Calcium phosphate nanoparticle delivery for medicine

Zuxriddinov Biloliddin
Year 1, Medical school, Central Asian University, Tashkent, Uzbekistan

Abstract. Calcium phosphate nanoparticles have been used for drug delivery in recent decades due to their biocompatibility, biodegradability, profitable characteristics, and strong bond formation with various biological substances and drugs. For instance, nucleic acids (DNA, RNA, etc.), proteins (albumin, immunoglobulins, osteocalcin, etc.), and drugs (cisplatin, carboplatin, doxorubicin, etc.). Synthetic methods of calcium phosphate nanocarrier preparation were the main issue during the last decades. Various methods are introduced to synthesize the nanocarriers, such as flame-spray pyrolysis, sol-gel synthesis, etc. However, the product of these methods appears to be toxic because of the organic solvents used during synthesis. In addition, these methods were not suitable for most biological molecules, because the medium during synthesis involves high temperatures. The issue lasted until the wet chemistry method was designed. The wet chemistry synthesis proceeds in aqueous solution and at room temperatures which makes it more biocompatible, economically profitable and nontoxic. This review intends to give general knowledge about calcium phosphate nanoparticles and a brief introduction to synthetic methods presented above. Therefore, the review introduces several classifications of the drug entrapment methods designed in the last few decades. Additionally, it introduces several calcium phosphate nanoparticles with different composition and their difference in medical applications. The review finishes with a short introduction to the action mechanism of the nanocarrier loaded with drug substances or biological molecules.

Key Words. Calcium phosphate nanoparticles, flame-spray pyrolysis, sol-gel synthesis, wet chemistry synthesis.

1. **Introduction.** A nanoparticle is referred to small-sized material from 1 to 100 nm. They have become objects of study in many fields of science because they have different chemical and physical properties. Properties like surface-to-volume ratio, solubility, melting point, etc., of nanoparticles are dependent on the size of the particle compared to bulky materials. In the field of medicine, one of the roles of nanoparticles is delivering substances to the targeted destination, eliminating their effect on non-targeted cells or tissues. The major benefit of nanoparticles is a rise in the solubility of substances carried by the nanoparticles. Additionally, the possibility to attach receptors that will initiate the effect of the drug, after it has connected to target cells or tissues, is crucial.

Several trials were made to synthesize or extract specific compounds from nature. Each case had advantages as well as disadvantages. For instance, Cyclodextrins – sugar-based cyclic molecules – have a strong affinity to catch drug substances in their inner cavity and high solubility **[1]**. In addition, sugar-based molecules are easily transformed to attach to specific receptors. On the other hand, the biodegradability of Cyclodextrins

is very poor, and the volume of the inner cavities of Cyclodextrins is not suitable for relatively small molecules. Calcium phosphate-based nanocarriers are introduced to overcome the biodegradability, biocompatibility, and economic efficiency of modern drug carrier agents. Calcium phosphate is a component of bones and teeth, which makes drug carriers based on calcium phosphate degradable in the body. Calcium phosphate-based nanocarriers are highly biocompatible, which is due to the fact that they are stable at neutral pH (e.g., blood circulation) but dissolve easily inside cells at lower pH.

This review highlights calcium phosphate nanoparticles as a drug carrier. First, we shall discuss different synthetic preparation methods of nanoparticles as well as drug entrapment methods. Furthermore, we shall look at features of the nanoparticles and their stability in different media. Finally, we shall discuss the action mechanism of the drug to apply it to drug design. The review intends to give a clear understanding of drug preparation and its effect on the action mechanism of the drug.

