90 km, is through reactions of ionic species and hence the increase in solar activity results in an increase in atomic nitrogen concentration as expected.

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# Solvent effect on fluorescence lifetime of 8-anilino-naphthalene-1-sulphonate

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**We report the fluorescence lifetime of 8,1-ANS in different solvents and compare them with the previously reported values. Effects of solvent viscosity and polarity on fluorescence lifetime have also been studied. A simple empirical relationship between fluorescence lifetime and solvent viscosity/polarity shows that the fluorescence lifetime is more sensitive to solvent polarity rather than viscosity.**

THE extreme sensitivity of the fluorescence parameters to the environment of 8-anilino-naphthalene-1-sulphonate (8,1-ANS) was first recognized by Weber and Laurence[1]. In water 8,1-ANS exhibits weak green fluorescence whereas an intense blue fluorescence is observed when bound to serum albumin or proteins[2-6]. The fluorescence decay time, quantum yield and fluorescence maximum of this molecule in solvents of varying polarity have been investigated by several workers[7-10]. The picosecond time resolved fluorescence of 8,1-ANS in a mixed solvent system[11], its photophysical decay pathways[12], the edge excitation red shift at liquid air temperature[13], time-dependent spectral shift at two different temperatures[7] have also been studied. In spite of the extensive work already reported on the spectroscopy and photophysics of 8,1-ANS, a

large inconsistency is found in the fluorescence decay times as reported by different authors. In this connection we report the fluorescence lifetime of 8,1-ANS in different solvents and compare them with previously reported values. Effects of solvent viscosity and polarity have also been studied.

Spectroscopic grade solvents (Aldrich Chemicals) were used after distillation. Aqueous solutions were prepared from triple distilled water. 8,1-ANS (Aldrich Chemicals) obtained as ammonium salt was purified by crystallizing it in $MgCl_2$ solution as described by Zadkowski and Fleming[8]. Freshly prepared solutions (ANS concentration $5 \times 10^{-5}$ M) were studied after bubbling nitrogen for 10 min. Lifetime was measured by degassing using thaw-freeze and pump method in several cycles (in n-propanol only). For fluorescence lifetime measurements a time correlated single photon counting[14] spectrometer was used with a coaxial flash lamp driven set-up with fwhm = 1 hs (Edinburgh Instruments 199 Spectrometer). Some measurements were carried out with a frequency doubled, cavity dumped dye laser synchronously pumped by a mode locked and frequency doubled Nd-YAG Laser (Spectra Physics) as the excitation source with fwhm 450 ps. Data were analysed with a PDP 11/2 micro computer by reconvolution method using a least-square fitting method. The goodness of the fit was estimated by reduced $\chi^2$, distribution of residuals and standard deviation.

The steady state spectra of 8,1-ANS recorded by us are similar to those reported earlier[7,8]. The fluorescence decay times of 8,1-ANS in various solvents at 25°C are given in Table 1 along with their viscosities ($\eta$) and solvent polarity parameters[15] [$E_T(30)$]. The fluorescence lifetime values (at 25°C) reported in the literature[7-10] have also been tabulated for comparison. The lifetimes reported by Chakrabarti and Ware[7] are exceptionally high, probably due to the inaccuracies in measurements. However, our values of under-gassed solutions resemble close to those obtained by other workers[8,9]. In an aerated solution the fluorescence lifetime is quenched by the presence of dissolved oxygen. Nakamura and Tanaka[10] have shown that if the solution is degassed by thaw-freeze method, the lifetime increases. However, our values of lifetime ($\tau_f$) (11.8 ns) after thaw-freeze and pump procedure (in n-propanol) did not match with those (14.5 ns) of Nakamura and Tanaka[10]. Our measurements for all other solutions have been done using the method of nitrogen bubbling for degassing.

From Table 1, it is evident that there is a large variation in lifetime in different solvents. In all the solvents, the fluorescence decay of 8,1-ANS exhibits a single exponential behaviour which remains invariant throughout the emission wavelength except in octanol where a double exponential behaviour[16] is observed

Table 1. Viscosity, solvent polarity and fluorescence decay times of 8,1-ANS in various solvents at 25°C and $\lambda_{em} = 475$ nm.

