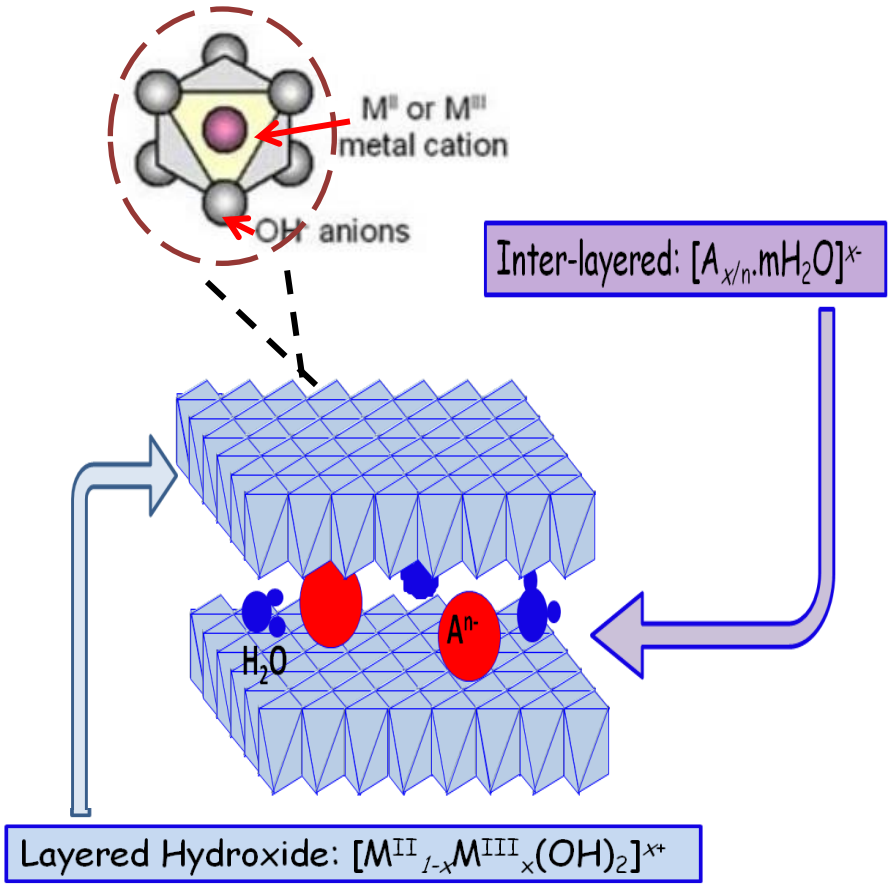
Mg-Al nitrate solution



Turmeric encapsulated
Layered Double Hydroxide

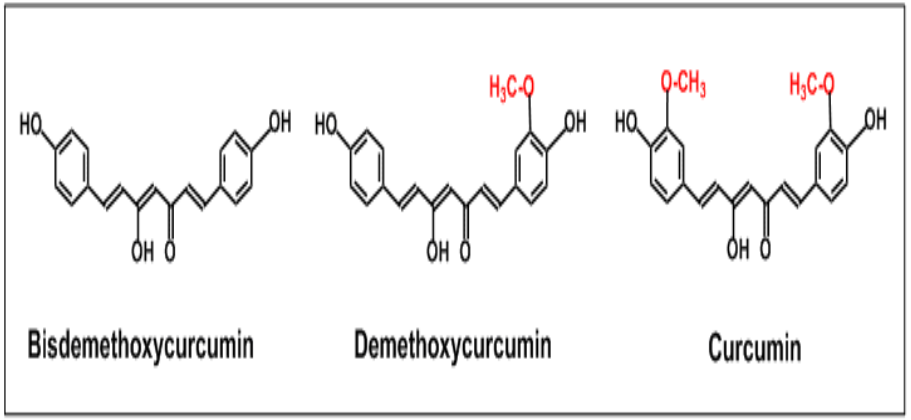
Layered Double Hydroxides (LDH)

Inorganic and organic anions exchanging clays.



Turmeric

Botanical name - *Curcuma longa*
Active ingredients – curcuminoids.



http://www.intechopen.com/books/new-advances-in-vehicular-technology-and-automotive engineering/nanocomposite-based-multifunctional-coatings

Objectives

1. Stabilization of turmeric within the nano layers present in layered double hydroxides.

2. Characterization and study the releasing kinetics of turmeric - LDH composites.

3. Investigation of anti-microbial properties of turmeric - LDH against bacteria and fungi species.

STEPS INVOLVED

Encapsulation of turmeric in to LDH (Turmeric - LDH nano composite)

