CHAPTER 1
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1.1. Concept of Controlled Release Drug Delivery

Over the years, the treatment of patients has been accomplished by administering drugs to the body via various pharmaceutical dosages like tablets. These conventional drug delivery systems are still commonly used in the pharmaceutical industry. To maintain the drug level in the body within the therapeutic range, it is often necessary to take this type of drug delivery systems several times a day which sometimes results in undesirable and harmful level of drug in the body [1].

In the last few years conventional dosage forms of drugs are rapidly being replaced by the new and the novel drug delivery systems. Amongst, these the controlled release dosage forms have become exceptionally popular in present-day therapeutics. Controlled release may be defined as the technique or approach by which active agents are administered to specified target at a rate and duration designed to achieve the proposed result [2]. A typical controlled release system is designed to delivers the drug or active agents at a predetermined rate, locally or systemically, for a specified period of time. [3]. The release of the active agent may be constant or cyclic over a long period of time. The main purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. [4]. Controlled release drug administration means not only prolongation of the duration of drug delivery, but the term also implies the predictability and reproducibility of drug release kinetics. With traditional formulations, the drug level in the blood follows the profile shown in Figure 1.1a, in which the drug blood level rises after each administration of the drug and then decreases until the next administration. With traditional drug administration the blood level of the drug exceeds toxic level immediately after drug administration, and falls down below effective level after some time. Controlled drug delivery systems are designed for long-term administration where the drug level in the blood follows the profile shown in Figure 1.1(b), remaining constant, between the desired maximum and minimum, for an extended period of time [4].
Nowadays, controlled release technologies are receiving immense attention in pharmaceutical industry, agricultural sector and academics. There is a growing awareness that substance in drugs and agricultural chemicals are highly toxic and sometimes, ineffective when applied by conventional methods. Controlled release technologies can provide a wide range of new therapeutic opportunities in pharmaceutical sectors like, product differentiation, market expansion and patent extension [5].

1.1.1. Need for Controlled Delivery Systems
Controlled drug delivery systems present numerous advantages compared to conventional drug delivery systems, which includes improved efficacy, reduced toxicity, and improved patient compliance and convenience [6]. Controlled drug delivery systems also increase the stability of drug by protecting it drug from hydrolysis or other derogative changes in gastrointestinal tract, it minimize the local and systemic side effects, reduces drug
accumulation with chronic dosing and most importantly, improve the bioavailability of some drugs [7,8].

Such systems often use synthetic or natural polymers as carriers for the drugs. All controlled release systems aim to improve the effectiveness of drug therapy [9,10]. This improvement can increase the therapeutic activity compared to the intensity of side effects, reducing the quantity of drug administrations required during treatment and eliminating the need for specialized drug administration.

1.1.2. Demerits of Controlled Release Systems

Controlled release systems have some disadvantages compared to conventional drug release systems which includes [11]

- Delay in commencement of drug action
- Possibility of dose dumping in the case of a poor formulation approach
- Greater dependence on gastrointestinal residence time of dosage form
- Likelihood of less precise dose tuning in some cases
- Cost per unit dose is higher when compared with conventional doses
- All drugs are not suitable for formulating controlled release systems.

In recent years, much advancement has occurred in the field of controlled delivery formulations. The polymers and fillers used in these systems have become much more sophisticated, with the capability to do more than simply extending the release period for a particular active agent (i.e. drug). Current controlled release systems can respond to changes in the biological environment and deliver or cease to deliver active agents based on these changes. Scientists are exploring the potential of the different technologies in the field of controlled release drug delivery.

1.2. Fundamental components of controlled delivery formulations

The most fundamental components of controlled delivery system include (a) the polymer matrix or matrices that regulate the release of the active components (b) the active agent i.e. drug, (c) reinforcing agent and (d) the crosslinking agents. Varieties of polymers,
active agents [12], and crosslinking agents are being used for the development of controlled delivery formulations.

1.2.1. Polymers

Polymers, both natural and synthetic, are highly beneficial in preparing controlled delivery formulations. Most of the drugs are low molecular weight compounds. Remarkable advancement in polymer science and technology has made it possible to combine a low molecular active agent species physically or chemically to a polymer. In controlled delivery technique, the active agent is allowed to release from the polymer-active agent combination over a period of time, most often to a specific target. In physical combinations, polymer acts as a rate-controlling device while in chemical combinations; it acts as a carrier for the active agent.

An important advantage of polymeric controlled delivery formulation is that the toxic natures of the chemicals are minimized. Many new drugs available are highly toxic. It poses risk to non-target organs also. But if it is encapsulated or distributed in a polymer, its toxicity will be much reduced, since the entire amount does not release at one time. Still another advantage is that the polymer combinations being solids are easy to handle [13].

The success of controlled delivery formulation relies on combining the active agent with the polymer in an economic manner alongwith time maintaining the desired release profile. These are often in opposition and one has to compromise in the ultimate cost/benefit ratio of controlled delivery formulations [14]. However there are many classes of polymers which can be effectively employed in controlled delivery formulations. The efficiency of controlled delivery formulations depends on the following polymer properties-

- Solubility and distribution characteristics with the active agent.
- Solubility and distribution characteristics with the environmental agents.
- Good compatibility with the environment i.e., it should be non-toxic.
- Good compatibility with the active agent i.e., it should not produce undesirable products.
- Stability in the environment i.e., it should not degrade during the course of action.
Degradation is preferable after the completion of desired function. The degraded products should not harm the environment.

Ease of fabrication.

