Journal of Chromatography B, 879 (2011) 1847–1850

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Surface energetics of bone mineral and synthetic hydroxyapatite using inverse gas chromatography

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ARTICLE INFO

Article history:
Received 20 January 2011
Accepted 21 April 2011
Available online 7 May 2011

Keywords:
Inverse gas chromatography
Hydroxyapatite coating
Biomaterial
Surface energy
Bone
Biological scaffolds

ABSTRACT

Surface energy is one of the important factors that govern protein adhesion and cell attachment on biomaterial surfaces. Inverse gas chromatography (IGC) provides an excellent method to measure the surface energetics of rough and porous biosurfaces. In this study IGC was used to characterize and compare the surface energetics of synthetic and biological hydroxyapatites (natural bone mineral). IGC experiments were performed on three samples: synthetic hydroxyapatites with two levels of purity (99% and 90%) and natural biological hydroxyapatite obtained from bovine trabecular bone. The Lifshitz–Van der Waals component of the surface free energy (γ_S^{LW}) and specific interaction parameter (ε_π) were determined by using homologous series of n-alkanes and alkenes as IGC probe molecules, respectively. The synthetic hydroxyapatite had values of γ_S^{LW} of 33.4 mJ m^{-2} at 99% purity and 53.3 mJ m^{-2} at 90% purity. Biological hydroxyapatite had a value of γ_S^{LW} of 45.7 mJ m^{-2}. For the synthetic hydroxyapatite, the values of π-bond specific interaction parameters, ε_π, were 0.95 mJ (99%) and 3.01 mJ (90%). The biological hydroxyapatite sample had a value of 2.44 mJ for ε_π. The results suggest that, as compared to the synthetic compounds, the biological apatite has considerable surface heterogeneity, either chemical (impurities) or structural suggesting a scaffold surface that is more conducive of protein adhesion and cell attachment.

1. Introduction

Calcium hydroxyapatite, (Ca_{10} (PO_4)$_6$ OH_2) is used extensively for various biomedical applications such as bone fillers, coatings, tissue engineering scaffolds, bone implants and dental composites due to its chemical structural similarity with inorganic component of bone, dentin and enamel [1]. Various methods such as element doping, surface modifications and compositional variations of synthetic hydroxyapatite have been used to increase protein adhesion and cell attachment [2–4]. All of these treatments induce changes in the surface chemistry, energetics, and nature of binding sites, which ultimately govern biological interactions. It is important to understand these surface properties in order to correlate them with biological performance to advance the development of biomaterials.

Biological apatite is superior in performance to synthetic apatite. Synthetic apatite is less osteoinductive, i.e. it does not support new bone formation. This difference in the biological performance of synthetic and biological apatites can be attributed to the physicochemical factors and surface properties of apatites [5]. Knowledge of surface energetics and chemistry of hydroxyapatite is therefore essential to engineer materials that have desirable biological interaction at the interface of biomaterials.

Wetting methods are easy and inexpensive techniques used for analyzing surface energy of biomaterials. However, simple interpretation of experimental results are hindered by artifacts such as surface roughness, chemical heterogeneity, adsorbed species, absorption into the structure or material dissolution that contaminates the liquid [6]. Other techniques that have been used to characterize surface energy include adsorption isotherm studies [7], atomic force microscopy [8], and inverse gas chromatography (IGC) [9]. In this investigation we used IGC to examine the surface energetics of synthetic and biological apatites by measuring the dynamic adsorption characteristic of probe gases. IGC is a suitable tool for this type of comparative study as it addresses many limitations of the wetting methods mentioned above.

In an earlier investigation, Harding et al. used IGC to study the surface phase transition in synthetic hydroxyapatite [10]. They found that the synthetic hydroxyapatite surface is highly reactive toward chlorinated organic probes and has a significantly higher value of the Liftshitz–van der Waals component of surface energy, of ~58 mJ m^{-2} at 100 °C. Smiciklas et al. [9] also used

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doi:10.1016/j.jchromb.2011.04.035

IGC to study hydroxyapatite surface using alkane adsorption. They observed strong adsorption of saturated alkanes on hydroxyapatite through nonspecific interaction with surface energy (γ_S^{LW}) values of ~70 mJ m^{-2} at 75 °C [9].

Since most of these studies were performed on synthetic apatites, there exists a need to compare these surfaces with biological apatites to further explore the differences in biocompatibility and bio-acceptability between the two apatite compounds. The goal of the present study is to compare the surface chemistry and energetics of synthetic and biological apatites using inverse gas chromatography. It is intended that such a comparison will help to assist in the identification of potential strategies for surface modification that will lead to the development of new biologically active synthetic hydroxyapatite materials.

