A ROUTE FOR THE PREPARATION OF [^{32}P]-SODIUM MONOFLUOROPHOSPHATE

B. Wierczinski[*], Z. I. Kolar, E. P. Sedlick[#]

Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, 2629 JB DELFT, The Netherlands

Summary

[^{32}P]-labelled sodium monofluorophosphate (Na$_2$FPO$_3$) was prepared according to a procedure, which is based on melting of a mixture of carrier-added [^{32}P]-phosphoric acid and ammonium bifluoride, both solid. Monofluorophosphate was precipitated as the silver salt from the reaction mixture, then it was converted to the sodium salt by using a cation exchange column in Na$^+$ form. The influence of the molar ratios of the reactants, and the reaction time on the final yield was studied. Powder X-ray diffraction showed a chemical purity of about 98 % and absence of NaF in the final product. After a reaction time of 2 hours and a molar ratio of the reactants of 1:1, the yield for the reaction was 30 % of the initial ^{32}P activity, corresponding to a specific activity of 56 MBq ^{32}P per mmol. An increase in yield is possible by increasing the reaction time, a higher specific activity may be obtained by a decrease of carrier phosphoric acid. The advantages of the procedure developed are the availability of [^{32}P]-phosphoric acid, and the simple preparation of the compound.

[*] corresponding author, e-mail: birgitw@iri.tudelft.nl
[#] Present address: Faculty of Applied Sciences, Delft University of Technology, Julianalaan 67, 2628 BC DELFT, The Netherlands

Introduction

The sodium salt Na_2FPO_3 of monofluorophosphate (MFP), has been known for more than 50 years[1]. Although its anticariogenic effect has been discovered at about the same time, it has not been used as an additive in dentifrices for another twenty years. Since then many research groups worldwide have been investigating the caries preventing effect of MFP. However, as yet no agreement has been reached on the mechanism involved. Several possible mechanisms have been proposed. One of them suggests that the exchange between phosphate groups of hydroxyapatite and FPO_3^{2-} is responsible for the reduced solubility of enamel[2], others imply, that MFP hydrolyses and the released fluoride ions subsequently incorporate into the hydroxyapatite lattice[3,4,5]. It is suggested that Na_2FPO_3 reduces the solubility of hydroxyapatite, and/or clogs the interprismatic areas, where ultrastructural changes occur first in the caries process[6].

A possible way to study these mechanisms is the use of radioactively labelled MFP as a radiotracer for FPO_3^{2-} or its components. This not only facilitates investigations of the caries preventing action of MFP, but also simplifies studies in other fields of research where MFP is used, namely osteoporosis[7,8], sarin detoxification[9], and others[10].

Labelling of MFP may be performed with three radioisotopes: ^{18}F ($t_{1/2} = 110$ min, β^+, $E_{max}=0.6$ MeV), ^{32}P ($t_{1/2} = 14.3$ d, β^-, $E_{max}=1.7$ MeV), and ^{33}P ($t_{1/2} = 25.3$ d, β^-, $E_{max}=0.2$ MeV). Comparison of the half-lives shows, that because of its longer half-life, ^{33}P is the most suitable of these three radioisotopes to perform physiological experiments, but ^{32}P is the most practicable one due to its general availability.

The labelled compound should be chemically and radiochemically pure, and have a sufficient specific activity. Furthermore, the preparation procedure should be as straightforward as possible and also not time-consuming. So far, no such procedures have been described to prepare radioactively labelled Na_2FPO_3 with the required properties. This article describes a novel approach to prepare $[^{32}P]$-Na_2FPO_3.

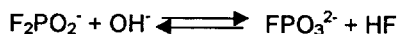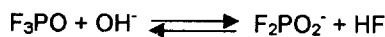## Evaluation of possible preparation routes for labelled MFP

Methods to label MFP with ^{32}P may be based on industrial and laboratory procedures for synthesising inactive MFP.

- For industrial production, a mixture of sodium metaphosphate and sodium fluoride is converted to Na_2FPO_3 [11,12]:

$$NaPO_3 + NaF \rightarrow Na_2FPO_3$$
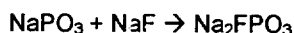
The reaction is carried out at 800°C and gives a yield of 90 %. Unfortunately, the final product is not very pure, and due to the high temperature phosphate polymerisation may occur; moreover [^{32}P]-$NaPO_3$ cannot be obtained commercially.

