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# A NOVEL BIODEGRADABLE NANO CELLULOSE-BASED DRUG DELIVERY SYSTEM (DDS) FOR pH-CONTROL DELIVERY OF CURCUMIN (TURMERIC).

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**ABSTRACT**

The main aim of this study was to targeted delivery and controlled release of drugs are attractive methods for avoiding the drug's leakage during blood circulation and burst release of the drug. We prepared a nano cellulose-based drug delivery system (DDS) for the effective delivery of curcumin (Tumeric). In the present scenario, the role of nanoparticles in fabricating the DDS is an important one and was characterized using various techniques. The drug loading capacity (DLE) was high as 50% at pH = 3.0 for acetate buffer, 48% at pH = 6.5 for phosphate buffer and also drug encapsulation efficiency (DEE) was 40% for acetate buffer and 38% for phosphate buffer.The prepared DDS was also examined for $H^1$ 1D NMR studies to assess its pharmaceutical field application and the investigation results recommended that it may serve as a potential device for targeted delivery and controlled release of Curcumin (Turmeric) for cancer treatment.

**KEYWORDS** – Nano cellulose, Targeted drug delivery, pH-responsive delivery.

## INTRODUCTION WITH LITERATURE SURVEY

In 2018, approximately 2.1 million new breast cancer cases and 0.6 million breast cancer deaths were reported. Literature enumerated that breast cancer has the highest incidence and mortality rates in women worldwide. For cancer treatment, chemotherapy is the most common method adapted. Chemotherapy cause infinite pain to patients and have some inevitable side effects. Conventional drugs were associated with severe side effects, including systemic toxicity and adverse side effects like nausea, constipation, dryness of mouth etc.

These downsides have led to tumor-targeted controlled drug delivery systems. Controlled drug delivery systems can beat many of the disadvantages faced by conventional drug delivery systems. For example, in cancer treatment, conventionally chemotherapeutic agents are used. It circulated non-specifically, destroying healthy cells and cancer cells, resulting in high toxicities and low effectiveness. Controlled DDSs would be outstanding carriers for chemotherapeutic agents because they guide the chemotherapeutic agents to the tumor site.Thereby preventing toxicity in normal cells and increasing the drug concentration in cancer cells.

Nanoparticles are a promising candidate for controlled drug delivery systems with a diameter of around 10–1000 nm. When nanoparticles are used as a DDS, they can improve the drug's efficacy by increasing drug half-life, release the drug in a controlled or sustained fashion, and improve the solubility for some hydrophobic drugs.

Among various Nano DDSs, polysaccharide-based material has gained considerable attention in the biomedical field. Polysaccharides are carbohydrate polymers, which comprise tens to hundreds to several thousand monosaccharide units. Polymer composite has unlimited usage in biomedical materials.

Among these, cellulose (Cell) is the most abundant naturally occurring polysaccharides. The properties include less expensive, low density, non-abrasiveness, combustibility, reproducibility, recyclability, non-toxicity,
biocompatibility, biodegradability, and environmental friendliness cellulose triggered significant focus on polysaccharides. It contains reactive hydroxyl groups, which makes it suitable for surface functionalization for use in different applications.

Compared to cellulose, nano cellulose materials are lighter in weight with a high surface area to volume ratio, higher strength and stiffness, renewability, biocompatibility, biodegradability, and outstanding mechanical properties that facilitate high levels of drug binding at the surface of Nano cellulose. The literature stated that Nano cellulose is an ideal material for biomedical applications. The introduction of Folic acid (FA), a water-soluble vitamin-B,is an interesting one, which binds selectively foliate receptor (FR), generally overexpressed
On the surface of a variety of human cancer cells and has a high affinity to FA binding. The utilization of FA increases the targeting efficiency of all type of DDS. FR is a well-known glycophosphatidylinositol-linked cell surface receptor, and also it is a high-affinity membrane folate-binding glycoprotein. Numerous studies have reported that the risk of certain human malignancies, including cancer, is decreased by including high folate status in dietary. Literature reported various FA-conjugated drug delivery carriers. Glycidyl methacrylate (GMA) was preferred for grafting onto FA-NC.