| Solvent | Viscosity $(\eta)$ in cp | $E_T (30)$ in kcal/mol | Fluorescence lifetime values $(\tau_f)$ in ns | | | |
| | | | Present experimental values | | | |
| | | | Undegassed | Nitrogen bubbled | Thaw–Freeze method | Literature values |
|---|---|---|---|---|---|---|
| Water | 0.890 | 63.1 | 0.193 ±0.017 | 0.223* ±0.010 | | 0.550[a],0.250[b], 0.200[c] |
| Methanol | 0.547 | 55.5 | 6.40 ±0.02 | 6.60 ±0.01 | | 6.05[a],5.9[b], 8.5[c],13.6[d] |
| Ethanol | 1.100 | 51.9 | 8.10 ±0.02 | 8.40 ±0.02 | | 8.85[a],8.3[b], 12.5[c],18.0[d] |
| n-Propanol | 2.000 | 50.7 | 10.20 ±0.03 | 11.20 ±0.03 | 11.80 ±0.02 | 10.2[a],14.5[c], 21.0[d] |
| Octanol | 8.800 | 48.3 | 11.80 ±0.04 | 12.40 ±0.03 | | 12.3[a] |

[a] Radda[9].
[b] Zadkowski and Fleming[8].
[c] Nakamura and Tanaka[10].
[d] Chakrabarti and Ware[7].
*The pulse source used is Nd–YAG mode locked, synchronously pumped and cavity dumped dye laser.

which varies across the emission wavelength $(\lambda_{em})$ but at $\lambda_{em} = 475$ nm, the decay is found to be monoexponential.

Tredwell and Kreary[17] have shown that viscosity $(\eta)$ is related to fluorescence lifetime $(\tau_f)$ by the relation

$$\tau_f = C\eta^\alpha$$

where $C$ is a constant and $\alpha$ shows the viscosity power dependence. Using this relation the log–log plot of fluorescence decay times and viscosity for different solvents gives a straight line (Figure 1) giving the value of $\alpha$ equal to 0.17. The temperature variation of 8,1-ANS in ethanol from 180 to 298 K where viscosity changes from 50 to 1.1 cp also gave a value of $\alpha = 0.18$. The value of $\alpha$ obtained from either variation of viscosity using different solvents or by variation of

temperature for a particular solvent comes out to be the same. The low value of $\alpha$ indicates that viscosity has a rather weak effect on the deactivation path of the excited ANS. Although Förster and Hoffman[18] have suggested that the viscosity dependence of fluorescence quantum yield $(\phi_f)$ follows a relation $\phi_f \alpha \eta^{2/3}$ and this dependence has been observed in several cases, yet a value of $\alpha = 1/3$ has been reported for malachite green[19]. Bagchi et al.[20] have shown that the fractional values of the exponent $\alpha$ ranges from 0.1 to 1 and that there is no theoretical restriction for $\alpha$ to be exactly equal to 2/3, in fact it is dependent on some natural parameters which are discussed in his model. Our value
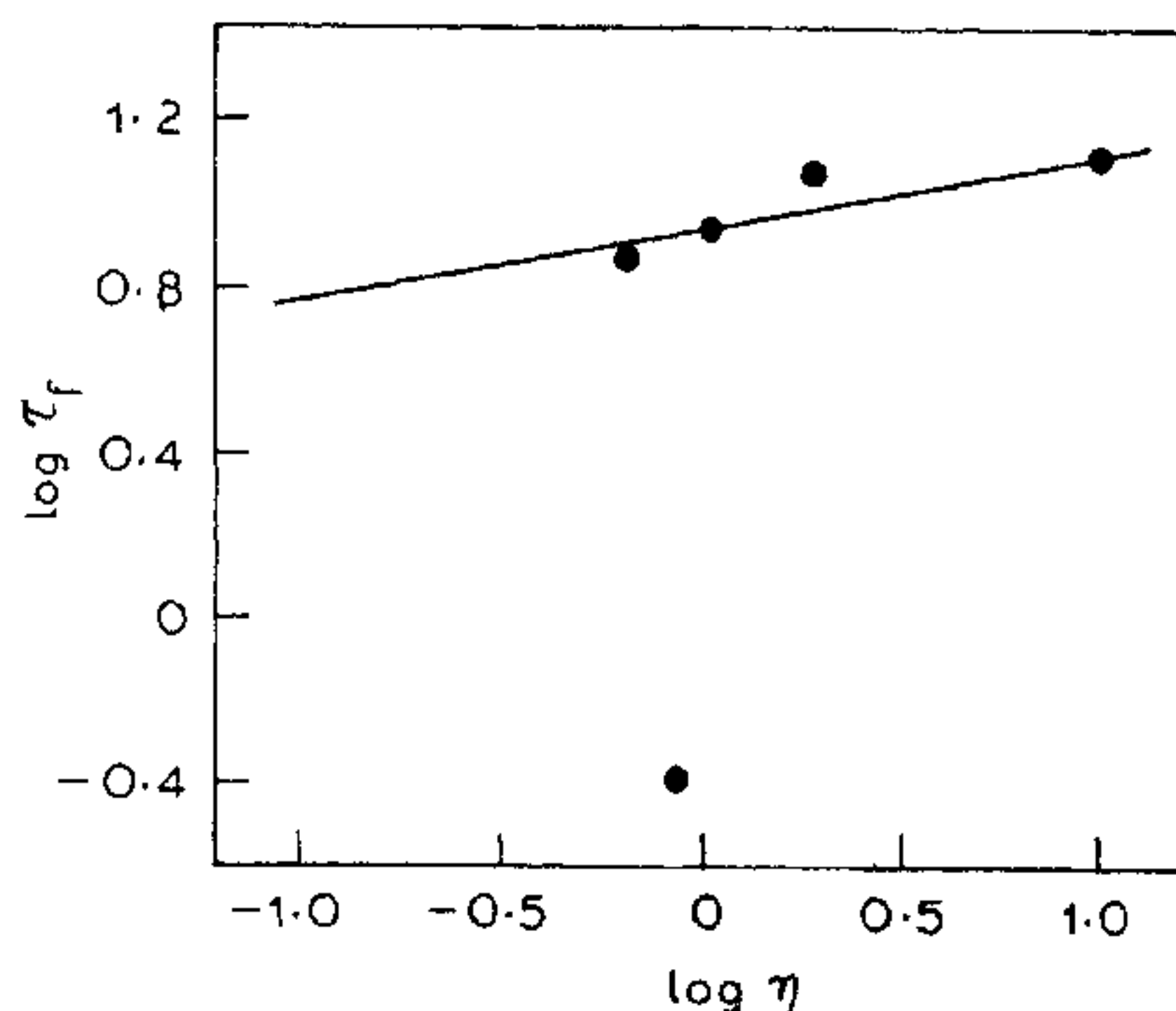