- Lange[13,14] found that monofluorophosphate can be obtained as an intermediate product in the hydrolysis of phosphoryl fluoride:

$$F_3PO + OH^- \rightleftharpoons F_2PO_2^- + HF$$
$$F_2PO_2^- + OH^- \rightleftharpoons FPO_3^{2-} + HF$$
$$FPO_3^{2-} + OH^- \rightleftharpoons PO_4^{3-} + HF$$

MFP can then be precipitated as its silver salt. Unfortunately, [^{32}P]-F_3PO is not commercially available, which excludes this method for a simple preparation of ^{32}P labelled MFP.

- The ammonium salt of MFP is obtained, when phosphorus pentoxide and NH_4F are melted together at 135°C[14]:

$$P_2O_5 + 3\ NH_4F \rightarrow NH_4PO_2F_2 + (NH_4)_2PO_3F.$$

If P_2O_5 is allowed to react with HF instead of NH_4F, the free acid is produced:

$$P_2O_5 + 3\ HF \rightarrow HPO_2F_2 + H_2PO_3F$$

Phosphate polymerisation is possible at the temperature used. Furthermore, the monofluoro compound is always obtained as a mixture with the difluorocompound which requires separation of the products.

- Free monofluorophosphoric acid can be obtained in a quantitative reaction by adding liquid HF (obtained by condensing gaseous hydrofluoric acid) to hot water-free metaphosphoric acid[15]:

$$HPO_3 + HF \rightarrow H_2FPO_3$$
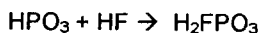
The reaction is rather slow and the method elaborate, and [^{32}P]-HPO$_3$ cannot be obtained commercially.

- One preparation method for MFP is based on the equilibrium of phosphoric acid and hydrofluoric acid[16]:

$$H_3PO_4 + NH_4F \cdot HF \rightleftharpoons H_2PO_3F + H_2O + NH_4F$$
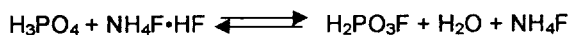
  This method requires [^{32}P]-H$_3$PO$_4$, which is commercially available in high specific activity.

- One publication describes the preparation of sodium monofluorophosphate labelled with ^{32}P and ^{18}F [17]. This preparation is based on melting [^{18}F]-NaF and [^{32}P]-NaPO$_3$ (the second compound is obtained by heating a mixture of NaH$_2$PO$_4$·H$_2$O and [^{32}P]-H$_3$PO$_4$ for 5 hours at 530ºC), and suffers again from the risk of phosphate polymerisation. Moreover, this publication does not describe a purification procedure for the final product, and appears rather time-consuming.

Taking into account the aspects of (i) availability of the radioactive reactants, (ii) the expected purity of the final product, (iii) the reaction time and temperature and (iv) ease of working with radioactive materials the method described by Lange[16] appeared the most promising. This method is based on the equilibrium between phosphoric acid and hydrofluoric acid and was adapted to the requirements of the labelled product by using solid phosphoric acid and ammonium bifluoride.

General outline of the preparation method

The preparation was finally carried as follows: An aqueous solution of carrier-free H$_3$^{32}PO$_4$ (1.9 GBq·ml^{-1}) is evaporated to dryness at 70°C in a centrifuge tube. Then 1 g solid (10 mmol) H$_3$PO$_4$ (98 %, m.p. 42.35°C) and 0.6 g (10 mmol) solid NH$_4$F·HF (m.p. 125.6°C) are added. The melt is stirred in the closed tube at 45°C for 2 hours with a magnetic stirrer. Following, the pH of the mixture is adjusted to 7 with ammonia (15 % in water) in an ice bath. A few drops of 3 mol·l^{-1} AgNO$_3$ are added to remove unreacted H$_3$PO$_4$, which precipitates as Ag$_3$PO$_4$. This procedure is repeated, until all H$_3$PO$_4$ is

precipitated as Ag_3PO_4. After centrifugation the supernatant is added to a saturated solution of $AgNO_3$ (about 3.5 g in 3 ml). Silver monofluorophosphate is now precipitated by addition of ethanol (96 %). After filtration through a filter ('Schleicher und Schüll Entkeimungsschicht'), the Ag_2FPO_3 is washed with ethanol (70 %), suspended in about 3 ml of water and subjected to ultrasonic treatment for 30 min. The suspension is centrifuged, the supernatant discarded and the procedure repeated. The Ag_2FPO_3 is dissolved in 50-ml water and impurities (Ag_3PO_4, Ag_2O) removed by filtration, using again a 'Schleicher und Schüll Entkeimungsschicht'. The solution is now loaded onto a cation exchange column in Na^+ form (Dowex 50 WX8, 1.9 meq/ml), and the silver MFP converted to sodium MFP. The conversion is completed, if the eluate does not yield a precipitate (Ag_2FPO_3) when added to a saturated solution of alcoholic $AgNO_3$. Reduction of the eluate to about 15 ml at 70°C and freeze drying yields [32P]-labelled sodium monofluorophosphate as a white powder.

