Dean R&D

Project completion report & UC are attached

Tarun Maji

(DoRD/Chem.Sc/TKM/20-94)

FORM – F

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
Human Resource Development Group
(Extra Mural Research Division)
CSIR Complex
Library Avenue, Pusa, New Delhi 110012

PROFORMA FOR PREPARING FINAL TECHNICAL REPORT

(Ten copies of the report must be submitted immediately after completion of the research scheme)

1. Title of the scheme

DEVELOPMENT OF NATURAL POLYMERIC SYSTEM FOR CONTROLLED DELIVERY OF ANTICANCER AGENTS	Scheme No.: 01(2502)/11/EMR-II Date of Commencement : 01/08/2011 Date of termination : 31/01/2015

2. Name and address of Emeritus Scientist

Prof. Tarun K. Maji Department of Chemical Sciences Tezpur University Napaam -784028

3. Name of sponsoring laboratory of CSIR (if applicable): **Not applicable**
4. Total grant sanctioned and expenditure during the entire tenure

	Amount Sanctioned	Expenditure
Staff	461097/-	461097/-
Contingency	350000/-	350000/-
Equipment	-	
Total	811097/-	811097/-

5. Equipment(s) purchased out of CSIR grant

Name	Cost
NA	NA

6. Research fellows associated with scheme

Name & Designation	Date of Joining	Date of Leaving
Nibedita Banik	03-05-2012	31-01-2015

S. Mahanty
06/10/16

7. Name(s) of the fellow(s) who received Ph.D. by working in the scheme, along with the title(s) of thesis:

Name of the fellow: **Nibedita Banik**

Thesis title: **"Development of Biopolymeric Nanoparticles for Controlled Drug Delivery"**

8. List of research papers published/communicated, based on the research work done under the scheme (Name(s) of author(s), Title, Journal, Volume number, Year and Pages should be given for each paper published and a copy of each of them should be enclosed; reprints/copies of papers appearing after submission of FTR should also be sent to CSIR):

1. N. Banik, M. Iman, A. Hussain, A. Ramteke, R. Boruah, T.K. Maji. Soy flour nanoparticles for controlled drug delivery: effect of crosslinker and montmorillonite (MMT). *New J. Chem.*, **37**, 3981-- 3990, 2013.
2. N. Banik, A. Hussain, A. Ramteke, H.K. Sharma, T.K. Maji. Preparation and evaluation of the effect of particle size on the properties of chitosan montmorillonite nanoparticles loaded with isoniazid. *RSC Advances* **2**, 10519--10528, 2012.
3. N. Banik, A. Hussain, A. Ramteke, T.K. Maji. Carboxymethyl chitosan-Montmorillonite Nanoparticles for Controlled Delivery of Isoniazid: Evaluation of the Effect of the Glutaraldehyde and Montmorillonite. *Polym. Advan. Technol.* DOI: 10.1002/pat.3406, 2013.
4. N. Banik, A. Hussain, A. Ramteke, T.K. Maji. Synthesis and Evaluation of the Properties of Chitosan-Cellulose whisker Microparticles for Controlled Release of Isoniazid. *ACS Sustainable Chem. Eng.* **(Under review).**
5. N. Banik, A. Hussain, A. Ramteke, T.K. Maji. Phosphorylated chitosan-Montmorillonite Nanoparticles for Controlled Delivery of Isoniazid: Evaluation of the Effect of the Glutaraldehyde and Montmorillonite, **(Communicated).**
6. N. Banik, A. Hussain, A. Ramteke, T.K. Maji. Preparation and evaluation of the effect of chitosan/montmorillonite nanoparticles for the controlled delivery of hydrophobic drug, Curcumin. **(Communicated).**

7. N. Banik, A. Hussain, A. Ramteke, T.K. Maji. A systematic study of carboxymethychitosan/montmorillonite nanoparticles for controlled delivery of Curcumin. **(Communicated).**
8. N. Banik, A. Hussain, A. Ramteke, T.K. Maji. Study of phosphorylated chitosan/ MMT nanoparticles as an anticancer agent and for the controlled delivery of Curcumin. **(Communicated).**

9. Details of new apparatus or equipment designed or constructed during the investigation: **Not applicable**

10. The likely impact of the completed work on the scientific/technological potential in the country (this may be attached as Enclosure-I): **Attached as Enclosure –I**

11. Is the research work done of some industrial or agricultural importance and whether patent(s) should be taken? Yes/No; if yes, what action has been/should be taken : **NA**

12. How has the research work complemented the work of CSIR Laboratory that sponsored your scheme: **NA**

13. Detailed account of the work carried out in terms of the objective(s) of the project and how far they have been achieved; results and discussion should be presented in the manner of a scientific paper/project report in about 5000 words; and this should be submitted as enclosure-II to this report : **Attached as Enclosure -II**

14. An abstract of research achievements in about 200-500 words, suitable for publication.

In the present scenario of pharmaceutical research, nanoparticles have evoked much interest for the delivery of drugs, peptides, proteins and genes due to their ability to protect these from degradation in the gastrointestinal region by proteolytic enzymes. Besides this, polymeric nanoparticles can control the release of a drug over an extended period of time either by forming matrices, membranes or by forming (nano) carriers, thereby avoiding repetitive dosing. Chitosan, a modified natural biodegradable polymer obtained from partial deacetylation of the biopolymer chitin, which is present in the shells of crustacean, such as crabs and lobsters gained impetus recently in drug delivery system due to their low toxicity, easy availability, mucoadhesivity and membrane permeation enhancing properties. Different water soluble derivatives of chitosan have been prepared.

