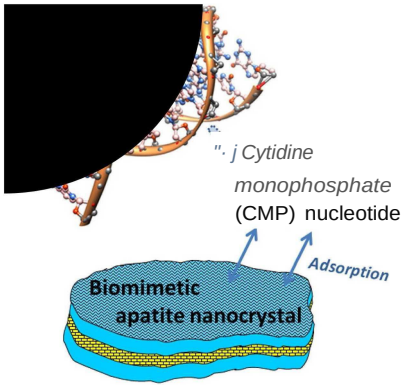
# Adsorption of nucleotides on biomimetic apatite: The case of cytidine 5' monophosphate (CMP)

Maëla Choimet[a,b], Audrey Tourrette[b], Christophe Drouet[a,*]

[a] CIRIMAT Carnot Institute, UMR CNRS/INPT/UPS 5085, University of Toulouse, Ensiacet, 4 allée E. Manso. 31030 Toulouse cedex 4, France
[b] CIRIMAT Carnot Institute, UMR CNRS/INPT/UPS 5085, University of Toulouse, Faculté des Sciences Pharmaceutiques, 35 Chemin des Maraîchers, 31400 Toulouse, France

## GRAPHICAL ABSTRACT




*Keywords:*
DNA
Adsorption
Biomimetic apatite
Cytidine monophosphate
Paleogenetics
Hydroxyapatite

## ABSTRACT

The chemical interaction between DNA macromolecules and hard tissues in vertebrate is of foremost importance in paleogenetics, as bones and teeth represent a major substrate for the genetic material after cell death. Recently, the empirical hypothesis of DNA "protection" over time thanks to its adsorption on hard tissues was revisited from a physico-chemical viewpoint. In particular, the existence of a strong interaction between phosphate groups of DNA backbone and the surface of apatite nanocrystals (mimicking bone/ dentin mineral) was evidenced on an Dexperimental basis. In the field of nanomedicine, DNA or RNA can be used for gene transport into cells.and apatite nanocarriers then appear promising. In order to shed some more light on interactions between DNA molecules and apatite, the present study focuses on the adsorption of a "model" nucleotide, cytidine 5' monophosphate (CMP), on a carbonated biomimetic apatite sample.The follow-up of CMP kinetics of adsorption pointed out the rapidity of interaction with stabilization reached within few minutes.The adsorption isotherm could be realistically fitted to the Sips model (Langmuir-Freundlich) suggesting the influence of surface heterogeneities and adsorption cooperativity in the adsorption process. The desorption study pointed out the reversible character of CMP adsorption on biomimetic apatite.This contribution is intended to prove helpful in view of better apprehending the molecular interaction of DNA fragments and apatite compounds, independently of the application domain, such as bone diagenesis or nanomedicine. This study may also appear informative for researchers interested in the origins of life on Earth and the occurrence and behavior of primitive biomolecules.

* Corresponding author.
*E-mail address:* christophe.drouet@ensiacet.fr (C. Drouet).

## 1. Introduction

After the death of an organism, its constitutive cells rapidly break up and release their contents, including DNA (deoxyribonucleic acid). The analysis of DNA extracts from body remains has led to much progress in domains such as paleogenetics, paleomicrobiology, animal and vegetal adaptations and even forensic sciences. Among the genetic markers studied are the uni- parental markers (e.g. located on mitochondrial DNA inherited from the mother, or on the Y chromosome inherited from the father), and the biparental DNA markers located on the autosomes. Ali these markers are very important for following evolutionary histories of populations [1] or revealing family relationships within funeral areas burials [2]. Another area of interest related to the analysis of DNA residues is paleo-microbiology. The analysis of lice (head bugs) found on soldiers from the Vilnius battle by Napoleon in 1812 is an illustration of this point application [3].

The development of molecular biology techniques has permitted to extract information from mineralized tissues (bone and teeth remains) that are the predominant vertebrates' vestiges found in archeological searches [4]. The study of DNA extracted from hard tissues then permitted to gain information on more and more ancient periods of time. Indeed, DNA seems to be better preserved in hard tissues than in soft tissues [5]. Today, DNA sequences dating of more than 1700000 years can be extracted from skeletal remains [6]. The preservation of DNA in such ancient specimens was at first surprising, because experimental data [7] only allowed one to expect *a priori* a much faster DNA degradation.

