COMPOSITION OF HUMAN BONE MINERAL BY FTIR AND ITS RELATIONSHIP TO THE AGE

D. S. Soejoko[1], Y. W. Sari[2], S. U. Dewi[2], Nurizati[2], K. Dahlan[2], and D. S. Atmadja[3]

[1.] *Department of Physics, Faculty of Mathematics and Sciences, University of Indonesia, Kampus UI Depok 16424, Indonesia*
[2.] *Department of Physics, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Jl. Meranti, kampus IPB Darmaga Bogor 16680, Indonesia*
[3.] *Department of Forensic Medicine and Medicolegal, Faculty of Medicine, University of Indonesia, Jl. Salemba Raya No.6 Jakarta Pusat 10430, Indonesia*

E-mail: djarwani@fisika.ui.ac.id

Abstract: Deproteinated human bone with hydrazine indicated that percentage of bone mass mineral increased with individual age in the range of child to adult, and then slowly decreased with ageing. Type of bone and sex also influenced the proportional of mineral in bone. Several information was obtained from infrared spectroscopy measurements. Calcium phosphate in bone mineral was a mixture of amorphous calcium phosphates and apatite crystals that rich of carbonates. Most crystals were carbonate apatite type B, with additional small amount of type A and AB. The splitting factor of υ4 phosphate bands indicated that crystalline degree was regulated by age, increases in the range of child to adult, then decrease up to a certain value (0.16) and finally almost constant. It is predicted that apatite crystals that was formed with less calcium phosphate will accompanied by the insertion of crystallization water in order to maintain the constancy of bone volume.

Keywords: *human bone mineral, apatite crystal, water crystallization*

Received 2 November 2013
Published 28 February 2014

I. INTRODUCTION

Although Glimcher et al (1981) argued the presence of amorphous phase during bone formation, current study conducted by (Olszta et al., 2007) supported previous observation of Termine and Posner (1966 and 1967) in which they called up the idea of both amorphous and crystalline phase were presence in bone mineral. Infrared spectroscopy indicated that besides phosphate, carbonate is also present in bone mineral which is incorporates with either amorphous or crystalline calcium phosphate. However, the existence of carbonate in an amorphous environment is still indeterminate. On the other hand, in calcium phosphate of bone mineral known as apatite crystal of which its structure is analogous to stoichiometric hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, carbonate ions may replace either OH^- or PO_4^{3-} ion and are designated as carbonate apatite type A and type B respectively. Two sets of infrared absorption bands at about 1545, 1450, 880 cm $^{-1}$, and 1465, 1412, 872 cm^{-1} correspond to carbonate apatite type A and type B (Termine and Lundy, 1973). In addition, there is also exist an unstable carbonate apatite which is introduced as type AB and characterized by the bands at 1452 - 1470 -1500 – 1545 – 1568 cm^{-1} (Rey et al., 1989, 1991).

In this paper, infrared spectroscopy of human bone mineral with differ individual age is reported. Obtaining the information of bone mass percentage, hydrazine was used to remove organic component from bone samples that most of which were taken from rib. Further investigation was carried out by Fourier Transform Infrared spectrometer (FTIR).

The *Invited Paper* section in *Journal of Medical Physics and Biophysics* serves in publishing selected works with merit. No review process was applied unto the works published in the section.

II. MATERIALS AND METHODS

Ten human bone samples were obtained from ten individuals who came to autopsy. It is believed that the subjects were in normal health prior to passed away. Those samples represented the age groups of child (1 day), adolescence (16 years), adult (21 - 36 years), and old (60 – 75) years. Most samples were selected from rib, and others were from femur, head, and tibia. First all samples were deproteinated to separate bone minerals from its organic compounds. Hydrazine was preferred than both ethylenediamine and hyphochlorite for deproteination as hydrazine did not alter the mineral phase in the treated sample (Termine, Eanes, Greensfield, Nylon, 1973 and Tomazic, Brown, Eanes, 1993). Samples were immersed into 10 ml of hydrazinium hydroxide for 1 hour at ambient temperature. Reimmersion was done subsequently for 2 hours at room temperature, 1 hour at 60 °C, and 24 hours at 60 °C. Then samples were diplo washed using 50, 75, 85, and 100 % ethanol and followed by aquadest. To remove adsorbed water, samples were heated at 110 °C for 12 hours.

