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Hydroxyapatite microcarriers for biocontrolled release of protein drugs

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Abstract

It is demonstrated that hydroxyapatite microcarriers suitable for biocontrolled release of protein drugs can be obtained by allowing hydroxyapatite to crystallize in the presence of protein. From tiny nuclei, radially grown star-like hydroxyapatite particles were formed at 100°C that were allowed to crystallize further at room temperature in the presence of BSA. After incubation for several weeks, the hydroxyapatite microparticles contained 5–10% BSA; 1–3% was incorporated into the crystalline hydroxyapatite microcarrier. It was impossible to release the incorporated BSA from the microcarriers even by extensive desorption procedures unless the hydroxyapatite crystals were dissolved. In vitro release of the incorporated BSA in a hydroxyapatite dissolving EDTA buffer of pH 5.9 proceeded gradually and reached completion in 3 weeks. At pH 6.9 the release rate of the incorporated BSA was 4-times lower, which is in line with the slower rate of dissolution of the hydroxyapatite at that pH. In vivo a hydroxyapatite microcarrier suspension was not detectable 1 month after injection in rabbit muscle, confirming the assumption that in soft tissue hydroxyapatite is fully resorbed.

Keywords: Hydroxyapatite; Seeded crystal growth; Controlled release; Drug inclusion

1. Introduction

Hydroxyapatite is the inorganic constituent of tooth enamel, dentine, bone and other hard tissues of vertebrates. Synthetic hydroxyapatite (HAP, $Ca_5(PO_4)_3OH$) is a bioactive, non-toxic ceramic material, being entirely biocompatible (Van Blitterswijk et al., 1990; Wilke et al., 1993). In early literature, hydroxyapatite prepared from aqueous solutions was amorphous initially, gradually changing to crystalline hydroxyapatite (Meyer, 1983). However, when phosphate and calcium chloride concentrations are kept sufficiently low, crystalline hydroxyapatite can be formed directly (Maniatis et al., 1991).

Porous hydroxyapatite ceramics have a considerable potential as carriers for controlled drug release (Kyoto Ceramic, 1980; Ducheyne, 1987; Asahi, 1990). Composites prepared by admixing hydroxyapatite powder, polylactic acid and an antibiotic were reported to be bioresorbable and osteocompatible and to result in sustained drug

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release (Ikada et al., 1985). In addition, hydroxyapatite beads have been used as carriers for antibiotic drugs (Yamamura et al., 1992).

In the neighbourhood of bone the bioactivity of hydroxyapatite involves the enhancement of calcified tissue formation. However, in areas where bone cells are not normally present hydroxyapatite does not promote the formation of calcified tissue (Ducheyne, 1987). Already in 1973 a process was claimed for preparation of aqueous suspensions of calcium phosphate gel particles which adsorbed vaccine into it. Injection of these vaccine-loaded calcium phosphate particles seemed to be without complications (Institut Pasteur, 1973). From these findings it was deduced that it should be possible to employ hydroxyapatite as a bioresorbable material for subdermal controlled release of protein drugs.

The reversible adsorption of proteins on hydroxyapatite particles is a well-known phenomenon, finding application, e.g., in protein chromatography (Bernardi, 1975). The interaction of proteins with hydroxyapatite appeared to depend on the overall protein charge, the number of acidic and basic groups, and the specific protein structure (Gurbunoff, 1984).

In the present study, the possibility was explored of incorporating protein drug during hydroxyapatite microcarrier formation by crystal growth, with the aim of bioresorption of the hydroxyapatite to be the main mechanism of drug release. This is particularly interesting, since bioresorption of hydroxyapatite is probably a much slower process than in vivo protein desorption. Bovine serum albumin (BSA), an acidic protein with an isoelectric pH of 4.7, has been chosen as the model compound, because acidic proteins are hardly desorbed from hydroxyapatite at phosphate concentrations below 0.05 M and are not desorbed in the presence of $CaCl_2$ (Gurbunoff, 1984). The phosphate concentrations applied during hydroxyapatite crystal growth are far below that needed for the desorption of acidic proteins. Basic proteins can be desorbed from hydroxyapatite already at relatively low $CaCl_2$ concentrations.

The investigations involved the preparation of crystalline hydroxyapatite particles of sufficient size and stability, the introduction of BSA into the particles, release studies as a function of pH, and finally a preliminary in vivo resorption experiment with injected aqueous hydroxyapatite suspensions.

2. Materials and methods

2.1. Materials

The following materials were used as obtained from the manufacturer: $CaCl_2 \cdot 2H_2O$ ('Baker grade', Baker, The Netherlands), KH_2PO_4, K_2HPO_4, KOH ('Baker analyzed'® reagents, Baker, The Netherlands), 0.1 N HCl, 0.1 N NaOH, phosphate buffer concentrate pH 7.00 ('Dilut It', Baker, Sweden), Na_4EDTA, 'Titriplex® solution', Merck, Germany), and BSA (Boseral, dem., Organon Teknika, The Netherlands).