Cost.

1.2.1.1. Chitosan

Among the natural polymers, polysaccharides have received increasing attention because of their outstanding physical and biological properties [15]. Chitin is the second most omnipresent natural polysaccharide after cellulose and is composed of β(1→4)-linked 2-acetamido-2-deoxy-β-D-glucose (N-acetylglucosamine) which was first identified by Henri Braconnot (Director of botanical garden in Nancy, France) in 1811 [16,17]. The name chitin is derived from Greek, connotation “tunic” or “envelope”. Chitin is structurally identical to cellulose, but has acetamide groups (NHCOCH$_3$) at the C-2 position. Chitin is a white, hard, inelastic, nitrogenous polysaccharide and occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. It is also produced by a number of other living organisms in the lower plant and animal kingdoms, serving in many functions where reinforcement and strength are required [18]. In 1843, Lassaigne demonstrated the presence of nitrogen in chitin. Depending upon the source from which it is obtained, chitin occurs as three different crystalline forms, namely α, β and γ forms [19-21]. It is found from studies that γ- chitin is a variant of α- chitin [22]. The strong inter- and intramolecular hydrogen bonding and crystalline structure are mainly responsible for limited solubility in common solvents.

After the discovery of chitin, the name “chitosan” came into sight. Rouget while experimenting with chitin first discovered it. Rouget observed that the compound of chitin could be maneuvered through chemical and temperature treatments for it to become soluble. Then, it was in 1878 when Ledderhose identified chitin to be made of glucosamine and acetic acid. By partial deacetylation under alkaline conditions, one obtains chitosan, which is the most important chitin derivative in terms of applications.
Chitosan plays an important role as drug delivery systems. In 1884, Rawls introduced chitosan as an attractive candidate for treating burns [23] Chitosan is a linear aminopolysaccharide composed of randomly distributed (1→4) linked D-glucosamine and N-acetyl-D-glucosamine units and is obtained by the deacetylation of chitin, a prevalent natural polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp [24]. It can also be obtained from some microorganisms and yeasts. Structure of chitosan is shown in Figure 1.2.

Chitosan is a mucoadhesive poly cation polymer at acidic pH which is not noxious and biocompatible [15, 25-27]. Chitosan has also fungicidal effect, wound healing properties and reduces cholesterol level [28]. Owing to its cationic nature chitosan has good mucoadhesive and membrane permeation enhancing properties [29]. Chitosan offer several advantages over other synthetic polymers and natural polymers.

- Like some plant fibers, it is not digestible; therefore it has no caloric value. This is a very important property for any weight loss product.
- Absorbs and binds fat and promote weight loss [30]
- Inhibits LDL cholesterol and boosts HDL cholesterol [31]
- Promote healing of ulcers/lesions [32,33]
- It has antibacterial and anticandida properties. It has also the ability to kill certain viruses [34-36]
- Acts as antacid [37,38]
- Inhibits the formation of plaque/tooth decay [39]
- Helps to control blood pressure
- May treat and prevent irritable bowel syndrome [40]
- Helps to prevent constipation [41]
- Helps to control blood pressure [42,43]
- Reduces uric acid level in blood [44]
- Anti-tumor action [45]
- Enzymatically biodegradable
- Non toxic and biocompatible

Beside biodegradability, natural polymer offers several advantages over synthetic polymers in terms of low cost, low density, low energy consumption, and wide availability.

So far, chitosan has been utilized in various fields of pharmaceutical technology, including the formulation of controlled release dosage forms, such as tablets, gels and microspheres, as mucoadhesive and/or permeation enhancing excipient for oral, nasal, ocular and buccal drug delivery and in non-viral gene delivery.

Even though chitosan has many advantages which make it useful in drug delivery system, it is also associated with the problem of solubility. Chitosan is a weak base and is insoluble in water, but soluble in dilute aqueous acidic solutions below pKa ~6.3, in which the glucosamine units (-NH2) gets converted into the soluble protonated form (-NH3+). To improve the solubility of chitosan, it is derivatized. The chitosan derivatives also have improved mucoadhesive and/or permeation enhancing properties in addition to improved solubility. The various derivatives which are used are trimethyl chitosan, phosphorylated chitosan, thiolated chitosan, etc. The strong cohesive properties of chitosan derivatives make them highly suitable excipients for prolonged controlled drug release dosage forms.

1.2.1.2. Carboxymethyl chitosan

Carboxymethyl chitosan is one of the water soluble derivatives of chitosan. Compared to other water-soluble derivatives of chitosan, carboxymethyl chitosan (CMC) has been widely studied because of its ease of synthesis, ampholytic character and possibilities of wide range of applications. The carboxymethylation procedure of both chitin and
chitosan has been reported by Muzzarelli [47]. Figure 1.3. shows the preparation process of CMC.

![Figure 1.3. Scheme of preparation of CMC from chitosan](image)

CMC has better solubility in water, superior antibacterial property [48] and enhanced biocompatibility [49,50]. CMC exhibits low toxicity [51].

1.2.1.3. Phosphorylated chitosan (PCTS)
Phosphorylated chitosan is one of the water soluble derivatives of chitosan which can be widely used in the field of drug delivery. Phosphorylated chitosan can be prepared by heating chitosan with orthophosphoric acid and urea in DMF or by the reaction of chitosan with phosphorus pentoxide in methane sulphonic acid [52-56]. Figure 1.4. shows the process of preparation of PCTS from chitosan. Phosphorylated chitosan of high degree of substitution (DS) was insoluble in water while those of low DS were soluble.

