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Review Article

# REVIEW ON CASEIN PRODUCTION AND CASEIN BASED NANO-FORMULATIONS

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**ABSTRACT**
Biodegradable systems have the ability to release the drug for a prolonged period of time and subsequently degrade which can be easily cleared from the body. This property makes use of them for the design of carriers for the controlled delivery of therapeutic agents, since it will release the entrapped drug over an extended period of time. Due to its non toxic, biocompatible and slow digesting nature it is an effective matrix in drug delivery. Milk has many proteins, including the nine essential amino acids. The two basic types of proteins in milk are called casein and whey. The ability of casein to modify drug dissolution from compacts was reported. The high tensile strength of casein film, favours its use as an acceptable film-coating for tablets. Casein floating beads were developed to increase the residence time of drugs in the stomach based on its emulsifying and bubble-forming properties. Casein-based microparticles bioactive molecules were prepared via emulsification-chemical cross linking Casein nanoformulations were also prepared to deliver nutraceuticals and synthetic drugs via enzymatic crosslinking, graft copolymerization, heat-gelation and polyelectrolyte ionic complexation. It can be concluded that casein-based formulations are promising materials for controlled drug delivery.
**KEYWORDS:** Biodegradable systems, controlled delivery, proteins, emulsification-chemical cross linking

## INTRODUCTION

Biodegradable polymers for the administration of pharmaceuticals and biomedical agents have increased dramatically. The most important biomedical applications of biodegradable polymers are in the areas of controlled drug delivery systems[1,2]. Drug delivery systems based on food proteins hold much promise because of their high nutritional value and excellent functional properties, including emulsification, gelation, foaming and water binding capacity as well as their applications as ingredients in the food industry[3,4]. In addition, proteins are metabolizable; hydrolysis of food proteins by digestive enzymes generates bioactive peptides that may exert a number of physiological effects in vivo, for example, on the gastrointestinal, cardiovascular, endocrine, immune and nervous systems. Systems based on proteins including gelatine, collagen, casein, albumin and whey protein have been studied for delivering drugs, nutrients, bioactive peptides and probiotic organisms. A wide variety of protein- 96 based delivery system formulations have been described, including films hydrogels , microparticles and nanoparticles. Casein, the major milk protein, is inexpensive, readily 101 available, non-toxic and highly stable. As a natural food product, this GRAS (generally recognized as safe) protein is biocompatible and biodegradable .Many of the structural and physicochemical properties of caseins facilitate their functionality in drug delivery systems[5]. These properties include binding of ions and small molecules, exceptional surface-active and stabilizing properties, excellent emulsification and self assembly properties together with superb gelation and water binding capacities. The pH-responsive gel swelling behaviour renders casein useful for programmable release. Casein could serve as a shield against radiation, particularly UV 120 light, utilizing its strong UV absorbance properties, around 200–300 nm 121[6].

### Distribution of Milk Protein

Milk has many proteins, including the nine essential amino acids. The two basic types of proteins in milk are called casein and whey. Table 1 represents different components of milk.

Table 1. Different components of milk

| Component | %Total | %Casein | %Whey |
|---|---|---|---|
| Casein | 83 | | |
| Alphas1 casein | 36 | 44 | |
| Alphas2 casein | 9 | 11 | |
| Beta Casein | 21 | 25 | |
| Kappa Casein | 12 | 14 | |
| Gamma Casein | 4 | 5 | |
| Whey | 17 | | |
| Beta-Lacto globulin | 10 | | 58 |
| Alpha-Lacto globulin | 2 | | 13 |
| Immunoglobulin | 2 | | 12 |
| Serum Albumin | 1 | | 6 |

### Production of Casein[6-8]

Production of casein is well described in Figure 1.

### Casein Structure

Casein comprises about 94% protein and 6% low molecular weight compounds collectively called colloidal calcium phosphate. Mainly four casein phosphoproteins, αS1-, αS2-, β-, and κ-casein, exist approximately in proportions of 4:1:4:1 byweight respectively all of the four caseins are amphiphilic and have ill-defined structures[7,8]. Figure 2 show Structure of Casein micelle. Caseins are proline-rich, open-structured. The proline peptides in the casein structure tend to interrupt alpha-helix and beta strands and disulphide bridges are absent in their structure Casein proteins have distinct hydrophobic and hydrophilic domains[7]. αS1-casein has a strongly acidic peptide of 40 amino acids that contains seven of the eight phosphate groups, twelve carboxyl groups andonly four positive groups. The highly charged N-terminal region of β-casein contains four of the five phosphates of the molecule, seven carboxyl groups and only two positive groups. The sialyated glycoprotein κ-casein has only one phosphate and fourteen carboxylic acid groups located in the hydrophilic C-terminal region called the glycoacropeptide41there is a growing interest in polymers of natural riginwhich possess high levels of biodegradability and aqueous solubility. The most common example is zein,