The step-wise modification of the original procedure[16] is described in the part 'Results and Discussion'.

Experimental

Chemicals and Equipment

Solid phosphoric acid (98 %), silver nitrate, ethanol (96 %), sodium chloride, and nitric acid were purchased in p.a. purity from Merck (Darmstadt, Germany). Ammonium hydrogen fluoride was obtained from Baker (Houston, USA), sodium monofluorophosphate used as reference material from Alfa (Karlsruhe, Germany). High specific [32P]-H_3PO_4 (1.9 GBq/ml) was purchased from New England Nuclear. All experiments were carried out in polyethene vessels.

Methods

Determination of the sodium content in the final sodium monofluorophosphate was performed by instrumental neutron activation analysis. Determination of fluoride in two of the inactive MFP preparations

was carried out after hydrolysis of MFP with 1 mol·l⁻¹ sulphuric acid by using an ion specific fluoride electrode with an Orion Ionalyzer 901 and compared with the reference material and the theoretical value[18].

In two inactive MFP preparations the phosphorus concentration was determined colorimetrically according to the method described by Fiske and Subbarrow[19] and compared to the amount of phosphorus of the commercially available MFP. In radioactive MFP preparations the ³²P quantity in Ag_3PO_4, Ag_2FPO_3 and Na_2FPO_3 was determined by dissolving samples of the compounds in 15 % NH_4OH and liquid scintillation counting in a Packard 2650 Liquid Scintillation Counter.

Powder X-ray diffraction (XRD) measurements were recorded using a Guinier de Wolff camera at Faculty of Chemical Technology and Materials Science, Delft University of Technology, Infrared (IR) spectra were recorded with a Beckman Accu Lab 6 IR-spectrometer. Both types of spectra were used to compare the chemical purity of the final product with the reference material.

For the yield determination of Ag_2FPO_3, the compound is washed with 70 % aqueous ethanol, and then dried at 105°C, the yield of Na_2FPO_3 was determined after evaporation or freeze-drying, respectively.

Results and Discussion

In the original publication[16], Lange uses 91.2 % phosphoric acid (liquid) and solid ammonium bifluoride for the reaction:

$$H_3PO_4 + NH_4F \cdot HF \rightleftharpoons H_2FPO_3 + H_2O + NH_4F$$
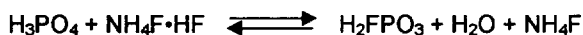
However, Devonshire and Rowley[20] mention, that monofluorophosphoric acid hydrolyses rapidly under strongly acidic or alkaline conditions or at elevated temperatures:

$$H_2FPO_3 + H_2O \rightleftharpoons H_3PO_4 + HF$$

In the present work evaporating the aqueous solution of [³²P]-H_3PO_4 to dryness at 70°C and adding solid ammonium bifluoride reduced the water content of the reactants. Adjustment of the pH-value in an ice bath was meant to avoid hydrolysis at elevated temperature.

In a first series of experiments, the yield of sodium monofluorophosphate was determined at different molar ratios of phosphoric acid (10 mmol) and ammonium bifluoride. The reaction was carried out at 45°C for 2 hours. Following Langes preparation[16], the precipitation of unreacted phosphoric acid was performed by addition of 1.7 g AgNO₃.

Table 1 summarises the yields obtained of unreacted phosphate (Ag_3PO_4), of the initially precipitated silver salt (Ag_2FPO_3) and the finally obtained sodium monofluorophosphate after cation exchange of the silver ions (Na_2FPO_3). The yields are based on the mass, calculated according to

$$yield(\%) = \frac{w}{M_w} \cdot \frac{100}{0.01} \qquad (1)$$

Table 1: Yields of Ag_3PO_4, Ag_2FPO_3 and Na_2FPO_3 obtained at different molar ratios of the reactants. Yields are based on masses, the number in parentheses gives the activity yield, the reaction time was 2 hours

Molar ratio	Yield (%)		
H_3PO_4 : $NH_4F \cdot HF$	Ag_3PO_4	Ag_2FPO_3	Na_2FPO_3
2:1		27.5	23.8
2:1	61.2 (14.3)		25.2 (4.9)
2:1	67.7 (36.6)		19.1 (8.7)
2:1	67.0 (74.1)	22.1 (27.1)	25.9 (26.8)
2:1	58.3	29.6	38.5
1:1	61.4 (58.5)	35.0 (25.0)	45.2 (25.2)
1:1	59.0	29.2	37.0
1:1	63.3	31.2	38.7
1:1	71.9	33.3	35.3
2:3	70.3 (69.9)	19.5 (22.1)	22.3 (20.4)
2:3	79.0	21.0	25.0
2:3	78.8	14.2	15.8

On the average, the highest Na_2FPO_3 yields are obtained for a molar ratio of 1:1, which is the ratio used in all later experiments. The difference

between mass and radioactive yield, which can be observed in table 1 was not present in later experiments; therefore it was not attempted to find an explanation for this observation.