![Figure 1.4. Preparation of phosphorylated chitosan from chitosan](image)
The insolubility of Phosphorylated chitosan of high DS may be accredited to the formation of inter- or intramolecular salt linkage between amino and phosphate groups due to the formation of poly-ion complex [57]. Phosphorylated chitosan has a wide range of applications. Phosphorylated chitosan has strong metal binding capacity. They have very high adsorption capacity for Uranium than that of any other heavy metal [54]. Phosphorylated chitosan has ability to form chelate rings with transition metal ions as well as Calcium ions [58]. Phosphorylated chitosan gel beads are also used in controlled release drug delivery applications [59-61]. Phosphorylated chitosan is used in the formation of biodegradable films, immobilization of enzymes, preservation of food from microbial deterioration, as additives (for clarification and deacidification of fruits and beverages, as emulsifying, thickening and stabilizing agents, for color stabilization etc.), and as dietary supplements. Phosphorylated chitosan is also used in tissue engineering as artificial bone scaffolds.

1.2.1.4. Soy flour

Soy (Glycine max (L.) Merr.) belongs to the leguminous family and contains nine different varieties. It was first used in United States in 1940 in bread formulations owing to its food and medicinal values [62]. Soybeans are classified as oil seeds, a stable food of nutritional value and a rich source of protein. Soy flour is made from soy beans by mechanically removing the hull, followed by extraction of the oil with hexane. Residual hexane is removed by flash desolventizer. The desolventized soy is then heat processed and ground to form the flour [63]. Soy flour is regarded as the most inexpensive vegetable protein of high quality which contains 50% protein and all of essential amino acids needed for human beings [64]. Soy flour has easy availability, good processability and is non toxic [65-68]. Soy flour lower cholesterol and thereby prevents heart attack, stroke and hypertension [69,70]. The protein and fibre in soybeans can prevent high blood sugar level and help in keeping blood sugar levels control. Soyabean promote serum insulin production, reduce bone loss that typically occurs after menopause in women and inhibit cancer development [71-73]. The uses of soy flour as a drug delivery device have not been explored much. Table 1.1. shows the composition of soy flour and Table 1.2. shows the amino acid content of soy flour.
Table 1.1. Composition of Soy flour [74]

<table>
<thead>
<tr>
<th>Content / 100 g</th>
<th>Unit</th>
<th>Content</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>kJ</td>
<td>1879</td>
<td></td>
</tr>
<tr>
<td>Protein, total</td>
<td>g</td>
<td>37.2</td>
<td>36.3 - 38.0</td>
</tr>
<tr>
<td>Fat, total</td>
<td>g</td>
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<td>20.6 - 23.8</td>
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<tr>
<td>saturated fatty acids</td>
<td>g</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>monounsaturated fatty acids</td>
<td>g</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>polyunsaturated fatty acids</td>
<td>g</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate, total</td>
<td>g</td>
<td>30.5</td>
<td>29.4 - 31.5</td>
</tr>
<tr>
<td>carbohydrate, available</td>
<td>g</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>added sugar</td>
<td>g</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>dietary fibre</td>
<td>g</td>
<td>10.4</td>
<td>9.8 - 11.0</td>
</tr>
<tr>
<td>Alcohol</td>
<td>g</td>
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</tr>
<tr>
<td>Vitamin A</td>
<td>RE</td>
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</tr>
<tr>
<td>retinol</td>
<td>µg</td>
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<td></td>
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<tr>
<td>β-carotene eq.</td>
<td>µg</td>
<td>66</td>
<td></td>
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<tr>
<td>Vitamin D</td>
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<td></td>
</tr>
<tr>
<td>D3 cholecalciferol</td>
<td>µg</td>
<td></td>
<td></td>
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<tr>
<td>D2 ergocalciferol</td>
<td>µg</td>
<td></td>
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<tr>
<td>25-hydroxycholecalciferol</td>
<td>µg</td>
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<td>Vitamin E</td>
<td>α-TE</td>
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<td></td>
</tr>
<tr>
<td>alpha-tocopherol</td>
<td>mg</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin K</td>
<td>µg</td>
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<td></td>
</tr>
<tr>
<td>Vitamin B1, thiamin</td>
<td>mg</td>
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<td></td>
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<tr>
<td>Vitamin B2, riboflavin</td>
<td>mg</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Niacin equivalents</td>
<td>NE</td>
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<td></td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>mg</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>
| Nutrient                  | Unit | Value
|--------------------------|------|-------
| Biotin                   | μg   |       |
| Folates                  | μg   | 800   |
| Vitamin B12              | μg   | 0     |
| Vitamin C                | mg   | 0     |
| L-Ascorbic Acid          | mg   |       |
| L-Dehydroascorbic acid   | mg   |       |
| Pantothenic Acid         | mg   | 1.8   |
| Sodium, Na               | mg   | 2     |
| Potassium, K             | mg   | 1936  |
| Calcium, Ca              | mg   | 150   |
| Magnesium, Mg            | mg   | 240   |
| Phosphorus, P            | mg   | 560   |
| Iron, Fe                 | mg   | 4     |
| Copper, Cu               | mg   | 1.6   |
| Zinc, Zn                 | mg   | 5     |
| Iodine, I                | μg   | 0.5   |
| Manganese, Mn            | mg   | 2.3   |
| Chromium, Cr             | μg   | 23    |
| Selenium, Se             | μg   | 11    |
| Nickel, Ni               | μg   | 390   |

