*Section A-Research paper*
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# Controlled Releases of Paclitaxel Drug investigation and Impact Analysis from Casein

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## Abstract:

This paper deals with the effects of Paclitaxel drug on casein nanoparticles and its used in control drug therapy. Such systems have shown potential in releasing drugs controllably chemotherapy. In the present investigation casein nanoparticles were synthesized by emulsion cross linking method and characterized by various techniques such as Fourier transform–infrared spectrometry, Transmission electron microscopy, XRD. The average diameter of prepared native casein nanoparticles is 6nm to 100nm through W/O emulsification-cross linking method. The release behavior of casein nanoparticles was studied as a function of various factors such as chemical composition of nano-carrier, pH, temperature, biological fluids. The results revealed that the casein nanoparticles prove to be an excellent option for controlled and targeted delivery of Paclitaxel. Therefore, there is a strong incentive to develop a new strategy for the synthesis of casein nanoparticles and investigated their properties.

Keywords: Casein, Nanoparticle, Paclitaxel, Polymer, Protein

## 1. Introduction:

The number of people suffering from cancer is projected to increase to 29.8 million in 2025 from 26.7 million in 2021. Cancer ranks either first or second among the leading causes of death before the age of 70 years, across 91 out of the 172 countries worldwide. The process of uncontrolled division of cells has been identified as the only responsible factor for the origin of cancer, which is a multi phasial disease and may appear in any organ or cell type [1].

Polymers are widely used as biomaterials due to their favorable properties such as good biocompatibility, easy design and preparation, especially in the field of smart drug delivery. Polymer plays a significant role as they can deliver therapeutical agents directly into the projected site of action with superior efficacy. Nanotechnology has applications in various fields one of which is in medical treatment. Owing to their specific size, shape and surface area, nanoparticles have revolutionized drug delivery in the field of medicine. [2, 3].

Different drug carrier systems in the micro- and nanometer size range have been developed and the number of patents and products in the drug delivery field has increased recently [4, 5**]**. The goal of the drug delivery systems is to render medicines to particular parts of the body. The application of nanotechnology for the treatment of cancer or other diseases has boosted during the last decades due to the possibility to precisely deliver drugs where needed, enabling less drug's side effects [6]. Nanoparticles constructed from Versatile polymers provide a span of structural arrangements which can be exploited for specific drug binding and delivery. One such specific polymer for designing carriers is milk protein casein.

Casein is a major milk protein and possesses many structural and physicochemical properties that facilitate its functionality in drug delivery systems [7, 8] Casein are biodegradable, highly natural, biocompatible and less immunogenic and produce harmless metabolites[9]. Thus, casein based nanoparticles have been proposed for delivering hydrophobic bioactive species and drugs including , thymol [10], curcuma [11], and paclitaxel [12].

Paclitaxel is one of the most effective chemotherapeutic drug ever developed and is active against a broad range of cancers, such as lung, ovarian, and breast cancers. Due to it slow water solubility, Paclitaxelis formulated in a mixture of Cremophor[13].

Paclitaxel (PX), isolated from the bark of Pacific Yew (Taxusbrevi folia),was first discovered by Mrs. Monroe E.Wall And Mansukh C.Wani, is a white crystalline powder with the melting point of~210°C [14]. It is one of the most effective chemo therapeutic drugs and is mainly used to treat lung, ovarian, breast cancer, etc. Novel drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimization of drug related side effects due to

controlled therapeutic blood levels instead of oscillating blood levels, improved patient adherence due to reduced frequency of dosing and the reduction of the total dose of drug administered [15]. Hence, the combination of both sustained release and control release properties in a delivery system would further enhance therapetic efficacy [16-18].Chemotherapy works through a number of different mechanisms, its major function includes in discriminately killing vigorously growing cells, including tumor and normal cells, which causes some serious side effects including bone marrow suppression, hair loss, and gastrointestinal reactions [19]

In the present study, casein nanoparticles have been synthesized and characterized. The swelling and diffusion controlled drug delivery-casein nanoparticles as a possible and potential carrier for mediated anticancer control drug delivery is proposed. The whole scheme of preparation of casein nanoparticles and its crosslinking reaction are depicted in Figure.1 The method is novel and different from the techniques where a simple blending of each type of nanoparticles is done to achieve the biopolymer nano-composite nanoparticles.

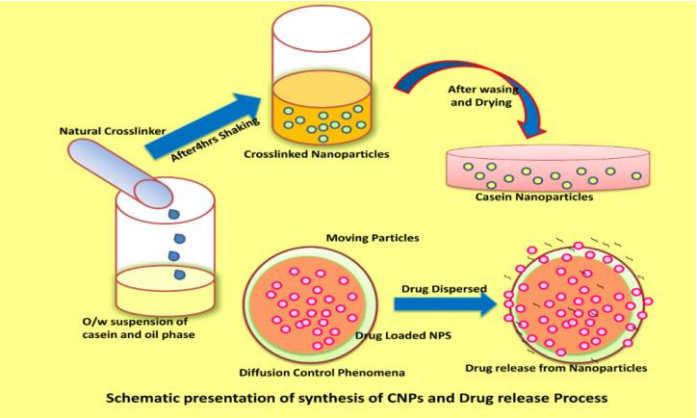


**Figure.1** Schematic representation of Preparation of casein nanoparticles and drug release profile

## 2. Experimental Section Materials:

Paclitaxel purchased from Mediclone Biotech Private, casein (soluble in normal distilled water) Mw=2061.98g/mol, was purchased from E.Merck, Mumbai (India) used as biopolymer and calcium chloride (Mw=110.98 g/mol) obtained from Merck, Mumbai, India was employed as a cross linker for casein without any pretreatment, Paraffin oil and Sodium hydroxide. Triply distilled water was used throughout the experiments.