Figure 1. A log–log plot of fluorescence lifetime $(\tau_f)$ against viscosity $(\eta)$ for 8,1-ANS in various solvents.
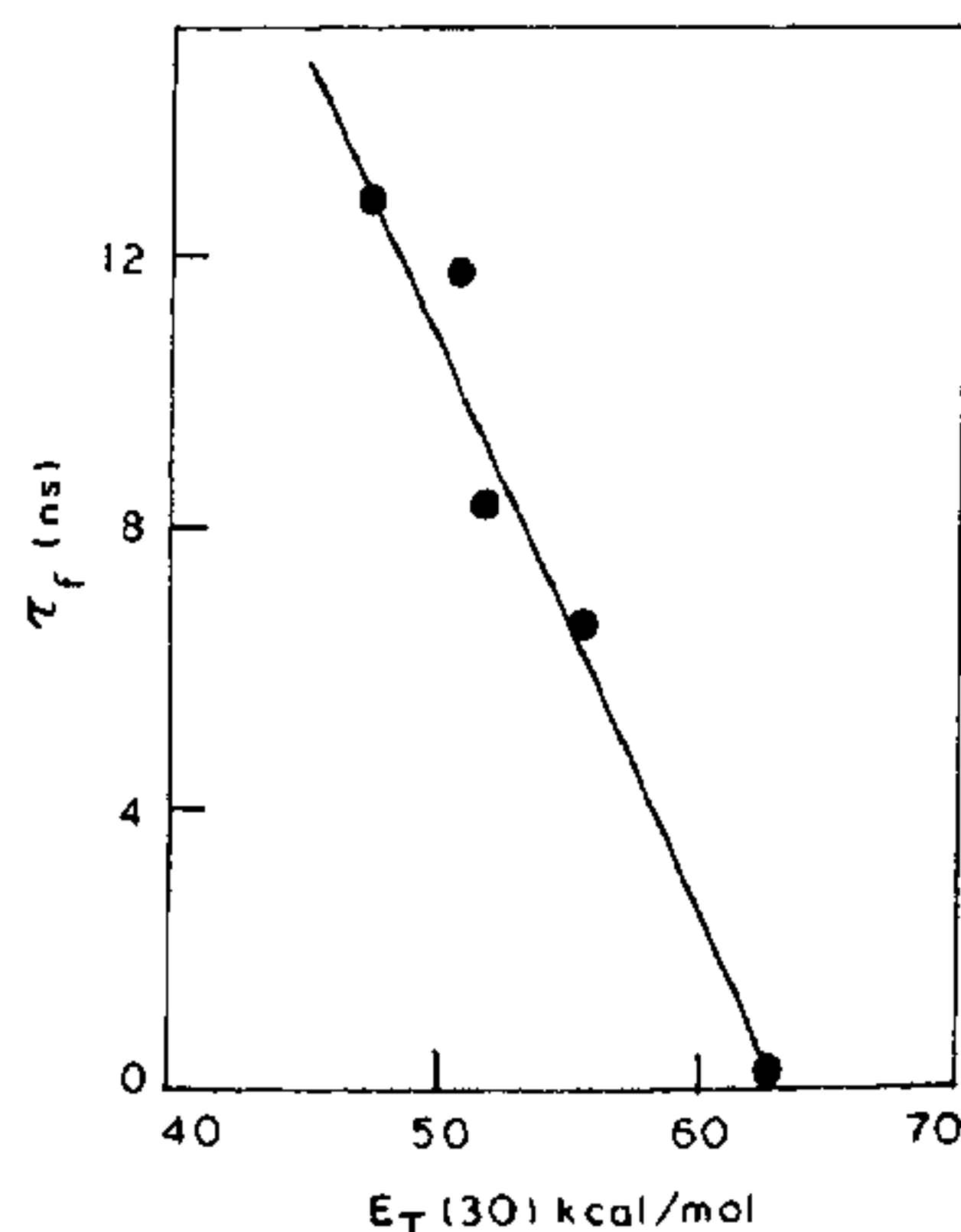