From the better conservation of DNA associated to bone and teeth arose the hypothesis of a possible role played by a specific interaction between DNA and the apatite contained in mineralized tissues. This hypothesis has long been used by paleo-geneticists (7], but without being actually demonstrated. Recently, a physico-chemical study [8] investigated the interaction between DNA fragments and biomimetic apatite analogous to bone mineral/dentin. By exploring DNA adsorption and desorption onto apatite nanocrystals, the existence of strong chemical links was evidenced: the existence of a clear interaction between phosphate groups of DNA backbone and the surface of apatite nanocrystals was indeed evidenced on an experimental basis [8]. The absence of desorption upon simple dilution was observed (while it was favored upon adding phosphate ions in the medium). The implication of DNA phosphate backbone in adsorption processes was also reported in other studies involving different substrates and for distinct applications [9-12].

In another domain, the adsorption of plasmid DNA or RNA on nanosystems such as colloidal nanoparticles can prove helpful for gene transfection/gene silencing applications [13-17]: since apatite-based nanoparticles have been shown to have a great potential in nanomedicine, the use of DNA- or RNA-loaded apatite nanoparticles for the transportation of extemal genes not naturally expressed by cells appeared indeed as an encouraging route.

DNA, however, is a complex macromolecule with altemating nucleotides organized in double-stranded helicoidal structure, where a nucleotide is the result of the association of a nucleic base (typically Cytosine, Adenine, Guanine or Thymine) with a pentose (deoxyribose) and a phosphate group. With the aim to inspect further the type of interaction that may occur between DNA and apatite, and independently of the application (paleogenetics, nanomedicine, . ..), the present contribution investigates the adsorptive behavior of a "model" nucleotide, cytidine 5' monophosphate (CMP), onto a biomimetic apatite substrate as previously used (see for example [8]). After summarizing the main characteristics of the apatitic substrate used in this work, the CMP adsorption kinetics and isotherm were investigated, at room temperature, and desorption was followed in the presence or in the absence of phosphate ions. These results could serve as a complementary instructive dataset in future DNA/apatite-related works, independently of the application field.

## 2. Materials and methods

### *2.1. Synthesis of the biomimetic apatite substrate*

The synthetic sample of carbonated biomimetic apatite used in this work was obtained by precipitation of a calcium nitrate $Ca(N0_3)z\text{-}4H_20$ solution (52.2 g in 750 ml of deionized water) with an ammonium hydrogenphosphate $(NH_4)zHP0_4$ solution and a sodium bicarbonate $NaHC0_3$ solution (90 g of each in 1500 ml of deionized water), at room temperature (20 °C). In the second solution, an excess of phosphate was used to buffer the pH of the solution at 7.2, close to the physiological values (7.4). The precipitate was then left to mature at room temperature for 1 week (7 days), filtered on Büchner funnel, and thoroughly washed with deionized water. The sample was then freeze-dried for 3 days, sieved in different size fractions, and stored at -18°C until further use. In this study, we worked with granulometry fraction below 100 µm. In the rest of the text, this 1-week matured sample will be referred to as "hac-lw".

### 2.2. *Physico-chemical characterization of the apatite substrate*

The physico-chemical characteristics of the hac-1w apatite were detailed in a previous study using several techniques [8]. Briefly, the total calcium and phosphate contents in the apatite phase were determined by complexometry and by spectrophotometry (Shimadzu UV 1800, Â. = 460 nm) respectively, while the carbonate titration was carried out by coulometric method (UIC, he. CM 5014 coulometer with CM 5130 acidification unit). The nature of the crystalline phase was determined by powder X-ray diffraction using an INEL diffractometer CPS 120 and the monochromatic Co Ka. radiation (,1. = 1.78892 A). Fourier transform infrared (Ff!R) spectroscopy analyses were achieved on a Thermo-Nicolet 5700 spectrometer with a resolution of 4 cm-1, using the KBr pellet method. A Microtrac BELSORP mini II apparatus (BET method based on nitrogen adsorption ) was used to determine the specific surface area Sw. Zeta potential (determined from electrophoretic mobility measurements) was determined at 25 °C using a Malvern Zetasizer Nano Analyzer apparatus. The apatitic substrate was studied after dispersion of the powder in water set at different pH values in the range 6-9.5 and filtration on micropore filter for avoiding the sedimentation of large aggregates (>1 µm) during the analyses.

### *2.3. Cytidine 5' monophosphate (CMP) adsorption and desorption*

Cytidine 5' monophosphate, in the form of di-sodium sait, was purchased from Sigma Aldrich; its chemical formula is given in Fig. 1. For ail experiments of adsorption or desorption, 20 mg of apatite powder (O < 100 µm) was dispersed in 5 ml of CMP solutions at room temperature. The CMP concentration present in the supernatant was evaluated using spectrophotometry (Shimadzu UV 1800, Â. = 272 nm). At any time, the adsorbed amount could be determined by difference of the initial quantity introduced in the medium and the residual amount in solution.