2. Theory

2.1. Inverse gas chromatography at infinite dilution

Inverse gas chromatography is used to determine various surface properties of the stationary solid phase by analyzing the dynamic interaction between polar/nonpolar gaseous molecules (probes) and the solid surface at various fixed temperatures [11–13]. The chromatographic column is filled with the solid material under investigation and small amounts of probe gases are injected into the carrier gas stream flowing through the column. In this investigation the values for t_N were calculated as the difference between the median values of the elution peak of the probe and non-interacting marker probe (methane).

The specific retention volume, based on the surface area of the stationary phase is related to net retention time by the equation:

$$V_S^0 = \frac{jFtN}{A_{SP}w} \tag{1}$$

where F is the temperature corrected flow rate of the high purity, inert carrier gas (nitrogen) and j is the James–Martin correction for compressibility. A_{SP} is the specific surface area and w is the weight of the stationary phase contained within the column. A more detailed discussion of the experimental approach is provided elsewhere [13].

At extremely low injection concentrations, known as infinite dilution or zero coverage, there is no interaction between probe molecules so that Henry's law for ideal gas can be applied. The specific retention volume is related to K_S, the Henry's law constant (partition coefficient) by the relationship

$$V_S^0 = \frac{K_S}{RT_C} \tag{2}$$

where T_C is the column temperature, and R is the gas constant. Due to energetic heterogeneity of the solid surface, K_S varies from site to site as a function of the acidic and basic groups on the surface. These may be characterized by testing probes that specifically interact, as Lewis acids or Lewis bases, with the surface sites.

The standard molar free energy of adsorption, ΔG_A^0, is determined from chromatographic retention volumes using the equation:

$$\Delta G_A^0 = -RT_C \ln V_S^0 + C \tag{3}$$

The integration constant, C, may be assumed constant for a given column packing and a homologous series of probes, as used in this study.

Surface free energy of the solid phase is a function of specific retention volume obtained from the chromatographic experiments. Surface free energy, γ_S, is comprised of the Lifshitz–Van der Waals (apolar) component, γ_S^{LW}, and acid–base (polar) component, γ_S^{AB}.

$$\gamma_S = \gamma_S^{LW} + \gamma_S^{AB} \tag{4}$$

When nonpolar molecules such as n-alkanes are used as the gas probes in IGC, surface energetic is dominated by γ_S^{LW}, and the polar interactions, γ_S^{AB}, can be neglected. The Lifshitz–Van der Waals contribution is the sum of three separate interaction energies: the London dispersive, γ_S^D, the dipole–induced dipole (Debye), γ_S^i, and the dipole–dipole orientation (Keesom), γ_S^μ, interactions.

$$\gamma_S^{LW} = \gamma_S^D + \gamma_S^i + \gamma_S^\mu \tag{5}$$

For low energy surfaces, the Debye induction and Keesom orientation components are negligible so that $\gamma_S^{LW} \approx \gamma_S^D$. However for high energy surfaces, such as mineral oxides, the induced dipole and orientation components may contribute significantly to γ_S^{LW} [13].

The Lifshitz–Van der Waals component of the surface free energy can be calculated using the method proposed by Dorris and Gray [14].

$$\gamma_s^{LW} = \frac{1}{\gamma_{CH_2}}\left[\frac{\Delta G_{CH_2}}{4Na_{CH_2}}\right]^2 \tag{6}$$

where ΔG_{CH_2} is the difference in the ΔG_A^0 value of two subsequent n-alkanes and is independent of the surface area and porosity of the solid adsorbent. N is Avogadro's number, a_{CH_2} is the cross-section area of the adsorbed group CH_2 group, 0.06 nm^2, and γ_{CH_2} is the surface energy of a hypothetical surface made up of CH_2 groups, e.g. polyethylene, $\gamma_{CH_2} = 36$ mJ m^{-2}. The incremental change of free energy per methylene group, ΔG_{CH_2}, is attained from the retention measurements of a homologous series of linear alkanes from the equation:

$$\Delta G_{CH_2} = -RT_C \ln\left(\frac{K_S^{n+1}}{K_S^n}\right) = -RT_C \ln\left(\frac{V_S^{n+1}}{V_S^n}\right) \tag{7}$$

The values for ΔG_{CH_2} are thus determined graphically from a plot of ΔG_A^0 vs. the length of the carbon chain in the alkane.