In a second series of experiments the amount of $AgNO_3$ was increased from 1.7 g to 3.5 g to ensure, that Ag_2FPO_3 is precipitated quantitatively. Table 2 shows the yields after the formation of Ag_2FPO_3 has been performed with 3.5 g $AgNO_3$ instead of 1.7 g.

Table 2: Yields of Ag_3PO_4, Ag_2FPO_3 and Na_2FPO_3 after increasing the amount of silver nitrate for the precipitation of silver MFP, the molar ratio of the reactants was 1:1, reaction time 2 hours

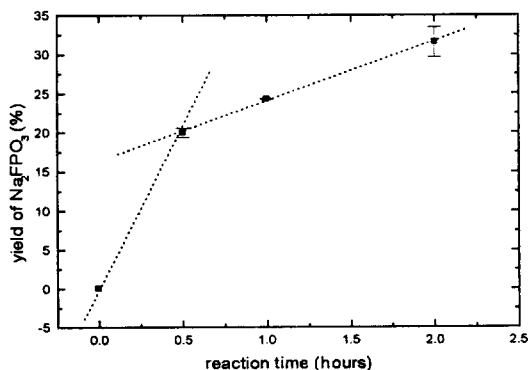| Yield (%)		
Ag_3PO_4	Ag_2FPO_3	Na_2FPO_3
55.0 (51.6)	26.6 (31.1)	31.3 (30.1)
54.7 (50.7)	39.4 (40.3)	49.8 (40.8)
60.0	40.9	51.0
65.7 (58.1)	32.4 (30.1)	40.4 (35.2)
61.9	34.9	43.2

Tables 1 and 2 show, that the mass yields are always higher for Na_2FPO_3, than for Ag_2FPO_3. Several possible explanations, such as fluoride containing inclusions in Ag_2FPO_3, the presence of other phosphate containing compounds, or the formation 'physical' impurities, such as aggregates, were discussed. However, after treating the Ag_2FPO_3 with ultrasound, the yields of Ag_2FPO_3 and Na_2FPO_3 were in good agreement (Table 3).

Table 3: Yields for Ag_3PO_4, Ag_2FPO_3 and Na_2FPO_3 after purification of Ag_2FPO_3 by ultrasonic treatment, molar ratio of reactants 1:1, reaction time 2 hours

| Yield (%)		
Ag_3PO_4	Ag_2FPO_3	Na_2FPO_3
71.3	31.6	31.6
53.4	33.6	31.7

In the last series of experiments, the parameter investigated was the reaction time. Figure 1 shows the Na_2FPO_3 yields obtained for 30 minutes, 1 hour and 2 hours.

Fig. 1: Dependence of Na_2FPO_3 yield on the reaction time, molar ratio of reactants 1:1, the yield of Na_2FPO_3 is based on the mass yield (dotted lines are drawn to guide the eye)

The results show, that the reaction is not complete after 2 hours and that it is very likely, that the yield may be increased.

For analysis of [^{32}P]-MFP, Infrared (IR) spectra of this compound and a commercially available reference material were recorded (figure 2).
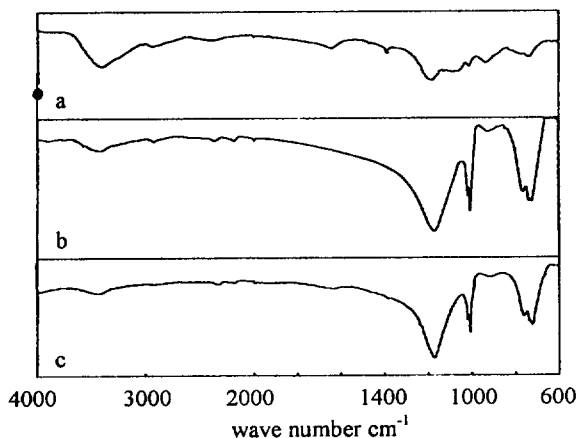
Fig. 2: IR spectrum of ^{32}P-labelled sodium monofluorophosphate after evaporation (a), of the reference material (b), of ^{32}P-labelled sodium monofluorophosphate after freeze-drying