an alcohol soluble protein obtained from corn, which has been used as an effective film former and coating agent[8]. Protein cross-linking can be achieved through the use of chemical agents such as formaldehyde or glutaraldehyde[8,9]. Casein films have been shown to exhibit high tensile strength making them suitable as coatings for tablets. Abu Diak et al.[8] who evaluated casein as a film former for tablet coating by the aid of different water soluble and insoluble plasticizers. In this study, diltiazemHCl (DZ) core tablets were coated with casein using a pan coater and the efficacy of four different plasticizing agents (glycerol, triethyl citrate, dibutyl sebacate and oleic acid) The percentage of drug released after 2 h in 0.1 N HCl from uncoated tablets exceeded 80% whereas the percentage of DZ release from casein- coated tablets was significant lower .

**Effect Of pH And Temperature On The Casein Micelle**

The casein proteins are associated into macromolecular complexes called casein micelles. Under the ionic environment found in milk, the casein micelles have an isoelectric point of about pH 4.6. The casein micelles will aggregate as the pH approaches this value if the temperature is above about $8^0$C. In milk, the native whey proteins are soluble at their isoelectric points; however, the denatured whey proteins are insoluble. The major whey protein b-lactoglobulin has an isoelectric point of about pH 5.3, which is markedly higher than that for caseins. For milk samples that are heated at pH 6.5, most of the denatured whey proteins are associated with the casein micelles[8-10]. This will alter the net charge characteristics of the casein micelles, and, as the isoelectric point of b-lactoglobulin is higher than thatof the native casein micelles, the association ofdenatured whey proteins with the casein micelles will increase the isoelectric pH of the micelles and aggregation will occur at a pH higher than that observed for unheated milk. As the heating pH is increased, lessdenatured b–lactoglobulin associates with the casein micelles. Although this will probably reduce the isoelectric pH of the casein micelles, it allows the serum phase denatured whey proteins to aggregate separately from the casein micelles. As the isoelectric point of the serum phase components will be higher than that of the casein micelles, the pH at which aggregation occurs will be progressively shifted to higher pH as the heating pH and the concentration of serum phase denatured whey proteins are increased. The dissociation of k-casein from the casein micelles may also contribute to the higher aggregation pH as the heating pH is increased, particularly above pH 6.7.Lower levels of k-casein on the micelles will reduce the density of the surface hairy layer. This may cause the surface hairy layer to collapse at a higher pH, or this layer may have a reduced efficiency in stabilizing the casein micelles[9].

**Casein-Drug Composites**

Watanabe et al.[12] observed that the release rate of phenytoin from a solid mass containing sodium caseinate and microcrystalline cellulose decreased gradually in comparison with its release from intact phenytoin powder. They related that decrease to the adsorption of sodium caseinate protein on the hydrophobic surface of phenytoin crystal. The dissolution characteristics of casein–ibuprofen compacts, over a range of compositions, have been investigated .The dissolution rate of acid casein was half that of the sodium salt form. The pH values being lower in acid casein than in sodium caseinate systems with values of 5.54 and 7.13 respectively. At lower pH ibuprofen (pka 4.59) being unionized, showed a decreased dissolution than in sodium caseinate systems[11,12]. An additional factor is the more viscous and more rigid nature of acid casein gels formed at the liquid–solid interface. Swelling and erosion experiments indicated that the presence of more casein decreased the extent of swelling of the matrices and accelerated the rate of erosion, while not altering the dissolution medium infiltration rate. Phase solubility analysis indicated that the solubility of the drug was also enhanced by sodium caseinate, consistent with complex formation between the drug and casein. From the previous studies, it can be concluded that casein can modify drug dissolution from compacts. The magnitude of the effect depended on the drug loading, the form of casein, i.e. salt or free acid, and the processing method, e.g. freeze dried or a physical mixture[12].