For infrared spectroscopy measurement a milligram of sample was mixed with about 100 mg KBr, grounded and pressed in the mould to produce transparent pellet. All infrared spectra were obtained using Brucker FTIR spectrometer, with the measurement range of 400 – 400 cm^{-1}. Wave numbers where transmission peaks occurred were recorded at each spectrum.

III. RESULTS AND DISCUSSION

Mass of bone minerals of 10 samples that was obtained from deproteination using hydrazinium hydroxide is presented in Table 1. These samples were originated from the rib (7 samples), the femur (1 sample), the head (1 sample), and the tibia (1 sample). Most samples were obtained from different individual and at different age, except 1 rib and 1 head samples were taken from the same male person at the age of 31 years. From these two samples, it is shown that the head has higher content of mineral mass than that of the rib. Figure 1 illustrates the relation between the bone mineral mass with the individual age. The mass percentage value for an individual age between16 to75 years is in the range of 20 -50 % with the tendency to decrease with the ripening.

Table 1 Mass of human bone minerals originated from various age of individual.

Category	Sex	Age	Type	Mass (% wt)
Child	Male	1 day	Femur	42,33
Adolescence	Male	16 years	Rib	50,65
Adult	Male	30 years	Rib	32,99
		31 years	Rib	48,12
		31 years	Head	65,05
		36 years	Tibia	56,27
Adult	Female	21 years	Rib	43,03
Old	Male	60 years	Rib	34,24
		65 years	Rib	25,47
Old	Female	75 years	Rib	23,85

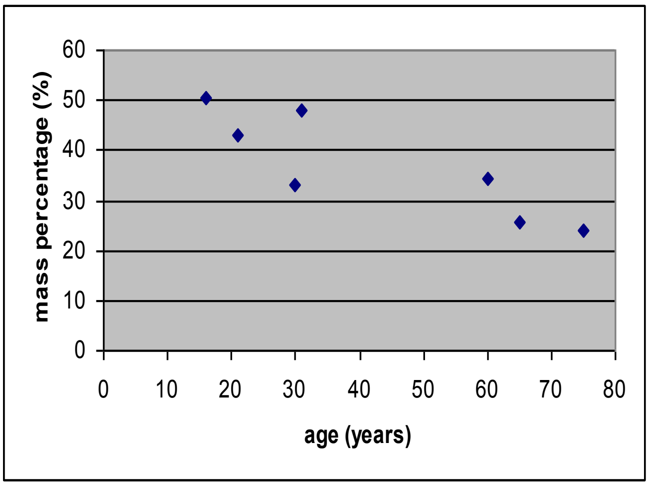

Figure 1. Percentage mass human bone minerals of the rib in relation with individual age.

The result of FTIR spectroscopy is illustrated in Figure 3. Band positions of recorded spectra are presented in Table 3. Of all appeared phosphate bands, there are two bands that occur in all spectra, the υ3 band in the range of 1000 – 1200 cm-1 and the υ4 band in the range of 500 – 700 cm-1. The υ3 band is a broad and asymmetry band with the peak at about 1032 – 1037. While the υ4 band is split into two peaks at 605 and 566 cm-1 which indicate that the sample contains apatite crystals. Besides these two phosphate bands, there is also υ1 phosphate band at about 962 cm-1 as a shoulder of the υ3 band.

The carbonate bands present at lower intensity compare to that of the phosphate bands. The υ_3 carbonate band is located at 1400 – 1600 cm^{-1}, and the υ_2 band is at about 873 cm^{-1}. All spectra contain υ_3 carbonate broad band with two peaks at about 1418 and 1453 cm^{-1}. Some spectra also exhibit additional small band with peaks at about 1542 and 1560 cm^{-1}. A Sharp υ_2 carbonate band can be seen clearly at about 873 cm^{-1} in all spectra. The bands at about 1545, 1450, and 880 cm^{-1} are attributed to carbonate apatite type A, and the bands at about 1465, 1412, and 872 are recognized as carbonate apatite type B (Termine and Lundy, 1973). The broad band with peaks at about 1418, 1453, 1542, and 1560 cm^{-1} is match with character most bands of type AB carbonate apatite (Rey et al. , 1989, 1991).