Spectra a and b are not identical. A possible explanation for the differences could be the occurrence of structural changes during the evaporation of the final radioactive product. Instead of evaporation, the final labelled product was freeze-dried to avoid structural changes and the IR spectrum of this product (c) compared to the reference material. The resulting spectrum was now in good agreement with the reference material (b). Both spectra show the expected bands at 1110-1180, 1000-1020, and 700-770 cm^{-1} [21]. Comparison of X-ray diffraction (XRD) spectra of the products also showed differences between the reference material and the evaporated labelled product, which could be completely eliminated after freeze-drying. An elemental analysis of [^{32}P]-Na$_2$FPO$_3$ and the reference material showed reasonable agreement with the theoretical values (Table 4).

Table 4: Phosphorus, sodium, and fluorine content of [^{32}P]-labelled MFP and the reference material (numbers in brackets show the deviation from the theoretical value)

	Na (%)	F (%)	P (%)
reference material	32.2 (+ 0.9)	13.6 (+ 3.0)	22.1 (+ 2.8)
^{32}P-labelled MFP	32.2 (+ 0.9)	13.2 (± 0.0)	21.2 (- 1.4)
theoretical value	31.9	13.2	21.5

Conclusion

Based on the reaction of a melt of phosphoric acid and hydrofluoric acid it was possible to develop a procedure, which is capable to prepare [^{32}P]-labelled sodium monofluorophosphate. The chemical purity of the compound was higher than 98 %, as determined by comparing XRD spectra of the radioactive compound with those of a reference material of 98 % purity. However, a test procedure to determine the exact chemical purity of the final product should be employed in future preparations.

The specific activity obtained was 56 MBq ^{32}P per mmol. However, since the [^{32}P]-labelled phosphoric acid has been diluted to a considerable amount with inactive phosphoric acid, a possible increase in specific activity is very

likely, when the ratio of active to inactive phosphoric acid is increased. The final yield for the preparation procedure was 30 % of the initial ^{32}P activity. This yield may be increased by using longer reaction times for the melting process.

[^{32}P]-sodium monofluorophosphate prepared according to the procedure described above has been successfully used in experiments to study the diffusion of MFP ions into whole bovine enamel[22].

Acknowledgement .

References

[1] Anonymous - Brit. Dent. J. 150: 285 (1981)
[2] G. S. Ingram - Caries Res. 6: 1 (1975)
[3] P. Grøn, F. Brudevold, R. Aasenden - Caries Res. 5: 202 (1971)
[4] Y. Ericsson - Acta Odont. Scand. 21: 341 (1963)
[5] E. D. Eanes - Caries Res. 10: 59 (1976)
[6] W. L. Jongebloed, I. Molenaar, J. Arends - Caries Res. 9: 388 (1975)
[7] A. Battmann, H. Resch, C. R. Libanati, D. Ludy, M. Fischer, S. Farley, D. J. Baylink - Osteoporos. Int. 7: 48 (1997)
[8] S. Patel, J. K. M. Chan, D. J. Hoskin - Bone 19: 53 (1997)
[9] N. Yoza, S. Nakashima, T. Nakazato - Chem. Lett. 1: 53 (1997)
[10] A. Rigalli, L. Esteban, L. Pera, R. C. Puche - Calcif. Tissue Int. 60: 86 (1997)
[11] O. F. Hill, L. F. Audrieth - Inorg. Synth. 3: 106 (1950)
[12] L. F. Audrieth, O. F. Hill - Inorg. Synth. 3: 109 (1950)
[13] W. Lange - Ber. Chem. Ges. 62: 786 (1929)
[14] W. Lange - Ber. Chem. Ges. 62: 793 (1929)
[15] W. Lange, R. Livingston - J. Am. Chem. Soc. 69: 1073 (1947)
[16] W. Lange - Ber. Chem. Ges. 62: 1084 (1929)
[17] Y. Ericsson - Int J. Appl. Radiat. Isot. 10: 177 (1970)
[18] A. F. Berndt, R. L. Stearns - Anal. Chim. Acta 74: 446 (1975)
[19] C. H. Fiske, Y. Subbarrow - J. Biol. Chem. 66: 375 (1925)
[20] L. N. Devonshire, H. H. Rowley - Inorg. Chem. 1: 680 (1962)
[21] R. A. Nyquist, R. O. Kagel - Infrared Spectra of Inorganic Compounds, Academic Press, New York and London, 1971.
[22] J. F. de Rooij, PhD Thesis, Rijksuniversiteit Groningen, 1981