The efficacy of these chitosan derivatives for controlled release applications have been tested using isoniazid as model drug. The prepared derivatives have been found to give satisfactory results. Encouraging from the study, efforts have been made to prepare curcumin, an anticancer drug, loaded chitosan-montmorillonite (MMT) nanoparticles to study the control release profile. Curcumin loaded chitosan-MMT, Carboxymethyl chitosan- MMT, Phosphorylated chitosan -MMT nanoparticles have been developed by ionic gelation method followed by chemical crosslinking. The nanoparticles have been characterized by fourier transform infra-red spectroscopy (FTIR), dynamic light scattering (DLS), X-ray diffractometry (XRD), scanning electron microscopy (SEM) and transmission emission microscopy (TEM). Drug loading and encapsulation efficiency, swelling property and the release of curcumin in different medium have been assessed. Finally, cytotoxicity, antiproliferative and apoptosis were studied using isolated human lymphocytes cultured *in vitro*.

The results demonstrated the successful preparation of curcumin loaded Chitosan-MMT nanoparticles with improved loading and encapsulation efficacy. The nanoparticles showed better swelling and drug release at pH 1.2. FTIR study indicated the interaction of the Chitosan polymer with MMT. The exfoliation of MMT layers was examined by XRD and TEM. SEM showed that the surface of the curcumin loaded chitosan-MMT, carboxymethyl chitosan- MMT, phosphorylated chitosan -MMT nanoparticles were less smooth compared to those of chitosan nanoparticles. Varying results of MTT, antiproliferative assay and apoptotic index clearly provided insight of control release of curcumin from the nanoparticles

The results imply that the curcumin loaded chitosan-MMT, carboxymethyl chitosan- MMT, phosphorylated chitosan -MMT nanoparticles can be exploited as a potential drug carrier for controlled release applications.

15. Mention here whether or not the unspent grant has been refunded to CSIR: **NA**

Date: 26/5/15

Signature of PI

Note: Final Technical Report is expected to be self-contained complete report of the work done. Please do not leave any column unanswered.

Enclosure-I

Impact of the completed work on the scientific/technological potential in the country

The research work takes a deep insight of the prospect of chitosan, its water soluble derivatives and soy flour in the field of drug delivery. The role of montmorillonite as reinforcing agent and genipin as crosslinker have been evaluated. Curcumin has been used as a model drug. Curcumin loaded nanoparticles were found to induce cell growth in normal cells. Moreover, the nanoparticles were highly cytotoxic to the cancer cell lines MCF-7 and HepG2. It is found that the developed nanoparticles can be effectively used for drug delivery. The study exhibits a remarkable improvement in cell viability of the normal lymphocytes as well as killing of cancer cells. Further studies in terms of repeatability and use of different cell lines are needed in order to authenticate the findings. Trials of the effect of the nanoparticles on animal model are needed for better understanding the anticancer activities and commercialization of the products.

All the material like polymer, filler, crosslinker and drug used in this work are from natural origin. Thus the whole system is ecofriendly, biodegradable and biocompatible with no toxicity to living organisms.

Enclosure II

(Final Progress report)

In this report, the following systems were studied for controlled drug delivery applications.

- Isoniazid loaded chitosan-MMT nanoparticles
- Isoniazid loaded soy flour-MMT nanoparticles
- Curcumin loaded Chitosan-MMT nanoparticles
- Curcumin loaded Carboxymethyl chitosan- MMT nanoparticles
- Curcumin loaded Phosphorylated chitosan-MMT nanoparticles

14.1. In this system, isoniazid was used as no other drug was available at that time for the purpose of our study. Chitosan-MMT-Isoniazid nanoparticles crosslinked with glutaraldehyde were characterized by FTIR, XRD, SEM and TEM. The swelling study and cumulative release study exihibited better results in gastric pH compared to intestinal pH. Moreover, both swelling and cumulative release decreased with the increase in MMT content. Cytotoxicity study showed that chitosan and MMT were not cytotoxic. But, isoniazid was cytotoxic and the cytotoxicity decreased on formation of the nanoparticles. Mucoadhesion test showed that chitosan had mucoadhesive properties. The mucoadhesion was more in gastric pH than in intestinal pH.

14.2. The system studied was Soy flour-MMT-Isoniazid nanoparticles crosslinked with glutaraldehyde for controlled drug delivery applications. We wanted to check whether our system work for other polymers or not. Soy flour-MMT-Isoniazid nanoparticles showed satisfactory results. These nanoparticles were also characterized by FTIR, XRD, SEM and TEM. The swelling and cumulative release study were found to be more in intestinal pH than in gastric pH. Cytotoxicity study showed that the nanoparticles were not cytotoxic.

14.3. The system studied was Chitosan-MMT-Curcumin nanoparticles crosslinked with genipin. Genipin is a natural crosslinker. Curcumin is an anti cancer drug. These nanoparticles were also characterized by FTIR, XRD, SEM and TEM. The swelling study and cumulative release were found to be more in gastric pH than in intestinal pH. The nanoparticles were not cytotoxic as revealed by cytotoxicity study.

