World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

In vitro microscopic study of calcium hydrogen phosphate dihydrate crystals growth patterns

Salman Ahmed[1], Muhammad Mohtasheemul Hasan[1*] and Zafar Alam Mahmood[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan
[2]Colorcon Limited – UK, Flagship House, Victory Way, Crossways, Dartford, Kent, DA26 QD-England

Received: 30-11-2015 / Revised: 24-12-2015 / Accepted: 30-12-2015 / Published: 01-01-2016

ABSTRACT

The purpose of the study is to explore the possible morphological features of calcium hydrogen phosphate dihydrate (brushite) crystals at a glass slide. The study was carried out on a glass slide under microscope to observe the growth patterns. As a result needle, platy, star shape, tetragonal bipyramidal shape crystals were observed. Different patterns of platy crystals like plates with spatial branches and radiating assemblage of platy crystals were also found.

Keywords: Brushite, calcium hydrogen phosphate dihydrate, crystallization, microscopic study, urolithiasis.

INTRODUCTION

The kidney contains mineral deposits in various phases of calcium salts such as calcium oxalate and calcium phosphate. Calcium hydrogen phosphate dihydrate ($CaHPO_4.2H_2O$) / brushite is a stable form of calcium phosphate which exist in the form of kidney and bladder stone. Brushite or CHPD belongs to monoclinic type crystal with unit cell dimensions: a = 9.973 Å, b = 7.288 Å, c = 6.293 Å and β = 106.87°. CHPD comes into the blood stream in the form of prepared supplements such as breakfast cereals, dog treats, enriched flour, poultry feeds, noodle products etc. Its journey in blood stream ends up forming crystal aggregates (stones). CHPD Crystal growth occurs due to the following reaction.

$CaCl_2 + H_3PO_4$ --------> CaHPO4 + 2HCl

The present study was carried out on glass slides by using reagents of single diffusion gel technique to observe the growth habits of calcium hydrogen phosphate dihydrate [1, 2]. The objective of the study was to provide detailed information about the morphology and aggregation patterns of brushite crystals and to develop an *in vitro* model for brushite crystals growth. In future, this will provide a unique path for different herbal extracts, infusions and decoctions will be applied to determine qualitative evaluation of their promoter or inhibitory effects on brushite type crystals.

MATERIALS AND METHODS

Apparatus and Instruments: Nikon Eclipse E 400 binocular microscope, Japan ; Ricoh CX4 Digital Camera, Japan ; Microscope slides 25.4 x 76.2 (1 " x 3 ") Universal Health Care Products, China ; Whatman filter paper # 02, Whatman International Ltd., England.

Chemicals and Reagents used: Calcium chloride dihydrate, orthophosphoric acid, sodium silicate solution (Merck, Germany).

Method of crystal growth: The different stages of calcium hydrogen phosphate dihydrate (brushite) crystal growth were studied under a compound microscope. Crystals were grown on glass slide at 26 ± 2 °C . A drop of gel media (1.06 g / ml sodium meta silicate solution + 1M orthophosphoric acid solution) in the pH range 4.99-5.09 was put in the middle of a glass slide. Gel media were allowed to form a good quality gel. After that a drop of the 1M calcium chloride solution was dropped to the gel. The glass slide was observed till it becomes completely dry.

RESULTS AND DISCUSSION

Different patterns of growing CHPD crystals were observed like elementary needles, needle clusters, an assemblage of needles with platy crystals, plates

***Corresponding Author Address: Muhammad Mohtasheemul Hasan,** Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan; E-mail: mohassan@uok.edu.pk*

with spatial branches, radiating assemblage of platy crystals, star shape, tetragonal bipyramidal, till 107 mins under 4x magnifications. After 108 mins CHPD crystals convert into cubical calcium oxalate monohydrate (COM) crystals (observed by 10x) also forms till 177mins (complete dryness of glass slide). CHPD crystals enable the nucleation of calcium oxalate monohydrate (COM) because it precipitates most readily in the urine environment at pH less than 6.9[2]. Biomineralization is considered to be very important in life sciences with respect to crystal deposition diseases. These diseases such as urinary stones and gall stones are associated with the presence of microcrystals which contribute to tissue damage and causing pain. Crystal deposition disease comprise of a cascade of simple mechanical effects, resulting blockage of ducts or hardening and weakening of flexible tissues by successive aggregation and crystal growth. Calcium oxalate and calcium phosphate are the commonly found calcium salts in kidney stone disease. Calcium phosphate minerals are considered as initiator for kidney and urinary bladder stone formation. Calcium hydrogen phosphate dihydrate (brushite) a stable form of calcium phosphate is found under various pathological conditions including kidney stones, some forms of arthritis, and caries[3]. Previous studies have shown different morphology of harvested CHPD crystals by single diffusion gel technique as a star, platelet[1, 4] needle[1], thin platelets of needle shape, blade shape with curved edges, and a coffin lid (prismatic nature) like[2], sword and leaf like[5]. CHPD acts as a precursor to form apatite [Ca_{10} (PO_4) $_6$ ($OH)_2$], an important bone forming mineral. It is found under various pathological conditions including kidney stones, some forms of arthritis, and caries[6]. brushite is considered as a transient precursor for octacalcium phosphate and hydroxyapatite phases. And thus, calcium phosphate minerals are thought to be the initiator of stone formation in the kidney and/or bladder, under favorable physiological environment[2]. *In vitro* investigations of the nucleation, growth and the kinetic studies of the crystalline components in the stones are very important to study different phases in case of growth inhibition and promotion. In the present work reagents of single diffusion method was used to grow brushite crystals by using sodium meta silicate (hydrogel). Crystallization of brushite crystals was investigated in silica gel on glass slide under compound microscope.