The splitting of the υ_4 phosphate band can be used for estimating the crystallization degree in mixtures of amorphous calcium phosphate and apatite crystal (Termine and Posner, Science, 1966). Evaluating the crystalline degree quantitatively, the splitting factor (SF) is defined as the ratio between AB and AC (Figure 2). The SF value of all samples is presented in Table 3. It can be noticed that SF is initially influenced by age on which its value is increase significantly from 0.18 (a born child /1 day) up to 0.25 (an adolescence/16 years), and then followed by a decline to 0.16 (adult/31 years). Afterwards, its value is relatively constant up to the age of 65 years. If SF at a born child (0.18) is assumed as a reference, at adolescence its value increases approximately up to 39% and at the age between adult and old its value increases only about 11 %. SF value as a function of age is presented in Fig. 4.

The existence of adsorbed water in all samples is indicated by the broad band at 3700 – 2500 cm^{-1}. Several sharp peaks that appear between 3300 – 1600 cm^{-1} are recognized as water of crystallization. Along this region, there are at least three distinct peaks, one peak in the region of 1600 – 1650 cm^{-1} and the two others in 2850 – 2930 cm^{-1}.

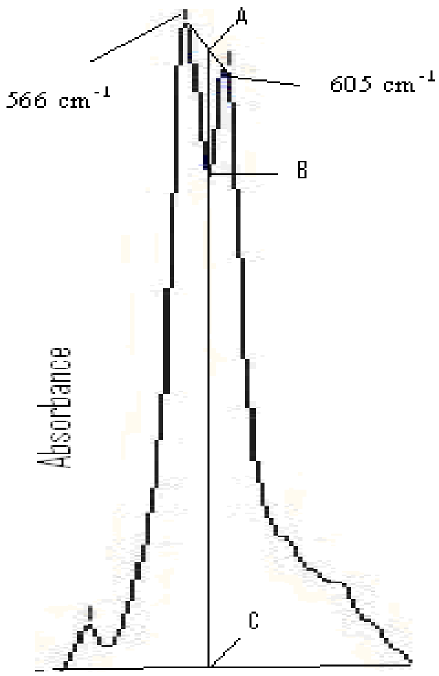

Figure 2. Illustration of splitting factor (SF= AB/AC) of υ_4 phosphate band.