2. Synthetic preparation methods and characterization of calcium phosphate nanoparticles.

2.1. Unsuccessful attempts to synthesize calcium phosphate nanoparticles.

In general, calcium phosphate synthesis requires high control over nanoparticle generation by temperature of reaction environment, molar ratio of reagents, reaction time, and solvent to achieve the expected size and shape of the product. For instances, methods like sol-gel chemistry, flame-spray pyrolysis, solid-state reactions etc. are used for the synthesis. The sol-gel synthesis is based on the reaction of calcium and phosphate sources on metallic implants **[2]**. The nature of the product is hydrophobic, highly soluble nanorods, which can be coated with ligands. The flame-spray pyrolysis is chosen for large-scale synthesis **[3]**. The method is based on the reaction of precursors in the flame at high temperature, forming nanoparticles.

Although the applied environment for the synthesis gives some control over particle size and shape, irreversible **particle agglomeration**[1], difficulty attaching drugs during synthesis, toxicity of solvents, and difficult purification significantly decrease the biocompatibility of the drug compared to reagents. However, these methods are not useless in the field of medicine. For example, they are used as structural biomaterials in bone and tooth implants.

2.2 Wet chemistry method to synthesize calcium phosphate nanoparticles.

The wet chemistry method is introduced to overcome toxicity and impurity of the nanocarriers **[4]**. The wet chemistry method is a chemical method for nanostructured material synthesis, such as calcium phosphate nanoparticles. This method is more economical and simpler than the methods discussed above, which require expensive equipment to achieve a constant high temperature. One of the refinement methods of wet chemical synthesis is coprecipitation method. This method involves pipetting a solution of $CaCl_2$ for around 20 seconds into another solution containing organic/inorganic regulators, stabilizers, and Na_3PO_4 in buffer (pH = 7.5). For instance, Kazunori Kataoka

[1] *Particle agglomeration – collision of nanoparticles due to gravitational force, forming bulky particles.*

et al. **[5,6]** synthesized a hybrid calcium phosphate nanocarrier. The nanocarrier system is composed of calcium phosphate, polyethyleneglycol (PEG), and charge conversional polymer (CCP). Despite the simplicity of the method, TEM (transmission electron microscopy) revealed synthesized nanoparticles with relatively homogenous shape and size of around 40 nm **[5]**. It allows the preparation of drugs containing nanoparticles attached to large biomolecules, and to avoid denaturation of these biomolecules due to the lower-temperature conditions during synthesis.

3. Development of a drug based on calcium phosphate nanoparticles.

3.1. Characterization of calcium phosphate nanoparticles.

Properties of calcium phosphate nanoparticles noticeably depend on the ratio of materials. The maintenance of the ratio of materials is essential to choose targets for the drug. Assuming that the drug is supposed to be consumed orally and react in the blood, the drug should be stable in the stomach (pH = 1.0-3.0) but unstable in the blood (pH = 7.3-7.5). Several nanoparticles and their pH stability range are presented in Table 1. Ca/P molar ratio appears to be directly proportional to the pH stability range. For instance, MCPM (Ca/P = 0.5) is stable at pH = 0.0-2.0, whereas HAp (Ca/P = 1.67) is stable at pH = 9.5-12.0. Current nanoparticles are prepared using NaH_2PO_4, Na_2HPO_4, Na_3PO_4 and $CaCl_2$ as the reaction below:

$10CaCl_2 + 6Na_3PO_4 + NaOH \rightarrow Ca_{(9+x/2)}(PO_4)_6(OH)_2 + (18+x)NaCl + (1-x/2)CaCl_2$

Table 1. Ca/P Molar Ration dependence on pH stability **[7]**.

Ca/P Molar Ratio	Name	Formula	pH Stability Range
0.5	MCPM	$Ca(H_2PO_4)_2 \cdot H_2O$	0.0-2.0
1.0	DCPA	$CaHPO_4$	2.0-5.5
1.0	DCPD	$CaHPO_4 \cdot 2H_2O$	2.0-6.0
1.3	OCP	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$	5.5-7.0
1.2-2.2	ACP	$Ca_xH_y(PO_4)_z \cdot nH_2O$ n=3-4.5	5.0-12.0
1.5-1.67	CDHAp	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ (0<x<2)	6.5-9.5
1.67	HAp	$Ca_{10}(PO_4)_6(OH)_2$	9.5-12.0