14.4. Curcumin loaded Carboxymethyl chitosan- MMT nanoparticles: Chitosan is widely used as polymer. But, its application is limited by its low solubility in neutral pH. To solve this problem, two water soluble derivatives of chitosan namely carboxymethyl chitosan and phosphorylated chitosan were prepared.

14.4.1. Synthesis of N-carboxymethyl chitosan (NCMC): The N-carboxymethyl chitosan derivatives were prepared by using standard procedure with little bit modification. Chitosan (1g) was swollen in 100 mL of water for 24 h, then monochloro acetic acid (4g) was added and mixture was stirred vigorously until all chitosan was dissolved in water to give a homogenous solution .Then the pH was adjusted to 8.0 by slowly adding 5% $NaHCO_3$ under vigorous stirring. The reaction medium becomes opaque due to precipitation of chitosan as the pH was raising but reverted to homogenous solution on heating at 90^0C.After heating at 90^0C for 6 h ,the solution was filtered and cooled to ambient temperature and pH was adjusted to 6.0 by using 1% (w/v) HCl solution. The precipitate was filtered off and washed with 90% ethanol solution. It was then dissolved in dilute sodium hydroxide solution to give the neutral Na-salt of N-carboxymethyl chitosan, which was separated by lyophillization.

14.4.2. Preparation of Curcumin loaded NCMC-clay nanoparticles: For preparation of curcumin loaded N-CMC –Clay microspheres ,CMC solution was made in distilled water(at 45^0C) where clay(MMT)(0%,1%,3%, 5%,w/w of the polymer) was dispersed by constant stirring for 24 h followed by sonication ,then desired concentration of drug was added to the medium. A little amount (0.2ml) of surfactant, Tween-80, was added for better dispersion. Precipitation was carried out by adding 1 wt% of $CaCl_2$ solution which was then crosslinked by using genipin. The precipitate was separated by centrifuge and then freeze dried.

14.4.3. Preparation of NCMC microparticles with varying the amount of clay (MMT): Temp: 45^0C; time: 24h; water: 100ml; Tween 80:0.2ml; genipin: 1ml of 0.01 w/v in 100 ml water,$CaCl_2$:1wt%.

Table 1:

Sample No:	CMC(gm)	MMT (gm)	Drug(gm)	Yield of nanoparticle (%)	Encapsulation efficiency (%)
A0	0.1	0.00	0.002	93.89	74.92
A1	0.1	0.001	0.002	93.23	74.02
A2	0.1	0.002	0.002	93.38	73.62
A3	0.1	0.003	0.002	92.99	73.79
A4	0.1	0.004	0.002	92.62	73.48

14.4.4. FTIR Study: Chitosan (CS) vs CMC:

Fig.1 presents the FT-IR spectra of CS, CMC. The absorption bands at 1654, 1593, 1323, 1381 cm^{-1} in the spectrum of CS assigned to amides I, II, III and $-CH_3$ vibration bends were appeared. Two strong peaks at 1603 and 1455.cm^{-1} (in CMC spectrum) were observed due to the asymmetrical and symmetrical stretching of COO– group. In the spectrum of CMC, the C–O stretching band at 1158 cm^{-1} corresponding to the primary hydroxyl group disappeared, verifying a high carboxymethylation of OH. The characteristic peak of second hydroxyl group at 1083 cm^{-1} was not changed. Thus CMC is successfully prepared from chitosan.

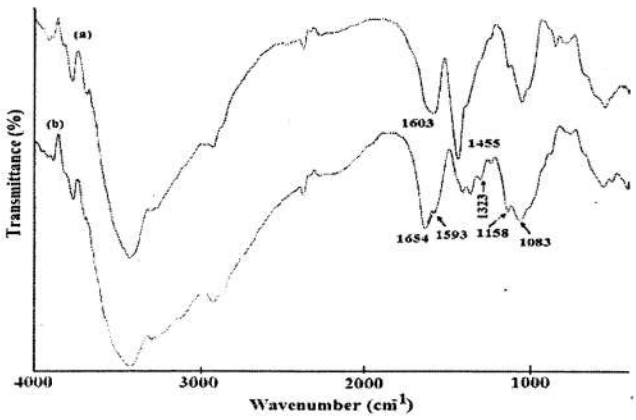

Figure 1: FTIR curves: (a) chitosan, (b) CMC

14.4.5 FTIR Study of CMC, Clay, Drug and nanonoparticles:

FTIR spectra of CMC (curve-a), MMT (curve-b), Curcumin (curve-c) and CMC-MMT nanoparticle loaded with isoniazid (curve-d) were shown in fig 2.

FTIR spectrum of CMC shows two strong peaks at 1603 and 1455 cm^{-1} due to the asymmetrical and symmetrical stretching of COO– group respectively which are also seen in the composite.

In the spectrum (shown as curve-c) of curcumin, the carbonyl absorption band (amide I band) appeared at 1657 cm^{-1}. The amide II band that occurred at 1552 cm^{-1} was due to N-H bending of the secondary amide group. Moreover, bands also appeared at 1328cm^{-1} and 1064cm^{-1} in the spectrum of curcumin. All the characteristic bands of curcumin appeared in the curcumin loaded nanoparticles (curve d), indicating the successful loading of curcumin in the composite.
Characteristic peaks of MMT clay appeared at 1050 (Si-O), 527 (Al-O), and 466 cm^{-1}(Mg-O) were also found in the spectrum of composite ensuring the presence of clay (MMT) in the same.