Table 1.2. Amino acid contents in Soy flour [74]

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>mg/100g</th>
<th>mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucin</td>
<td>1800</td>
<td>280</td>
</tr>
<tr>
<td>Leucine</td>
<td>3200</td>
<td>490</td>
</tr>
<tr>
<td>Lysine</td>
<td>2600</td>
<td>400</td>
</tr>
<tr>
<td>Methionine</td>
<td>520</td>
<td>80</td>
</tr>
<tr>
<td>Cystine</td>
<td>650</td>
<td>100</td>
</tr>
</tbody>
</table>
1.2.2. Active Agents

The substance to be encapsulated or loaded is called active agent. It may be virtually any substance, natural or synthetic, that is entirely or partially soluble in the reaction medium or solvent. It may be solid, a hydrophobic or hydrophilic liquid, or a mixture of a solid and a hydrophobic or hydrophilic liquid. The major active agents include drugs, food products, agrochemicals and vaneties of oils [75]. The loading substance may include a purified or partially purified substance depending on the requirements of application.

1.2.2.1. Isoniazid

Isoniazid (or 4-Pyridinecarboxylic acid hydrazide or isonicotinic acid hydrazide or INH), a first line drug used for tuberculosis chemotherapy [Figure 1 5]. Its molecular formula is C6H7N3O. Isoniazid (INH) is one of the primary chemotherapeutic and prophylactic drugs used against *Mycobacterium tuberculosis*, the causative agent of tuberculosis, which is one of the leading cause of death due to an infectious agent throughout the world [76].
Isoniazid is soluble to the extent of 125 mg/mL of water at room temperature. Solubility in water varies as ~14% at 25°C, ~26% at 40°C; in ethanol: ~2% at 25°C, ~10% in boiling ethanol; in chloroform: ~0.1%. It is almost insoluble in ether and benzene. It has melting point of 171.4°C. Isoniazid is one of the key active pharmaceutical ingredients (API) used in the combination treatment of tuberculosis (TB) recommended by the World Health Organization (WHO).

The WHO recommends a dosage range from 4 to 6 mg/kg, with the maximum daily dose not to exceed 300 mg. The 300 mg maximum daily dose is also used as preventive therapy for populations at high risk. At this dose, the antibiotic is well tolerated.

The most commonly occurring adverse effect in the treatment with isoniazid is hepatotoxicity. Serious toxic symptoms have been reported to occur at doses of 2–3 g or higher in adults. Doses of 10–15 g may be fatal without appropriate treatment [77].

1.2.2.2. Curcumin

Cancer is a familiar disease of old age. It is anticipated that the process of tumorigenesis initiates approximately at the age of 20 and detection of cancer is normally around the age of 50 or later. So, the estimated incubation time is around 20–30 years. Current studies indicate that in any given type of cancer 300–500 normal genes get modified somehow to result in the cancerous phenotype.

The ineffectiveness, lack of safety, and high cost of therapies have led to a lack of faith in anticancer approaches. Many plant-based products, however, achieve multitargeting naturally and, in addition, are low-priced and safe compared to synthetic agents. However, because pharmaceutical companies are not usually able to secure intellectual property rights to plant-based products, the development of plant-based anticancer
therapies has not been prioritized. Nonetheless, curcumin, a plant-based product, has shown significant promise against cancer and other inflammatory diseases. Curcumin is one of the active components of Turmeric plant (Curcuma longa). Turmeric is a perennial herb of the Zingiberaceae family and is cultivated extensively in south and southeast tropical Asia [78].

Figure 1.6. Pharmaceutical properties of curcumin

Turmeric, i.e., the ground rhizomes of Curcuma longa, has a long history of use in food as a spice, mainly as an ingredient in many diverse forms of curry powders and sauces, where curcumin is the main colouring substance. Curcumin is first identified in 1910 by Lampe and Milobedzka [79]. Curcumin has numerous pharmacological activities, including antioxidant, antimicrobial properties, anti-inflammatory effects and anti-cancer activities [80-82]. Some other pharmaceutical properties of curcumin are described in figure 1.6 [83]. Chemically, curcumin is bis-α,β-unsaturated β-diketone (commonly called diferuloylmethane), which shows keto–enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium [84,85].
Commercial curcumin contains approximately 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin (Fig. 1.7.) [86].

![Figure 1.7. Composition of curcumin](image)

The pharmacological safety and efficacy of curcumin makes it a budding compound for treatment and prevention of a wide variety of human diseases. But, in spite of all these advantages curcumin is not considered as the therapeutic agent due to its low bioavailability. The reasons for low bioavailability of curcumin within the body are poor absorption and high rate of metabolism. Animal studies have shown curcumin is rapidly metabolized, conjugated in the liver, and excreted in the feces, therefore having limited systemic bioavailability. A 40 mg/kg intravenous dose of curcumin given to rats resulted in complete plasma clearance at one hour post dose. An oral dose of 500 mg/kg given to rats resulted in a peak plasma concentration of only 1.8 ng/mL, with the major metabolites identified being curcumin sulfate and curcumin glucuronide [87]. To solve the problem of bioavailability curcumin can be complexed with other substances, such as alkaloid piperine, phospholipid, etc. [88,89] or curcumin can be incorporated in nanoparticles.