3.2. Attachment of a drug substance to calcium phosphate nanoparticles.

The basis of the drug attachment to the nanocarriers is ionic bond formation between the Ca^{2+} ion and functional groups of the drug, which can act as a ligand, as shown in Figure 1. Common functional groups in biological compounds are phosphate group ($-PO_3H^-$, nucleic acids), carboxyl group (-COOH, hormones), amino group ($-NH_2$, proteins), etc. However, the presence of additional ions around the nanoparticles may result in particle agglomeration or dissolution. Therefore, theoretically allowed biomolecules should be checked for possible errors. Despite the disadvantages, calcium

phosphate nanocarriers appear to be suitable for surface functionalization, which is necessary to achieve the expected therapeutic effect.

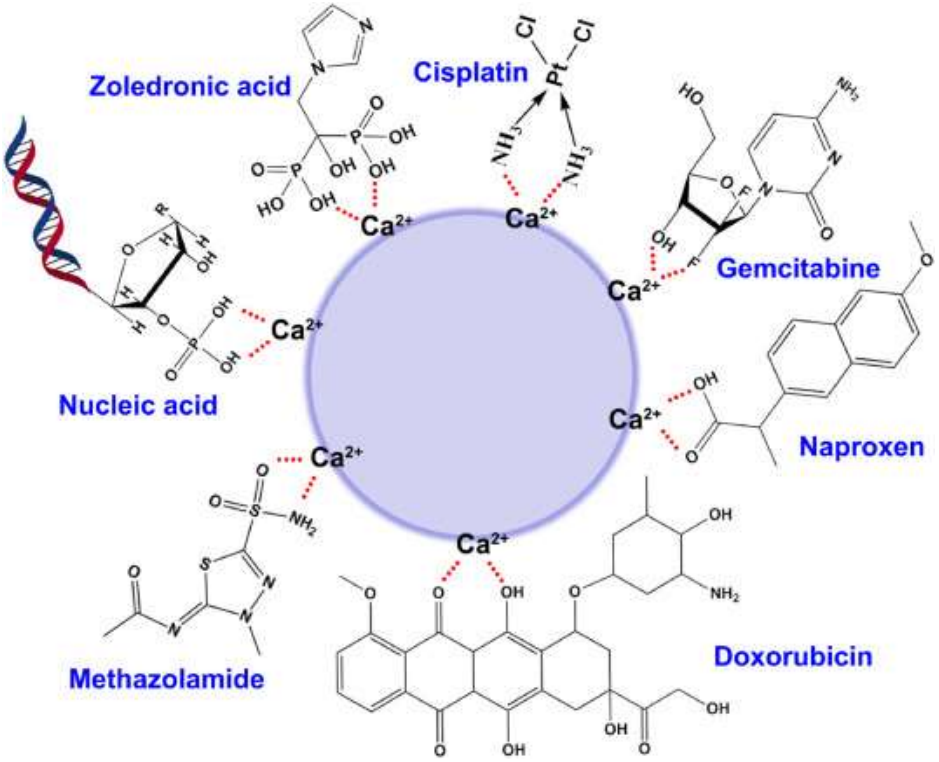

Figure 1. Illustration of the ionic bond between Ca^{2+} of nanocarriers and different biological molecules **[7]**.

Various methods of drug entrapment in the nanocarrier were classified into four main groups, and an illustration of these methods is shown in Figure 2: (1) To prepare a "Mix-CaP" as in Figure 2A, the Ca^{2+}, PO_4^{3-} aqueous solutions, and drug or nucleic acid were rapidly mixed. The product is a calcium phosphate nanoparticle containing the drug and nucleic acid in the inner spaces; (2) The product of Figure 2A is first prepared as the starting point. Other materials are then coated on the surface of the nanoparticle with a "Polymer Shell" (figure 2B) or "Lipid Shell" (figure 2C). The product is coated with an additional outer layer, which can be modified with receptors or antigens; (3) A "Multi-Layer" (figure 2D) method involves precipitation of Ca^{2+} and PO_4^{3-} multiple times. The product has multiple layers which can be modified in different manners; (4) A "CaP-Shell" (figure 2E) method involves precipitation of Ca^{2+} and PO_4^{3-} on the prepared core containing drug molecules. In addition, a combination of other methods with this method is also possible to prepare a complex nanocarrier.