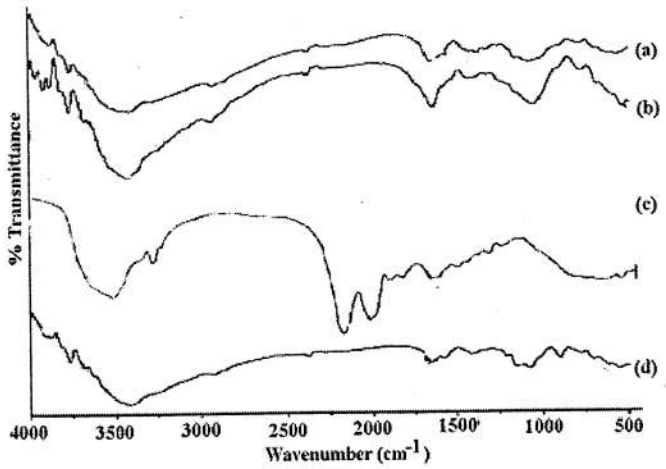

Figure 2: FTIR curves: (a) CMC, (b) MMT, (c) Curcumin, (d) CMC-MMT nanoparticle loaded with isoniazid

14.4.6. XRD study

From XRD micrograph, CMC showed its characteristic peak at 2θ=20, and MMT showed its characteristic peaks at 2θ= 8.6°. The disappearance of the peak in the nanoparticles loaded with curcumin suggests that either the full expansion of MMT gallery occurred which was not

possible to detect by XRD or the MMT layer became delaminated and no crystal diffraction peak appeared .

XRD diffraction graph reveals that curcumin showed multiple sharp peaks at 2θ varying from 12 to 50°, which is because of the crystalline nature of curcumin. However, these peaks were not observed in the diffractograms of curcumin-loaded nanoparticles indicating the occurrence of a molecular level dispersion of curcumin in the curcumin-loaded nanoparticles.

14.4.7. SEM study

SEM photographs of curcumin loaded chitosan-nanoparticles with MMT (Fig.3 a) and without MMT (Fig.3 b) are shown. The surface of the chitosan- MMT nanoparticles without MMT (fig.3b) appeared more agglomerated and smooth compared to curcumin loaded nanoparticles with MMT (fig. 3a) Curcumin loaded chitosan-MMT nanoparticles were spherical shape. The SEM of the curcumin loaded chitosan nanoparticles showed that the nanoparticles had solid dense structure with rough spherical shape.

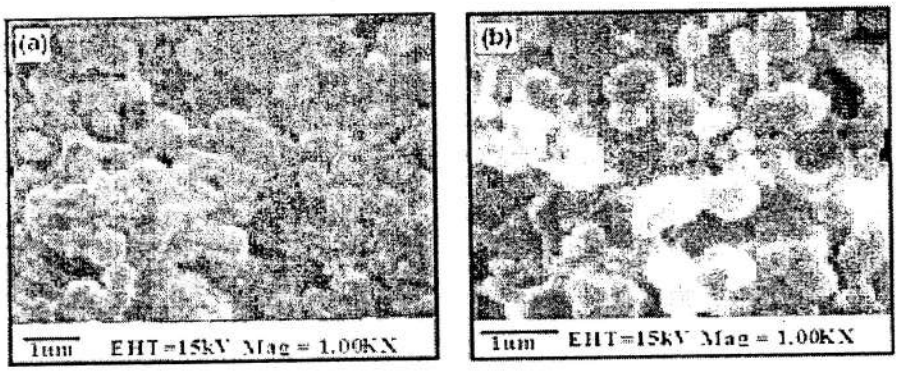

Fig. 3: SEM study of (a)curcumin loaded CMC nanoparticles with MMT: (b) without MMT

14.4.8. Swelling studies:

Swelling experiments were performed in buffer solution of pH 7.4 and 0.1N HCl solution of pH 2. The weighed mass of dry nanoparticles was dipped in the swelling medium. At predetermined time interval, the nanoparticles were removed from the swelling medium and blotted with filter paper to remove excess swelling medium from the nanoparticles surface and weight of the swollen nanoparticles were taken. The percentage of swelling was determined as

$$S = (W_1 - W_2)/W_2 \times 100\ \%$$

Here, W_1 = weight of swollen microsphere

W_2 = weight of dry microsphere

The swelling of nanoparticles was more in gastric pH than in intestinal pH and increased with decrease in MMT.