### 2.1 Preparation of Native Casein Nanoparticles:

In the present study crosslinked protein casein nanoparticles were synthesized by emulsion polymerization method, the aqueous phase was prepared by dissolving 2g casein powder in 1% NaOH solution and paraffin oil was used as oil phase. The crosslinking solution was prepared by dissolving 1gm of calcium chloride in 3mL ethylalcohol (absolute) solution. The crosslinker solution was added drop wise added in to the aqueous phase of casein solution in continuous stirring by magnetic stirrer (3000 rpm) at room temperature for 3h. The crosslinking reaction was completed in 3h.The prepared nanoparticles were separated from the liquid medium by washing thrice with toluene and twice with acetone solvents. The dried particles were stored at room temperature.

**2.2 Loading of Paclitaxel drug onto protein NPs:**

Paclitaxel was loaded onto nanoparticle by the post-loading method, in which the drug is absorbed after formation of nanoparticles by incubating the carrier with a concentrated drug solution. For loading of drugs in the nanoparticles, a known volume of drug Paclitaxel was diluted with an appropriate amount of PBS (Phosphate Buffer saline) and shaken vigorously for mixing of drug and PBS solution. Drug-loaded nanoparticles were prepared by allowing 0.1 g of nanoparticles to swell in freshly prepared drug solution (10 mL) until equilibrium swelling was reached. The percentage loading of drug onto nanoparticles was calculated by the following equation:

$$\% \text{ Loading} = \frac{Wd - Wo}{Wo} \times 100 \qquad \text{.....(1)}$$

Where Wd and Wo are the weights of loaded and unloaded nanoparticles respectively. The percentage loading of drug provides a quantitative measure of the amount of drug present in the nanoparticles. For determining drug encapsulation efficiency [20,21]. A varying degree of drug loaded nanoparticles were prepared by allowing 0.1g of nanoparticles to swell until equilibrium in freshly prepared drug solutions (10mL).

**3. Physicochemical Characterization-**

**3.1 FTIR spectral analysis:**

FTIR spectral analysis was carried out for the identification and characterization of functional groups present in native casein, Paclitaxel loaded casein nanoparticles and Pure Paclitaxel. FTIR spectra were recorded on a IRAffinity-1S FTIR –A221356 (00917) spectrophotometer (Shimadzu) in the range of 400-4000cm-1 (Perkin-Elmer, 1000

Paragon)

### 3.2 TEM analysis:

TEM is a powerful technique to determine morphology and size of the nanoparticles, which are the key properties of nanoparticles to decide their stability [22].

The size analysis of innative casein, Paclitaxel loaded casein were investigated by recording the image by transmission electron microscope JOEL from Lab, Jiwaji University, Gwalior, Madhya Pradesh, India.

### 3.3 X-ray diffraction analysis

The X-ray diffraction studies of Casein nanoparticles and Paclitaxel loaded nanoparticles were carried outon Bruker-D2 advance phaser X-ray powder diffractometer. The diffraction data were collected from 10 to 100 theta value and values with a step size of 0.02 and counting time of 2s using a wavelength of 1.54 A˚. The average crystallite size of particles was calculated by using Debye scherrer's equation.

### 3.4 Swelling studies in nanoparticles

Swelling of native casein nanoparticles and Paclitaxel loaded casein nanoparticles was studied by a usual gravimetric method. In the experiment, 0.1g of nanoparticles were allowed to swell in a definite volume (10mL) of phosphate buffer saline (PBS) taken in a pre-weighed sintered glass crucible (pore size 1-5 µm) and weighed after definite time periods by removing excess of phosphate buffer saline by vacuum filtration. The swelling of nanoparticles was monitored continuously up to 15 min after which no weight gain of swollen nanoparticles was recorded. This clearly indicated the arrival of equilibrium swelling conditions. The amount of water imbibed by the nanoparticles was calculated by the following equation **[23, 24].**

$$\text{Swelling Ratio} = \frac{\text{Weight of swollen particles (Ws)}}{\text{Weight of dry particles (Wd)}} \qquad \text{.....(2)}$$

The amount of water sorption capacity to imbibe increasing number of water molecule by the sample provides information about the hydrophilic nature of the material.

### 3.5 *In-vitro* Release Experiments-

*In-vitro* release study of the Paclitaxel loaded nanoparticles (0.1g) was carried out by placing in a test tube containing a definite volume (10mL) of phosphate buffer saline

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(PBS) as the release medium (pH=7.4) (1.2mM $KH_2PO_4$, 1.15mM $Na_2HPO_4$, 2.7mM KCl, 1.38mM NaCl).The resulting suspension was gently shaken for a definite time period (1.5h) and 5mL aliquot was withdrawn at pre-determined time intervals (15min) from the suspension medium replacing it with the same volume of fresh PBS. The amount Of Paclitaxel released from the polymeric nanoparticles was measured spetrophotometrically at 298 nm (Shimandzu 1700 Pharma Spec.) and the released amount of drug was determined from the calibration plot.

The release of Paclitaxel from biopolymeric nanoparticulate systems is usually considered as combination of Fickian(diffusion) and non-Fickian movements of drug molecules through polymer chains. Release from nanoparticulate system is pre dominantly by diffusion controlled method. In the present study the kinetic data were analyzed with the help of the following equation [**25**], which could be helpful in determining the possible mechanisms of their release process,

$$\frac{W_t}{W\infty} = Kt^n \qquad \text{.....(3)}$$

For evaluating the diffusion constant of loaded drugs, the following equation can be used:

$$\frac{W_t}{W_\infty} = \left(\frac{Dt}{\pi L^2}\right)^{0.5} \qquad \text{.....(4)}$$

Where, D is the diffusion constant of the drug and L being the diameter of the dry.

Where $W_t / W_\infty$ is fraction of drug released at time t, k is the rate constant and n is the release exponent. The n value is used to correlate Diffusion and solute release mechanism according to given range in Table.1[26].