Identification & Characterization of turmeric - LDH nano composite

Structural orientation

- PXRD
- FTIR
- TEM

Stability test

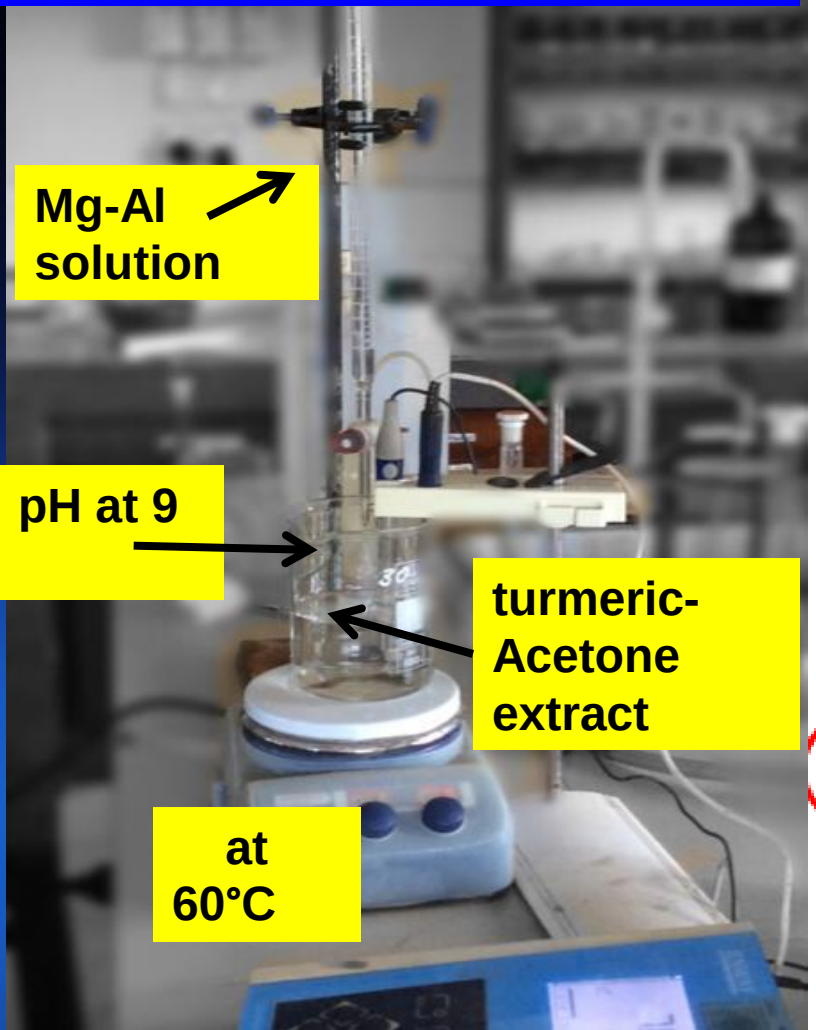
- UV analysis
- TGA

Release kinetics of turmeric - LDH

Antimicrobial test

Synthesis of Turmeric-LDH Composites

Co-precipitation method



$Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.6H_2O$

60°C

Drop wise + Vigorous stirring

pH at 9

Turmeric in acetone

Slurry

60°C

Stirred overnight

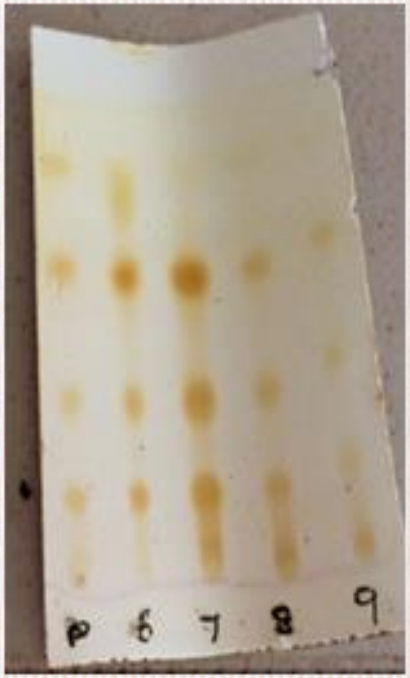
Dried at 90°C

Filtered

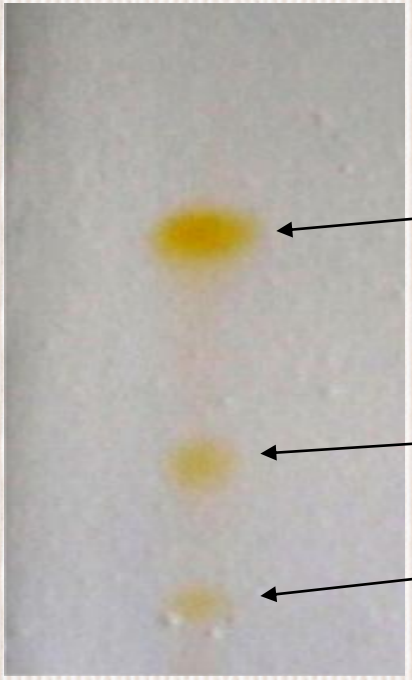
Turmeric-LDH composite

back to steps invoved

Separation of Curcuminoids by TLC



Turmeric in acetone



Turmeric - LDH in acetone

Solvents
Chloroform : Methanol
9 : 1

Curcuminoids	R_f values
Curcumin	0.75
Demethoxy curcumin	0.55
Bisdemethoxy curcumin	0.27

selective encapsulation of curcuminoids

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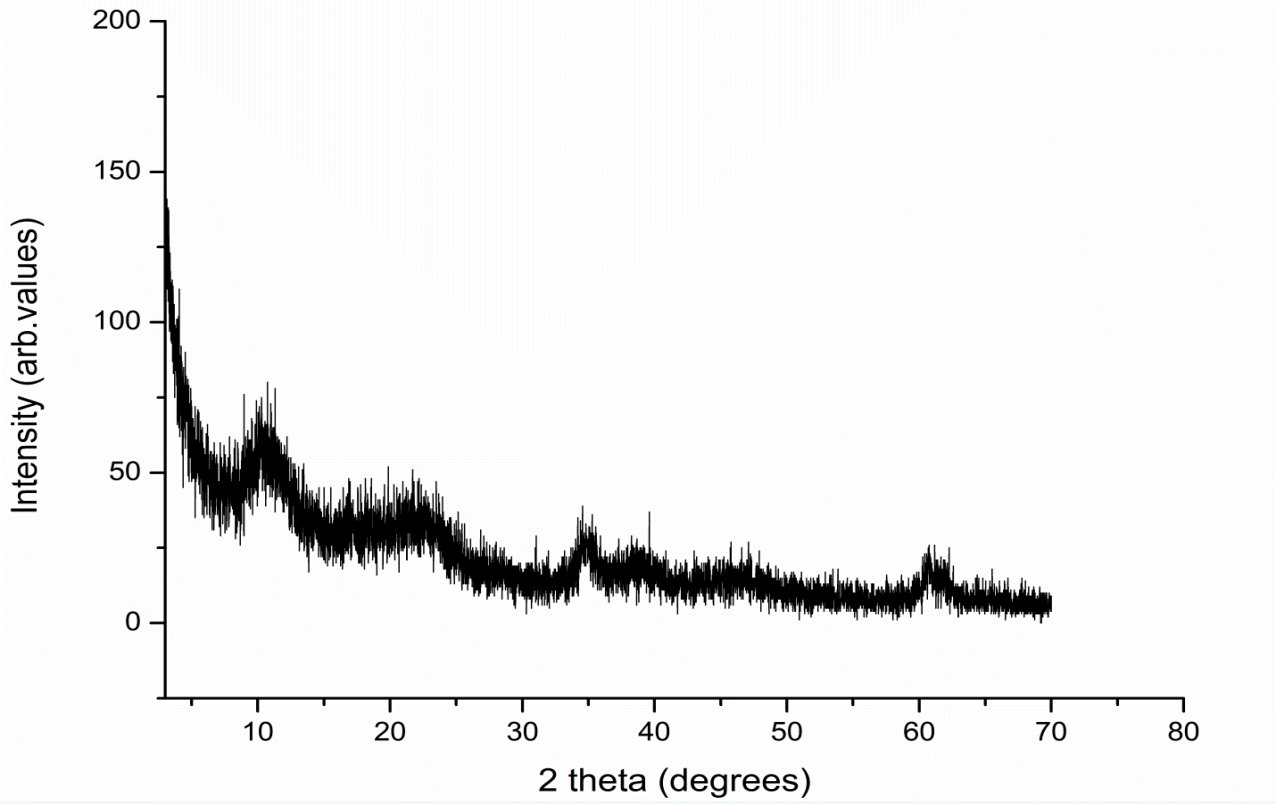
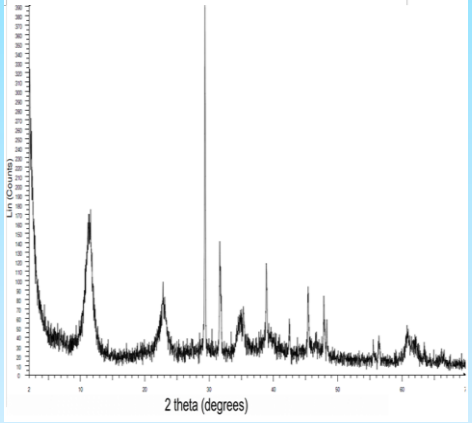
Powder X-ray diffraction (PXRD) analysis



Tumeric - LDH



Literature Curcuminoid - LDH

- **The pattern confirms that LDH has been succesfully synthesized**
- **Both pattern coicide well with respect to the basal and non-basal reflections related to LDH.**
- **In both the basal reflection (003) appear at 2 theta value of 11.5°.**
- **The corresponding basal spacing is 0.76 nm.**

Source : Samindra, S.; Kamkanam, M.; Kottegoda, N., Encapsulation of curcumin into layered double hydroxides. Nanotechnology Reviews 2014, 3 (6), 579-589.