Adsorption kinetics was investigated by following the amount of adsorbed CMP versus time. For these experiments, a CMP solution of 300 mg/l in I<Cl solution (0.01 M) was used, at room temperature and physiological pH. Different contact times ranging from 5 min to 3 h were followed so as to evaluate the adsorption

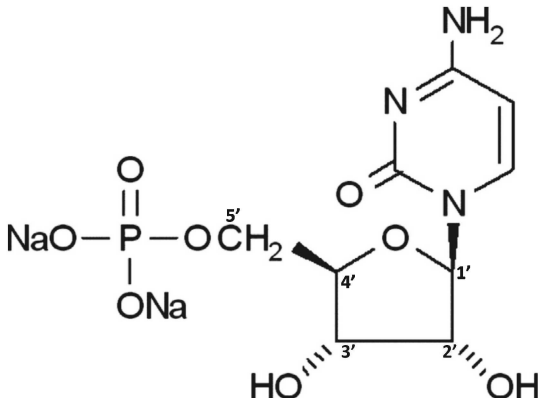


Fig. 1. Chemical formula of cytidine 5' monophosphate (CMP) di-sodium sait.

equilibrium of CMP on the apatite substrate. After the selected contact time, the mixture was centrifuged for 2 min at 5000 rpm and the supernatant was withdrawn and analyzed by spectrophotometry as indicated above. The CMP adsorption isotherm was then built by following (after 1h of contact time) the adsorbed amount versus the equilibrium concentration in solution, varying from 0 up to 1200 mg/!.

When mentioned in the text. the amount of phosphate and calcium ions present in the supernatant after adsorption were respectively evaluated by spectrophotometry and atomic absorption (Analytik jena ContrAA300, $N_20$/acetylene flame, .1.=422.67 nm).

The eventual desorption of CMP was investigated for some datapoints corresponding to the isotherm (250, 600 and 1000 mg/L). After 1 h of adsorption, a known amount of supernatant was withdrawn and replaced by KCI solution (0.01 M). After the same immersion time, the solution was analyzed by spectrophotometry. The effect of an addition of phosphate ions on the release of CMP was also followed by the addition of $KH_2P0_4$ at a final concentration of 18 mM.

## 3. Results and discussion

### *3.1. Physico-chemical characterization of apatite substrate*

As mentioned above, the biomimetic apatite substrate, hac-1w, used in this work was previously characterized (see [8] for details); for the sake of completeness however its main features are summarized hereafter.

The apatitic nature of the sample was confirmed by powder X-ray diffraction (XRD) analysis, complemented by Fourier-Transform lnfrared (FTIR) spectroscopy. ln particular, the XRD pattern showed the absence of detectable secondary phases (Fig. 2a), and the biomimetic character of this sample can be assessed by the similarity of crystalline features to those of bone specimens (see Supporting Information, Fig. SI 1). The broadness of the diffraction peaks can be attributed bath to the existence of microstrains in the apatitic network and to nanosized constitutive crystals. The application of Scherrer's formula to diffraction lines (002) and (310) suggested, in a first approximation, a mean crystallite length of around 16 nm and a mean width/depth close to 5 nm, underlining the nanometer dimensions of this hac-1w sample, similarly to those of bone minerai [18]. The specific surface area of the granulometry fraction selected in this work reached 122 $m^2$/g. For additional information on the physic-chemical characteristics of the apatitic substrate used in this work, its zeta potential was also measured, in a wide pH range from 6 to 9.5. It was found to be negative throughout this pH range, and close to -30 ± 2 mV. Such negative values are consistent for such