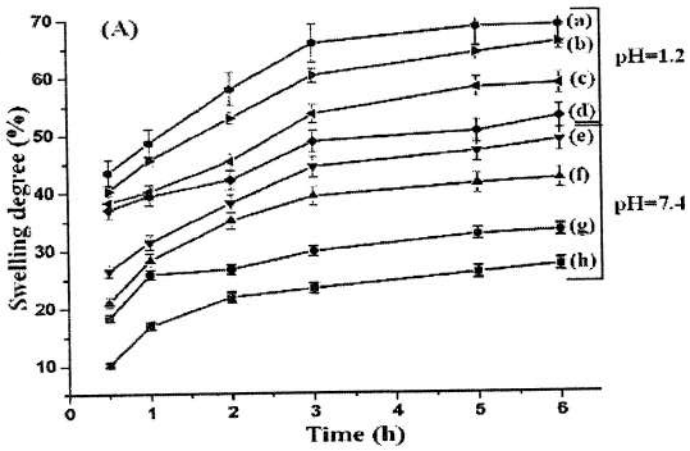

Figure 4: Swelling study of the nanoparticles in buffer solution of pH 1.2 and 7.4 for 30min, 1hr, 2 hrs, 3hrs and 6 hrs and 8 hrs

14.4.9. In vitro drug release studies:

To study the release profile for the curcumin loaded nanoparticles, dried drug loaded test samples were immersed in a solution of different pH value, stimulating gastrointestinal tract conditions. At scheduled time interval, 5mL solution was withdrawn and equal amount of same dissolution medium was added back to maintain a constant volume. The amount of drug released from the matrix was determined by UV-Visible spectrophotometer measurement at 430 nm and calculated from the calibration curve which was previously made with different concentration of drug solution. The release of curcumin was more in gastric pH than in intestinal pH. The release of drug increased with the decrease in MMT content.

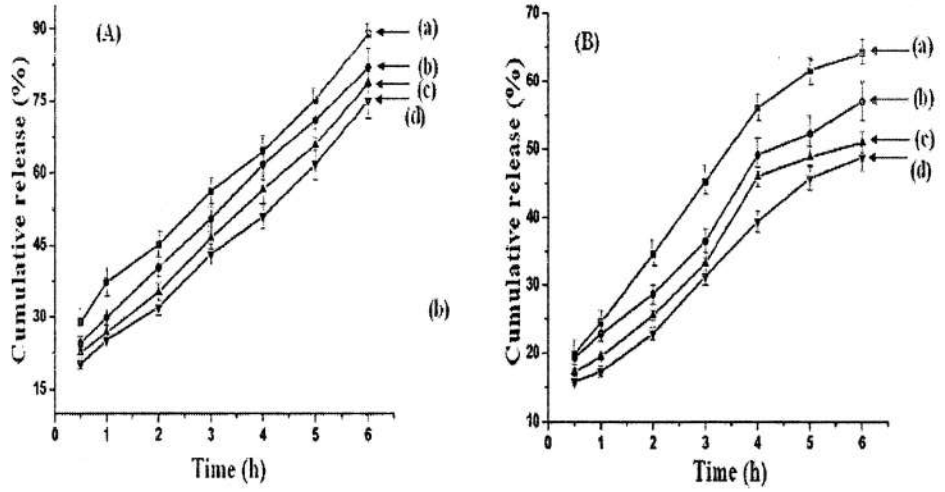

Figure 5: cumulative release study of the nanoparticles in buffer solution of pH 1.2 and 7.4 for 30min, 1hr, 2 hrs, 4hrs and 6 hrs.

14.4.10. Cytotoxicity experiments

Cytotoxicity assay were performed by measuring the viability of cells according to the standard method as described by Denizot and Lang (1986). The key component (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) was dissolved in suitable solvent. The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material. Briefly, after treatments, cells were treated with 10 % of MTT for 2 h and after which formazan crystals were dissolved in solvent and its absorbance were measured at 570 nm. The absorbance of control cells at 6 h, 12 h and 24 h were separately set as 100% viability and the values of treated cells were calculated as percentage of control.

The cell viability was more for curcumin loaded carboxymethylchitosan nanoparticles with 0.004g MMT. The cell viability decreased with the decrease in MMT concentration because of release of more drugs causing harm to the cells. The cytotoxicity study on MCF-7 and Hep G2 cell line showed around 40% killing of the cancer cells.

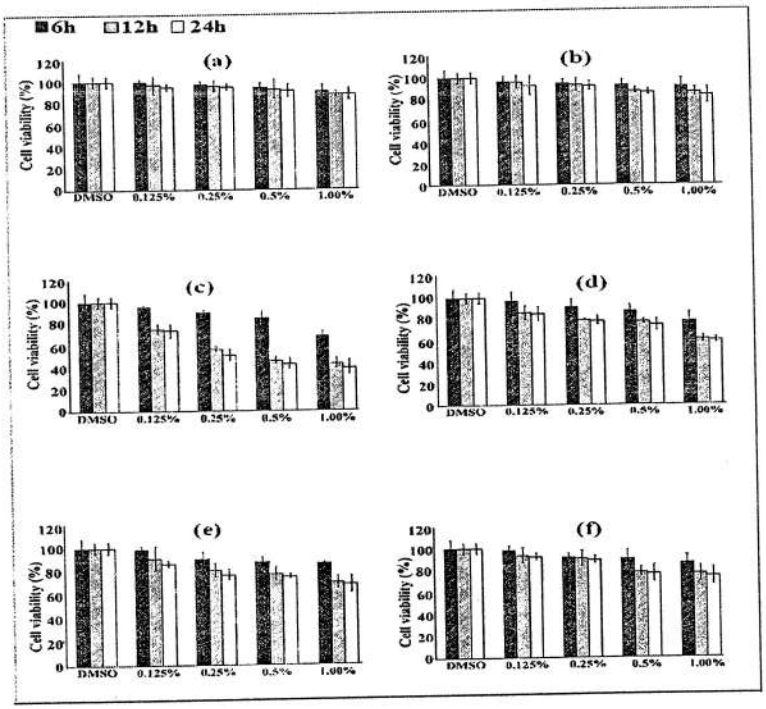

Fig.6: Cell viability study with variation of (a) CMC, (b) MMT, (c) curcumin, and (d) nanoparticle having 1% MMT(e) Having 3% MMT (f) having 5% MMT at 6 h, 12 h, and 24 h

14.4.11. Mucoadhesion Study

Mucoadhesion study showed that CMC was mucoadhesive and the mucoadhesion was more in gastric pH than in intestinal pH.