1.2.3. Reinforcing agents

In general, the reinforcing agent or the reinforcement is the discontinuous phase in the nanoparticles or microparticles which are much stronger and stiffer than the polymer
matrix. The reinforcing agents can either fibers, particles, laminae, whiskers, or flakes and they can either be organic, inorganic, metallic or ceramic materials. They are structural constituents. They determine the internal structure of the nanoparticles and provide strength and modulus to the nanoparticles. In controlled release drug delivery the properties of the nanoparticles can be controlled by varying the quantity of the reinforcing agent. The reinforcing agents used for the present study are montmorillonite and cellulose whisker.

1.2.3.1. Montmorillonite (MMT)
Montmorillonite was first described in 1847 for an occurrence in Montmorillon in the department of Vienne, France by Mauduyt [90]. He named the compound as “montmorillonniste”. The name “montmorillonite” or the German form “Montmorillonit” appears to be first used by Naumann in 1850 [91]. Montmorillonite are sheet structured hydrous silicates which are referred to as phyllosilicates. Montmorillonite falls in the category of 2:1 smectite clay. The general formula for the chemical structure of this group is (Na,Ca)_{0.3}(Al,Mg)_{2}Si_{4}O_{10}(OH)_{2}·n(H_{2}O) [Figure 1.8.]. Montmorillonite has layered structure where each layer is composed of two types of structural sheets: octahedral and tetrahedral. The tetrahedral sheet is composed of silicon-oxygen tetrahedra linked to neighboring tetrahedra by sharing three corners, resulting in a hexagonal network [92]. The remaining fourth corner of each tetrahedron forms a part to adjacent octahedral sheet. The octahedral sheet is usually composed of aluminum or magnesium in six-fold coordination with oxygen from the tetrahedral sheet and with hydroxyl.

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![Structure of montmorillonite](Figure 1.8. Structure of montmorillonite)
The two sheets together form a layer, and several layers may be joined in a clay crystallite by interlayer cations, Van der Waals force, electrostatic force, or by hydrogen bonding. The presence of charge in tetrahedral and octahedral sheets influences the layered structure. Isomorphous substitution (i.e., the replacement of an element with another element in mineral crystal without modifying its chemical structure) in clay mineral mainly results in the charge development. For example, Al<sup>3+</sup> can replace Si<sup>4+</sup> in tetrahedral coordination, and replacement of Al<sup>3+</sup> is possible by Mg<sup>2+</sup>. Fe<sup>2+</sup> in octahedral coordination. Montmorillonite is a hydrophilic clay with an aspect ratio of 1000:1 [93]. Montmorillonite can be used in pharmaceutical fields in creams and powders, baby powders, and as face packs and therapeutic muds [94]. Montmorillonite is used as a food additive for health and stamina [95] and for antibacterial activity against tooth and gum decay [96]. It can be used for the treatment of irritable bowel syndrome, prevention of constipation and prevents intestinal adsorption of cholesterol [97]. In controlled drug delivery applications, montmorillonite can control the release of the therapeutic agents as well as adsorb dietary toxins [98]. It has antibacterial effect and is non-toxic. Due to its biomedical properties, montmorillonite is also known as “medical clay” [99].

1.2.3.2. Cellulose whiskers (CW)

Cellulose is the most abundant biopolymer available on earth, and is present in a large variety of living species, such as animals, plants and bacteria. It is a renewable polymer which is biodegradable and biocompatible, and is being exponentially considered as a green alternative to fossil-fuel based polymers. Cellulose is a fibrous, tough, water-insoluble substance, which is found in the protective cell walls of plants, particularly in stalks, stems, trunks and all woody portions of plant tissues.

Figure 1.9. Structure of cellulose
Cellulose in the plants is composed of 1,4-β-glucopyranose units associated by hydrogen bonding [Figure 1.9.] and forming a semicrystalline structure where highly ordered regions (the crystallites) are distributed among disordered domains (the amorphous phase) [Figure 1.10.].

![Figure 1.10. Schematic diagram showing the hydrogen bonding within and between cellulose molecules along with the amorphous and crystalline region in cellulose](image)

Cellulose exists as four different polymorphs. They are cellulose I, II, III and IV. Cellulose I is the form usually found in nature and it occurs in two allomorphs Iα and Iβ. Cellulose II is the crystalline form that appears after re-crystallization with aqueous sodium hydroxide, and it is thermodynamically the most stable crystalline form [100]. Cellulose IIIα and IIIβ are obtained by a liquid ammonia treatment of cellulose I and II, respectively. Cellulose IV is obtained from heating cellulose III, the alteration being usually partial [101]. Cellulose has very high elastic modulus as well as high specific strength which make it an ideal candidate to reinforce polymer matrices in the form of macroscopic fibers.

Cellulose whiskers (CW) are usually obtained from cellulose fibers through an acid treatment combined with sonication. This process involves an acid hydrolysis of the fibers using concentrated sulfuric acid (H₂SO₄), which removes disordered regions of cellulose and leaves crystalline regions intact. After this treatment, rod-like shaped cellulose nanofibers, having anionic sulfate ester groups at their surface, are produced.
Introduction

The geometrical dimensions of CW depend on the starting cellulose source, resulting in values for width varying from 5 to 20 nm, and for length from 100 nm to 1–2 μm [102]. CW has high specific strength, modulus and aspect ratio. Some other advantages of CW are their low density, renewable nature, abundance, biodegradability, and relatively low cost. As reinforcing agent, CW can significantly improve the mechanical properties of the polymers at low loading level [103]. Different types of value-added nanomaterials could be produced from CW. CW can be used to make aerogels, emulsion and foam stabilizer in food industry, DNA hybrid material, films, adhesives etc. [104-107]. CW is used in bone regeneration, artificial liver, regenerated cartilage, and suppression of action of matrix metalloproteinases (MMPs) in wound healing [108-110]. But much research has not been done in application of CW in controlled release drug delivery applications.