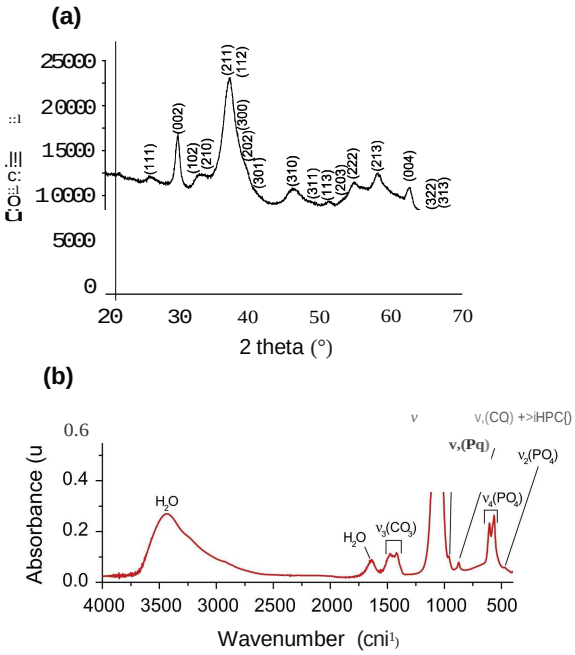



Fig. 2. (a) XRD pattern (2 theta between 20° and 70°) of the biornirnetic carbonated apatite sample hac-lw (maturation of 1 week) used in this work, with *( hk l)* indexation after JCPDS file #09-432 relative to hydroxyapatite, and (b) FTIR spectrum for hac-1w with phosphate, carbonate and water band attributions.

nonstoichiometric nanocrystalline apatites as previously reported [19]. Note however, that this measurement only gives a global value of the surface charge and does not imply that only negatively charged surface sites are present as bath calcium and phosphate (protonated) ions coexist.

The carbonation of this sample was confirmed by the presence of carbonate bands observable by FTIR (Fig. 2b), in the ranges 1350-1550 $cm^{-1}$ ($v_3(C0_3)$ domain) and 840-910 $cm^{-1}$ ($v_2(C0_3)$ domain). Bath direct coulometric measurements and FTIR carbonate titration using a recently reported method (20] revealed a carbonate amount of 3.6 wt.%. The Ca/( P + C) overall molar ratio determined by complementary chemical titrations led to 1.36. This value, noticeably Iower than 1.67 (characteristic of stoichiometric hydroxyapatite), points to the nonstoichiometry of this compound, as for bone samples (18], confirming again the biomimetic nature of this apatite compound.

The above results thus substantiate the choice of this apatite sample for the present study, aiming at inspecting the interaction of the CMP nucleotide with a substrate close to bone minerai.

### *3.2. Investigation of CMP adsorption and desorption*

CMP adsorption onto hac-1w was investigated here in two ways. First, the sorption kinetics was followed by varying the CMP/apatite contact time, so as to determine the conditions of achievement of thermodynamic equilibrium. Then, for a selected contact time, the adsorbed amount was measured for increasing CMP concentrations in solution, in order to define the adsorption isotherm (at room temperature ).

#### *3.2.1. Adsorption kinetics*

Fig. 3 shows the adsorption kinetics of CMP on hac-lw (for an initial concentration of 300 mg/l), by plotting the amount of CMP adsorbed as a function of contact time. The thermodynamic equilibrium was reached very quickly after Jess than 10 min. Stabilization occurred at a concentration of ca. 224 mg/I,

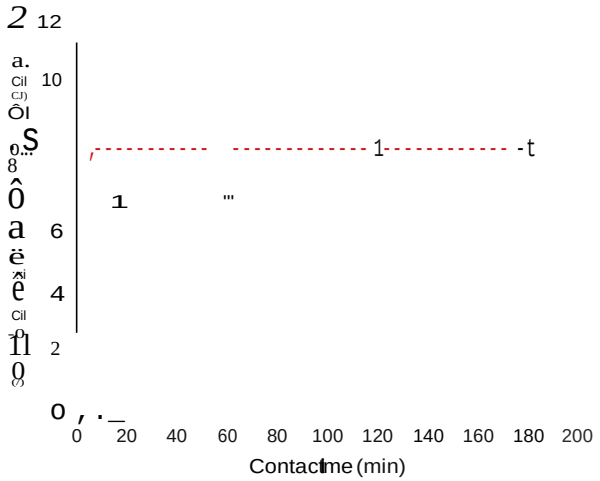



Fig. 3. Kinetics of adsorption of CMP onto biomimetic apatite hac-1w (room temperature).

corresponding to an adsorbed amount of ca. 7.5 mg/g of apatite (0.19 µmol/m²).

Taking into account the rapidity of this adsorption process, probably painting to a high affinity of CMP molecules for the surface of biomimetic apatite, it was not possible to draw advanced conclusions relatively to the type of kinetic mode!.

*3.2.2. Adsorption isotherm*

The adsorption isotherm of CMP on apatite is shown in Fig. 4. The shape of the plot indicates a progressive rise of the adsorbed amount (Q,ctsl for increasing equilibrium concentrations (Ceq). ln order to explore further the adsorption mechanism implied in the CMP/biomimetic apatite system, the experimental datapoints were then tentatively fitted to various mathematical models often encountered in adsorption studies, including with apatite substrates (e.g. [8,21,22]).