14.5. Curcumin loaded Phosphorylated chitosan-MMT nanoparticles

14.5.1 Synthesis of phosphorylated chitosan:

Phosphorylated chitosan derivatives were prepared as described by Pramanick et. al. A mixture of approximately 1 g chitosan powder, 100mL of 2% acetic acid solution, 3 g of orthophosphoric acid was taken in a three naked-round bottomed flask equipped with a stirrer, condenser, thermometer, and nitrogen gas inlet tube. The mixture was heated up to 80^0C under constant stirring until dissolution of chitosan powder and then heated to reflux. After 2 h, the resultant solution was cooled and precipitated in excess methanol. Again, the precipitated gel was dissolved in water and all unreacted phosphate and acetic acid were removed by repeated

reprecipitation in excess methanol. Finally, the gel was collected and dried under vacuum oven at 80^0C for 24 h. The successful preparation of the phophorylated chitosan was confirmed by NMR and FTIR study.

14.5.2: Preparation of Curcumin loaded phosphorylated chitosan-MMT nanoparticles:

For preparation of Curcumin loaded phosphorylated chitosan –MMT nanoparticles, first a phosphorylated chitosan solution was made in distilled water (at 45°C) where MMT (0%, 1%, 3%, 5%, w/w of the polymer) was dispersed under constant stirring for 24 h followed by sonication, then desired concentration of drug was added to the medium. A little amount (0.2ml) of surfactant Tween-80 was added for better dispersion. Precipitation was carried out by adding 1 wt% of potassium tripolyphosphate solution which was then crosslinked by using genipin. The precipitate was separated by centrifuge and then freeze dried.

Preparation of phosphorylated chitosan nanoparticles with varying the amount of clay (MMT): Temp: 45^0C; time: 24hrs; water:100ml; Tween 80:0.2ml; genipin:1ml of 0:1 w/v in 100ml water,TPP:1wt%.

Table 2:

Sample No:	Phosphorylated chitosan(gm)	MMT % w/w w.r.t phosphorylated chitosan (amount in g in 50 mL water)	Drug(gm)	Yield of nanoparticle (%)	Encapsulation efficiency (%)
A0	0.1	0 (0.00)	0.001	95.89	78.65
A1	0.1	1 (0.005)	0.001	94.23	77.01
A2	0.1	3 (0.015)	0.001	91.38	76.34
A3	0.1	5 (0.025)	0.001	92.87	76.09

14.5.3. FTIR study of Phosphorylated chitosan, MMT, Curcumin and nanonoparticles:

FTIR spectrum of phosphorylated chitosan showed characteristic peaks at 2934 and 2850 cm^{-1} for asymmetric and symmetric stretching of methylene (–CH2–) groups, respectively. Bands at around 1544 and 1620 cm^{-1} were for N–H stretching. The bands at around 1088 and 1047 cm^{-1} were attributed to the C–O–P stretching and phosphorylated hydroxyl group, whereas, the characteristic peaks at around 991 and 493 cm^{-1} were attributed for P–OH groups in CSP polymer. The bands at around 1100 to 1250 cm^{-1} were due to P–O, P=O stretching of phosphate group.

In the spectrum of Curcumin, the carbonyl absorption (amide I) band appeared at 1657 cm^{-1}. The amide II band that occurred at 1552 cm^{-1} was due to N-H bending of the secondary amide group. Moreover, bands also appeared at 1328cm^{-1} and 1064cm^{-1} in the spectrum of curcumin. All the characteristic bands of curcumin appeared in the curcumin loaded nanoparticles, indicating the successful loading of curcumin in the composite.

Characteristic peaks of MMT clay appeared at 1050 (Si-O), 527 (Al-O), and 466 cm^{-1} (Mg-O) which were also present in the spectrum of composite ensuring the presence of clay (MMT) in the same.

14.5.4. XRD study

Phosphorylated chitosan showed its characteristic peak at $2\theta=20°$, and MMT showed its characteristic peaks at $2\theta= 8.6°$. The disappearance of the peak in the nanoparticles loaded with curcumin suggests that either the full expansion of MMT gallery occurred which was not possible to detect by XRD or the MMT layer became delaminated and no crystal diffraction peak appeared . Curcumin showed multiple sharp peaks at 2θ varying from 12 to 50°, which is due to the crystalline nature of curcumin. However, these peaks were not observed in the diffractograms of curcumin-loaded nanoparticles indicating the occurrence of a molecular level dispersion of curcumin in the curcumin-loaded nanoparticles.

14.5.5. SEM study and TEM study

SEM photographs of curcumin loaded chitosan-nanoparticles with MMT (Fig.7a) and without MMT (Fig.7b) are shown.