### 3.6 Chemical stability of drug

In order to check the stability of entrapped drug in different release medium, the UV spectral study (Shimandzu 1700 Pharma Spec) was performed. The UV spectra of native casein nanoparticles and released fractions at different pH and different time periods (The pure drug and released drug in release media (i.e. at pH 7.4) were recorded. The drug molecules significantly absorb between 300 to 500 nm respectively. The UV

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spectral curve for native drug and entrapped drug exhibit a peak at 298nm and pH 7.4. It is clearly observed from the outcome that the spectra were almost identical and suggests no significant changes in biochemical activity of the drug in loading and release studies.

## 4. RESULTS AND DISCUSSION:

### 4.1 FTIR spectral analysis

The FTIR study of (a) Native casein nanoparticles,(b)Paclitaxel loaded casein Nanoparticles (c) Pure drug was carried out to confirm the presence of characteristic functional groups of the components as shown in Figure.2 (a, b and c respectively). The FTIR spectra of native casein are shown in Figure. 3(a) In casein nanoparticles shows the appearance of strong peak at 3498 and 3200$cm^{-1}$indicates the presence of OH group, the amide A band at 3,400 $cm^{-1}$and amide B at 3,150 $cm^{-1}$are observed, peaks at 1628 $cm^{-1}$and 1683 $cm^{-1}$confirm the presence of C=O stretching and C=N stretching confirms the crosslinking reaction in casein polymeric chain. The presence of carboxylic group (O-C-OH) in casein particles is confirmed by the peak at1415$cm^{-1}$. In the FTIR spectra of pure Paclitaxel (2c) peak at 3867 $cm^{-1}$ indicates the presence of OH stretching and the functional group of hydroxyl alcohol and Phenolic C-OH group. Peak at2951$cm^{-1}$ is due to C-H stretching. 1556, 1572 $cm^{-1}$ gives the peak of N-O asymmetric stretch. 1392, 860, 549$cm^{-1}$ indicates the presence of N-O symmetric stretching. Thus, the observed absorption bands clearly confirms the presence of native casein, Paclitaxel loaded casein nanoparticles. **[27]**.

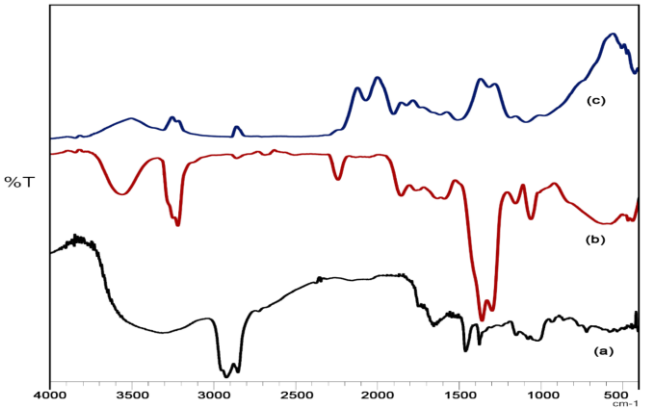


**Figure.2** FTIR spectra of (a) Native casein nanoparticles (b) Paclitaxel loaded casein nanoparticles (c )Paclitaxel drug

### 4.2 Transmission Electron microscopy:

In order to investigate the size and morphology of prepared nanoparticles, transmission electron micrograph (TEM) images were recorded as shown in Figure.3(a, b, c, d, e, f) respectively which represent the size of nanoparticles. It is clear from the images (a, b and c) that the size of native casein and Figure.3 (d, e, f) Paclitaxel - casein nanoparticles vary from 2nm to100nm, respectively. The images also indicate that the shape and size of nanoparticles are not uniform.

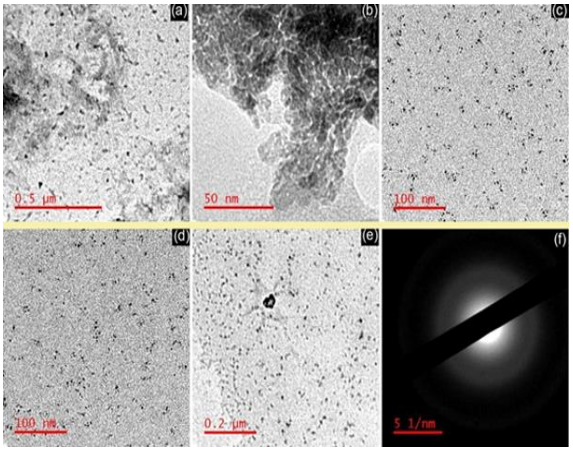


**Figure 3.**Transmittion electron microscopy image of (a,b,c) – Native casein Nanoparticles and (d,e,f) Paclitaxel loaded casein Nanoparticles

### 4.3 Powder X-ray diffraction (XRD) analysis

The characterization of the crystalline nature of –casein nanoparticles was carried out by using XRD analysis,which is an essential tool for the determination of crystallinity of the material. The well-defined X-ray diffraction patterns indicate the formation of less crystalline nature of nanoparticles **[28]**. The XRD pattern of the prepared native casein nanoparticles and Paclitaxel loaded Casein nanoparticles is shown in Figure.4(a, b),

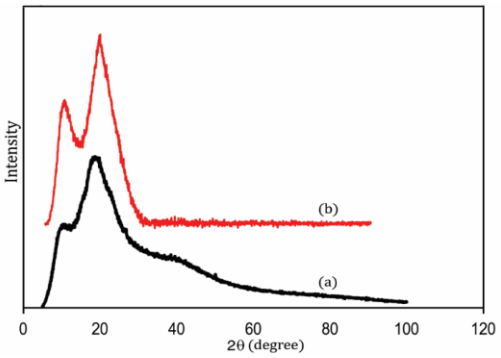


**Figure.4** XRD pattern of (a) Native casein (b)Paclitaxel loaded casein nanoparticles

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respectively. The structural pattern and characteristic peaks at 7.23° , 21.40 and 18.76°, for native casein particles is shown in Figure.4 (a) and the characteristic peaks at 9.88°, 19.23° and change in shape of peaks in Figure 4(b) clearly reveal the incorporation of Paclitaxel in casein nanoparticles The mean grain size was calculated using Debye- Scherrer formula [30], as shown in the equation(5);

$$d=\frac{k\lambda}{\beta\cos\theta} \quad \text{.....} \quad (5)$$

Where *d* is mean grain size, *k* is the shape factor (0.9), β is broadening of the diffraction angleandλ is diffraction wavelength (1.54 Å).The estimated average grain size of casein nanoparticles , and Paclitaxel casein nanoparticles was found to be 1.74 and 0.75nm.