CONCLUSION

Calcium hydrogen phosphate dihydrate are formed as elementary needles, needle clusters, an assemblage of needles with platy crystals, plates with spatial branches, radiating assemblage of platy crystals, star shape crystals, tetragonal bipyramidal. It was a preliminary study and doesn't have any quantitative and statistical analysis. Now, the authors are focusing different other scientifically based authentic aspects of the same study.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this paper.

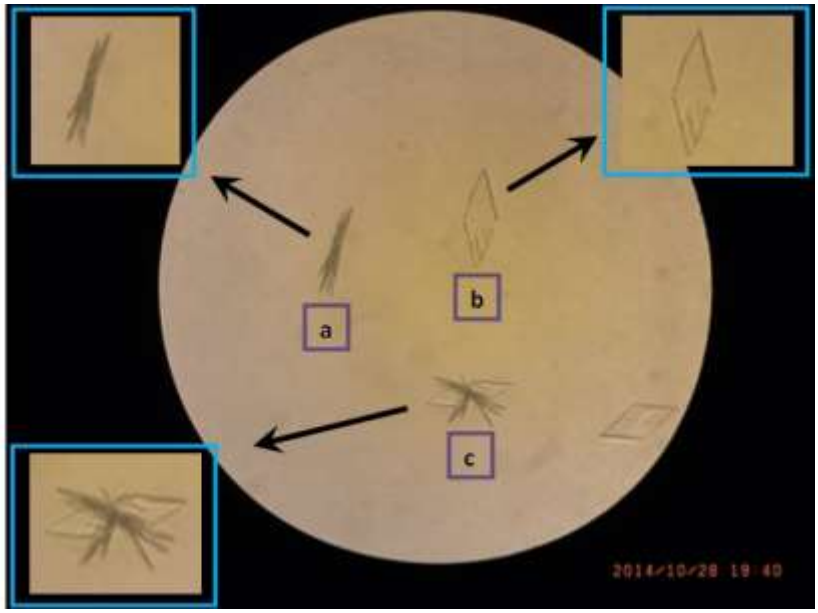

Figure 1.1: Formation of CHPD crystals. a. needle clusters ; b. platy crystals c. aggregation of platy and needle crystals.

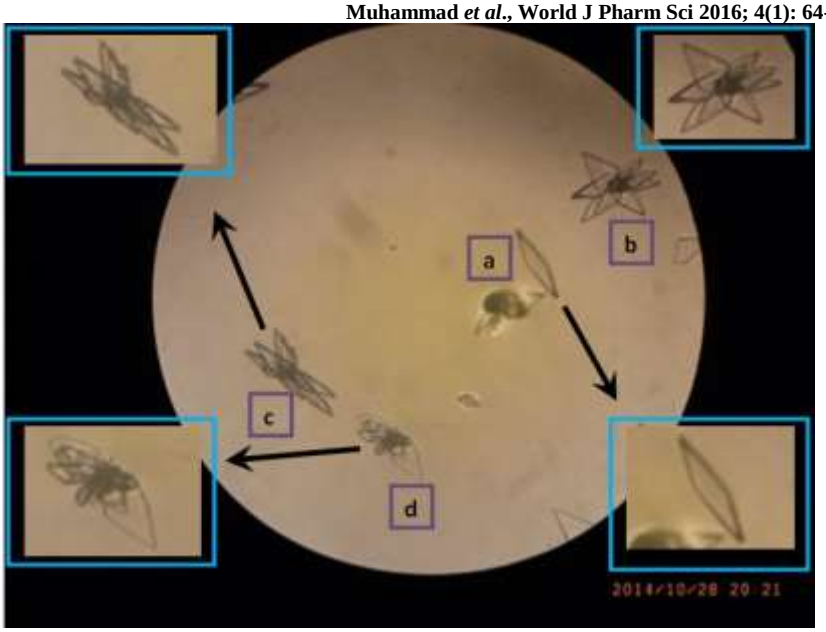

Figure 1.2: Formation of CHPD crystals. a. tetragonal bipyramidal ; b. star shape; c and d. plates with spatial branches.

Figure 1.3: Formation of CHPD crystals. a. tetragonal bipyramidal ; b. star shape.

Figure 1.4: Conversion of CHPD tetragonal bipyramidal crystals into COM cubical crystals.

REFERENCES

1. Joseph K et al. Inhibition of growth of urinary type calcium hydrogen phosphate dihydrate crystals by tartaric acid and tamarind. Curr Sci 2005; 88(8): p. 1232-8.
2. Rajendran K, Keefe CD. Growth and characterization of calcium hydrogen phosphate dihydrate crystals from single diffusion gel technique. Cryst Res Technol 2010; 45(9): 939-45.
3. Selvaraju R, Vasuki G. Growth of calcium hydrogen phosphate dihydrate (CHPD) crystal and characterization studied by spectral method. Int J Current Adv Res 2013; 3(10):40-2.
4. Parekh BB, Joshi M. Crystal growth and dissolution of brushite crystals by different concentration of citric acid solutions. Indian J Pure Appl Phys 2005; 43(9) : 675-8.
5. Diana K, George K. Urinary stone formation: Efficacy of seed extract of *Ensete superbum* (Roxb.) Cheesman on growth inhibition of calcium hydrogen phosphate dihydrate crystals. J Cryst Growth 2013;363:164-70.
6. Joshi VS et al. Inhibition of the growth of urinary calcium hydrogen phosphate dihydrate crystals with aqueous extracts of *Tribulus terrestris* and *Bergenia ligulata.* Urol Res 2005; 33(2):80-6.