The surface of the chitosan- MMT nanoparticles without MMT (figure.7b) appeared more agglomerated and smooth compared to curcumin loaded nanoparticles with MMT (figure7a). Curcumin loaded phosphorylated chitosan-MMT nanoparticles have spherical shape. The SEM of the curcumin loaded chitosan nanoparticles showed that the nanoparticles had solid dense structure with rough spherical shape. TEM study shows that phosphorylated chitosan nanoparticles with MMT contain black patches or lines.

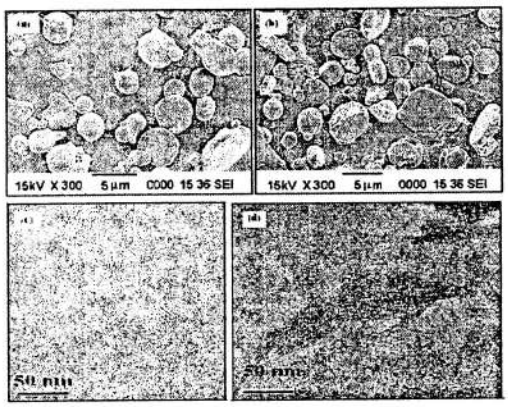

Fig. 7: SEM study of (a) curcumin loaded phosphorylated chitosan nanoparticles with MMT: (b) without MMT and TEM study of (c) curcumin loaded phosphorylated chitosan nanoparticles without MMT and (d) with MMT

14.5.6. Swelling studies:

Swelling experiments were performed in buffer solution of pH 7.4 and 0.1N HCl solution of pH 1.2. The swelling of nanoparticles was more in gastric pH than in intestinal pH and increased with the decrease in MMT content.

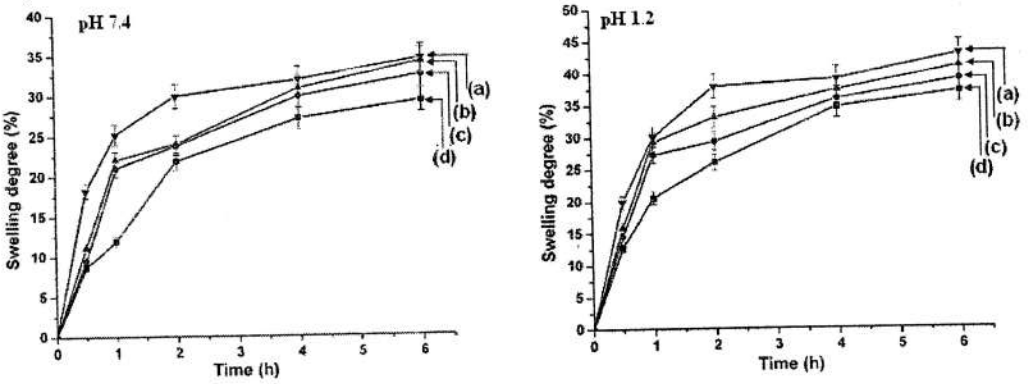

Figure 8: Swelling study of the nanoparticles in buffer solution of pH 1.2 and 7.4 for 30min, 1hr, 2 hrs, 4hrs and 6 hrs

14.5.7. In vitro drug release studies:

To study release profile for the curcumin loaded nanoparticles, dried drug loaded test samples were immersed in a solution of different pH value, stimulating gastrointestinal tract conditions. The release of curcumin was more in gastric pH than in intestinal pH. The release of drug increased with decrease in MMT.

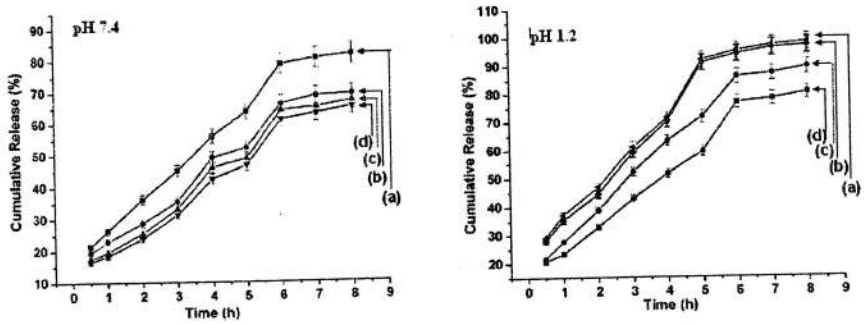

Figure 9: cumulative release study of the nanoparticles in buffer solution of pH 1.2 and 7.4 for 30min, 1hr, 2 hrs, 4hrs and 6 hrs.

14.5.8. Cytotoxicity experiments

Cytotoxicity assay was performed by measuring the viability of cells according to the method as described by Denizot and Lang (1986). The cell viability was more for curcumin loaded phosphorylated chitosan nanoparticles with higher MMT content due to release of less drug in the dissolution medium causing less harm to the cells. The cytotoxicity study on MCF-7 and Hep G2 cell line showed around 40% killing of the cancer cells.