The amorphous and crystalline nature of the Paclitaxel-casein nanoparticle was determined by degree of crystallinity. The numerical formula to calculate the percent crystallinity of nanoparticles is given in the following equation:

$$Xc\ (\%) = (Ac/\ Aa+Ac)x100 \quad \text{.....} \quad (6)$$

Where Ac and Aa are the area of crystalline and amorphous phases, respectively [32]. The crystallinity of the material has been calculated using the equation formula given in equation (6).The % crystallinity of native casein was found to be [29, 24] while for Paclitaxel casein nanoparticle was about 37.7. These results are quite obvious to confirm the amorphous nature of theparticles.

### *4.4* Effect of swelling and % loading of Paclitaxel*:*

The mechanism of water transport into the polymer is particularly important for suitability of nanoparticles as drug delivery system, as the amount of drug released is dependent on the rate and transport mechanism of water diffusing into the polymer network [30].When a dry nanoparticle is placed in contact with a PBS solution, fluid begins to enter the dry particles, solidMatrix network by diffusion and the particles begin to diffuse into the fluid and reaches equilibrium with the fluids and an interface is formed between the dry and swollen nanoparticles.When all the fluid has been absorbed, the nanoparticles reach chemical equilibrium and the fluid single interface is again achieved between the nanoparticles and the fluid [31,32].

**Table-1 Diffusion Mechanism**

| Release exponent (n) | Overall solute diffusion mechanism |
|---|---|
| 0.45 | Fickian diffusion |
| 0.45 < n < 0.89 | Anomalous (non-Fickian) diffusion |
| 0.89 | Case-II transport |
| n> 0.89 | Super case-II transport |

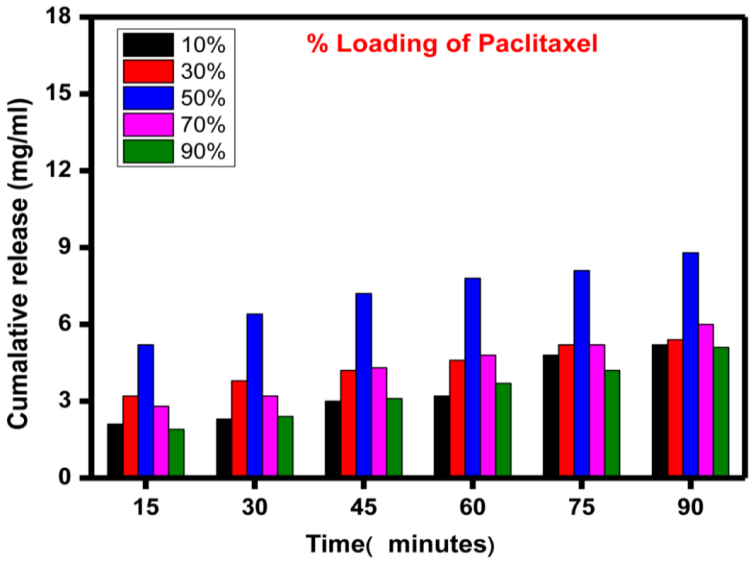


**Figure.5:** Effect of percent loading on the release of Paclitaxel casein nanoparticles of definite composition casein=2g, calcium chloride=1.0g.

The % Loading results are shown in Figure.5, which clearly indicate that initially upto 50% solution of Paclitaxel loading cumulative release increases, while beyond this there is a fall in the release rate, which can be justified as high drug loading fast and enhanced intake of physiological fluids. The observed increase in drug release is due to the reason that higher loading of drug facilitates a faster movement of the solvent that penetrates the surface of the loaded nanoparticles. From the release profile it can be summarized that higher drug loading value result in better maintenance of drug levels during drug release process [33].

### 4.5 Effect of Casein on release of Paclitaxel

The effect of casein on the amount of drug release has been investigated by

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varying its amount in the range of 1.0 to 4.0 g in the feed mixture. The release and swelling results are displayed in Figure.6 which clearly indicates that the cumulative release of Paclitaxel increases with increasing casein content in the range of 1.0-2.0 g and, thereafter, a decrease in release was noticed. This may be attributed to the fact that casein is a hydrophobic protein contains 35-40% non-polar amino acids which are highly hydrophobic it contains high number of sulphur and phosphate amino acids and its increased amount in the particles would increase the hydrophobicity of the nanoparticles and thus a higher release is expected [34]. The observed release study are consistent also it is clear that the release amount increases due to increase hydrophobicity of nanoparticles and beyond 4g of casein content the observed value decrease in release could be attributed to enhanced compactness of the nanoparticles due to greater interaction between casein nanoparticles as shown in the Figure.6