1.2.4. Crosslinking agents

Crosslinking is the formation of chemical links between molecular chains to form a three dimensional network of connected molecules. The crosslinking is used to control and enhance the properties of the resulting polymer system or interface. Crosslinking of polymers improves the mechanical properties as well as control release behaviour of the active agents. Depending on polymer and active agent properties, suitable crosslinkers are chosen for crosslinking. A large number of crosslinking agents, both natural and synthetic, are known to crosslink different natural and synthetic polymers. Synthetic crosslinkers such as formaldehyde, glutaraldehyde, glyceraldehydes [111-113], glyoxal [114,115], epichlorohydrin [112], sulfuric acid [111], sodium hexametaphosphate [114], sodium tripolyphosphate [116], disocyanate, carbodiimides, tannic acid etc. All these chemical crosslinking agents are relatively cytotoxic. Nowadays, biocompatible cross-linking agents have received much attention in the field of biomedical application. For example, enzyme-catalyzed cross-linking methods have been developed to crosslink some biomaterials [117]. Genipin is a natural crosslinking agent, which is both non toxic and biocompatible [118]. Genipin can be obtained from its parent compound geniposide, which may be isolated from gardenia fruits. It has been reported that genipin can spontaneously react with amino acids or proteins to form dark blue pigments [119, 120]. In the present study, in order to improve the controlled release behaviour, synthetic
crosslinker, glutaraldehyde and natural crosslinker, genipin have been used for crosslinking of polymers. Figure 1.11. shows the structure of some of the available crosslinkers.

**Figure 1.11. Structures of several crosslinking agents**

1.3. **Role of micro- and nanoparticles in controlled release drug delivery**

Microparticles and nanoparticles are of considerable interest in the field of modern day drug delivery. Several methods are available for the preparation of nanoparticles and microparticles. The current situations, therefore, provides a practical basis for developing such type of controlled drug delivery systems for further applications. Although various applications of these micro and nanoparticles, e.g., for encapsulation of drugs, enzymes or fragrances, are discussed in some literatures [121] they are yet to be applied practically. Since the majority of the proposed applications are located in the
pharmaceutical field, most of the polymers used till now are not well-suited and nanoparticles composed of biocompatible and biodegradable natural polymers are required. Generally, many of the described approaches are rather unproductive (e.g., they require very low concentrations) and hence a further challenge will be to widen up the production of the nanoparticles.

The use of micro- and nanoparticles in drug delivery is also known as particulate drug delivery systems. Particulate drug delivery systems have traveled a long way from being used for research purposes to clinical applications in the last few decades. The terms "microparticle" and "nanoparticle" refer to particles where the dimensions of the particle are measured in micrometers and nanometer respectively. Most biologically active macromolecules and agents such as viruses, membranes and protein complexes are natural nanostructures; it is believed that nano-sized structures will be capable of enhanced interaction with cell membrane and proteins [122]. Owing to their very small size, micro- and nanoparticles drug delivery systems are easy to inject in the body, can be used for inhalation as dry powders or can be used for oral drug delivery purposes [123]. Nanoparticles were first developed around 1970 and were first formulated as carriers for vaccines and anticancer drugs [124]. Later on, nanoparticles were used for ophthalmic and oral drug delivery. The main advantages of nanoparticles in biomedical applications are listed below [125]

- Nanoparticles can improve the solubility of hydrophobic drug (e.g. Curcumin)
- Nanoparticles with dual functionality can be used for diagnostic and therapeutic purposes (e.g., Fe₂O₃-Pt Nanoparticles)
- Nanoparticles can target tumors and can be used to reduce toxicity of the therapeutic drug
- Nanorobots can be used for drug release (e.g., photo- or pH-triggered drug release), thermal ablation, and hyperthermia.
- Increased surface area results in a faster dissolution of the active agent in the human body. Faster dissolution generally equates greater absorption and bioavailability

Polymer based micro-/nanoparticles are submicron size polymeric colloidal particles in which the active agents can be encapsulated within the polymeric matrix or adsorbed
onto the surface of the polymer [126]. These nanoparticles act as an excellent vehicle for delivery of a number of biomolecules, drugs, genes and vaccines to the specific sites. The prime advantages of polymeric nanoparticles are given below [127,128]

- Increases the stability of any volatile active agents
- Can be easily and cheaply fabricated in large quantities by a variety of methods
- Offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness
- The choice of polymer and the ability to amend the release of active agents from polymeric nanoparticles have made them ideal contender for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics

1.4. Fabrication techniques of nanoparticles for controlled delivery formulations

Within the broad category of nanoparticles ‘nanospheres’ refer to spherical particles and ‘nanocapsules’ applies to particles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid or even gas. Nanoparticles usually refer to a homogeneous mixture of the polymer and active agent, whereas nanocapsules have at least one discrete domain of active agent. There are many methods of preparation of nanoparticles. Some methods for the preparation of nanoparticles include two main steps. The first step constitutes the preparation of an emulsified system while the nanoparticles are formed during the second step of the process. The second step is achieved either by the precipitation or the gelation of a polymer or by polymerization of monomers. In general, the principle of this second step gives its name to the method. In some cases, the nanoparticles form in the same time than the starting emulsified system. Suitable emulsified systems can be emulsions, mini-emulsions, nano-emulsions and microemulsions.