The models of Langmuir, Freundlich and Temkin were the first to be tested; being respectively described by the following equations:

$$Q_{ads} = Q_m \left( \frac{K_L \cdot C_{eq}}{1 + K_L \cdot C_{eq}} \right) \quad \text{Langmuir} \qquad (1)$$

$$Qads = KF \cdot C!" \quad \text{Freundlich} \qquad (2)$$

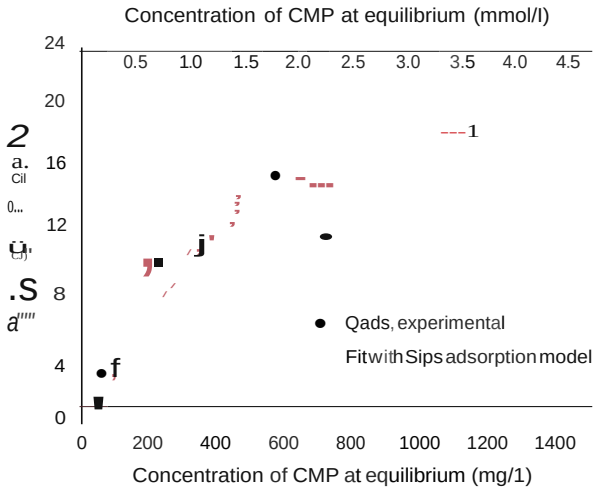



Fig. 4. CMP adsorption isotherm (-20 °C) on hac-1w biomimetic apatite (concentrations given in mmol/l and in mg/l).

$$Q.ds = B \cdot \mathrm{Ln}(A) + B \cdot \mathrm{Ln}(Ceq) \quad \text{Temkin} \qquad (3)$$

where KL. Nm.Kf, *n*, *A* and *B* are the related constants, at a given temperature. The application of these models to the experimental data led to the following correlation parameters (adjusted $R^2$): 0.9916, 0.9637 and 0.9453. These findings suggest that pure Freundlich or Temkin adsorptive behaviors cannot adequately explain the experimental variation observed here. Instead, an improved fit was found with the Langmuir equation (with = 32.33 ± 2.41 mg/g (0.82 ± 0.06 µmol/m²) and KL = 0.0017 ± 0.0002 1/mg). A recent study on the adsorption of tetracycline on biomimetic apatite [23] however pointed out the interest to test also the adequacy of another mode!, known as Sips equation [24,25] (also known as "L angmuir-Freundlich" isotherm) which appears as a modified version of the Langmuir mode! by incorporating the exponent "m" as follows:

$$Q.cts,e = Qm \cdot \frac{Ks \cdot C\eta}{1 + K_{s} \cdot eq} \quad \text{Sips isotherm} \qquad (4)$$

The application of this equation to our experimental points led to the adjusted $R^2$ correlation parameter 0.9933, indicating that this mode! is the most relevant, among the four tested, to describe adequately the obtained datapoints. The corresponding Sips parameters are *m* = 1.20 ± 0.11, = 26.1± 2.6 mg/g (0.66 ± 0.07 µmol/m²), and *Ks* = 0.00052 ± 0.00027. In particular, the value of "m", noticeably greater than unity, confirms that the isotherm should not be considered as fully Langmuirian.The maximal adsorbed amount (monolayer coverage in our experimental conditions) thus reaches about 26.1 mg/g (0.66 µmol/m²), which is equivalent, in micromoles per gram, to 80.8 µmol/g. This value is roughly of the same order of magnitude as the one found for tetracycline on a 1-day matured [23] (the concept of maturation of nanocrystalline apatites being explained elsewhere [26]) carbonated apatite sample ( 82 µmol/g). On the other hand, it is difficult to directly compare this maximal adsorbed amount to that found for much larger macromolecules such as DNA (of the order of0.49 µmol/g [8]), due to very different molecular sizes and dynamics. Nonetheless, the adsorption of CMP molecules on apatite probably involves at least an interaction via the exposed phosphate end-group which is known to have a high affinity for apatitic surfaces, as was also suggested by other studies (e.g. bisphosphonates [27,28] or DNA [8]).