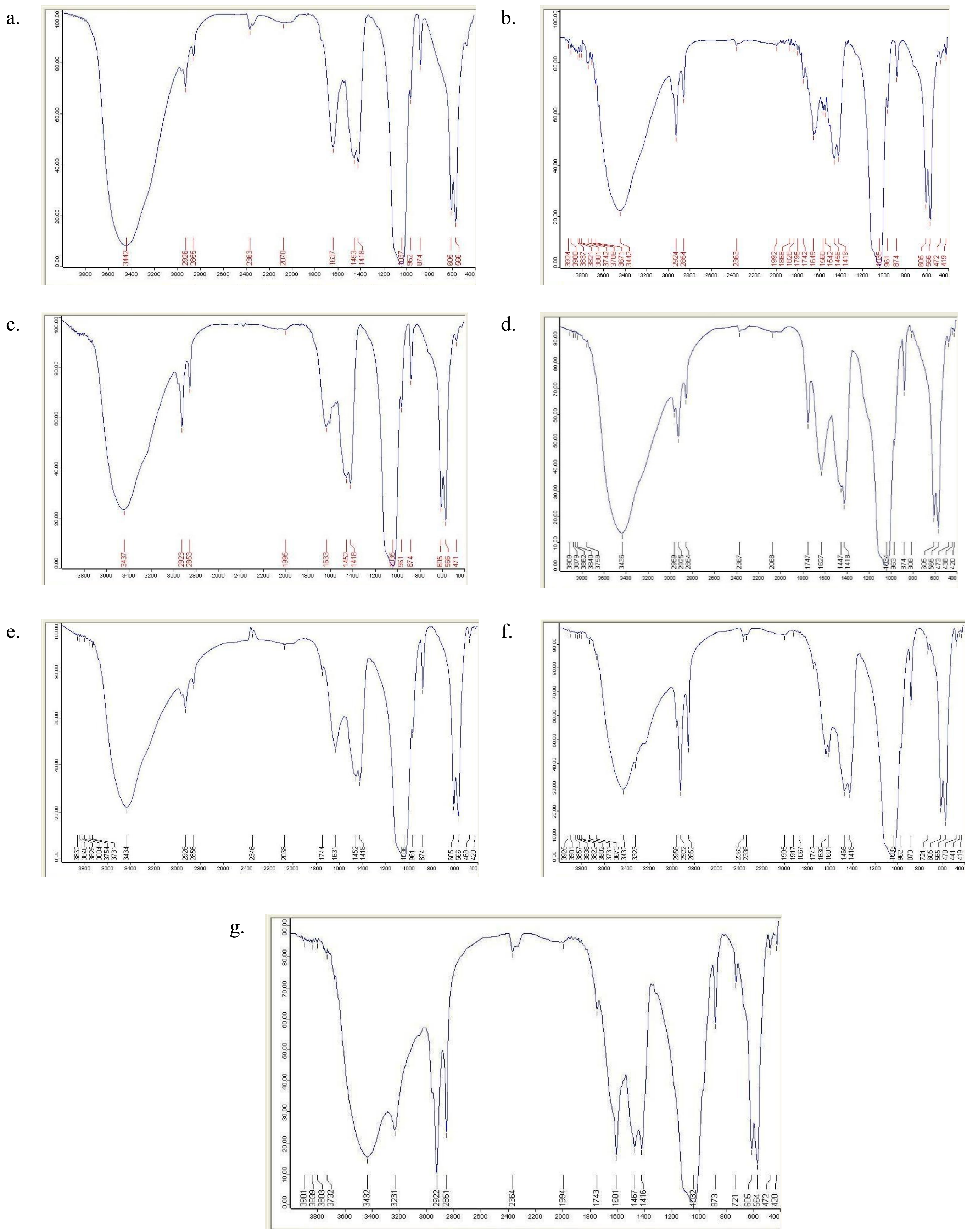

Figure 3. Infrared spectra human bone minerals of individual with the age of a) 1 day, b) 16 years, c)30 years, d) 31 years, e) 36 years, f) 60 years, and g) 65 years.

Table 2. Position of absorbed bands for phosphates, carbonates, and water crystals in infrared spectra of human bone minerals.

Age	Type	Phosphate (cm^{-1}) υ_1	Phosphate (cm^{-1}) υ_3	Phosphate (cm^{-1}) υ_4	Carbonate (cm^{-1}) γ_2	Carbonate (cm^{-1}) υ_3	Carbonate (cm^{-1}) υ_4	Crystallization water	Crystallization water
1 day	Femur	962	1037	605 566	872	1453 1418	-	1637	2926 2866
16 yrs	Rib	961	1035	605 566	874	1419 1456 1542 1560	-	1649	2924 2854
30 yrs	Rib	961	1035	605 566	874	1452 1418	-	1633	2923 2863
31 yrs	Rib	963	1034	605 566	874	1447 1418	-	1627 1747	2925 2864 2959
36 yrs	Tibiae	961	1036	605 566	874	1452 1418	-	1631 1744	2925 2856
60 yrs	Rib	962	1033	605 565	873	1457 1417 1559	721	1630 1601	2922 2852
65 yrs	Rib	-	1032	605 564	873	1457 1416	721	1601 1743	2922 2851 3231

Table 3. Splitting factors of the υ4 phosphate bands in infrared spectra of human bone mineral.

Age	AB (cm)	AC (cm)	SF
1 day	0.90	4.9	0.18
16 years	1.1	4.4	0.25
30 years	0.95	4.4	0.22
31 years	0.70	4.5	0.16
36 years	0.70	4.5	0.16
60 years	0.75	4.7	0.16
65 years	0.70	4.6	0.15

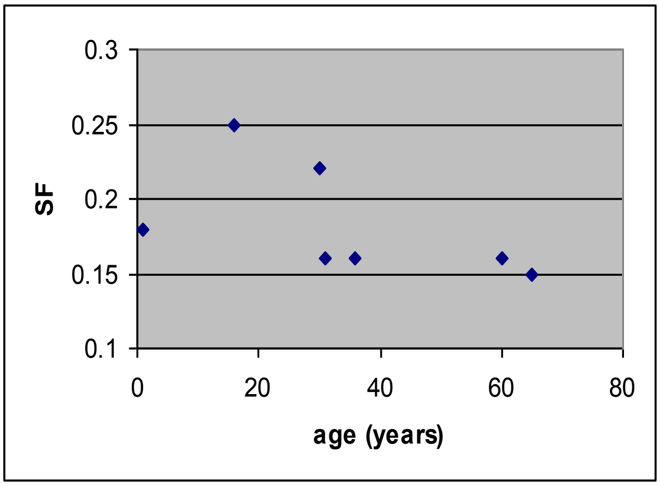

Figure 4. Splitting factor in related with individual age.