**Casein Hydrogels**

In particular, hydrogels based on natural and synthetic polymers are of special interest in controlled release applications because of their soft tissue biocompatibility, the ease with which the drugs are dispersed in the matrix and the high degree of control achieved by selecting the physical and chemical properties of the polymer network. Caseins possess a number of favourable characteristics suitable for the development of hydrogel biomaterials, such as high hydrophobicity, good biocompatibility-particularly in oral delivery applications, lack of toxicity and availability of reactive sites for chemical modification. Genipin is found in traditional Chinese medicine and extracted from the gardenia fruit[11,13]. Casein-based hydrogel for the controlled release of bovine serum albumin (BSA) has been prepared for the first time using genipin to crosslink casein protein in an aqueous system. At pH 1.2, the swelling ratio of the hydrogel and the amount of BSA released were relatively low. The release behavior could be related to crosslinking and swelling degrees of the hydrogel networks formed by various amounts of genipin [13,14,19]. Song et al.[19,20] used microbial transglutaminase (MTGase) to assist the gelation of an aqueous casein system for controlled drug release. An increase of MTGase amount resulted in a decrease of the gelation time and an enhancement of the hydrogel strength. Compared with the casein hydrogel without the added MTGase, the enzyme- induced casein hydrogel is characterized by a more complex network structure with a higher fractal dimension. By means of this enzyme assisted gelation, Vitamin B12 as a model drug was incorporated into casein hydrogel matrix undermild conditions. This hydrogelwas found 344 to have a potential use as a new matrix for the entrapment and controlled release of Vitamin B12.

**Casein Floating Beads**

Among the different dosage forms for prolonged gastric residence, many studies have been dedicated floating systems. Such systems seemto be useful for drugs acting locally in the proximal gastrointestinal tract and drugs that are unstable in intestinal fluids but well absorbed in the stomach[19,20]. Casein has been used to prepare biodegradable microspheres by an emulsification technique .The microspheres obtained by this technique do not show any floating properties. However, casein foaming properties causes the incorporation of air bubbles that act as air reservoirs in the floating systems Casein emulsifying properties caused air bubble incorporation and formation of large holes in the beads. The percentage of casein in the matrix increased the drug loading of both low and high porosity matrices, with the loading of high porosity matrices

being lower than that of low porosity ones. The drug release rate increase in high porosity matrix, in comparison with beads without cavities, is due to the rapid diffusion of the drug through water filled pores. Both floating and non-floating systems were suitable to control drug release being biodegradable, therefore they can be proposed as carriers for the oral administration of active agents according to therapy requirements. Oil-entrapped floating casein/alginate microbeads prepared using the emulsion gelation metho were optimized by a factorial design. A polymer ratio of 1.5:0.5 (casein: sodiumalginate) by weight, and 15% w/v of oil (mineral oil/castor oil) and 1 M calcium chloride solution were selected as the optimized processing conditions for the desired buoyancy and physical stability[20,21].

**Casein Microparticles**

microspheres based on serum albumin are particularly promising for clinical use because of their inherent long term stability and their relatively high drug payloads casein microspheres may be advantageous to be used as an alternative to albumin as a matrix for microsphere drug carriers. Casein microspheres are relatively inexpensive; they have better amphiphilicity and good dispersibility in aqueous systems, and they form uniform spherical structures[22-24]. Propranolol HCl is used as a model drug which is loaded by the principle of steric stabilization**.** Morphological characterization reveals that the microparticles are in the range of 0.5-5µm in diameter. *In-vitro* release studies of Propranolol HCl loaded casein microparticles were performed using simulated gastric and intestinal fluids. The initial drug release seems to be governed mainly by diffusion, and it is expected that the remaining is released due to the degradation of the polymeric matrix. In this study it was demonstrated that

casein microparticles containing Propranonol HCl can be successfully prepared and that these microparticulate systems seem to be quite promising for controlled release applications[15,22].

