Journal of Chromatography A, 1468 (2016) 116–125

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Inverse liquid chromatography as a tool for characterisation of the surface layer of ceramic biomaterials

Karol Kadlec*, Katarzyna Adamska, Zuzanna Okulus, Adam Voelkel

Poznań University of Technology, Institute of Chemical Technology and Engineering, ul. Berdychowo 4, 60-965 Poznań, Poland

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 9 September 2016
Accepted 14 September 2016
Available online 15 September 2016

Keywords:
Inverse liquid chromatography
Linear free energy relationship
Ceramic material
Solid/liquid interface
Structure – property relationship

ABSTRACT

The novel technique for ceramic biomaterials surface characterisation was proposed. The examined bone substitute materials were two orthophosphates: hydroxyapatite, β-tricalcium phosphate and the mixture of these two – biphasic calcium phosphate. The aim of this work was characterisation of the ceramic biomaterials surface expressed via the values of parameters *e*, *s*, *a*, *b*, *v* considered in linear free energy relationship. The values of these parameters reflect the ability of stationary phase to occur in different types of interactions. The sorption phenomena occurring on the bone substitute materials surface are responsible for the process of the multiplication of the osteoblasts. Thus the detailed description of this phenomena may contribute to the better understanding of bone loss regeneration mechanism. The data required for characterisation by using LFER model was collected by means of inverse liquid chromatography with the use of five different mobile phases: 98% ethanol, ethanol/water (50/50), water, 0.2 M NaCl and SBF. The determination of the ceramic orthophosphates surface properties in SBF solution allowed to observe the behaviour of biomaterials in "natural environment" – in living organism.

1. Introduction

Calcified tissues constitute the major component of mammals bones and teeth. In recent years, many synthesis procedures have been developed. They enable to obtain calcium orthophosphates, showing biological and chemical conformity with living tissue [1–3]. Hence the bone loss is replaced by ceramic materials such as synthetic hydroxyapatite (HA) and β-tricalcium phosphates (β-TCP), which provide appropriate osteoinductivity, osteoconductivity and biodegradability [4–6]. These two compounds, mixed in a ratio 6 to 4, are commonly used as a biphasic calcium phosphate (BCP), that is currently available under dozens of trademarks, e.g. 4Bone (MIS, Israel); Ceraform (Teknimed, France); Indost (Polystom, Russia) [7]. Moreover, the commercial fabrication methods allow to obtain macroporous BCP scaffold with channel-shaped pores, similar to those in calcified tissue [8–10]

β-TCP with a general formula $Ca_3(PO_4)_2$ and HA ($Ca_{10}(PO_4)_6(OH)_2$) might be categorised as resorbable or bioactive material, respectively [11]. However, these two materials, applied as a single-phase bioceramic scaffold, do not exhibit desired surface properties compared to BCP. The multiplication and adhesion processes of osteoblasts are carried out in more natural way on BCP surface than that on pure HA or β-TCP [12]. The implantation of bi-, tri- or multiphasic calcium orthophosphate scaffolds, prevents encapsulation of implanted material by a fibrous tissue and isolation them from the surrounding bone [13,14]. Thus, the surface properties of bone-substituted material should be investigated in details, taking into consideration the changes in sorption properties caused by the influence of the environment, in which the biomaterial normally "works".

The current investigations have focused only on more detailed description of the phenomena occurring on the HA surface. Based on FTIR spectroscopy investigation, Ishikawa et al. have suggested, that HA surface possesses three kinds of P-OH group acting as adsorption sites for CO_2, CH_3OH, CH_3I and H_2O [15]. The study on retention mechanism between protein and HA chromatographic stationary phases allowed to formulate the thesis, that there is a cation-exchange phosphonate group and an anion-exchange calcium group [16]. However, the precise analysis of the β-TCP and BCP physicochemical surface properties has still not been developed.

Despite many techniques, that can serve for surface characterisation, including FTIR, Raman spectroscopy, X-ray diffraction, contact angle measurements and others, all of them do not allow to observe the influence of liquid environment on surface properties [17–19]. In the case of bone-substituted biomaterials, it is vital to know this type of a relationship. Calcium orthophosphates,

* Corresponding author.
E-mail addresses: karol.k.kadlec@doctorate.put.poznan.pl, celdak@gmail.com (K. Kadlec).

http://dx.doi.org/10.1016/j.chroma.2016.09.032
0021-9673/

implanted into a living organism, are surrounded by body fluid, which contributes to the changes of their sorption ability. Knowing the surface physicochemical characteristics of biomaterials and determining their overall reactivity appears to be useful, e.g. to design a material with desirable surface properties. The detailed characterisation of the surface layer of biomaterials may be essential, e.g. in the selection of appropriate modifiers of the surface of biomaterial or assessing its ability for cell adhesion.

The inverse liquid chromatography (ILC) seems to be a very helpful tool for carrying out this type of characteristic. The principle of measurements is based on the determination of the retention factors for the test solutes, having specified physicochemical properties. These analytes are introduced into the chromatographic system separately. The column is filled with the examined material, which in this case would be one of the calcium orthophosphates. Depending on the force of interactions between the test solute and the examined surface, the solutes leave the column with different retention times, which is the basis for physicochemical characterisation. The ILC technique can be a useful tool for biomaterials characterisation, because, by changing the composition of the mobile phase, we are able to recreate conditions similar to those present in a living organism. In such case the behaviour of the surface functional groups may be similar to that in a real system.