It incorporates carbon-carbon –Π bonds onto the macromolecules'
structure and allows it to undergo a gelling process through radical
crosslinking polymerization. It also offers the possibility of subsequent functionalization on the polymer chain. For the flexible movement of the polymer chain, GMA will provide spacer groups.

GMA grafting increases the material's chain length, which helps increase the drug's higher encapsulation from the aqueous medium onto the material and increases the biocompatibility.
Another monomer used is 2-hydrox-yethyl methacrylate (HEMA). It has a great interest due to its excellent properties such as good chemical and hydrolytic stability, excellent biocompatibility, and physicochemical properties similar to those of living tissues. This makes it an ultimate material for biomedical applications.
The drug used for anticancer activity in our work is curcumin (CUR) because it has a wide variety of properties, including analgesic, insecticidal, anti-inflammatory, larvicidal, anti-carcinogenic, anti-mutagenic, anticoagulant, antioxidant, anti-infective effects, and antiseptic activity. CUR's downsides, such as poor water solubility, low permeability, and low bioavailability, have led to Nano carriers' development for effective delivery. Encapsulation of curcumin in suitable carrier systems will decrease the drug's downsides to get the drug's full potential towards the disease. The present investigation deals with a systemic strategy for synthe-sizing functionalized cellulose-based Nanocarriers with targeted delivery and controlled release behaviors and improved biosafety features; for this purpose, nano cellulose was synthesized by acid-alkali treatment on cellulose. Then the Nano cellulose was conjugated with folic acid. The folic acid conjugated

Nano cellulose (FA-NC) was polymerized with glycidyl methacrylate (GMA) and hydroxyethyl methacrylate (HEMA) using ceric ammonium sulfate (CAS) as initiator and ethylene glycol dimethacrylate (EGDMA) as a crosslinker for the controlled delivery of curcumin (CUR).

As a result, FA-NC/GMA-HEMA/EGDMA drug delivery system was formed. The anticancer drug CUR was successfully loaded onto the carrier. From the cell viability assay, it was established that the prepared CUR-loaded DDS is a potent killer of cancer cells. The results obtained from various studies demonstrated that the prepared DDS is a promising material for the safe loading of CUR.

**Nuclear magnetic resonance spectroscopy ?**

**Nuclear magnetic resonance spectroscopy**, most commonly known as **NMR spectroscopy** is a spectroscopic technique to observe local magnetic fields around atomic nuclei. The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers. The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups. As the fields are unique or highly characteristic to individual compounds, in modern organic chemistry practice, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.

The principle of NMR usually involves three sequential steps:

1. The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field $B_0$.
2. The perturbation of this alignment of the nuclear spins by a weak oscillating magnetic field, usually referred to as a radio-frequency (RF) pulse.
3. Detection and analysis of the electromagnetic waves emitted by the nuclei of the sample as a result of this perturbation.

Similarly, biochemists use NMR to identify proteins and other complex molecules. Besides identification, NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The most common types of NMR are proton and carbon-13 NMR spectroscopy, but it is applicable to any kind of sample that contains nuclei possessing spin.
NMR spectra are unique, well-resolved, analytically tractable and often highly predictable for small molecules.

Different functional groups are obviously distinguishable, and identical functional groups with differing neighboring substituents still give distinguishable signals. NMR has largely replaced traditional wet chemistry tests such as color reagents or typical chromatography for identification. A disadvantage is that a relatively large amount, 2–50 mg, of a purified substance is required, although it may be recovered through a workup. Preferably, the sample should be dissolved in a solvent, because NMR analysis of solids requires a dedicated magic angle spinning machine and may not give equally well-resolved spectra. The timescale of NMR is relatively long, and thus it is not suitable for observing fast phenomena, producing only an averaged spectrum. Although large amounts of impurities do show on an NMR spectrum, better methods exist for detecting impurities, as NMR is inherently not very sensitive - though at higher frequencies, sensitivity is higher.

# MATERIALS

## I. Chemicals

All the chemicals and reagents were bought from sigma Aldrich. the chemicals used were Glycidylmethacrylate, Hydroxylethyl methacrylate , Ethyleneglycol dimethacrylate , Ceric ammonium sulfate , Folic acid , Ethylene glycol , (3-(3-dimethylaminepropyl) -N ethyl carbodimide hydrochloride , (N-hydroxy succinimide) . All the solvents were obtained from Merck, Mumbai.