**PREPARATIONS OF CASEIN MICROSPHERES**

**(1) Emulsification-chemical crosslinking**

Microcapsules (5–100 µm) through interfacial crosslinking of various proteins (human serum albumin, lysozyme, haemoglobin, casein and pepsin) with glutaraldehyde or terephthaloylchloride following w/o emulsion formation. Chen et al.[5,20] compared albumin with casein Compared with albumin, the surface charge of the casein system was more negative and the microspheres (14–38 µm) exhibited a slower in vitro release of drug. Cummings et al.[21], where two forms of doxorubicin could be distinguished chromatographically after digestion and solubilisation of microspheres with trypsin. A freely diffusible extractable formand also an immobilized non-extractable form covalently bonded to protein matrix. These bridgeswere suggested to have occurred during the glutaraldehyde crosslinking step although covalent binding of the drug was stated to occur for drug incorporated both during microsphere manufacture and by post-manufacture drug adsorption Quigg et al.[22,23] described the preparation of albumin, casein and sodium caseinate microspheres containing methotrexate and mitoxantrone by an emulsion crosslinking process. In vitro drug release was characterized by a rapid release within 1–2 h of 10–15% of the total drug loaded. In addition to cytotoxic drugs, glutaraldehyde-crosslinked casein microspheres can also be a useful alternative to the expensive synthetic polymer-based contraceptive products by reducing the overall cost of the product and treatment. An injectable system composed of the biodegradable levonorgestrel-loaded casein microspheres was developed as a monthly contraceptive system to replace the synthetic ones The manufacture of sodium caseinate microspheres loaded with hydrochlorothiazide, eosin, patent blue violet and sodium salicylate was described by Millar and Heelan and Corrigan Release of the incorporated agents from microspheres was rapid with over 90% drug release in each case within 1–2 h without degradation of the protein matrix and was attributed to the loss of surface and loosely bound drug. Further drug release occurred over the following few days at a greatly reduced rate with a certain proportion of the drug loading appearing to be indefinitely entrapped within some of themicrospheres. Addition of 0.1% trypsin to the release vessel resulted in a rapid release of the remaining drug over a period of about 2 h.[22].

(2) **Enzymatic crosslinking**

Casein is particularly an excellent substrate for enzymatic crosslinking due to its open tertiary structure. Therefore, Huppertz et al.[20,22] prepared biocompatible micro-gel particles from casein micelles crosslinked with transglutaminase (TGase) enzyme.

**(3) Coacervation and electrostatic complexation in aqueous systems**

The use of lactic acid (coacervating agent) plus hydroxypropyl cellulose (thickener) and gelatin (plasticizer) as a coacevating solution resulted in the formation of homogenous appearance of casein microparticles. The potential of those casein microcapsules as sustained release systems was assessed using acetaminophen as a model drug[23]. A novel type of protein-walled microparticles was developed by adding 1% aqueous protamine solution (pH 10) to 100 ml of a 1% 6 aqueous casein solution (pH 11) under homogenization. At the casein: protamine ratio of 5:2 (w/w), phase separation manifested in the form of white floccules which were condensed by 12 ml of 1 N NaOH[23,24]. Microencapsulation process via aqueous coacervation was appropriate for the preparation of microencapsulated systems for sustained drug delivery in a less harmful formulation.

**(4) Casein nanocarriers**

In recent years, casein-based nanovehicles were utilized for drug and nutraceuticals delivery applications varying from nanosized micelles to enzymatically crosslinked nano-gel particles, or nanoparticles prepared by graft copolymerization, heat gelation, or polyelectrolyte ionic complexation. Casein nano-sized micelle Casein micelles have received much attention in many fields such as food, cosmetics and medicine. Approximately, caseins can be thought of as block copolymers consisting of blocks with high levels of hydrophobic or hydrophilic amino acid residues. Therefore, caseins exhibit a strong tendency to self-assemble into spherical casein micelles[25]. The spherical casein micelle has a hydrophobic interior, surrounded by a hydrophilic kappa casein “hairy” layer that stabilizes the micelle through steric and electrostatic effects . The complexation of the poorly soluble chemopreventive agent curcumin with the natural nanostructure of bovine casein micelles and its application in drug delivery to cancer cells was investigated In a recent study, the interaction of caseinmicelles (CM) with gold nanoparticles (GNPs) was studied .The results showed that GNPs bind to CM surface, leading to the formation of GNP–CM conjugates, via reaction with the carboxylate or amine groups on CM

surfaces, but not by electrostatic action. Casein micelles impart stability to GNPs towards the salt concentration and pH mainly by steric interactions. Beta casein (β-CN) micelles have also been studied as nanovehicle for hydrophobic bioactives. The critical micelle concentration (CMC) of 702 β-CN ranges between 0.05% and 0.2% (w/v), depending on temperature pH, solvent composition, and ionic strength[26]. β-CN nanoparticles may serve as effective oral-delivery nano vehicles for solubilization and stabilization of hydrophobic drugs. Mandelbaum and Danino, focused their research on encapsulation of celecoxib and budesonide, both have low bioavailability into nanostructured β-casein assemblies

**(5) Graft copolymerization**

Pan et al.[27], grafted dextran to casein to increase its hydrophilicity through the Maillard reaction, which is a nontoxic.Spherical nanoparticles with a hydrodynamic diameter about 200 nmwere formedwith casein and β-carotene core and dextran shell. The hydrophilic dextran shells made the particles stable and dispersible in the pH range of 2–12. The encapsulated β-carotene can be released by pepsin or trypsin hydrolysis. This is a green process of simultaneous nanoparticle formation and encapsulation driven by hydrophobic interaction between casein and β-carotene.