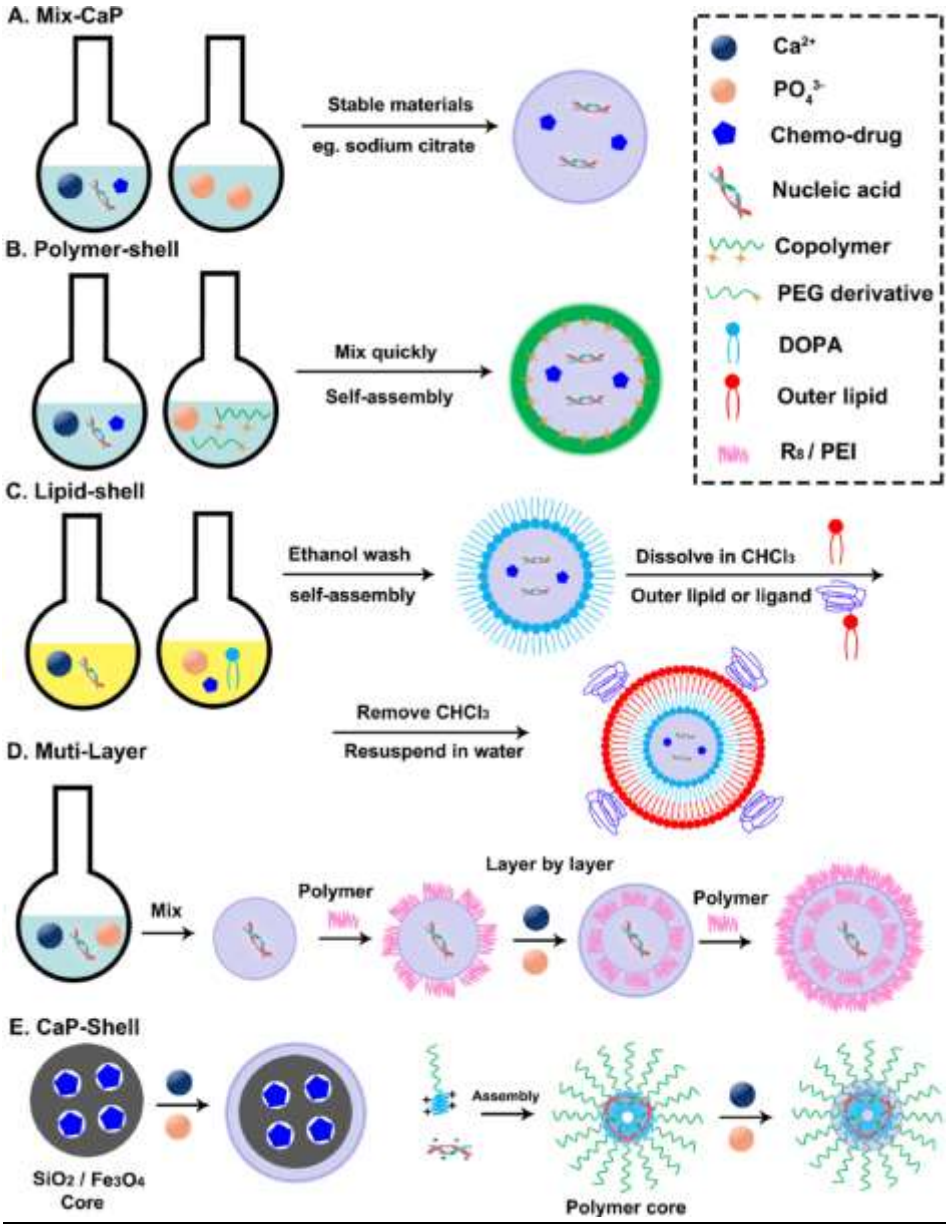

Figure 2. Four main methods of drug entrapment into nanocarriers **[7]**.