## II. Instrumentation

The morphology of the DDS were determined by nuclear magnetic resonance spectroscopy (NMR).

## III. Preparation of Solution

### A. Phosphate buffer –

1. Prepare 800 mL of distilled water in a suitable container.
2. Add 20.214 g of Sodium Phosphate Dibasic Heptahydrate to the solution.
3. Add 3.394 g of Sodium Phosphate Monobasic Monohydrate to the solution.
4. Adjust solution to final desired pH using HCl or NaOH.
5. Add distilled water until the volume is 1 L.

### B. Acetate buffer –

1. Prepare 800 mL of distilled water in a suitable container.
2. Add 5.772 g of Sodium Acetate to the solution.
3. Add 1.778 g of Acetic Acid to the solution.
4. Adjust solution to desired pH using 10N HCl (typically pH ≈ 5.0).
5. Add distilled water until the volume is 1 L

## METHODS

- Sugarcane bagasse was the starting materials for the drug delivery system (DDS).

**Synthesis of nanocellulose (NC) :-**

**Isolation of cellulose –**

Sugarcane bagasse was dried in sunlight and then cut into small pieces.The cut bagasse was milled to become powder. The powder of bagasse was bleached with 250 ml of 0.735% (w/v) sodium hypochlorite for 6 hours with constant stirring at 45 °C to remove the lignin.The residue was washed with distilled water until a neutral pH. The neutral residue was refluxed with 150 ml of 17.5% sodium hydroxide for 3 hours with constant stirring at 45 °C to remove hemicellulose.The residue of this process was also washed until reach a neutral pH, and it was dried at room temperature for 2-3 days.

**Preparation of nanocellulose –**

Dried cellulose (5.0 g) was added to 250 mL distilled water and 140 mL conc.H2SO4 was dropped to it without cause heating. After complete addition, the mixture was heated at 50 ◦C for 2 h. The hot reaction mixture was quenched using crushed ice, and the obtained Nano cellulose (NC) was centrifuged.