As part of biological system, bone mineral is in a relation with metabolic activity. Bone mineral is being continuously deposited and resorbed. Parallel with slowing down of metabolic activity, the bone mineral content also declines with increasing of individual age. This tend can be seen noticeably from the rib samples (Table 1) of which the percentage bone mineral of an individual from adult (16 years) up to old (65 years) is decrease from 50.65% up to 25.47%. This found support the statement that bone mass of long bone is reduced as much as 60% for women and 50% for men during ageing (Larry L. Hench and Julian R. Jones, 2005). Type of bone is also influenced the proportion of mineral in bone. Compared to the rib, both the head and tibia bones have a higher mass percentage. This relates with their structure. On one site, the head bone is a flat bone completely composed of compact bone. A tibia is a long bone that has two regions, diaphyses and epiphyses. Diaphysses is constructed by compact bone while epiphyses, as an extremity of long bone consist of spongy bone. On another site, rib is constructed by two layers, a compact bone in the outer and a spongy bone in the inner site.

The most stable calcium phosphate is hydroxiapatite (HAP) crystal, $Ca_{10}(PO_4)_6(OH)_2$. Both complex and simple ions in the vicinity of calcium and phosphate ions able to produce biological apatite in bone mineral in similar structure with HAP, but diverse in composition and stoichiometry. The formula of biological apatite can be written as follows

$$(Ca, M)_{10}\ (PO_4, Y)_6\ (OH_2, X)$$

with M represents simple ion such as Mg, Na, and K, and Y represents complex ions such as carbonate and sulphate, whereas X represents either complex or simple ion such as carbonat, fluor, and chlor.

Infrared spectra of bone mineral indicates that most crystal apatite is carbonate apatite type B and some amount of type A, with addition of type AB. Type A and B carbonate apatite is produced by substitution of OH^- and PO_4^{3-} with CO_3^{2-} ion, respectively,. While type AB carbonate apatite is not a simple addition of both type A and B but it is predicted that it has a correlation with a poorly crystalline phase (Rey et al., 1989). The presence of carbonate type B is obvious; it is indicated by the appearance of a strong band at about 873 cm^{-1}. Contrary, the presence of carbonate apatite type A is an ambiguous as there is a disappearance of the peak at 880 cm^{-1} which is one of its characteristics band. Nevertheless, the absence of OH^- bands at about 3572 and 630 cm^{-1} as characteristic bands of hydroxyl in hydroxyapatite indicates that there is a carbonate substitution to hydroxyl group in hydroxyapatite which result a carbonate type A. The appearance of carbonate type AB is clearly seen in samples with the age of 16 and 60 years.

Apatite crystal in bone mineral includes in metabolism activity. New apatite crystals are always formed, the existed young crystals growth to mature, and the mature crystals come to old. Naturally, the resorption will occur among the oldest, the same trend is also happen in apatite crystal. Therefore bone mineral always contain of apatite crystals with different ages. Using SF of the υ_4 phosphate band, it can be inferred that crystalline degree of bone mineral is a function of time from the new born up to adolescence. This parameter further decrease up to a certain degree and then inclines up to a constant value during adult to old period. Compare to bone mass percentage, this study suggest that there is no correlation between bone mass percentage and crystalline degree. As an example, though both bone mass percentage from the rib of 30 and 60 year old person has a nearly value (32.99 and 34.24 %, respectively), there is a difference in crystalline degree which is indicated by a varied SF value (0.22 and 0.16, respectively).

All infrared spectra indicated the existence of crystallization water by appearing OH^- bands in the range of 1601 – 1747 cm^{-1} and in the range of 2922 – 3231 cm^{-1} that further will be called the first and second range. One sharp band at the peak at about 1630 cm^{-1} always appears clearly in the first range, and two sharp bands with the peaks at about 2860 and 2925 cm^{-1} are well observable in the second range. The band at 2925 cm^{-1} is in higher intensity compare with the band at 2860 cm^{-1}. There are other bands with low intensity which are located between the two ranges. All of these bands indicated that more than one type of water environment exist in the crystals. It is interesting to note that intensity of the band in the first range is much higher than those in the second range at the spectra from femur of a new born child (1 day) and tibia of an adult (36 years). A slight different is found at the rib spectra; the bands in the first and second range are comparable in intensity particularly which come from adult (16, 30, and 31 years). A great different appeared at the rib spectra from old individual (60 and 65 years), the bands in first and second range are at high intensity, and moreover those from 65 years person are relatively higher. This is an indication that bone mineral from old individual contains high concentration of crystallization water.