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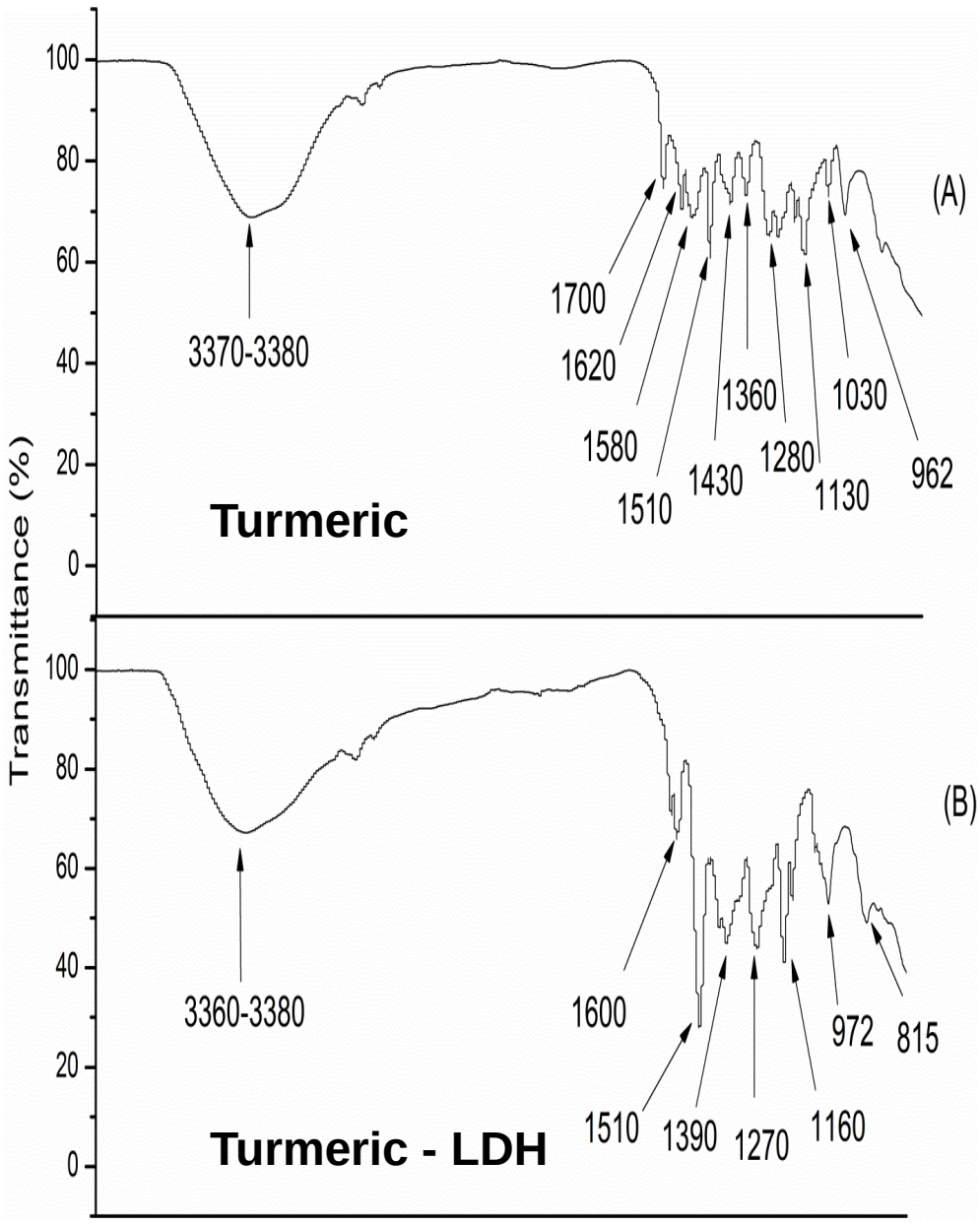
FTIR Analysis of Turmeric & Turmeric - LDH



Peak assignment	Turmeric	Turmeric - LDH
	Peak position cm^{-1}	Peak position cm^{-1}
Phenolic OH stretching vibrations	3370-3380	3360 - 3380
Carbonyl stretching	1700	1600
Mixed C=C and C=O stretching	1510 (Weak peak)	1500 (Strong sharp peak)
Aromatic C-C stretching	1430	1390
C-O stretching of methoxy groups	1280	1270