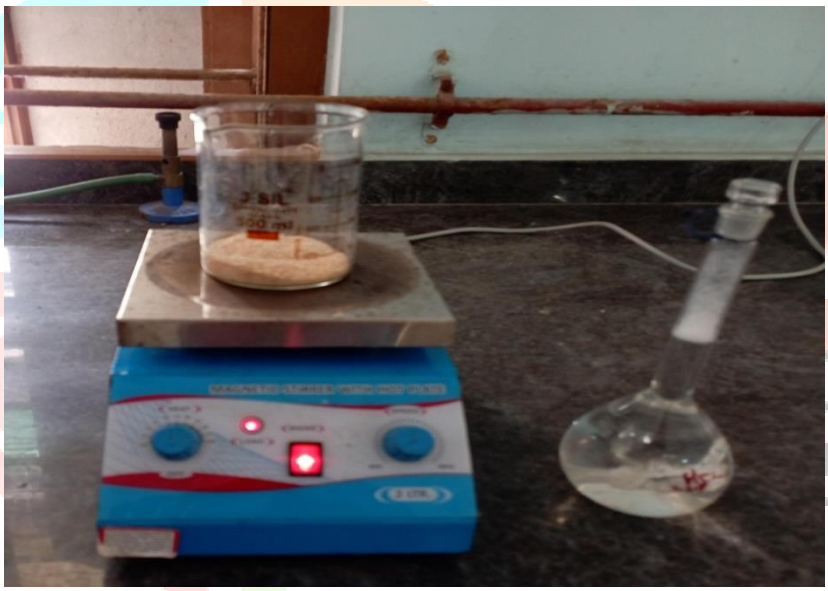

**Figure 1 : Setup for Isolation of cellulose**

**Figure 2 : Hemicellulose**

**Figure 3 : Washed Hemicellulose**

**Figure 4 : Residue was washed until reach a neutral pH**

**Figure 5 : Residue was dried at room temperature for 2-3 days**

**Figure 6 : In preparation of nanocellulose – the mixture was heated at 50 ◦C for 2 h**

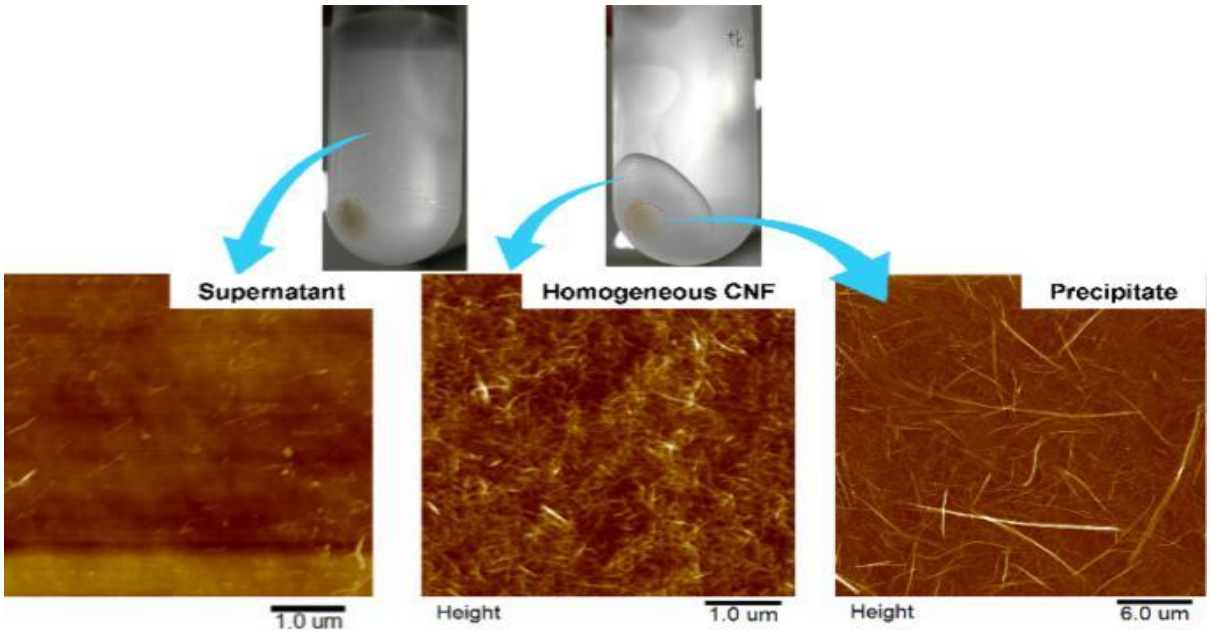


**Figure 7 : The obtained Nano cellulose (NC) was centrifuged**

**Periodate oxidation of NC -**

About 4.0 g of dry NC, 5.33 g NaIO4, and 15.6 g NaCl were added into 266 mL deionized water. The reaction was accomplished at room temperature and stirred at a speed of 105 rpm for 42 h. The reaction vessel was concealed with aluminum foil to prevent the photo-induced decomposition of the periodate. The reaction was controlled by adding 1 mL of 4.0% ethylene glycol per gram of NC to quench the residual properties.