**(6) Enzymatic crosslinking**:

Casein nanogel particles were more stable to heat-induced coagulation but less stable to acid-induced coagulation than native casein micelles. Casein nanogel particles offer applications not only in traditional dairy products but also in applications where the integrity and biocompatibility of the nanogel particle is important such as their use in drug delivery applications[27,28].

**(7) Heat-gelation method**

As many globular food proteins, lysozyme has good gelation properties; so upon heating a lysozyme solution a gel was obtained Pan et al. , studied the self-assembly of β-casein and lysozyme, having isoelectric points of pH 5.0 and 10.7 respectively, to form polydisperse electrostatic complex nanoparticles. The absolute values of the charges of lysozyme are respectively more and less than the absolute values of the charges of β-casein when the pH is lower and higher than 8.0[28,29].

(8) **Polyelectrolyte ionic complexation**

The pH and the ionic strength are themost important factors influencing the formation of these complexes. Both factors affect the number of charges present on the biopolymers, thus influencing the intensity of the electrostatic interactions[28,29].

## CONCLUSION

A number of in vitro and in vivo studies showed that casein is a suitable material for efficient drug delivery. Casein may be degraded by the digestive enzymes proteases. Crosslinked casein microspheres are resistant to proteolytic enzymes, and thus stable in the casein appears to be a promising carrier for the delivery of many orally as well as parenterally administered drugs. A number of casein-based colloidal systems such as micelles and nanoparticles are promising carriers for bioactive molecules. Casein formulation prepared by different techniques like nano carrier, microparticles, emulsification, crosslinking etc. By this we can understand mechanism of action of casein formulations.

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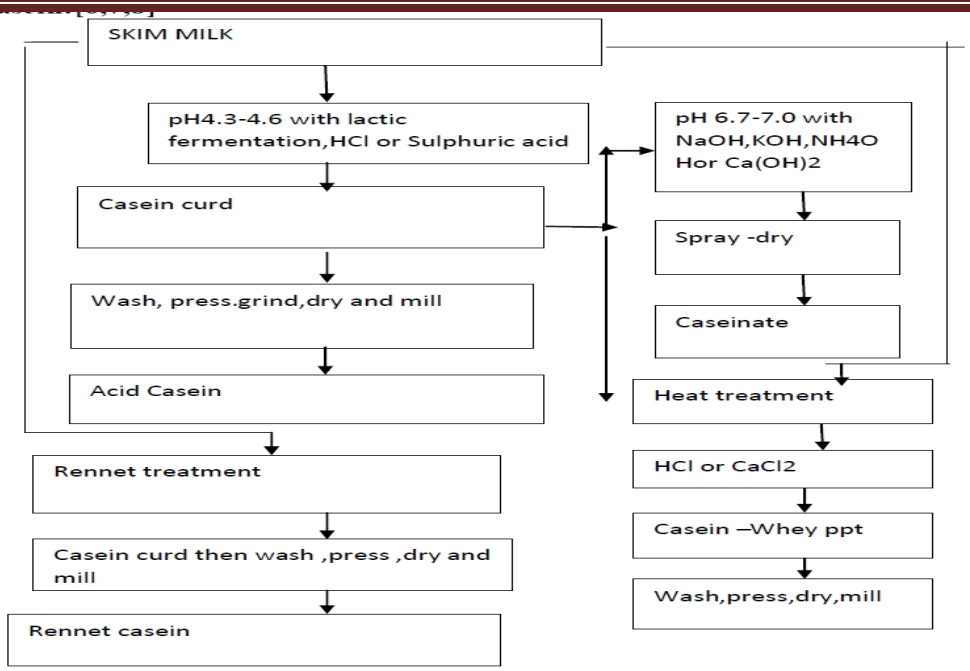


Figure 1. Production of casein

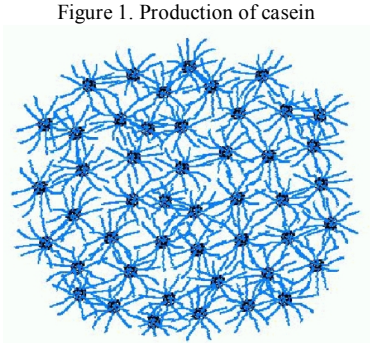

Figure 2. Structure of Casein micelle