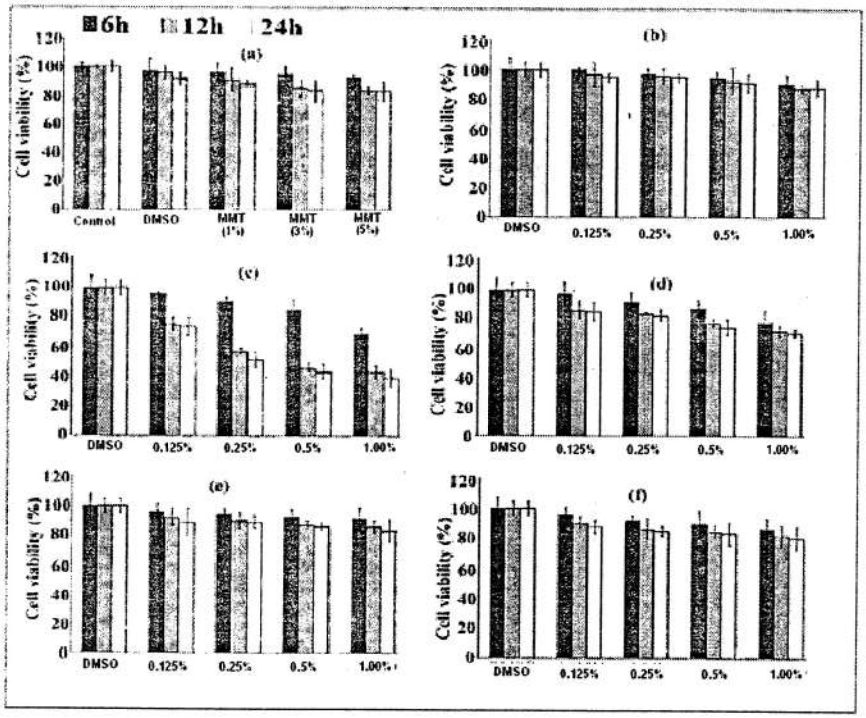

Figure 10. Cell viability study with variation of (a) MMT, (b) phosphorylated chitosan, (c) curcumin, and (d) A0, (e)A1, (f) A5 at 6 h, 12 h, and 24 h

14.5.9. In vitro wash-off test for evaluation of mucoadhesive property and *Ex vivo* mucoadhesive test

In vitro wash off test and ex vivo mucoadhesion test showed that phosphorylated chitosan is a mucoadhesive polymer. Furthermore, the nanoparticles were mucoadhesive too and the mucoadhesion was more in gastric pH than in intestinal pH.

तेजपुर विश्वविद्यालय
(केंद्रीय विश्वविद्यालय)
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TEZPUR UNIVERSITY
(A Central University)
Napaam, Tezpur - 784 028, Assam, India

UTILISATION CERTIFICATE

This is to certify that the expenditure claimed under different heads has actually been incurred and utilized properly during the period (1.08.2011 to 31.01.2015) and further that the grant has been exclusively utilized for the purpose for which it was sactioned.

22/4/15

Signature of Finance Officer
(with stamp)

5.2015

Signature of Internal Audit Officer
(with stamp)

Internal Audit Officer
Tezpur University

Signature of PI
(with stamp)

फोन / Phone : 03712-267007 - 9 (3 lines) ● फैक्स / Fax : 03712-267005, 267006 ● ई-मेल / e-mail : admn@tezu.ernet.in

Consolidated statement of Accounts

From the date of commencement: 1st August 2011 till 31st Jan 2015

Scheme Number : 01(2502)/11/EMR-II

Title of the Research scheme : "*Development of Natural Polymeric System for controlled Delivery of Anticancer Agents*"

Name of the Investigator : Prof. Tarun Kumar Maji

Date of Commencement : 01.08.11 Date of Termination: 31.01.15

Receipts (particulars of grant received)								Payment (particular of grant spent)						
Period (ending 31 Jan)	Cheque No., date & Amount	Stipend	Contigency	Scientist Allowance (for Emeritus Scientist Scheme Only)	Equipment Grant	HRA + MA	Total	Stipend	Contigency	Scientist Allowance (for Emeritus Scientist Scheme Only)	Equipment Grant	HRA + MA	Total	Balance
01/08/2011 to 31/01/2015	DD No. 014539 dated 21/02/2012 for Rs. 3,33,667/- and Voucher No. 3006 dated 18/11/2013 for Rs. 2,54,097/-, Dated 22.12.2014 for Rs. 2,23,333/-	Rs. 2,42,000/- + Rs. 79097/- + Rs.1,40,000/-	Rs. 91,667/- + Rs. 1,75,000/- + Rs. 83,333/-	Nil	Nil	Nil	Rs. 8,11,097/-	01/08/2011 to 31/03/2012=Nil 01/04/2012 to 02/05/2012=Nil 03/05/12 to 31/03/13 =Rs.1,53,097/- 01/04/13 to 31/03/14=Rs. 1,68,000/- 01/04/15 to 31/01/15= Rs. 1,40,000/-	01/08/11to 31/03/12=Rs. 66,666/- 03/05/12 to 31/3/13=Rs. 1,00,018/- 01/04/13 to 31/03/14=Rs.1,00,001/- 01/04/14 to 31/01/15= Rs. 83,315/-	Nil	Nil	Nil	Rs. 8,11,097/-	Nil

Signature of Registrar
With stamp

Siagnature of Finance Officer
With stamp

Signature of Internal Audit Officer
With stamp

Signature of PI
With stamp