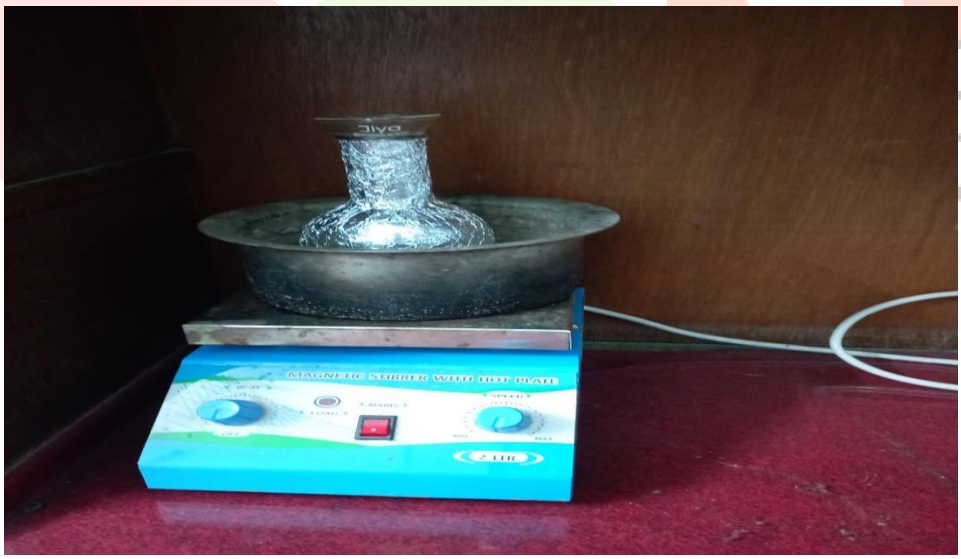

**Figure 8 : Periodate oxidation of NC**

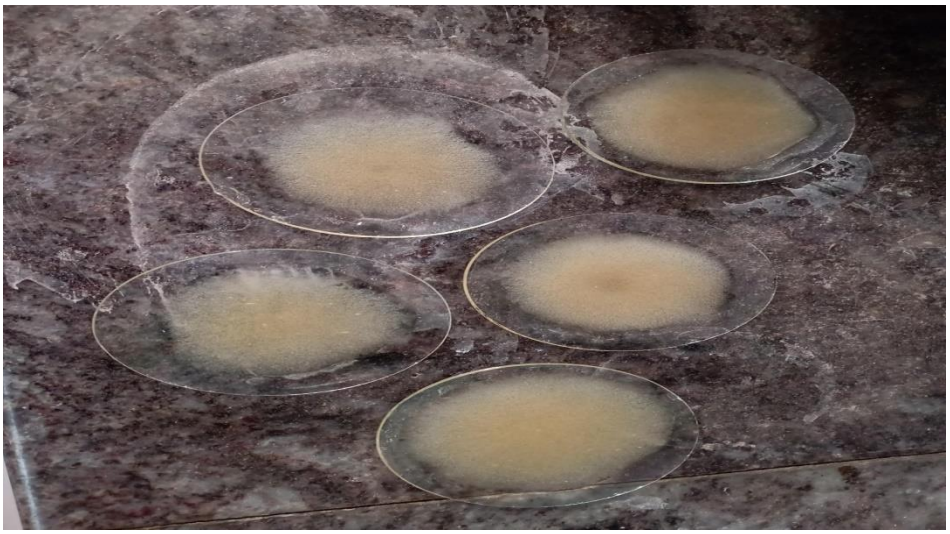

**Figure 9 : Periodate oxidation of NC – obtained Product was kept for drying**

**Synthesis of folic acid conjugated NC –**

The FA (1 mg/mL) can be conjugated with NC by EDC-NHS coupling. Take FA (1 mg/mL), 0.78 mg of N-(3-dimethyl aminopropyl)-N-ethyl-carbodiimidehydrochloride (EDC) and 0.98 mg of N-hydroxysuccinimide (NHS). At room temperature, the mixture was allowed to stir for 2 h. Twenty-five milliliters of NC (5 mg/mL) were added drop-wise to the above mixture and stirred overnight.