For the preparation of hydroxyapatite particles distilled and filtered water was used (standard Milli-Q water system, Millipore Corp., U.S.A.).

2.2. Preparation of primary hydroxyapatite particles

Hydroxyapatite BSA-loaded microcarriers were prepared according to a two-step manufacturing procedure. In the first step, primary hydroxyapatite particles were formed in boiling water. In the second step, these primary particles were further grown at room temperature in the presence of BSA.

Primary hydroxyapatite particles were prepared at 100°C according to the procedure given below. After nucleation, star-shaped hydroxyapatite particles were obtained, consisting of radially grown whiskers.

To prepare 1.055 g of hydroxyapatite whiskers, 250 ml of an aqueous solution of $CaCl_2 \cdot 2H_2O$ (42.00 mmol/l), 250 ml of an aqueous solution containing 1.554 mmol KH_2PO_4 and 4.746 mmol K_2HPO_4, and 250 ml 0.040 N KOH were added dropwise (flow rate 50 ml/h) to 3 l of boiling H_2O, using a three-channel peristaltic pump for synchronized dosing. The reactants were dosed under stirring in a N_2 atmosphere. The hydroxya-

patite particles obtained were cooled to room temperature and used as seeds for crystal growth in the presence of the protein BSA. In order to optimize the hydroxyapatite formation process, the rate of dosage, concentration of the reactants, pH and impellor speed were varied, resulting in the protocol given above.

2.3. Seeded crystal growth / encapsulation of BSA

Crystal growth was performed at 22°C according to the following reaction:

$$5Ca^{2+} + aHPO_4^{2-} + bH_2PO_4^- + cPO_4^{3-} + H_2O \rightarrow Ca_5(PO_4)_3OH + xH^+ \quad (1)$$

$$a + b + c = 3$$

$$x = 1 + a + 2b$$

The coefficients a, b, c and x depend on the pH of the solution.

At 25°C they are (Inskeep and Silvertooth, 1988): (pH 7.4) $a = 2.25$; $b = 0.73$; $c = 0.02$; $x = 4.71$; (pH 8.2) $a = 2.75$; $b = 0.15$; $c = 0.10$; $x = 4.05$.

The initial solution for crystal growth at pH 7.40 (reaction volume 3500 ml, 1.00 g of seeds) contained 0.600 mmol $CaCl_2 \cdot 2H_2O$/l, 0.0888 mmol KH_2PO_4/l, 0.2712 mmol K_2HPO_4/l and 85.7 mg BSA/l (30 wt% of the seeds). Crystal growth is monitored by recording the amount of 0.1 N KOH added by the titration device of the pH-stat system. For increasing the growth rate the Ca^{2+} and phosphate concentrations were restored periodically to 150% of the initial concentrations from feed stock solutions (42.00 mmol $CaCl_2 \cdot 2H_2O$/l and 25.20 mmol (KH_2PO_4/K_2HPO_4)/l, flow rate 50 ml/h). Additional 1% BSA solution was supplied periodically to restore the initial BSA/hydroxyapatite ratio (flow rate 100 ml/h).

2.4. Equipment and auxiliaries

2.4.1. Reaction vessel

The preparation at 100°C was carried out in a glass 6 l reaction flask provided with a glass mechanical stirrer, N_2 inlet, N_2 outlet, water-cooled condenser, three reservoirs containing the reagent solutions and three entries for dropwise synchronized addition of these solutions through a three-channel peristaltic pump (P3, Pharmacia, Uppsala, Sweden; pump tubing diameter 1.0 or 3.0 mm; connecting capillary tubing PTFE).

Crystal growth experiments near 22°C were carried out in the same vessel, now provided with a pH stat system (entries for the calomel/glass-Ag electrode and titration device) and two entries for the dropwise synchronized dosing of the $CaCl_2$ and KH_2PO_4/K_2HPO_4 solutions through the peristaltic pump. Another entry was used for the manual dropwise addition of the BSA solution from a connected reservoir.

2.4.2. pH stat system

The pH stat system consists of the following combined devices (Metrohm AG, Herisau Switzerland): Digital pH-meter E 632 with calomel/glass-Ag electrode, Dosigraph 625, Impulsomat 614, and Multi Dosimat 655.

2.4.3. Filtering system

For filtration of suspensions, a stirred ultrafiltration cell (Amicon 8400, Amicon Div., W.R. Grace & Co., U.S.A.) and polycarbonate membrane filters (pore size, 1.0 or 3.0 μm; diameter, 76 mm; Poretics Corp., U.S.A.) were used.