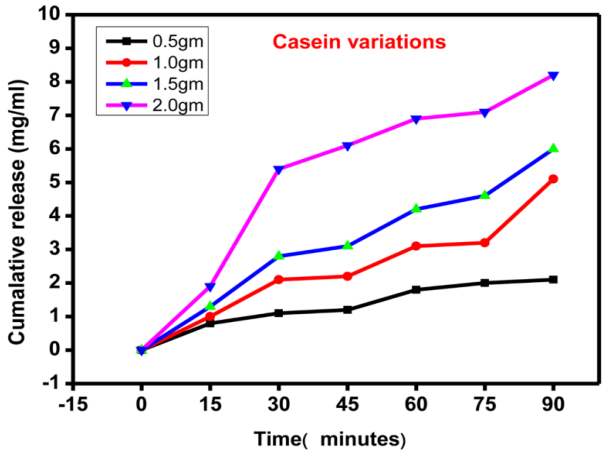


**Figure.6** Effect of varying amount of casein on the cumulative release of Paclitaxel the composition of $CaCl_2$=1g

### 4.6 Effect of cross linker on release of Paclitaxel:

In the present study, calcium chloride was used to crosslink in casein nanoparticles, the concentration range used was0.5mL to 2.0 mL in the feed mixture. There salts revealed that upto the addition of 1 mL crosslinker, the release of Paclitaxel constantly increases while beyond this concentration a drop in swelling capacity of nanoparticles was noticed. The observed increase could be attributed to the fact that calcium chloride is crosslinked by reacting with the hydroxyl groups of casein at its two terminals. Thus, a crosslinked casein network could be imagined as molecular sieve that contains wide pore size in its structure and therefore, possesses an abnormal capacity of

accommodating Paclitaxel molecules in to the network [35].Thus, capacity to imbibe increasing number of Paclitaxel molecules result in an increased cumulative release which is clearly shown in Figure.7. The obtained increase in release permits a greater number of molecules to diffuse out of material and pass into the release medium. However, beyond 1g of calcium chloride, the network of casein is highly crosslinked, which consequently reduces the free volume accessible for the penetrations of water molecules.

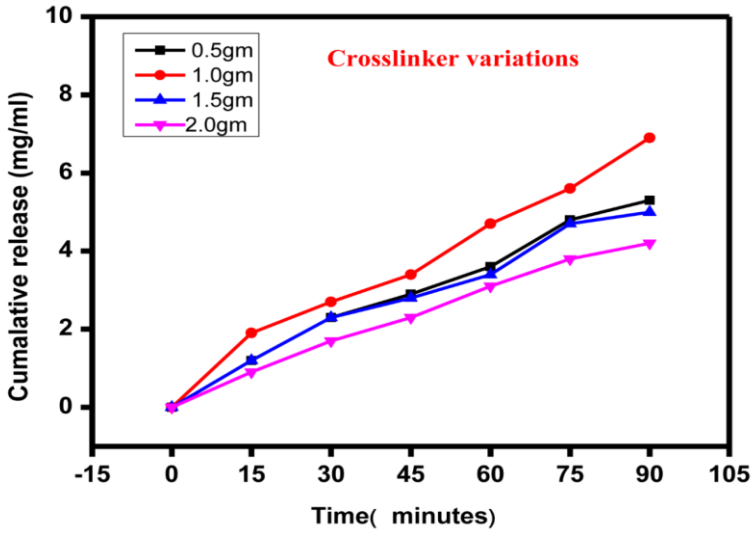


**Figure.7** Effect of varying amount of calcium chloride on the cumulative release of the composition of casein is =2g.

### 4.7 Effect of pH

pH responsive nanoparticle devices have been commonly used to design controlled formulations for oral administration of drug which remains the most clinically acceptable way of drug delivery [32]. In the present studies, the influence of pH on the drug release ratio of Paclitaxel loaded casein nanoparticles has been studied by adjusting the pH of swelling medium at 1.8, 7.4 and 8.6 respectively. In the present work, the release dynamic of nanoparticles has been studied under varying pH condition. The results are shown in Figure.8 which show that swelling ratio of particles constantly increases with increasing pH. The reason behind is casein's cationic in nature and therefore a change in pH of the swelling medium also affects the charge profiles of casein nanoparticles [33]. The swelling results of nanoparticles indicate a swelling behavior on different pH values. The results reveal that at pH 1.7, a minimum swelling ratio was observed, because casein exists in the form of –COOH at low pH medium (pH 1.7) and the macromolecular nanoparticle network of the hydrogen bonding produced by -COOH

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groups of casein led to the stronger interaction between polymer chains. Accordingly, the swelling ratio in pH 1.7 is relatively lower. At higher pH, the carboxylic groups get ionized and acquire $-COO^-$ form. Thus, weak hydrogen bonding between biopolymer chains and electrostatic repulsion between $-COO^-$groups result in the higher swelling ratio[36, 37]. The release of Paclitaxel was observed at pH 1.7,7.4,and 8.6, which represent the pH of different part of our body fluids respectively it is clear that at pH 8.6, the release of Paclitaxel is maximum while in the other release media of pH 1.8 and 7.4 a lower amount of drugs is released.