Polar molecular probes such as n-alkenes are generally used to analyze the specific interaction by comparison with n-alkanes.

$$-\varepsilon_\Pi = \Delta G^{alkene}(n) - \Delta G^{alkane}(n) \tag{8}$$

where ε_π is the specific interaction parameter, and ΔG^{alkane} is the value of the ΔG_A^0 of the n-alkane probe treated as a dispersive interacting reference. Alkene double bond (π electrons) is a weak nucleophile and interacts with the Lewis acid sites on the solid surface. This polar interaction results in an additional contribution to the surface free energy. Specific interaction parameter obtained using this method is a measure of the electron accepting capacity of the solid surface.

3. Materials and methods

IGC experiments were conducted on three samples. A 90% pure synthetic hydroxyapatite was obtained from Riedel-de Haën, Germany. The impurity composition assay is given in Table 1. A 99% pure synthetic hydroxyapatite was obtained from Fluka, Switzerland, the assay of which indicated only trace mineral impurities. Both samples were prepared for analysis by heating at 500 °C for 2 h under a nitrogen sweep. The bone sample was obtained from bovine femur that was heated under nitrogen at a rate of 10 °C/min to 700 °C and held at temperature for 2 h. All samples were placed in a constant humidity chamber at 65% RH for at least 16 h.

A Hewlett Packard Model HP 5890 series II gas chromatograph was used for IGC experiments. A flame ionization detector (FID) was

Table 1
Chemical assay of the chemical impurities contained in the 90% purity hydroxyapatite, as provided by the manufacturer (Riedel-de Haën).

Sample	Anionic traces (mg kg^{-1})	Cationic traces (mg kg^{-1})
Chloride (Cl^-)	≤500	
Sulfate (SO_4^{2-})	≤500	
Cadmium (Cd)		≤5
Cobalt (Co)		≤5
Copper (Cu)		≤20
Iron (Fe)		≤400
Nickel (Ni)		≤5
Lead (Pb)		≤10

used to detect the elution of the hydrocarbons. About 0.5–1.0 g of sample was packed into a stainless steel column that was 25 cm long and had an inside diameter of 2.3 mm. To maintain an operable pressure drop across the GC column, samples were formed into course aggregated particles. The humidified powders were pressed into pellets that were crushed and sieved to create particles with diameters from 150 to 600 μm. The columns were packed and preconditioned at 200 °C with a 5 cm^3/min nitrogen sweep for 16 h. The temperature was then reduced to the operating temperature of 100 °C.

The elution of the probes was recorded into a computer at 0.1 s intervals. The retention times were determined from the median values of the elution peaks. The repeatability of retention time measurement was under 1% between injections. The reproducibility of retention time for different columns was less than 3% (relative). Therefore, the error for individual points is less than the symbol size for the graphs presented in the results.

Specific retention volumes for selected alkanes and alkenes were used to determine the apolar component of surface free energy (γ_S^{LW}) and specific interaction parameter (ε_π) values of the samples. High purity linear hydrocarbon probes (HPLC grade, Fisher Chemical) of different carbon chain length, from C_5-C_9, were used in this study. Probes were selected for each sample so that retention time was between 1 min and 1 h. The specific surface area, A_{sp}, of each sample was measured by multi-point BET (Brunauer, Emmett and Teller) nitrogen adsorption using an ASAP 2000 surface analyzer (Micromeritics Instrument Corp.).

Table 2
Summary of IGC results. Due to presence of high impurity and surface defect sites, trabecular bone sample has much higher surface free energy and electron exchange capacity as compared to 99% pure synthetic hydroxyapatite sample, and it is comparable to that of 90% pure synthetic hydroxyapatite.

Sample	Surface area (S) (m^2 g^{-1})	ΔG_{CH_2} (kJ mol^{-1})	γ_S^{LW} (mJ m^{-2})	ε_π (mJ)
Hydroxyapatite 99% pure	69.5	2.93	33.44	0.95
Hydroxyapatite 90% pure	38.2	2.32	53.32	3.01
Trabecular bone sample	38.2	2.71	45.71	2.44

4. Results and discussions

Fig. 1 shows a graph of the standard free energy of adsorption, ΔG_A^0, of the n-alkanes as a function of the alkane carbon chain length for the three samples. All three samples exhibit linearity on this plot. The value of ΔG_A^0 increases with carbon number, and the slope, ΔG_{CH_2} is a direct function of surface free energy, γ_S^{LW}.