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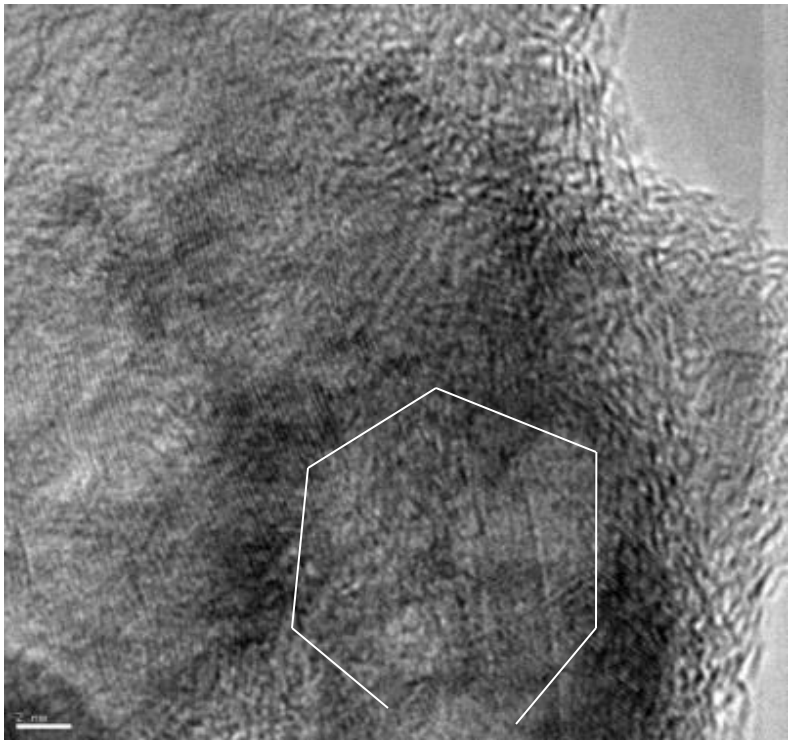
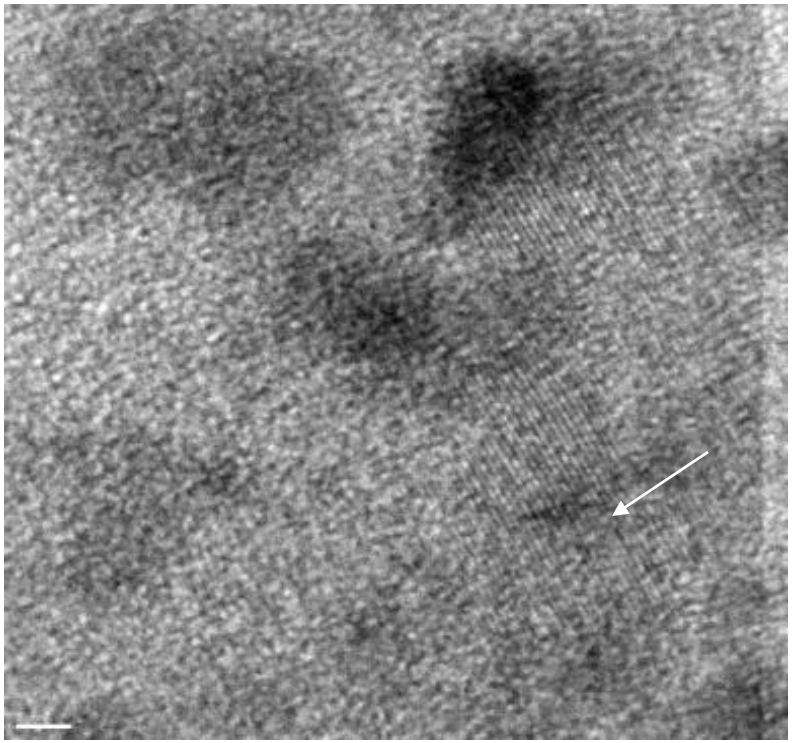
TEM Analysis of Turmeric - LDH



Resolution 2 nm

(a)
Typical hexagonal plate-like structure



Resolution 2 nm

(b)
Layered nature

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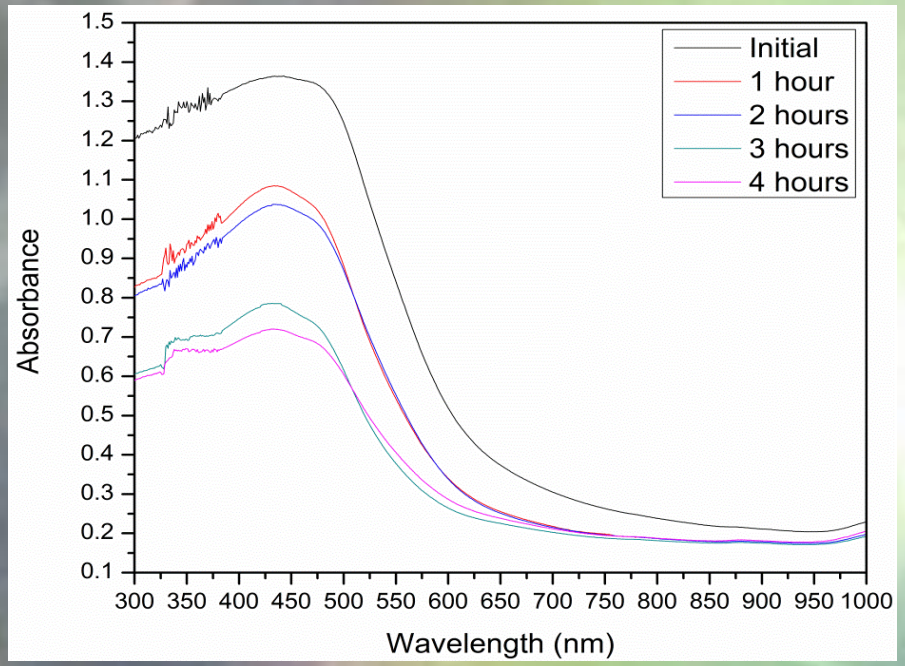
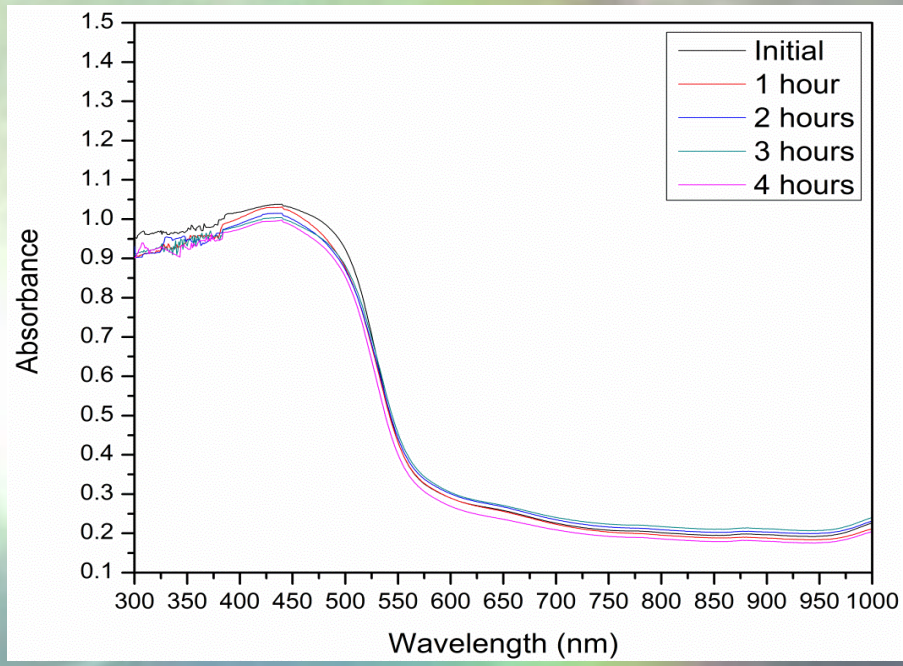
Photo stability analysis of turmeric-LDH

Solid state absorbance spectra





Turmeric	Turmeric - LDH
Max absorbance wave length (λ_{MAX}) has gradually shifted to lower wave length	Negligible decrease in the wave length (λ_{MAX}) of turmeric-LDH
The absorbance has decreased at λ_{MAX} with time of UV exposure	Negligible decrease in the absorbance at λ_{MAX} of turmeric-LDH