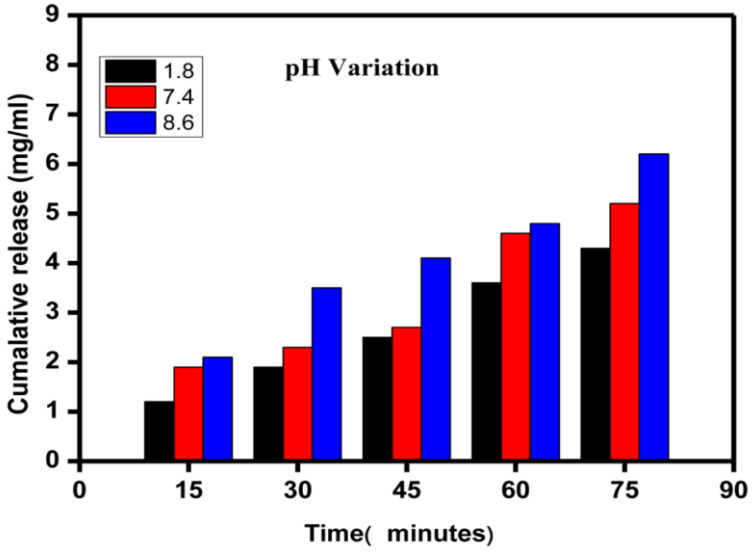


**Figure.8** Effect of pH on the cumulative release of Paclitaxel the composition of casein is =2g, $CaCl_2$=1g

### 4.8 Effect of temperature on drug release

In the present study, the temperature of release medium was varied in the range 15-42° C and its effect on release ratio was investigated. The results are displayed in Figure.9 which clearly indicates that with increasing temperature, the release of nanoparticles increases in the whole studied range. The observed increase in the Paclitaxel loaded casein nanoparticles can be explained by the fact that a rise in temperature enhances rate of water diffusion and segmental mobility of macromolecular chains which combined results in a greater release. Moreover, with increasing temperature the H-bonds between the Paclitaxel molecules and network chains are broken, thus converting bound water into free water [38]. This result in a higher concentration of Paclitaxel, consequently, an increase in the released amounts is also observed.

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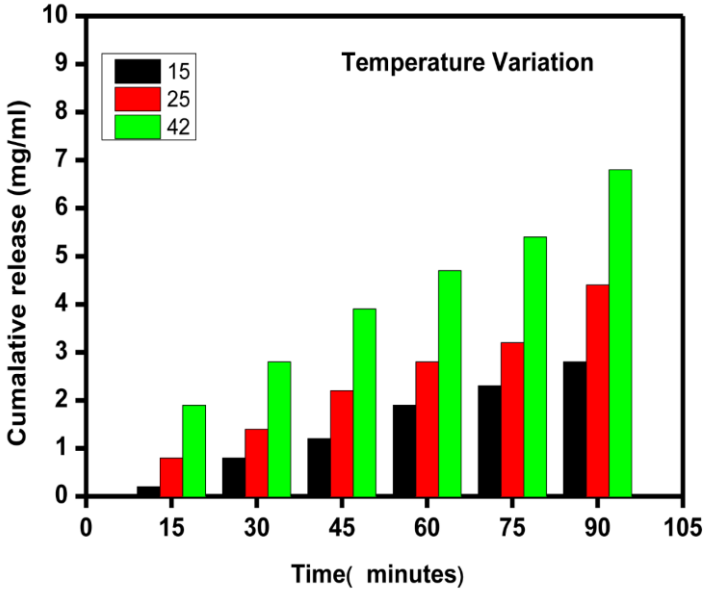


**Figure.9** Effect of temperature on the cumulative release of Paclitaxel the composition of casein is =2g, $CaCl_2$=1g

### 4.9 Effect of Biological Fluids:

The effect of the nature of biological fluids on the swelling of Paclitaxel loaded casein nanoparticles has been investigated by performing swelling experiments in various simulated physiological fluids. The results are depicted in Figure.10 which show an increase or decrease in swelling behavior of the Paclitaxel in loaded casein nanoparticles. The effect of biological fluids was examined by performing swelling experiments in the presence of urea, D-glucose, and potassium iodide, saline water and PBS. The results are summarized in Figure.10, which clearly show that the presence of solute suppresses the swelling ratio of these nanoparticles. The possible reason for the observed lower swelling of paclitaxel casein particles in these fluids may be due to the presence of salt ions in the release medium which lowers the osmotic pressure of the swelling system thus resulting in a lower swelling of loaded casein nanoparticles.

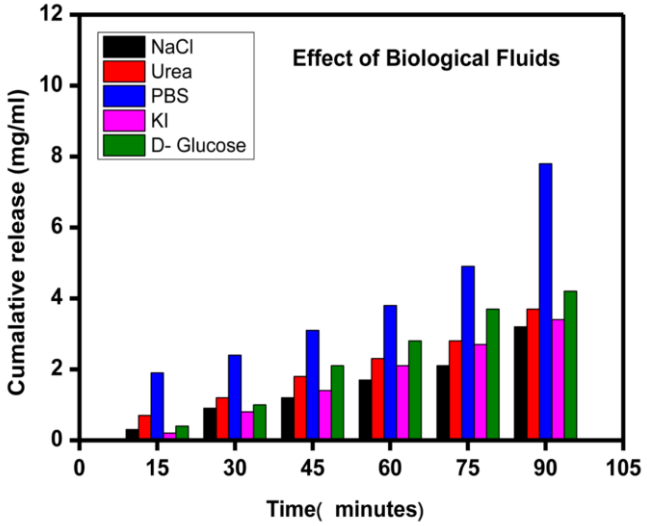


**Figure.10** Effect of stimulated Biological Fluids on the cumulative release of Paclitaxel the composition of casein is =2g, $CaCl_2$=1g

**4.10 Drug release study:**

The Paclitaxel casein nanoparticles may be visualized as a three dimensional network of casein macromolecules containing drug molecule which occupy space available between the network chains, when such nanoparticles are allowed to swell in a release medium the solvent (PBS) molecule enter into the nanoparticles matrix and subsequent relaxation of polymer chains take place. The Paclitaxel molecules release out through water permeation channels present in the macromolecular network, the diffusion of drug molecules and relaxation of nanoparticles chains determine the type of mechanism being followed by the drug molecule. It has been laid down by Highuchi equation [39, 40, 41] that if n=0.43,the release is diffusion controlled(Fickian), when n=0.84 the release is non Fickian (case-II) and for when in some cases n has been found to exceed 0.84 and the mechanisms known as super case(II), the value of diffusion coefficient (D) and release exponents (n) have been calculated as described above and summarized in Table-2 along with the value of D and n is the respective value of regression coefficients ($R^2$) have also been expressed inTable-2.It is clear from the data that the value of $R^2$ are always great or than 0.99 and therefore suggested for a good applicability of release data to equation –respectively.