A few other methods do not require the preparation of an emulsion for obtaining the nanoparticles. They are based on the desolvation or precipitation of a polymer in conditions of spontaneous dispersion formation or by self-assembly of macromolecules to form nanogels or polyelectrolyte complexes from a polymer solution. The methods are explained below.
1.4.1. Evaporation or Extraction of Solvent Based Process

In these methods the solvent in which the polymer is dissolved is eliminated. This elimination can be achieved by evaporation or by extraction. The formation of an emulsion is a necessary requirement. Aqueous and oily phases can be present, according to the nature of the continuum phase of the formed emulsion. The polymer is contained in the organic phase and the emulsifier is present in the aqueous phase. The emulsified organic drops containing the polymer and the active agent form micro-/nanoparticles by the elimination of the organic solvent [129,130].

1.4.1.1. Coacervation phase separation method

Coacervation method is first developed by The National Cash Register (NCR) Corporation for carbonless copy paper as well as many other applications in 1950. This method involves the phenomenon of formation of liquid rich in polymer phase in equilibrium with another liquid phase. According to IUPAC, coacervation is defined as the separation into two liquid phases in colloidal systems. The phase more concentrated in colloid component is the coacervate, and the other phase is the equilibrium solution [131]. There are two methods of coacervation available, viz. simple coacervation and complex coacervation. Both the methods are almost identical except for the process in which phase separation is carried out.

1.4.1.1.1. Simple Coacervation Method

Simple coacervation is achieved when chemical compounds having high affinity for water such as salts and alcohols are added to the aqueous polymer solution. Simple coacervation can be brought about in any aqueous polymer solution if the pH, temperature, solvent and salt are properly chosen and adjusted [132]. The added compound cause two phases to be formed, one polymer rich phase and the other one poor. The whole process can be explained by the following three steps [133].

1. Dispersion of the core material in aqueous solution of polymer
2. Creation of insufficiency of water for hydrophilic colloid and the deposition of the coacervate around the core
3. Gelation of the coacervate and hardening of the nanoparticles
Figure 1.12. illustrates the preparation steps of nanoparticles by simple coacervation method. Microcapsules with different natural polymers have been produced by this method.

![Figure 1.12. Schematic diagram showing important steps in simple coacervation method: (a) Dispersion of the core material in aqueous solution of polymer, (b) deposition of the coacervate around the core and (c) hardening of the nanoparticles](image)

**1.4.1.1.2. Complex Coacervation Method**

Complex coacervation commonly refers to the liquid-liquid phase separation that results when solutions of two oppositely charged polymers are mixed, resulting in the formation of a dense polymer-rich phase, the precursors of which are soluble complexes [134]. The encapsulation process in complex coacervation involves four major steps:

1. Preparation of the hydrophilic colloid solution
2. Addition of second hydrophilic colloid solution of opposite charge to induce coacervation
3. Deposition around the core
4. Gelation of the coacervate and hardening of the nanoparticles

The core material (usually oil) is first dispersed into a polymer solution (e.g., a cationic aqueous polymer). The second polymer (water soluble, anionic) solution is then added to the prepared dispersion. Deposition of the shell material onto the core particles occurs when the two polymers form a complex. This process is initiated by the addition of salt or by changing the pH, temperature or by dilution of the medium. The shell thickness can be obtained as desired by controlled addition of the second polymer. Finally, the prepared
nanoparticles are stabilized by crosslinking, desolvation or thermal treatment. Complex coacervation is used to produce nanoparticles containing fragrant oils, liquid crystals, flavors, dyes or inks as the core material. Porous nanoparticles can also be prepared using this technique. When using this technique, certain conditions must be met to avoid agglomeration of the prepared capsules [135]. Figure – shows the steps of complex coacervation methods [136].

1.4. 2. Emulsion Based Process
1.4.2.1. Single emulsion
The particulate carriers of natural polymers, i.e. those of proteins and carbohydrates are prepared by single emulsion technique. In the first step the polymers are dissolved or dispersed in aqueous medium followed by dispersion in the non aqueous medium eg. Oil. In the second step, cross linking of the dispersed polymeric globule is carried out either by means of heat or by using chemical crosslinkers. The chemical cross linking agents used are gluteraldehyde, formaldehyde, terephthalate chloride, diacidchloride, etc. [137,138] Crosslinking by heat is accomplished by adding the dispersion to previously heated oil. Heat denaturation is not suitable for the thermolabile drugs while the chemical cross-linking suffers disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation [139].

Figure1.13. Example of complex coacervation involving (a) dispersion of the core, (b) initial coacervation after addition of coacervation agent, (c) coacervation on the surface of the core and (d) formation of the cross-linked shell by reticulation of the interface.
This process involves oil-in-water (o/w) emulsification. The o/w emulsion system consists of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase containing a dissolved surfactant. Once the emulsion is formed, it is subjected to solvent removal by either evaporation or extraction process to solidify the polymer droplets. In the case of solvent removal by evaporation, the emulsion is maintained at a reduced pressure or at atmospheric pressure and the stir rate is reduced to enable the volatile solvent to evaporate.

A major problem with this technique is a poor encapsulation efficiency of moderately water soluble and water soluble compounds, which partitioned out from the organic dispersed phase into the aqueous continuous phase. Successfully entrapment of drug within the microspheres is thus highly dependent on solubility in the aqueous phase.