The value of "m" in Sips equation is also informative on the type of interaction existing in the adsorbent/adsorbate system, as was discussed previously [25]: it was in particular related to the existence of lateral interactions between adsorbed molecules (which is not considered in the Langmuir theory). For $m > 1$, "positive" cooperativity between adjacent molecules is hypothesized, while $m < 1$ would correspond to "negative" cooperativity. In the present case, *m* 1.20 could thus suggest a favoring role of adsorbed molecules on the adsorption process. The fact that Sips isotherm fits better the experimental data as compared to Langmuir may also highlight the heterogeneous nature of the surface of apatites.

The Sips constant *Ks* also carries some thermodynamic meaning as it was proposed to be linked to the change in standard Gibbs free energy of adsorption through the following equations [24] (with $K_S$ recalculated with Ceq expressed in mol/!):

$$K_S = \exp\left( -\frac{\Delta G^{\circ}_{ads}}{RT} \right) \qquad (5)$$

In the present case, this leads to the negative value Gacts° -19 kj/mol (uncertainty estimated to ±1 kj/mol), indicating a favorable adsorption of CMP on biomimeti c apatite in standard conditions. For information, this value is of the same order as the one (-22 kj/mol) found for tetracycline adsorption [23].

In order to inspect further the adsorption mechanism, the amounts of calcium and phosphate ions potentially released in the medium after adsorption were measured. lndeed, previous studies have shown that the adsorption of some molecules, such as bisphosphonates [21,29], were accompanied by the concomitant release of ions from apatite nanocrystals surfaces, leading to a physical "anchoring" of the molecule on the substrate. ln the present case, the titration of Ca and P in the supernatants showed however their presence only in small quantities after adsorption. After subtraction of the amounts of calcium and phosphate ions naturally released due to the partial dissolution of the apatite substrate in the medium (as any ionic compound ), it is then possible to examine whether any additional release is observed due to the adsorption process itself. For <lacts varying from 0 to 24 mg/g (see isotherm Fig. 4; corresponding in moles to the range 0-75 µmol/g), the amount of phosphate released remained low and comprised between 0 and 6 µmol/g, while calcium was found to vary around a mean position of 1OO µmol/g. No clear trend was observed in function of the adsorbed amount. Thus, these findings suggest that the adsorption of CMP on nanocrystalline apatite is not accompanied by a significant simultaneous release of surface ions.

### 3.2.3. Desorption study

In continuation with the adsorption study, the possible desorption of adsorbed CMP molecules was followed by dilution of the medium (see dilution details in experimental section). Severa( desorption points were examined, corresponding to various degrees of surface coverage, as shown in Fig. 5. On this figure, the "hypothetical" points calculated by supposing the absence of desorption upon dilution are indicated in parentheses , for information. For each point studied, dilution of the medium led, in contrast, to partial CMP desorption as marked by the curved arrows. ln the assumption that this adsorption process would be fully reversible, desorption should occur in a way that the point would follow the adsorption isotherm in the opposite way. ln the case of nanocrystalline apatites, however, the adsorption of some molecules was found to be irreversible upon dilution; and this observation was related to a concomitant ion release from the surface of the nanocrystals: for example, the adsorption of bisphosphonates cited above was shown to occur simultaneously to the release of surface phosphate ions, with no clear release of calcium, thus leading to an "anchoring" of the molecule on the nanocrystal surface, in a "surface phosphate" position.

ln the present case, the experimental desorption of CMP, another phosphate-bearing molecule, (see square "D" datapoints in Fig. 5) are found to clearly approach the isotherm (although without being strictly superimposed to it); therefore one can conclude that the CMP/apatite adsorption process is for the most part "reversible" upon simple dilution. This conclusion seems to be corroborated by the fact that the addition of phosphate ions in the diluted medium (in the form of dissolved $KH_2P0_4$ sait, see star "*" datapoints in Fig. 5) does not promote desorption. Also, this is in good agreement with our above results (see previous subsection) indicating that the adsorption process was not accompanied by a significant phosphate release in the supernatant.

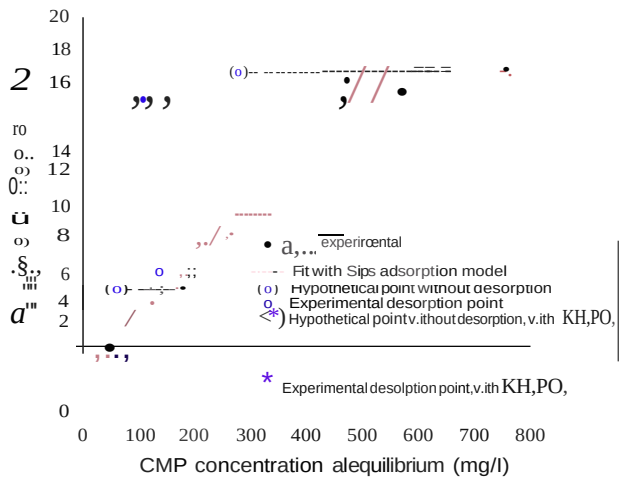



Fig.5. Desorption study upon dilution, and effect of the addition of phosphate ions in the medium ($KH_2P0_4$).