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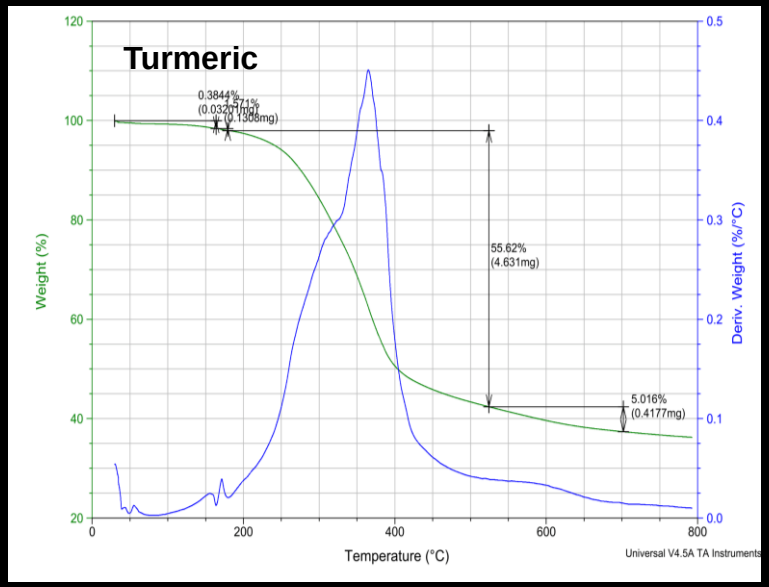
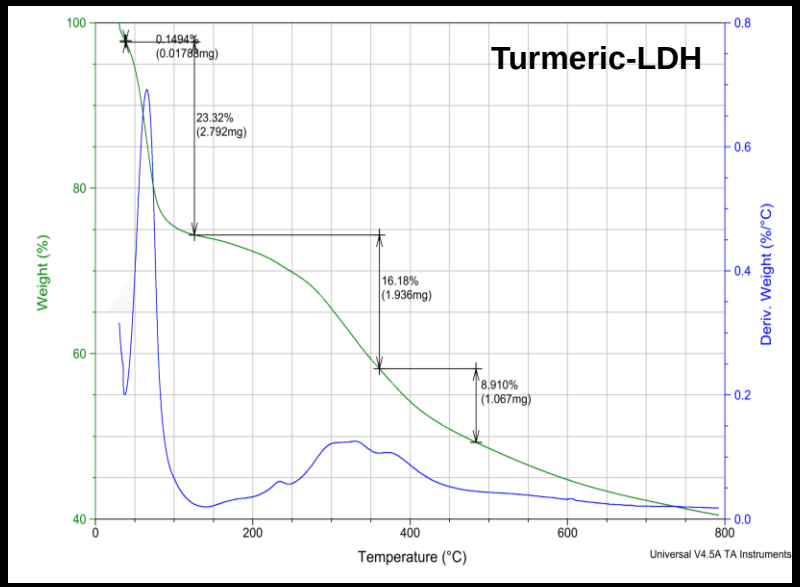
Thermal Analysis





PROCESS INVOLVED	INVOLVED TEMPERATURE/° C	
	TURMERIC	TURMERIC-LDH (48.56%)
Removal of water	174	7O and extends up to 125 (23.32%)
Complete decomposition	360 (sharp peak)	200-450 (16.18%) (broad)

- **In turmeric a clear deomposition peak is obseved at the temperature maximum at 360 ° C**
- **In tur-LDH a broad decomposition peak is observed at the range of 200 ° C**
- **This observation confirms that the intercalation of turmeric into the layered matrix has increased the thermal stability of the turmeric as it provides protection for the intercalated anions over thermal combustion.**

back to steps invoved

Percentage Release of Turmeric from Turmeric - LDH

Turmeric-LDH (2 g) was suspended in phosphate buffer solution (pH 3 & 5) and left over night