Figure 2. A plot of fluorescence lifetime $(\tau_f)$ against solvent polarity parameter $E_T (30)$ (in kcal/mol) for 8,1-ANS in various solvents.

of $\alpha$ is in close agreement with those of Zadkowski and Fleming[8] and Kosower and Kanety's[12] results who reported fluorescence lifetime to have little correlation with viscosity.

Kosower and Kanety[12] have reported that in 8,1-ANS, the radiative rate constant $(k_r)$ is mildly sensitive to solvent polarity and the variation in lifetime in solvents of different polarity is mainly due to intramolecular electron transfer from the charge transfer state. Such type of intramolecular electron transfer is recently proposed to be a barrierless process[21]. The activation energy calculated from the temperature-dependent studies is found to be 0.23 kcal/mol, indicating a very small barrier for the deactivation process and thus can be treated as a barrierless process.

The plot of fluorescence lifetimes against solvent polarity, $E_T$ (30), gives a straight line indicating that the fluorescence parameters in 8,1,-ANS are polarity-dependent (Figure 2). Summarizing, the deactivation process in 8,1-ANS has been found to be polarity-dependent whereas viscosity has rather insignificant effect indicating the absence of any large amplitude internal motion.

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# Preparation of octacalcium phosphate and its conversion to hydroxyapatite: An electron microscopic investigation

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Octacalcium phosphate has been prepared by a precipitation technique and characterized by X-ray, IR and electron microscopy. Its hydrolysis to hydroxyapatite has been investigated, the products are characterized by electron microscopy. A mechanism of hydrolysis is suggested.

OCTACALCIUM phosphate, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, OCP, is considered as a precursor in the formation of bones, teeth, renal stones, dental calculus etc.[1,2]

It has been synthesized earlier by the hydrolysis of Brushite[3], $\alpha$-tricalcium phosphate[4], and gel-diffusion method.[5] The precipitation of OCP at 60°C by Newsely[6] was found to be contaminated with hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, (HAP). We, therefore report here a new method of preparation of OCP under suitable conditions (temperature, 37°C and pH 7.2). Its formation is confirmed by X-ray, IR and electron microscopy. The mechanism of its conversion to hydroxyapatite has been proposed.

The chemicals used were AR (BDH or E. MERCK) grade. All solutions were prepared in $CO_2$-free double distilled water. A solution of calcium acetate, 9.9 mM/l and another of ammonium dihydrogen phosphate, 5.9 mM/l maintained at pH 7.2 were dropped simultaneously at a slow rate of 40 drops $min^{-1}$ to 400 ml of $CO_2$-free water taken in a flask maintained at 37°C. The precipitation was done at a constant ionic environment of 0.9 (Wt/Vol) NaCl and $CO_2$-free atmosphere. The precipitate was left in contact with the mother liquor at 37°C for 2 h to facilitate the ripening and homogeneity of the crystals. It was then washed with water, acetone and desiccated for seven days. The yield was about 60% of the theoretical. The crystals were flakey and lustrous white.

Calcium and phosphorus in the samples determined complexometrically gave Wt% CaO: 46.28 and $P_2O_5$: 43.03 and Ca/P g atom ratio 1.295. The number of water molecules was estimated by substracting CaO and $P_2O_5$ amounts from the total amount of sample weighed for analysis which corresponded to $5H_2O$ in the sample. The density of the sample was determined[7] to be 2.65 g $cm^{-3}$ (literature value[3] = 2.673 g $cm^{-3}$).

The X-ray diffractogram recorded using $CuK_\alpha$ radiation indicated that the sample obtained is Octacalcium phosphate[8]. The electron microscopic pattern of the sample obtained on a Simens Elmiskop is composed of blade-like habit (Figure 1a) and contained no extraneous phase confirming the purity of the sample. The