### 3.3. Concluding remarks

In this contribution, the adsorption of a nucleotide, CMP, onto a nanocrystalline apatite substrate mimicking bone minerai was investigated in detail. The adsorption isotherm, established for a contact time permitting to reach equilibrium, was found to follow a Sips (Langmuir-Freundlich) model, and data analysis suggested a cooperative adsorption of CMP molecules. The study of the desorption upon dilution and the analysis of supernatants allowed us to insinuate that CMP adsorption on nanocrystalline (biomimetic) apatite was highly reversible and not accompanied by a concomitant ion release from the apatite substrate (despite the presence of a terminal phosphate group on the molecule). This type of analysis, on a single nucleotide – forming one "building black" of DNA or RNA macromolecules – will have to be extended to polynucleotide subunits and compared to data recently obtained on the adsorption of DNA on biomimetic apatite [8]. Nonetheless, it already provides some additional data in view of better comprehending the interaction between apatite compounds and DNA, e.g. in post-mortem settings (study of bone diagenesis) or for nanomedicine applications.

By extrapolation, this work may also appear informative for researchers interested in the origins of life on Earth, where the occurrence and preservation of primitive biomol ecules such as nucleotides is of prime relevance. Indeed, as in the case of DNA (see Ref. [8]), the adsorption of nucleotides onto minerai substrates constitutive of the Earth's geochemistry such as apatites (e.g. present in metamorphic rocks [30] of pelitic, carbonate, basaltic, and ultramafic composition) may provide protection against premature degradation by environmental factors. Also, in the adsorbed state, nucleosides/nucleotides may then undergo surface reactions (possibly catalytic) to promote chemical modifications and/or their combination into polynucleotides of biological relevance [31]. Therefore, better apprehending adsorption processes involving nucleotides and minerais like apatites could participate to better understanding how "primitive" biomolecules succeeded to evolve toward more elaborate systems.


## Acknowledgment

The authors wish to thank the CIRIMAT carnot Institute for internai funding of Maela Choimet's PhD thesis.


## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/jjcis.2015.06.021.

## References


[1] C. Keyser, C. Bouakaze, E. Crubezy, V.G. Nikolaev, D. Montagnan, T. Reis, B. Ludes, Hum. Genet. (2009) 126.
[2] C. Keyser-Tra cqui, E. Crubezy, B. Ludes, Am. j. Hum. Genet. (2003) 73.
[3] D. Raoult, O. Dutour, L. Houhamdi, R.jankauskas, P.E. Fournier, Y.Ardagna, M. Drancourt, M. Signoli, V.D. La, Y. Macia, R. Aboudharam, j. Infect. Dis. (2006) 193.
[4] E. Hagelberg, B. Sykes, R. Hedges, Nature (1989) 342.
[5] P. Gill, P.L. Ivanov, C. Kimpton, R. Piercy, N. Benson, G. Tully, 1. Evett, E. Hagelberg, K. Sullivan, Nat. Genet. 6 (1994).