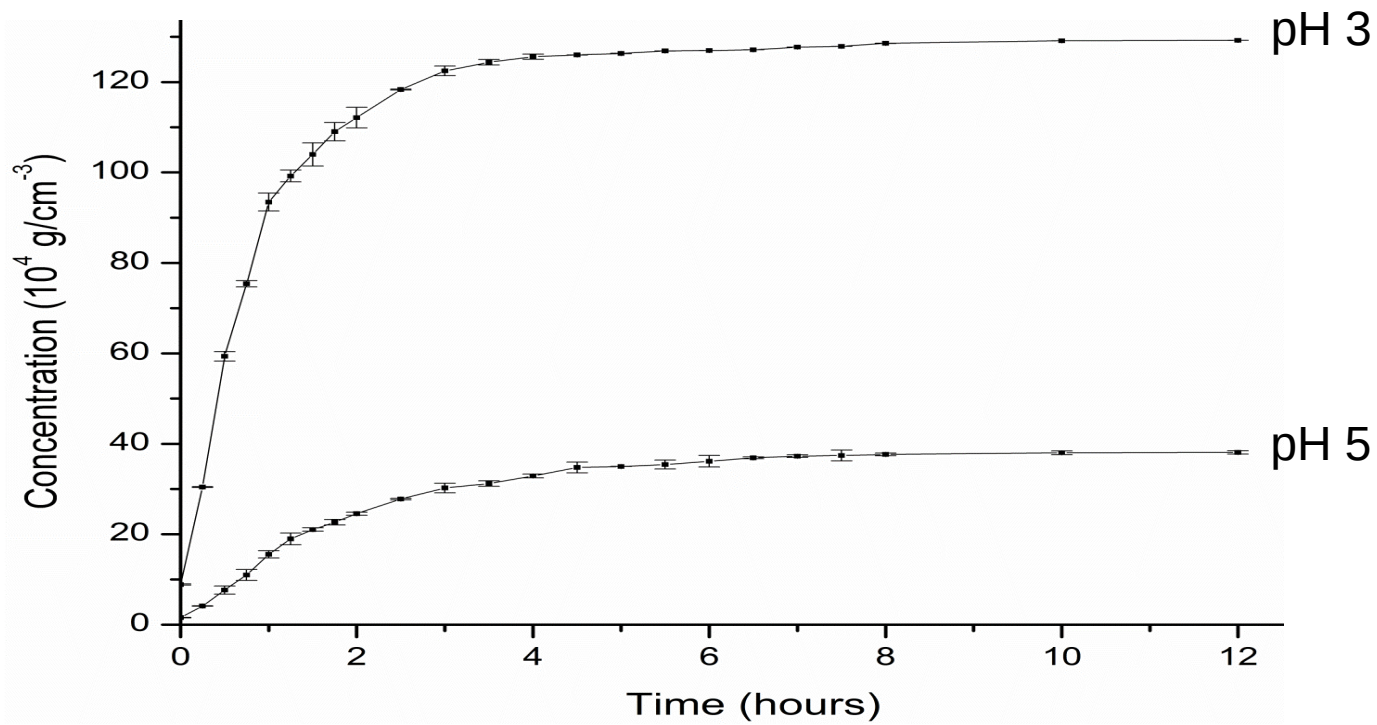
→

After 24 hrs the filtrate was measured under UV-Vis spectrometer

43 % in pH 3

12% in pH 5

Release Study of Turmeric- LDH

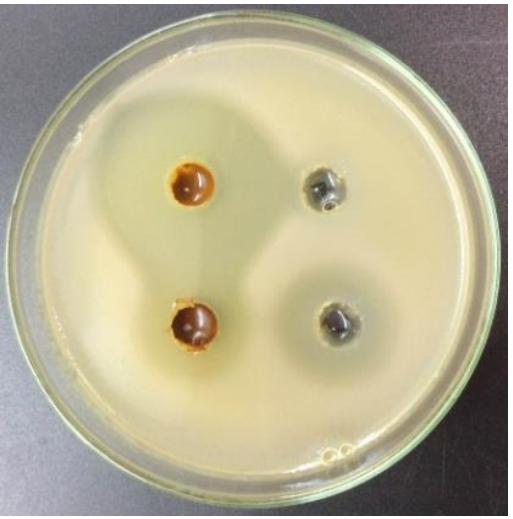


Release behavior of turmeric-LDH (a) pH 3, (b) pH 5

- The release profile shows a high initial drug release rate in the first 3 hours and reaches almost constant level afterwards over a longer period
- Meantime, no measurable release was observed for pure turmeric in aqueous medium due to its very low solubility.
- Such a release profile is characteristic of a diffusion-controlled release process.
- This confirms the slow and sustained long term release of the drug from layered matrix.

back to steps invoved

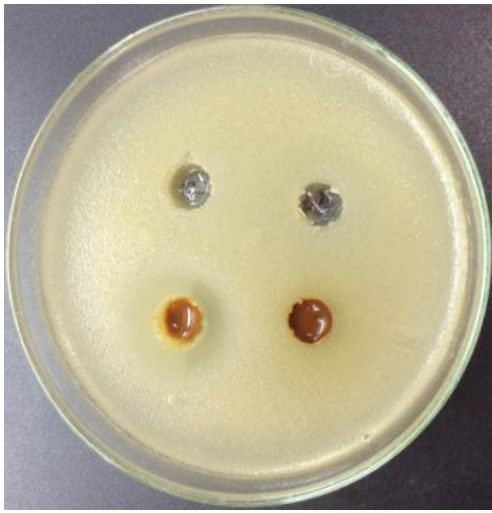
Antimicrobial properties against bacteria and fungi species



- Agar well diffusion method

- Bacterial species used ;
  - *Staphylococcus aureus* (ATCC 25923)
  - *Escherichia coli* (ATCC 25922)
  - *Pseudomonas aeruginosa* (ATCC 27853)

- Fungal species used ;
  - *Candida albicans* (ATCC 10231)
  - *Candida dubliniensis* (clinical isolate)



- **Positive Control - yeast species- Flucanozole; bacterial species- Vancomycin & Gentamicin**
- **Negative Control – acidic water (pH 3,4 & 5)**

Determination of Antimicrobial Properties of Turmeric - LDH

Substance tested	*Staphylococcus aureus* ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 25922	*Candida albicans* ATCC 10231	*Candida dubliniensis* Clinical isolate
Positive control	11 mm	11 mm	23 mm	11 mm	21 mm
Negative control	-	-	-	-	-
Pure turmeric extract	-	-	-	-	-
Turmeric-LDH (at pH 3)	17 mm	19 mm	27 mm	21.3 mm	8.3 mm
Turmeric-LDH (at pH 4)	15.3mm	13mm	7.3 mm	-	-
Turmeric-LDH (at pH 5)	8 mm	10 mm	-	-	-

The results suggest that turmeric-LDH has an improved slow release property against tested microorganism.

In summary,

-]The PXRD and FTIR data revealed the successful selective encapsulation of natural curcuminoids into the nanolayers of the LDH.
- TEM images confirmed the typical hexagonal morphology and the layering pattern of the resulting nanohybrid.
- TGA and UV exposure data propose the stabilization of the curcuminoid molecules within the nanolayers thus, making it suitable for potential practical application.
- Slow and sustained behavior of encapsulated curcuminoids was observed in acidic pH values thus proving its applicability in antimicrobial skin formulations.
- Improved and sustained activity of the novel nano hybrid has been proved antimicrobial activity against 3 bacteria species and 2 candida species.
- **The turmeric - LDH nano composites can provide a powerful route to develop new efficient drug delivery system with suspended release rate.**

Suggestions for further studies

- Evaluation of advanced therapeutic properties of curcumin-LDH nanocomposites and determining the corresponding toxic dose, lethal dose, margin of safety values under animal trails.

- Novel product development using the principle of turmeric encapsulation of layered double hydroxides.

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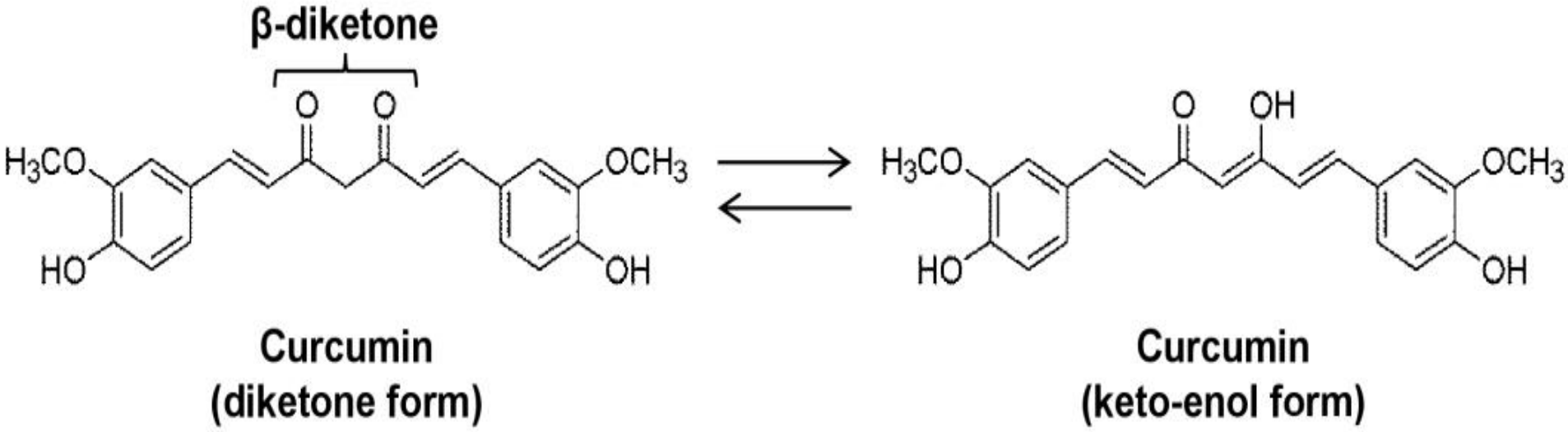
- Mr. Sudhair James
- Mr and Mrs. James
- Mr and Mrs. Megalathan