3.3. Medical application of calcium phosphate nanoparticles.

The major uses of nanocarriers loaded with drug molecules are to target cancer cells, liver cells, and bone tissues. However, there were cases when nanocarriers targeted epithelial cells, stem cells, neurons, etc. The main mechanism of cellular uptake by cells is endocytosis followed by several steps until drug is released from the nanocarrier to the cellular medium. First, the nanocarrier should reach the targeted destination to start cellular uptake. Subsequently, the nanocarrier enters the cell through endocytosis and moves towards the lysosome. Lysosome fuses with the endosome containing the nanocarrier to start nanocarrier dissolution due to low pH inside the lysosome, releasing the drug. Excessive Ca^{2+} and HPO_4^{2-} ions create osmotic pressure inside the lysosome, rupturing the lysosome. As a result, drug substances, Ca^{2+}, and HPO_4^{2-} ions are consumed by the cell, or excessive ions are pumped out **[8]**.

4. Limitations and future directions.

In general, calcium phosphate nanocarriers are very useful in drug delivery due to the reasons discussed previously. However, they have some limitations due to chemical properties. First, constant consumption of drugs on the basis of calcium phosphate nanocarrier will noticeably increase the concentration of Ca^{2+} and HPO_4^{2-} ions in the target cells and blood stream. High concentration of Ca^{2+} ions in the bloodstream will affect heart rhythm, causing bradycardia (slow heart rhythm), ventricular arrhythmias (heartbeat without repeating units), etc. Secondly, irreversible agglomeration of the calcium phosphate nanocarriers in physiological solutions prevents them from cellular uptake. Trials to reduce agglomeration by decreasing the concentration of calcium phosphate nanocarriers will result in low efficiency of the drug. Additionally, nanocarriers show low ability to bind protein molecules (receptors, antigens, etc.) due to the size ratio of nanocarriers and protein molecules. A possible solution to increase the dose of the drug will result in the drawback mentioned earlier. Lastly, calcium phosphate nanocarriers are easily detected and attacked by the immune system (macrophages, liver, spleen) of the organism. It results in low efficiency of the drug to target cells and a short time of active dose in the blood.

The future direction of calcium phosphate nanocarriers is related to drug design to reach the expected destination without being detected by the immune system of the organism. It will allow a low dosage of the drug while keeping the high efficiency of therapy. Possibly, cancer therapy is one of the directions, considering that the possibility of toxins attacking healthy cells is eliminated.

5. Conclusion.

In human history, nanoparticles are the only material of an era with a similar composition to previous eras. The difference between bulky and nanosized materials occurs in various properties. This phenomenon created many opportunities for material scientists to synthesize nanoparticles of different substances. One of the successful examples of nanoparticles is calcium phosphate nanoparticles, discussed in this article. There were several attempts to synthesize the calcium phosphate nanoparticle, but the product appeared to be toxic due to the use of organic solvents. Additionally, these methods demanded constant high temperatures which made them expensive. However, the issue was addressed when wet chemistry synthesis was discovered. It requires room temperature and proceeds in aqueous solution. The wet chemistry method was used to synthesize different types of calcium phosphate nanoparticles. Subsequently, their characteristics were studied to find suitable nanoparticles for drug design.

The drug design begins with searching suitable nanoparticle and its action mechanism for the expected effect of the drug. For instance, ACP can be used to carry nucleic acid, because it only degenerates when nanoparticle reaches lysosomes inside the cell due to low pH. Therefore, its content is released to the cytoplasm and consumed by the cell/nucleus. It follows with the drug load to the nanoparticles. There are several classes of loading, which can be used for drug entrapment as well as receptor/antigen attachment.

Disclosure of Interests. The author has no competing interests to declare that are relevant to the content of this article.

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