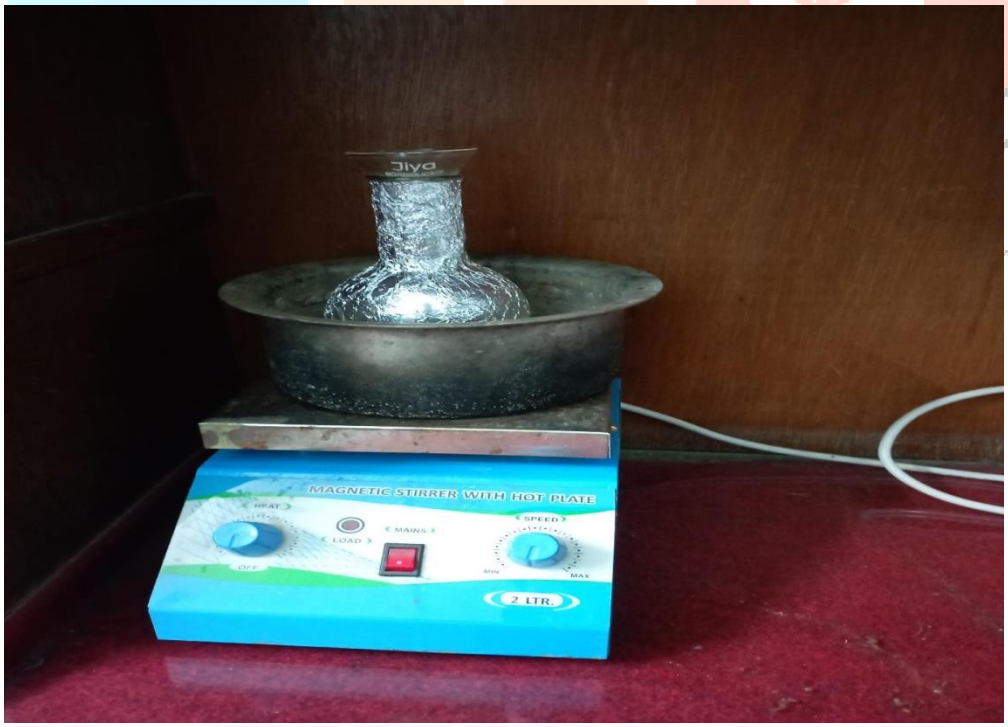


**Figure 10 : Synthesis of folic acid conjugated NC – Stirred overnight**

**Figure 11 : Obtained Product was kept for drying**

**Synthesis of DDS –**

Exactly 0.50 g of GMA and 0.50 g of FA-NC were dispersed in 50 mL methanol in a RB flask fitted with a magnetic stirrer and reflux condenser. Then 0.02 g of ceric ammonium sulfate was added. Then add 0.50 g of HEMA and 50 wt% EGDMA into the flask. The vessel was heated to about 60–70 ◦C for 3 h. The polymeric material was separated by centrifugation after cooling to room temperature, washed with water to remove unreacted products, and dried in an air oven at 50 ◦C to obtain the desired DDS.