The determination of the surface energy or excess adsorption isotherm, the assessment of hydrophobicity or silanol activity, the retention of aromatic sulfonic acids – all these methods involve the liquid chromatography for surface characterisation [20–23]. However, in each case only one parameter is obtained as a result, for instance, the value of free energy or the concentration of the solute in the stationary phase for adsorption isotherms. In this study, the linear free energy relationship (LFER) was used, which involves five independent parameters characterising physiochemical properties of the examined surface [24,25].

In this mathematical relationship, the retention parameter depends on the solute solvation process, which has been identified and dissected into four types of solute-solvent interactions: cavity formation-dispersive interaction, dipolarity-polarizability interaction and acidity as well as basicity hydrogen bonding interaction. In the case of liquid chromatography, these types of interactions are observed not only between the solute and the stationary or the mobile phases but also take place between the solvent and the stationary phase. All these interactions have a major impact on the observed retention parameter. One of the widely accepted symbolic representation of LFER model in the form of multiple linear regression equation was presented by Abraham:

$$\log k = c + eE + sS + aA + bB + vV \quad (1)$$

where: *log k* is the logarithm of the solute retention factor, c is the linear regression coefficient. The capital letters *E*, *S*, *A*, *B* and *V* correspond to the solute descriptors independent from the mobile/stationary phase used; *E* is the excess molar refraction; *S* – dipolarity/polarizability descriptors, *A* and *B* correspond to the solute hydrogen bond acidity and basicity, respectively, while *V* is the McGowan volume of the solute. The lowercase letters *e*, *s*, *a*, *b*, *v* are the system parameters reflecting the difference in the solute interactions with the mobile and the stationary phase.

It is worth to notice, that there is a limited number of publications involving ILC as a method for surface characterisation of non-commercially available stationary phases. Most of them are the studies of porous materials, directly destined for a column chromatography. In our investigation ILC technique served for the surface properties determination of HA, β-TCP and BCP. All examined solids find an application in implantology and do not possess any features typical for chromatographic stationary phases – high surface area and low particle diameter. However, the knowledge of surface properties of these materials, investigated in a real system, should be useful in the estimation (prediction) of their biocompatibility, stability in various conditions and potential future applications.

Table 1
Descriptors of the test solutes [28–31].

Test solute	Descriptor				
	E	*S*	*A*	*B*	*V*
1,3-diaminopropane	0.446	0.610	0.430	1.140	0.731
1,3-propanediol	0.397	0.91	0.77	0.85	0.6487
1,4-dioxane	0.329	0.750	0.000	0.640	0.6810
1-propanol	0.236	0.420	0.370	0.480	0.5900
Acetic acid	0.265	0.64	0.62	0.44	0.4648
Acetonitrile	0.237	0.90	0.07	1.739	0.4042
Acetophenone	0.818	1.010	0.000	0.480	1.014
Aniline	0.955	0.960	0.260	0.500	0.8162
Benzonitrile	0.742	1.110	0.00	0.330	0.8710
Butanone	0.166	0.700	0.000	0.510	0.6879
Caffeine	1.500	1.600	0.000	1.330	1.3632
Cyclohexanone	0.403	0.860	0.000	0.560	0.8611
Cyclohexanol	0.460	0.540	0.320	0.570	0.904
Diethyl ether	0.041	0.250	0.000	0.450	0.7309
Ethyl acetate	0.106	0.620	0.000	0.450	0.7466
Geraniol	0.513	0.630	0.390	0.660	1.4903
N,N-dimethylformamide	0.367	1.31	0.00	0.74	0.6468
Phenol	0.805	0.89	0.6	0.3	0.775
Propylamine	0.225	0.350	0.160	0.610	0.631
Pyridine	0.631	0.840	0.000	0.452	0.6750
Tetrahydrofuran	0.289	0.520	0.00	0.48	0.6223

2. Experimental

2.1. Materials and instruments

Hydroxyapatite (purity ≥ 97%) and β-tricalcium phosphate (purity ≥ 96%) applied as a column filling were purchased from Sigma-Aldrich. Biphasic calcium phosphate (BCP) was prepared by mixing HA and β-TCP in a ratio 6:4 [26]. The water used for preparing mobile phase was purified in water deioniser Sartorious Stedim Biotech (Germany). Ethyl alcohol (98%) and dichloromethane were purchased from Avantor (Poland). The inorganic salts needed to prepare simulated body fluid (SBF) were: sodium chloride, potassium chloride, calcium chloride, sodium sulphate and tris-hydroxymethyl aminomethane (POCH, Poland); sodium hydrogen carbonate, di-potassium hydrogen phosphate trihydrate and magnesium chloride hexahydrate (Sigma Aldrich) – purity ≥95%. The SBF preparing procedure was precisely described in Ref. [27]. The test solutes were at least of analytical grade: caffeine, phenol, tetrahydrofuran, benzonitrile, 1,3-propanediol, 1-propanol, 1,4-dioxane, cyclohenanone, cyclohexanol, geraniol, *N,N*-dimethylformamide (Sigma Aldrich); acetonitrile, diethyl ether, 1,3-diaminopropane, propylamine, butanone, acetic acid, ethyl acetate (POCH, Poland); pyridine, aniline, acetophenone (Fluka). Test compounds used in the experiment with values of their descriptors are listed in Table 1.

The chromatographic experiments were carried out by using the liquid chromatograph Dionex Ultimate 3000LC System equipped with a refractive index detector (Shodex, Ltd.USA), a chromatographic liquid pump with maximum operating work pressure 600 bar, a column oven and a degasser – all from Dionex (currently Thermo Scientific, USA). The stainless steel column (2.1 mm i.d. × 250 mm) was purchased from Applied Research Europe, Germany.

2.2. Column filling preparation

The incompressibility and high specific surface area are one of the most important properties, which have to be revealed by