Values of γ_S^{LW} for the three samples were calculated using the Dorris and Gray method, Eq. (6) and are provided in Table 2. This table also in includes the BET specific surface area results. The apolar component of the 90% hydroxyapatite was 53.3 mJ m^{-2}. By extrapolation, this compares favorably with the results previously reported by Simciklas et al. [9] for synthetic hydroxyapatite for column temperatures from 70 °C (71.3 mJ m^{-2}) to 80 °C (62.9 mJ m^{-2}). The high purity hydroxyapatite had $\gamma_S^{LW} = 33.4$ mJ m^{-2} which is much lower than the sample that had contaminants. Small amounts of contaminants can contribute significantly to the measured apolar component of surface free energy. A similar observation was previously observed by Keller and Luner [15] for calcium carbonates. Several authors have reported values of γ_S^{LW} for typical mineral surfaces such as silicates in the range of 55–60 mJ m^{-2} at 100 °C [16,17]. Natural trabecular bone hydroxyapatite had $\gamma_S^{LW} = 45.7$ mJ m^{-2} with a significant amount of impurity. The apolar component of surface energy is considerably higher than the high purity synthetic hydroxyapatite, which can contribute to increased adsorption, adhesion and wettability.

Retention time obtained for polar gaseous probes such as 1-alkenes, was used to determine the specific interactions with surface acidic sites on the samples. In Fig. 2, standard free energies of adsorption of 1-alkenes and n-alkanes are plotted against the

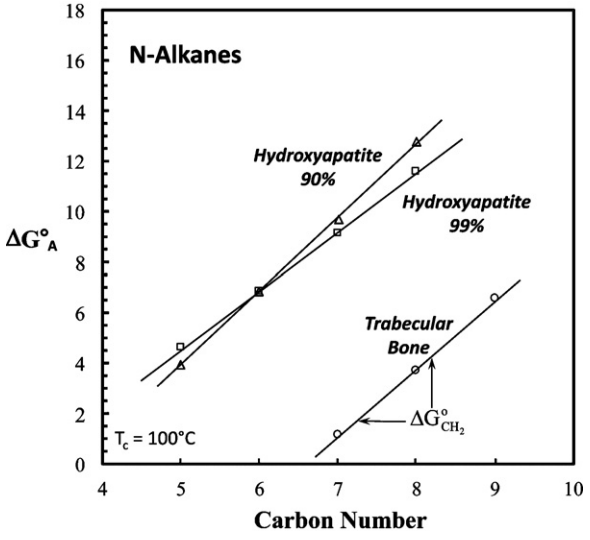

Fig. 1. IGC results of 90%, 99% pure synthetic hydroxyapatite, and trabecular bone using n-alkane gaseous probes. Surface free energy (γ_S^{LW}) is a function of (ΔG_{CH_2}) values obtained from these graphs and can be calculated using equation (7).

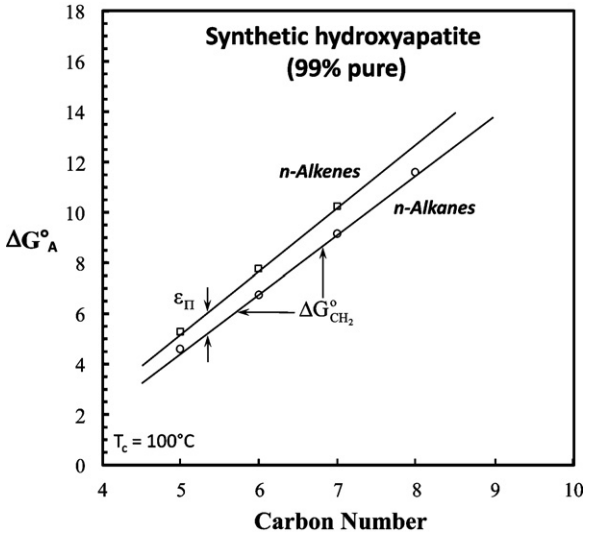

Fig. 2. IGC results of 99% pure synthetic hydroxyapatite with n-alkanes and 1-alkenes gaseous probes showing separation factor (ε_π), a measure of electron accepting and donating capacity of the surface.