**Table. 2**

Data showing the release exponents and diffusion coefficients obtained under varying experimental conditions.

| S.No. | Casein | $CaCl_2$ | pH | $Dcm^{-2}S^{-1}$ | N | $R^2$ | Mechanism |
|---|---|---|---|---|---|---|---|
| 1 | 1.0 | 1.0 | 7.4 | 10.98 | 0.46 | 0.973 | Non Fickian |
| 2 | 2.0 | 1.0 | 7.4 | 11.67 | 0.48 | 0.988 | Non Fickian |
| 3 | 3.0 | 1.0 | 7.4 | 12.58 | 0.58 | 0.993 | Non Fickian |
| 4 | 4.0 | 1.0 | 7.4 | 19.34 | 0.76 | 0.923 | Non Fickian |
| 5 | 2.0 | 0.5 | 7.4 | 17.14 | 0.73 | 0.893 | Non Fickian |
| 6 | 2.0 | 1.5 | 7.4 | 16.14 | 0.62 | 0.998 | Non Fickian |
| 7 | 2.0 | 2.0 | 7.4 | 12.58 | 0.46 | 0.973 | Non Fickian |
| 8 | 2.0 | 1.0 | 1.8 | 19.61 | 0.77 | 0.994 | Non Fickian |
| 9 | 2.0 | 1.0 | 8.6 | 18.05 | 0.85 | 0.993 | Non Fickian |

**CONCLUSIONS:**

In the present study, casein nanoparticles were synthesized using an emulsion crosslinking method. It is found that % loading of Paclitaxel concentration and crosslinker nanoparticles has great influence on the release profile of drug. It is also found that with increase in % loading of drug, the released amount of drug constantly increases and after 50% its decreases.

It is observed that the effect of nanoparticles on the release of Paclitaxel increases when the amount casein is increased from 1g to 2.0g whereas the extent and release decreases beyond 2gm casein due to casein being hydrophobic in nature. The effect of crosslinker addition upto 1gm. Results in the increase of drug released where as decreased paclitaxel release was observed on addition of $CaCl_2$beyond1gm.

It is found that the extent of swelling of nanoparticles plays a vital role in regulating the cumulative release of drug. An optimum release is obtained at basic pH while lower release is observed in acidic pH and in physiological fluids. Basic medium and physiological pH 8.6 support the extent of release of drugs.

The chemical stability of Paclitaxel carrying nanoparticles is confirmed by UV spectral analysis. The prepared nanoparticles are suitable as swelling controlled drug

delivery system and its follows the peppas model and show a non-fickian transport mechanism.

**REFERENCE:**

1. K. Sathishkumar, S. Leburu, T. Ramamoorthy, P. Mathur et.al Burden of cancers in India - estimates of cancer crude incidence. Indian Journal of medical Res.527, 2022.
2. L. Zitvogel**,** L. Apetoh, F. Ghiringhelli, G. Kroemer, Immunological aspects of cancer chemotherapy. Nature reviews immulology. 8(1),59-73, 2008.
3. F. Fontana, D. Liu, J. Hirvonen, and H.A.Santos, Delivery of therapeutics with nanoparticles: Rev. Nanomed. Nanobiotechnol. 9,14-21, 2017.
4. S. Jinjun, R. Alexander, O. Votruba, C. Farokhzad, R. Langer, N. Lett, Nanotechnology in Drug Delivery and Tissue Engineering: From Discovery to Applications. 8, 10(9), 3223–3230, 2010.
5. J.K. Patra, G. Das, L.F. Fraceto, E.V.R. Campos, Nano based drug delivery systems, recent developments and future prospects. J Nanobiotechnology. 16(1), 71, 2018.
6. N. Amreddy, A. Babu, R. Muralidharan, R.J. Panneerselvam, A. Srivastava, A Ahmed, R., et al. Recent advances in nanoparticle-based cancer drug and gene delivery. Adv. Cancer Res. 137,115–170, 2018.
7. B. Bahrami, M. HojjatFarsangi, H. Mohammadi, E. Anvari, G. Ghalamfarsa, M.Yousefi, et al. Nanoparticles and targeted drug delivery in cancer therapy. *Immunol. Lett.*190, 64–83, 2017.
8. M. Jerry, J. Laughlin, A novel mechanism for the control of clinical cancer: inhibition M of the production of adenosine triphosphate (ATP) with a standardized extract of paw paw*(Asimincz triloba*, Annonaceae*) m. Chem. Soc*., 114, (26),10203–10213, 2010.
9. N. Arora, T. Garg, A. Bilandi, review on casein production and casein-based nano-formulations, International research journal of pharmacy. 3(1),41-45, 2012.
10. E. Semo, E, Kesselman, D. Danino, Y.D. Livney, Casein micelle as a natural nano-capsular vehicle for nutraceuticals, Food Hydrocolloids. 21(5), 936- 942, 2007.
11. X. Pan, M. Mu, B. Hu, P. Yao, M. Jiang, Micellization of casein-*graft*-dextran copolymer prepared through Maillard reaction, Biopolymers. 81(1),29-38, 2006.
12. H.R. Rahimi, R. Nedaeinia, A.S. Shamloo, N. Shima, K.R.K Oskuee, Novel delivery system for natural products: Nano-curcumin formulations, Avicenna J

Phytomed.6 (4),383–398, 2006.