It seems that there is a tendency in which high crystalline degree or high SF value will be accompanied by low degree of water crystallization, vice-versa. These phenomena can be understood since the insertion of water molecules into the crystal lattice will influence the packing arrangement. Metabolism rate of an old individual is relatively lower compare with that happen in young individual, therefore the formation and growth of crystals in bone mineral is not balanced with the body's resorption of calcium from bone. To keep a constant volume of the bone, water molecules might enter crystal lattice and form a hydrogen bond to substitute the crystal destruction.

IV. CONCLUSION

Human bone mineral relates with metabolism activity, and the mass tends to decrease with ageing. From the male rib bone samples, the changes is clearly appeared, in the interval between 16 – 65 years (adolescence to old individual) the bone mass was found 50.65% and 25.47%. The decrease is started at about individual age of 60 years. Even with limited samples it can also be seen that the percentage bone mass is also influenced by type of bole and sex of individual.

From infrared spectra it is shown that composition of amorphous calcium phosphates and apatite crystals in human bone is also affected by metabolism activity. Most apatites were type B with additional small amount type A and AB. The splitting factor of υ_4 phosphate bands illustrated the crystalline degree of bone mineral. From the rib samples SF decrease from 0.25 to 0.16 that was found at the range of age from 16 to 31 years of individual age. After 31 years of age, the SF value was relatively constant. At any instant bone mineral contains newly formed apatite crystals, young and mature crystals. It was predicted that for maintaining bone volume, crystallization water play a significant role. Apatite crystals which were formed with less of calcium phosphates will be rich of crystallization water in order to maintain the constancy of bone volume.

V. ACKNOWLEDGMENTS

We thanks to dr. Swasti Hertian, Sp.F (The Head of Department), Department of Forensic Medicine and Medicolegal, Faculty of Medicine, University of Indonesia, Jakarta, for the permission to get the human bone sample from autopsy material, and to dr. Evi Untoro for helping us in collecting the human bone sample from autopsy material.

REFERENCES

[1] Termine J. D. and Lundy D. R., Hydroxide and carbonate in rat bone mineral and its synthetic analogues, Calc. Tiss. Res. 13, 73-82, 1973

[2] Rey C., Collins B., Goehl T., Dickson I. R., and Glimcher M. J., The carbonate environment in bone mineral: A Resolution enhanced Fourier Transform Infrared Spectroscopy Study, Calc. Tiss. Int. 45, 157-164, 1989.

[3] Termine J. D., Posner A. S., Infrared analysis of rat bone: Age dependency of amorphous and crystalline mineral fractions,Science 153, 1523-1525, 1966.

[4] Termine J. D., Posner A. S., Amorphous/crystalline interrelationships in bone mineral, Calc. Tiss., Res., 1, 8-23, 1967.

[5] Termine J. D., Eanes E. D., Greensfield D. J., and Nylon M. U., Hydrazine deproteinated bone mineral, Calc. Tiss. Res., 12, 73-90, 1973.

[6] Hench L. L., The skeletal system, in *Biomaterials, artificials organs and tissue engineering* (L. L. Hench and J. R. Jones, Editors), Woodhead Publishing Limited, Cambridge, 2005.

[7] M.J. Glimcher, L.C. Bonar, M.D. Grynpas, W.J. Landis, A.H. Roufosse, Recent Studies of bone mineral- is the amorphous calcium phosphate theory valid, J. Cryst. Growth 53 (1981) 100-119.

[8] Olszta M.J, et al, Bone Structure and Formation : A new perspective, Materials Science and Engineering R 58 (2007) 77-116.

[9] Rey C, V. Renugopalakrishan, M. Shimizu, B. Collins, and M.J. Glimcher. A resolution-enhanced Fourier Transform Infrared Spectroscopic Study of the environment of the CO32- ion in the mineral phase of enamel during its formation and maturation. Calcif Tissue Int (1991) 49: 259-268.

[10] Tomazic BB. Brown WE, Eanes ED (1993) A Critical evaluation of the purification of biominerals by hyphochlorite treatment. J Biomed Mater Res 27: 217-225