Water soluble drugs (e.g. caffeine and salicylic acid) could not be entrapped within the poly (lactic acid) (PLA) microsphere using an Oil/Water emulsion method, while drugs with low water solubility, such as Diazepam, Hydrocortisone and Progesterone were successfully retained within the microspheres [140].

In order to increase the encapsulation efficiency of water soluble drugs, an oil-in-oil emulsion method was developed [141]. In this method, the drug may be dissolved or suspended in the oil phase before being dispersed in another oil phase. The processing scheme for nanoparticle-preparation by single emulsion technique shown in Figure 1.14.[142].

![Figure 1.14. Processing scheme for nanoparticle-preparation by single emulsion technique](image-url)
1.4.2.2. Double emulsion technique

This method for preparation of nanoparticles was reported to overcome the problem of low encapsulation efficiency of water soluble drug prepared by conventional water/oil emulsion solvent evaporation method.

This method involves the formation of the multiple emulsion or double emulsion of type w/o/w. It is best suited to water soluble drugs, peptides, proteins and vaccines. This method can be used with both the natural as well as the synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is then subjected to the homogenisation or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in formation of a double emulsion. Emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction process. The solvent evaporation is carried out by maintaining emulsion at reduced pressure or by stirring the emulsion so that the organic phase evaporates out. The emulsion is then added to large quantity of water into which organic phase diffuses out. The solid microspheres are subsequently obtained by filtration and washing with n-hexane, acetone or any organic solvent to remove traces of oil from the surface [143,144]. The Processing scheme for nanoparticle-preparation by double emulsion technique is shown in Figure 1.15. [142]

![Processing scheme for nanoparticle-preparation by double emulsion technique](image-url)

Figure 1.15. Processing scheme for nanoparticle-preparation by double emulsion technique
1.4.3. Ionotropic Gelation Technique

Ionotropic gelation is based on the ability of polyelectrolytes to crosslink in the presence of counter ions to form hydrogel beads known as gelispheres. Gelispheres are spherical crosslinked hydrophilic polymeric entities capable of extensive gelation and swelling in simulated biological fluids and the release of drug through it controlled by polymer relaxation. The hydrogel beads are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations. The cations diffuse into the drug-loaded polymeric drops, forming a three-dimensional lattice of ionically crosslinked assembly. Biomolecules can also be loaded into these gelispheres under mild conditions to retain their three-dimensional structure [145, 146]. In ionotropic gelation technique, there are natural polymers can be used as drug carriers due to their biocompatibility and biodegradability. The natural or semisynthetic polymers such as Alginates, Gellan gum, Chitosan, Pectin and Carboxymethyl cellulose are widely used for the encapsulation of drug by this technique [147]. These natural polyelectrolytes contain certain anions/cations on their chemical structure which forms network structure by combining with the counter ions and induce gelation by crosslinking. The important steps of ionotropic gelation are shown in figure 1.16 [148].

### Figure 1.16. Important steps of ionotropic gelation

<table>
<thead>
<tr>
<th>Polyelectrolyte solution</th>
<th>Countercation solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Sodium Alginate (-)/Gellan gum (+)/CMC (+)/Pectin (+)/Chitosan (+) + Drug]</td>
<td>[Calcium chloride solution (+)/Sodium tripolyphosphate (-)]</td>
</tr>
<tr>
<td>Added drop wise under magnetic stirring by needle</td>
<td></td>
</tr>
</tbody>
</table>

Gelispheres
1.4.4. Desolvation method

Desolvation technique is mainly used for the preparation of nanoparticles for protein and polysaccharides [149]. Desolvation is a thermodynamically driven self assembly process for polymers to prepare nanoparticles. The polymer from an aqueous phase can be desolvated by pH change or change in temperature by suitable counter ions. Crosslinking can be done in the desolvation step. Desolvation technique involves three main steps: polymer dissolution, polymer aggregation and polymer deaggregation. Sodium sulphate, acetone, isopropanol, ethanol etc. can be added as desolvating agents. Both hydrophobic and hydrophilic drugs can be entrapped in nanoparticles using this technique [150]. The desolvation technique is illustrated in Figure 1.17.

![Schematic diagram of desolvation technique](image)

**Figure 1.17. Schematic diagram of desolvation technique**

1.4.5. Spray Drying

Spray drying is a technique used in pharmaceutical industry to produce dry powder from liquid phase by spraying in hot drying medium [151]. This technique has also been
employed as a microencapsulation method because it can be adapted to the development of different systems, microspheres or microcapsules, depending on the initial aqueous formulation, a solution, a suspension or an emulsion [152]. It is a continuous process which involves several steps, such as, atomization, mixing of spray with drying gas, evaporation and nanoparticle separation. The steps in spray drying is depicted in Figure 1.18. [153].

Polymers capable of forming a film over the surface of drying droplets have been included in formulations for spray drying to form microspheres. Among the applications, the suitability and ability of the polymer to modify the release characteristics by spray drying process can be identified as, for nasal drug delivery [154], modify mineral release in food fortification [155] and developing mucoadhesive delivery systems [156].

![Figure 1.18. Schemation diagram of spray drying technique](image)

1.4.5. Other fabrication techniques

Some other methods of nanoparticle preparation are also available which includes sol-gel method, pan coating, layer by layer (LBL) techniques, electrostatic encapsulation etc.
References


[44] Maekawa, A, & Wada, M Food containing chitin or its derivatives for reduction of blood and urine urea acid Jph Kokai Tokkyo Koho, 1991


[74] National Food Institute- Technical University of Denmark (DTU), Danish Food Composition Databank – ed 7.01, 2009