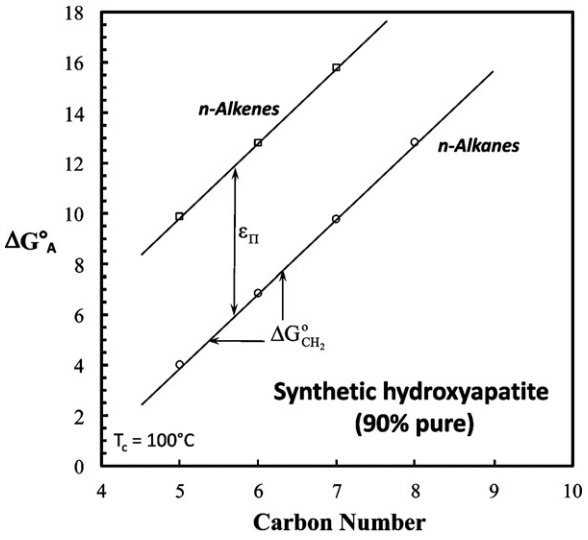

Fig. 3. IGC results of 90% pure synthetic hydroxyapatite with n-alkanes and 1-alkenes gaseous probes showing separation factor (ε_π), a measure of electron accepting and donating capacity of the surface.

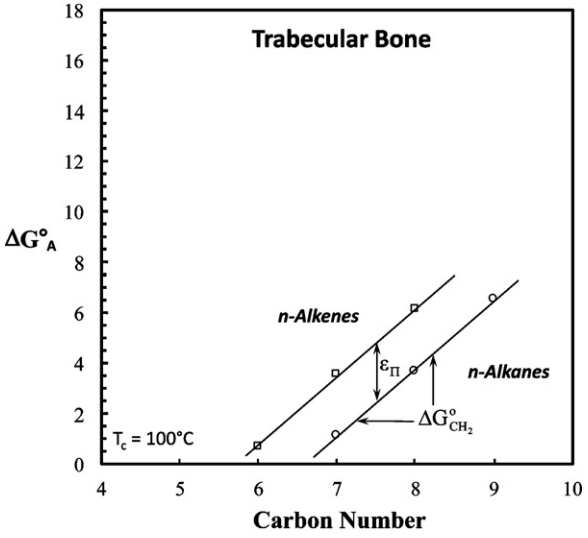

Fig. 4. IGC results of trabecular bone sample with n-alkanes and 1-alkenes gaseous probes showing separation factor (ε_π), a measure of electron accepting and donating capacity of the surface.

carbon number for 99% synthetic hydroxyapatite. The vertical separation between the lines shown on the graph is referred to as the specific interaction parameter (ε_π). It is an energetic measure of the interaction of π-bonded electrons of the alkenes with acid sites on the surface. Figs. 3 and 4 show analogous plots for synthetic hydroxyapatite at 90% purity and the trabecular bone sample, respectively. The ε_π value for the 90% sample is relatively high (3 mJ), in indication of the presence of impurity while that for the 99% sample is 0.95 mJ, as might be expected. The bone mineral has much higher ε_π value (2.4 mJ), than that of the 99% sample, which is consistent with the likely presence of impurities. However, it is also consistent with the fact that bone mineral is not stoichiometric hydroxyapatite, but rather has lower concentrations of calcium and hydroxyl groups within the material [18,19].

The results are consistent with the following model. The 90% sample has (elevated concentrations of iron, chloride and sulfate according to its assay, cf. Table 1., which introduce defects and high energy sites on the surface. The iron sites will especially interact with the alkene probes as indicated by the increased values of ε_π that were observed. The 99% sample has fewer defect sites and thus the value of γ_S^{LW} is lower than that for the 90% sample. The value for the bone sample is higher than that for the 99% sample. This suggests that bone mineral also contains mineral impurities and surface defects which act as a high energy sites. These sites play a very important role, facilitating cell adhesion and improving performance of the implant material.

These results are extremely important in light of the fact that protein adhesion and cell spreading increases with an increase in the surface free energy [6]. Based on this information, it is desirable to increase the surface free energy of synthetic hydroxyapatites by introducing impurities and surface defects to improve protein adhesion and cell spreading on the surface. These modifications may involve various techniques such as chemical treatment, heat processing, elemental doping. Thus, IGC can be used to characterize prospective surface modification strategies to mimic bone mineral properties by "engineering" synthetic hydroxyapatite.

5. Conclusion

Inverse gas chromatography results demonstrated that the presence of impurities and surface defects in bone mineral results into higher surface free energy, when compared with that of synthetically pure hydroxyapatite. Strong interaction between π bonded electrons of the alkenes and bone mineral indicates interaction with charged domains on the surface. Presence of these high energy domains could enhance protein adhesion and cell spreading significantly. IGC analysis can be used to study potential surface modification strategies for "engineering" synthetic hydroxyapatite to mimic natural bone mineral. IGC serves as an excellent technique to analyze the surface energetics of biomaterials at the molecular level.

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