(6) L. Orlando, A. Ginolhac, G. Zhang, D. Froese, A. Albrechtsen, M. Stiller, M. Schubert, E. Cappellini, B. Petersen, 1. Moltke, P.L.F. Johnson, M. Fumagalli, j.T. Vilstrup, M. Raghavan, T. Korneliussen, A.-S. Malaspinas,]. Vogt, D. Szklarczyk, C.D. Kelstrup, J. Vinther, A. Dolocan, J. Stenderup, A.M.V. Velazquez, j. Cahill, M. Rasmussen, X. Wang, j. Min, G.D. Zazula, A. Seguin-Orlando, C. Mortensen, K. Magnussen, J.F. Thompson, J. Weinstock, K. Gregersen, K.H. Roed, V. Eisenmann, Cj. Rubin, D.C. Miller, D.F. Antczak, M.F. Bertelsen, S. Brunak, K.A.S. Al-Rasheid, O.Ryder, L. Andersson , j. Mundy, A. Krogh, M.T.P. Gilbert, K. Kjaer, T. Sicheritz-Ponten, L.j. Jensen, j.V. Olsen, M. Hofreiter, R. Nielsen , B. Shapiro, J. Wang, E. Willerslev, Nature 499 (2013) 74-78.
[7] T. Lindahl, Nature 362 (1993).
[8] A. Grunenwald, C. Keyser, A.M. Sautereau, E.Crubezy, B. Ludes, C. Drouet, Appl. Surf. Sei. 292 (2014) 867-875.
(9) B. Liu,]. Liu, Langmuir 31 (2015) 371-377.
(10) X. Zhang, F. Wang, B. Liu, E.Y. Kelly. M.R. Servas, ]. Liu, Langmuir 30 (2014) 839-845.
(11) B. Liu,]. Liu, Chem. Commun. 50 (2014) 8568-8570.
(12) R. Pautler, E.Y. Kelly, P.-j.J. Huang, J. Cao, B. Liu, J. Liu, ACS Appl. Mater. Interfaces 5 (2013) 6820-6825.
(13) T. Welzel, 1. Radtke, W. Meyer-Zaika, R. Heumann, M. Epple, j. Mater. Chem. 14 (2004) 2213-2217.
[14] S. Hossain, A. Stanislaus, MJ. Chua, S. Tada, Y.-1. Tagawa, E.H. Chowdhury, T. Akaike, j. Contrai. Release 147 (2010) 101-108.
(15) S. Hossain, S. Tada, T. Akaike, E.H.Chowdhury, Anal. Biochem.397 (2010) 156-161.
(16) E.H. Chowdhury, T. Akaike, Curr. Anal. Chem. 1 (2005) 187-192.
[17] E.H. Chowdhury, Expert Opin. Drug Deliv. 4 (2007) 193-196.
(18) A. Grunenwald, C. Keyser, A.M. Sautereau, E.Crubezy, B. Ludes, C. Drouet, Anal. Bioanal. Chem. 406 (2014) 4691-4704.
(19) 1. Rodriguez-Ruiz, ]. Manuel Delgado-Lapez, M.A. Duran-Olivencia , M. Iafisco, A. Tampieri, D. Colangelo, M. Prat, J. Gomez-Morales, Langmuir 29 (2013) 8213-8221.
(20) A. Grunenwald, C. Keyser, A.M. Sautereau, E. Crubézy, B. Ludes, C. Drouet, ]. Archaeol. Sei. 49 (2014) 134-141.
(21) P. Pascaud, P. Gras, Y. Coppel, C. Rey, S. Sarda, Langmuir 29 (2013) 2224-2232.
(22) L. Benaziz, A. Barroug, A. Legrouri, C. Rey, A. Lebugle, ]. Colloid Interface Sei. 238 (2001 ) 48-53.
(23) S. Cazalbou, G. Bertrand, C. Drouet,]. Phys. Chem. B 119 (7)(2015) 3014-3024.
(24) Y. Liu, Sep. Purif. Technol. 61 (2008) 229-242.
(25) LI<. Koopal, W.H. Vanriemsdijk,j.C.M. Dewit, M.F. Benedetti,].Colloid Interface Sei. 166 ( 1994) 51-60.
(26) N. Vandecandelaere, C. Rey, C. Drouet, J. Mater. Sci.-Mater. Med. 23 (2012) 2593-2606.
[27] P. Pascaud, F. Errassifi, F. Brouillet, S. Sarda, A. Barroug, A. Legrouri, C. Rey,]. Mater. Sci.-Mater. Med. 25 (2014) 2373-2381.
[28] F. Errassifi, S. Sarda, A. Barroug, A. Legrouri, H. Sfihi, C. Rey, J. Colloid Interface Sei. 420 (2014) 101-111.
(29) A. Al-Kattan, F. Errassifi, A. Sautereau, S. Sarda, P. Dufour, A. Barroug, 1. Dos Santos, C. Combes, D. Grossin, C. Rey, C. Drouet, Adv. Eng. Mater. 12 (2010) B224-B233.
[30] F.S.Spear,j.M. Pyle, Apatite, monazite, and xenotime in metamorphic rocks, in: MJ. Kahn, J. Rakovan, j.M . Hughes (Eds.), Phosphates: Geochemical, Geobiological, and Materials Importance, vol. 48, Reviews in Mineralogy and Geochemistry, Mineralogical Society of America, Washington, D.C., 2002, pp. 293-335.
[31] F.G. Burton, M.W. Neuman, W.F. Neuman, Biosystems 3 (1969) 20-26.