13. P.J. Ma, R. Mumper, Paclitaxel Nano-Delivery Systems: A Comprehensive Review, Journal of nanomedicine & nanotechnology. 18, 4(2):1000164, 2013.
14. N.C. Kampan,M.T. Madondo,O.M. McNally, M. Quinn, Plebanski, Paclitaxel and Its Evolving Role in the Management of Ovarian Cancer, M. Biomed Res Int., 413076,2015.
15. W. Lohcharoenkal, L. Wang , Y.C. Chen and Y. Rojanasakul, Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy , Bio Med Research International. 180549,12, 2014.
16. A. K. Mitra, V. Agrahari, A. Mandal,K.Cholkar, C. Natarajan et.al, Novel Delivery Approaches for cancer therapeutics, J Control Release. 10, 219: 248–268, 2015.
17. K. Vikas, A. Sharma, G. Joshi, G, D. Vipasha, Recent Advances In Ndds (Novel Drug Delivery System) For Delivery Of Anti- Hypertensive Drug, Int. J. Drug Dev. & Res. Jan-March. 3 -1,252-259, 2011.
18. D. Wang, B. Ma, Z. Wang, Y. Zhao, Y. Sun, Y. Luan , J. Wang, Preparation and characterization of β-casein stabilized lipopeptide lyotropic liquid crystal nanoparticles for delivery of doxorubicin**,** Soft Matter, 28;15(44):9011-9017,2019.
19. S. Gandhi, I. Roy, Doxorubicin-loaded casein nanoparticles for drug delivery: Preparation, characterization and in vitro evaluation, Int. J Biol Macromol**,** 1216-12, 2019.
20. Xia W, Zhu Z T B, Zhang W, Liu C, Chen S, Song M ,Targeted Delivery of Drugs and Genes Using Polymer Nano carriers for Cancer Therapy. *Int. J. Mol. Sci. 22*(17), 9118, 2021.https://doi.org/10.3390/ijms22179118
21. S. G. N. David, K.J. N.K. Chou, Kinetics of water swelling and development of porous structure in ionic poly (acrylonitrile-acrylamide-acrylic acid) hydrogels, Polymer, 37(6),1019-1025,1996.
22. S. Mahobia, J. Bajpai, A.K. Bajpai, An *In-vitro* Investigation of Swelling Controlled Delivery of Insulin from Egg Albumin Nano carriers, Iran J Pharm Res.15 (4),695–711,2016.
23. Y. Lu, S. Proch, M. Schrinner, M. Drechsler, M. Kempe, R, Ballauff, Thermo sensitive core-shell microgel asa–nano reactor |for catalytic active metal nanoparticles, *Mater, J. Chem.*.19,3955, 2009.
24. R. Chouhan, A.K. Bajpai, Real time *in vitro* studies of doxorubicin release from PHEMA nanoparticles, J. Nano biotechnology. 7, 5,2009.
25. S. Chairam, E. Somsook, Starch vermicelli template for synthesis of magnetic iron oxide nano clusters, Journal of Magnetism and Magnetic Materials. 320(15),2039-2043, 2008.

26. M. H. Shoaib, J. Tazeen, H. Merchant, H, A, Yousuf, Ismail, R, , Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC, Pak J Pharm Sci. 19(2),119,2006.

27. A. Singh, J. Bajpai, A. Tiwari, A.K. Bajpai, Designing casein-coated iron oxide nanostructures (CCIONPs) as super paramagnetic core–shell carriers for magnetic drug targeting, Prog Biomater. 4,pages39–53, 2015.

28. M. Sandra, R. Londoño, J.C. Rodrigo, E.M.R. Beatriz, Munoz, R. García, Effect of the Nano Crystal Size on the X-ray Diffraction Patterns of Biogenic Hydroxyapatite from Human, Bovine, and Porcine Bones, *Scientific Reports* , Vol. 9 ,5915,2019.

29. A.K. Bajpai, H. Bundela, Designing of hydroxyapatite-gelatin based porous matrix as bone substitute: Correlation with biocompatibility aspects, eXPRESS Polymer Letters, 2008, Vol.2, No.3, 201–213.

30. S. Likhitkar, A.K. Bajpai, Magnetically controlled release of cisplatin from super paramagnetic starch nanoparticles , Carbohydrate polymers, 87, 300- 308,2012.

31. A. Bajpai, S. Shukla, R. Saini, A. Tiwari, Stimuli responsive drug delivery system. I Smithers, 2010,143-183.

32. A. M.Vargason, A. C. Anselmo,and S.Mitragotri, The evolution of commercial drug delivery technologies, Nature Biomedical Engineering. 5, 951–967,2021.

33. F. Rehana, N. Ahemada, M. Gupta, Casein nanomicelle as an emerging biomaterial—A comprehensive review. Colloid and surfaces B: Bio interfaces, vol 179,280-292, 2019.

34. A. Shipra, I. Davidson, N. Avni, Y G, Assaraf, Y D. Livney, β-Casein nanoparticle-based oral drug delivery system for potential treatment of gastric carcinoma: Stability, target-activated release and cytotoxicity.8, 2,298-305,2012.

35. A.S. Luebbe, C. Alexiou, C. Bergmann, Clinical applications of magnetic drug targeting, J Surg Res, 95,200-6,2001.

36. A. Singh, J, Bajpai, A.K, Bajpai, Investigation of magnetically controlled water intake behavior of Iron Oxide Impregnated Super paramagnetic Casein Nanoparticles (IOICNPs), Journal of nano biotechnology, 12, 38, 2014.

37. M.K. Gupta, J. Bajpai, A.K. Bajpai, Optimizing the release process and modelling of in vitro release data of *cis*-dichloro di amino platinum (II) encapsulated into poly(2-hydroxyethyl methacrylate) nano carriers, Materials Science and Engineering. vol 58,852-862, 2016.

38. A. Singh, J. Bajpai, A.K. Bajpai, Investigation of magnetically controlled water intake behavior of Iron Oxide Impregnated Super paramagnetic Casein Nanoparticles (IOICNPs). J. Nano biotechnol. 12,38,2014.

39. S. Likhitkar, A.K. Bajpai, investigation of magnetically enhanced swelling behaviour of super paramagnetic starch nanoparticles, Bull. Mater. Sci. 36, 1, 15-

24,2013.

40. J. Choubey, A.K. Bajpai, Design of gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate, Journal of Material science. Material inMedicine, 21,1573–1586, 2010.

41. J. Siepmann, N.A. Peppas, Mathematical modeling of controlled drug delivery Adv. Drug Del. Review. 48,139–157,2001.