**Figure 12 : Synthesis of DDS**

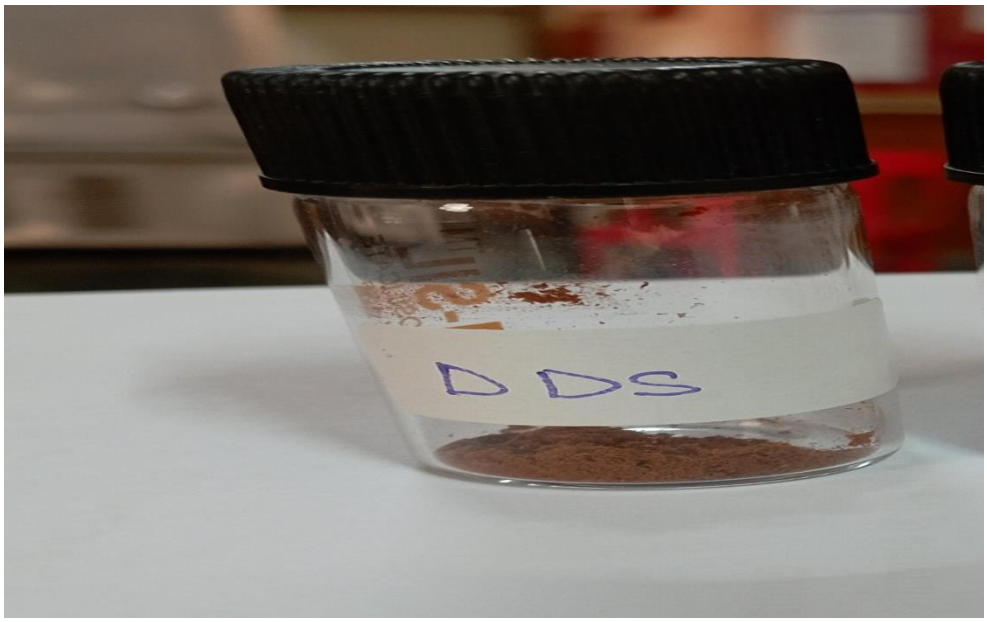


**Figure 13 : Dried and Obtained DDS**

**Drug loading efficiency (DLE) and drug encapsulation efficiency (DEE) -**

A stock solution of Curcumin (Turmeric) in DMSO with a concentration of 2.7 mm was prepared. 0.1 g of FA-NC/GMA-HEMA/EGDMA was added to 50.0 mL of Curcumin (Turmeric) solution of known concentration (75.0 mg/L) and stirred for 24 h to Obtain maximum drug loading. The drug loading efficiency (DLE) and drug encapsulation efficiency (DEE) of CUR in FA-NC/GMA-HEMA/ EGDMA have been measured with a UV- visible spectrophotometer λ max = 432 nm. The material's loading efficiency was calculated at different pHs, containing 50 mL acetate buffer (pH 2.0–5.5) and phosphate buffer (pH 6.0–8.0) for 24 h at 30 ◦C in different stoppered bottles and stirred. The solution was centrifuged, and the supernatant solution was collected. After finishing the reaction, to remove the unreacted CUR, the suspension was centrifuged at 8000 rpm for 30 min. The resulting drug delivery system was dried under a vacuum. The values of DEE and DLE of CUR in FA-NC/GMA-HEMA/EGDMA were calculated using the following Eqs. (1) & (2).

**For Acetate buffer at pH 3.0 , Temp. 28°C , UV at 432 nm = 0.801**

**DEE (%)** = The total amount of CUR − Free CUR / The total amount of CUR × 100 ……. (1)

= 0.1 – 0.050 ÷ 0.1 × 100

= 40 %

**DLE (%)** = Amount of loaded drug / Amount of polymer × 100 ……. (2)

= 0.050 ÷ 0.1 × 100

= 50 %

**For Phosphate buffer at pH 6.5 , Temp. 29°C , UV at 432 nm = 0.889**

**DEE (%)** = The total amount of CUR − Free CUR / The total amount of CUR × 100 ……. (1)

= 0.1 – 0.048 ÷ 0.1 × 100

= 38 %

**DLE (%)** = Amount of loaded drug / Amount of polymer × 100 ……. (2)

= 0.048 ÷ 0.1 × 100

= 48 %

**Figure 14 : Drug loading efficiency (DLE) and drug encapsulation efficiency (DEE)**

**RESULT AND DISCUSSION –**

The synthesis of the drug-loaded drug carrier and synthesis of drug carriers involves the modification of cellulose.

First, cellulose is converted into Nano cellulose by acid-alkali treatment. Periodate oxidation of Nano cellulose leads to the formation of dialdehyde Nano cellulose. It is then coupled with folic acid to get the folic acid conjugated NC to achieve the DDS targeting ability. GMA and HEMA were introduced to the FA-NC polymer chain by radical polymerization using EGDMA as a crosslinker. Curcumin (Turmeric) gets loaded into the drug carrier through hydrogen bonding interaction. FA-NC/GMA-HEMA-EGDMA expedites the controlled and maximum release of CUR at pH 3.0 and pH 6.5. The modification of FA-NC with GMA, HEMA, and EGDMA makes the material more biocompatible and ensures maximum drug encapsulation efficiency.

## CONCLUSION

In summary, this work illustrated a novel drug carrier's synthesis using the polysaccharide – cellulose by polymerization technique for the controlled and targeted delivery of curcumin (Turmeric). The long-time sustained release of the drug, pH-responsive release behavior, and too high drug- loading capacity demonstrating its potential for utilization as a novel drug carrier. We used instrumentation techniques such as NMR studies for the material's complete characterization. The increased encapsulation and loading efficiencies of CUR are attributed to the hydrogen bonding interactions between the polymeric carrier and CUR.These results point out that the FA-NC/ GMA-HEMA/EGDMA is a promising drug carrier for targeted delivery and controlled release of anticancer drugs.

## FURTHER WORK

Further work can be done such as In vitro drug release study – To determine the drug release profile of the DDS, the CUR-loaded DDS were treated with both acidic tumor pH (5.0), at 1.2 and simulated intestinal fluid (SIF) pH (7.4) and Cell viability assay – to determine anticancer activity of free CUR, FA-NC/GMA-HEMA/EGDMA, and CUR-FA-NC/GMA-HEMA/EGDMA was assessed in MDA MB-231 cell by MTT (3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay in MDA MB-231 (Human Breast Adenocarcinoma) cells.

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**Biography**

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