THANK YOU

Keto-enol tautomerism



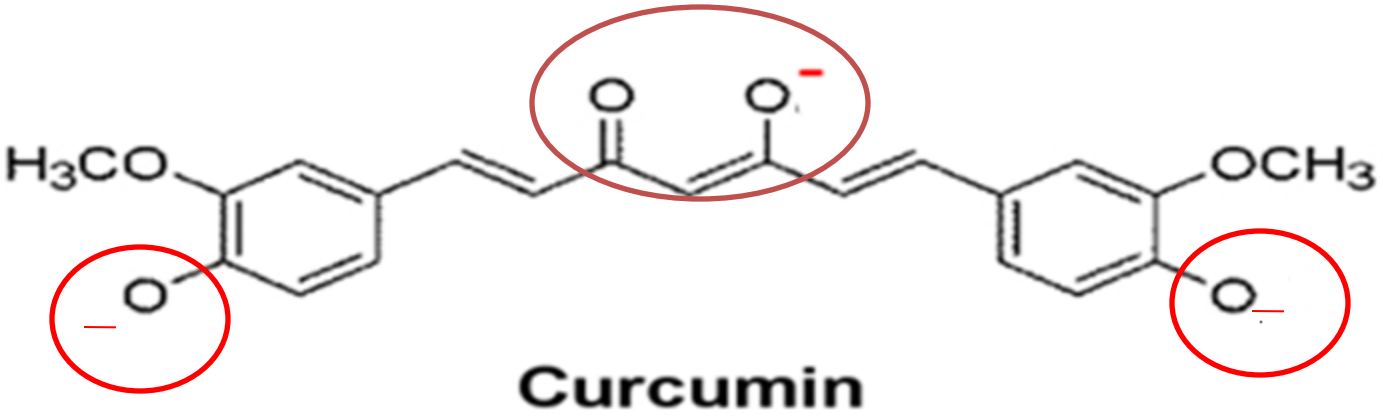
Keto form

Acidic medium

Enol form

Basic medium

Bo Yuan et al, Cytocidal Effects of Polyphenolic Compounds, Alone or in Combination with, Anticancer Drugs Against Cancer Cells: Potential Future Application of the Combinatory Therapy, Apoptosis and Medicine, 2012



Curcumin

Basic medium

↓

High affinity towards the positively charged cation layers of LDH

↓

Interlayer spacing reduces

↓

Flat molecular arrangement of curcumin within LDH

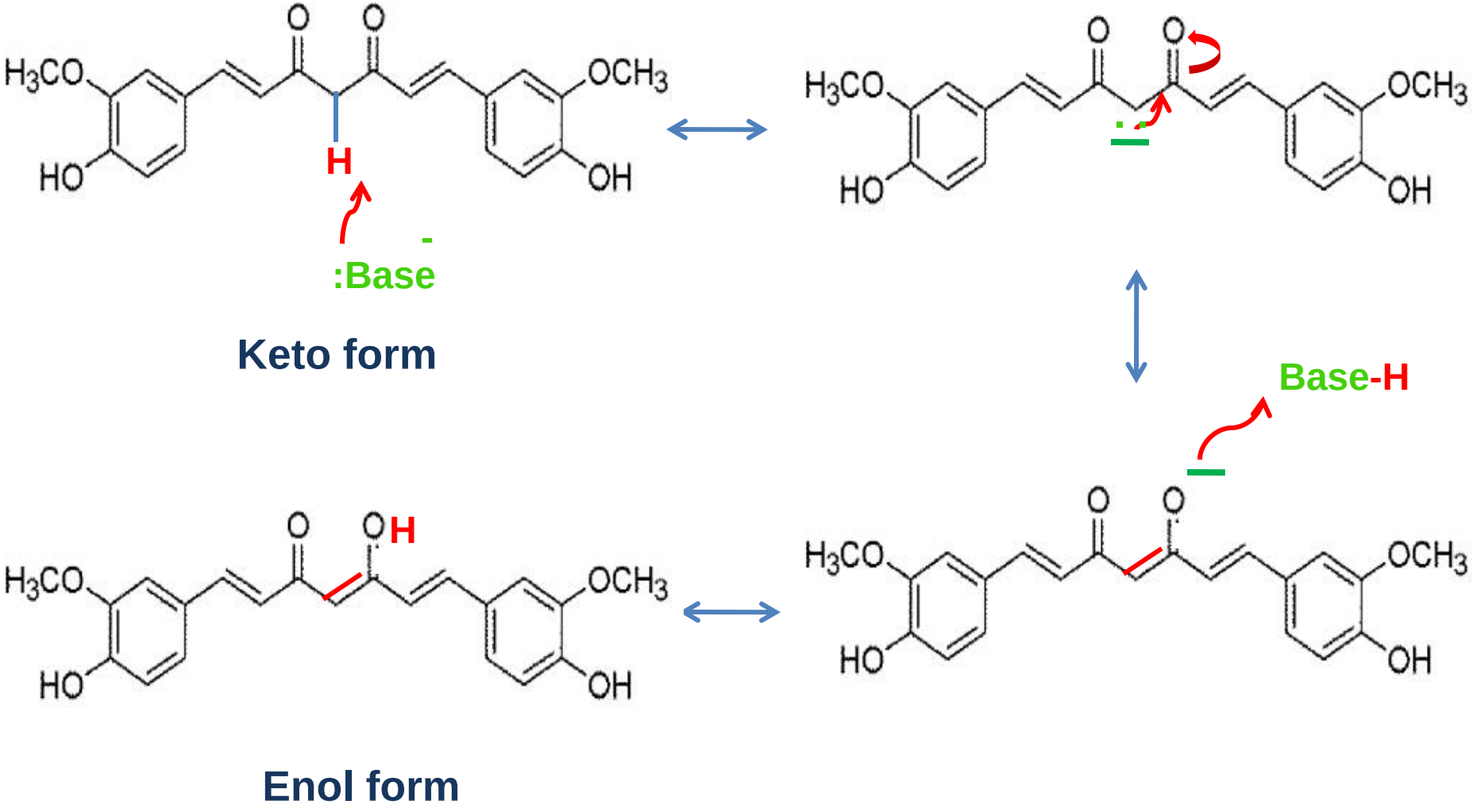
• pH 3 and pH 5 medium consists more H^+ ions than OH^- ions. Thus keto-enol tautomerisation is restricted in acidic medium.

• Hence LDH - turmeric composite is destabilized in pH 2 and pH 5.

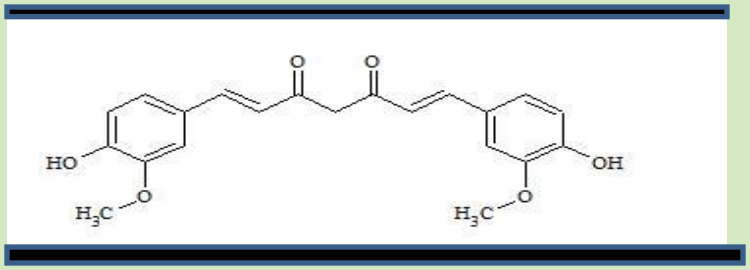
• According to the kinetic study of slow releasing properties of turmeric intercalated LDH and turmeric-LDH-cotton, zero order model was more applicable.

• Under low pH conditions it showed a significantly higher and linear release due to destabilization between LDH and turmeric

The mechanism of keto-enol tautomerism



Flat molecular arrangement



Plane of curcumin perpendicular to the cation layer

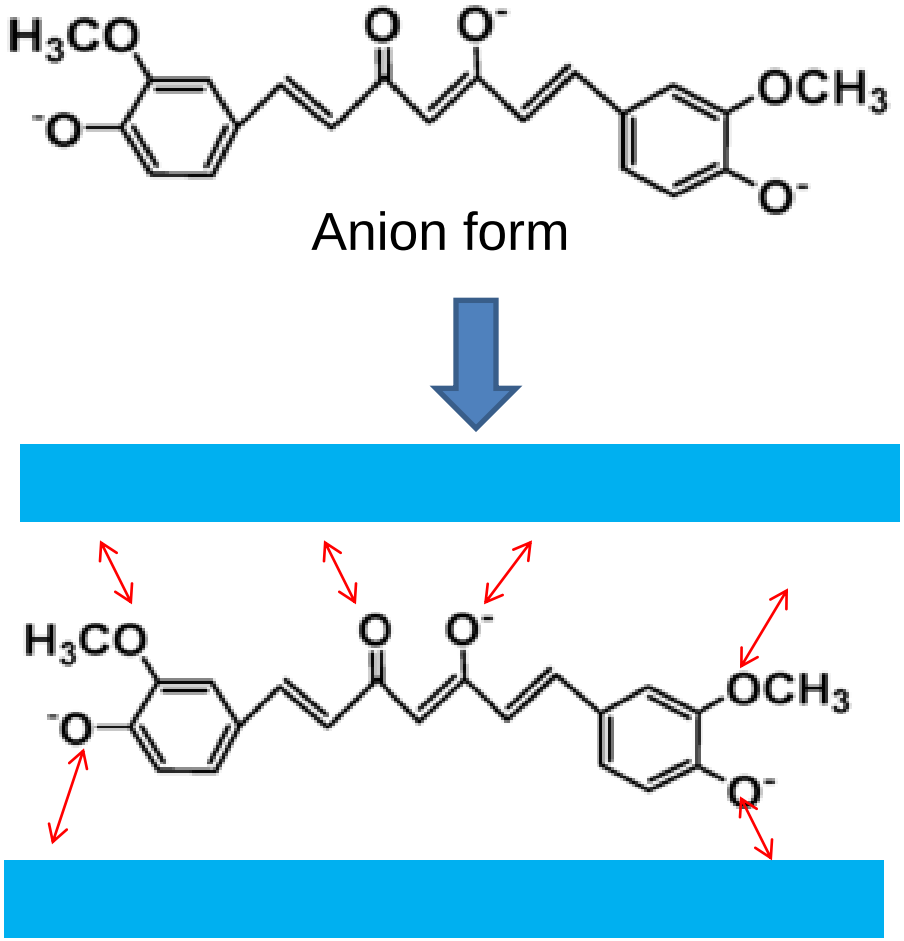
Plane of curcumin parallel to the cation layer

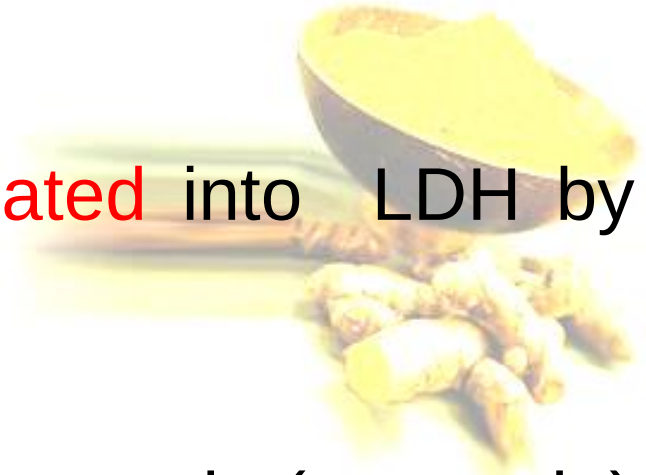
Width of curcumin is approximately 6.9 Å and compared to the basal spacing it is less feasible to insert a molecule in perpendicular arrangement.

Hence parallel conformation is the most stable configuration

Protection of Turmeric by LDH (Photo Stability)

Alkaline Conditions





- Turmeric was successfully intercalated into LDH by co-precipitation method.

- High affinity of negatively charged turmeric (curcumin) towards the positively charged cation in the LDH was shown by the reduction of basal spacing from 8.75 Å to 7.66 Å.

- At pH 2 and pH 5, it obeyed the slow releasing kinetics of zero-order model. Therefore, it can be used as a therapeutic composite.

- Zero-order model - transdermal systems …..medication applied topically (to th e skin), matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.
- First order model- describes the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices
- Higuchi model - transdermal systems and matrix tablets with water soluble drugs
- Hixsonñ Crowell model- pharmaceutical form such as tablets, where the dissolution occurs in planes that are parallel to the drug surface.

- pH of the intact skin is 4.8-6
- The wound bed pH of chronic venous leg ulcers (varicose) and pressure ulcers (coma and diabetics) was found to be alkaline or neutral when compared to intact surrounding skin.
- Protease(destroys the cell) activity is extremely pH sensitive. Protease activity peaks at between pH7 to pH8 and decreases rapidly in the presence of acidity. Below pH4, some proteases are permanently inactivated. The pH of wounds is neutral to alkaline whereas the pH of normal skin is acidic: pH5.5. When a wound is kept in an acidic condition, the fibroblasts proliferate more actively and the wound's healing process is stimulated more than when it is in a neutral or alkaline condition.

http://www.smith-nephew.com/belgique/produits-old/cadesorb-/cadesorb--simple-science/the-relationship-between-ph-and-wound-healing/

Experimentation for Kinetic Study of Turmeric - LDH Composites

Turmeric - LDH composite (5.0 g) was placed in a beaker

100.0 cm^3 Buffer solution

(slowly added along the wall of the beaker)

Measured kinetic release using UV-Visible spectroscopy

15 mins time interval for 1 hr
followed by
30 min time interval for 12 hrs

well diffusion

- One milliliter of test inoculum was inoculated on the solidified MHA (Oxoid, England) plate in order to obtain a confluent growth. Using a sterile cork-borer, 9 mm wells were cut on each MHA plate. The bottoms of the wells were sealed by adding a drop of molten agar in to wells using a sterile pipette.

- The pH of SEC-LDH composite was adjusted (pH 3, 4 and 5). Wells were loaded with 180µl of the SEC-LDH composite using a micropipette. Fluconazole, Vancomycin and Gentamicin were used as positive controls and sterile acidic solvents (pH 3, 4 and 5) were used as negative controls. Plates were kept outside for nearly 10 minutes and finally the plates were incubated aerobically at 37 0C and observed after 24 hours