2.5. Analyses

2.5.1. X-ray diffraction

Diffractographs were recorded in air using a Philips PW1050 reflection diffractometer equipped with a graphite monochromator, using CuKα radiation. Reference patterns were taken from the ICPDS Data Base (International Center for Diffraction Data).

The average crystallite size of the prepared hydroxyapatite samples was determined using the Scherrer equation (Klug and Alexander, 1974):

$$l_c = K \cdot \lambda / \beta \cdot \cos\theta$$

in which l_c is the average crystallite size (Å), K denotes the shape factor ($K = 1$ assumed), λ is the X-ray wavelength (for CuKα $\lambda = 1.5418$ Å), β represents the peak at half width (in rad), and

θ is the Bragg angle of the peak (002 reflection of hydroxyapatite at $2\theta = 25.8°$).

2.5.2. BSA analysis

Hydroxyapatite microparticles were dissolved in EDTA buffer, a mixture of 60 ml Titriplex solution (Na_4EDTA) + 180 ml 0.1 N HCl + 760 ml phosphate buffer, pH 7.0. The pH of the solution was adjusted by addition of 0.1 N HCl and/or 0.1 N NaOH. In 1 l of EDTA buffer 0.5 g of hydroxyapatite can be dissolved.

A Bio-sil sec 250 size exclusion column (length 60 cm) and a Pharmacia-LKB 2150 HPLC pump were used. BSA was detected at 210 nm with an Applied Biosystems 757 absorbance detector. Before injection the sample was filtered over a 0.22 μm Millex-GV filter (Millipore Corp.).

2.5.3. Loss on ignition

The hydroxyapatite content of a sample was determined as the residual weight after standardized combustion of 150 mg of a sample in a quarz vessel, using a Teclu burner. The sample was heated for 10 min at 700°C with interruptions of 15 s at time 2, 4 and 6 min for additional air access (out of the flame). After cooling to room temperature, the sample was stored in a desiccator (23°C, $SiO_2 \cdot nH_2O$) and the mass was determined until it remained constant.

2.5.4. Microscopy

For an impression of the appearance of the hydroxyapatite particles and the determination of their size range, samples were examined under a Leitz ortholux optical microscope.

For scanning electron microscopy a Camscan-S4 Scanning Electron Microscope was used.

2.5.5. In vivo analysis

The right sural muscle of each of two rabbits was injected with 0.25 ml of a hydroxyapatite suspension (without drug). The left sural muscle of each of the rabbits was injected with the reference suspension liquid (placebo). After 1 and 4 months the injected muscles were isolated, fixed in Bouin (month 1) or in a 4% aqueous formalde-

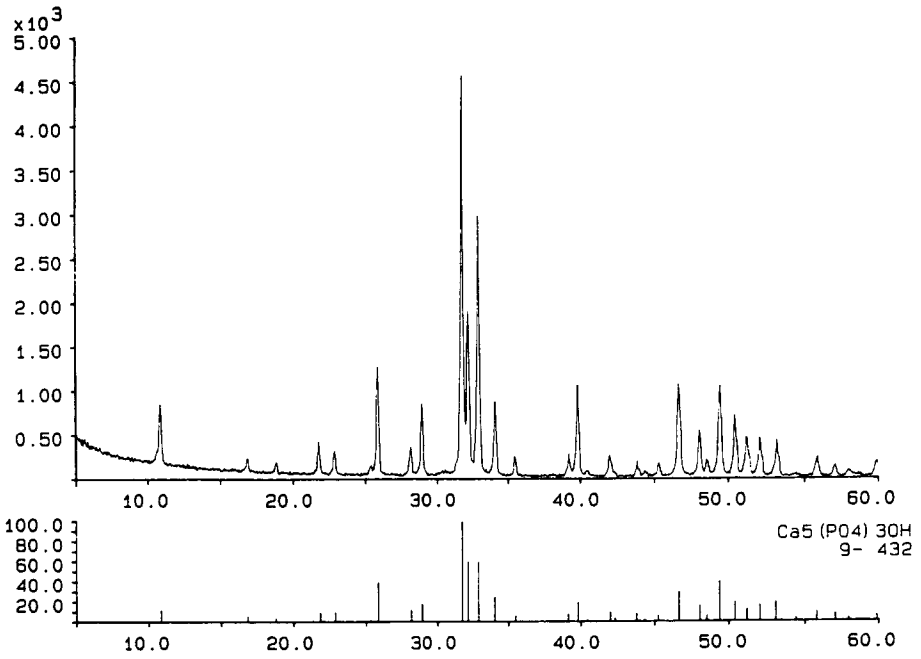

Fig. 1. X-ray diffraction pattern of hydroxyapatite whiskers obtained for particles from batch II. The calculated average crystallite size is 0.05 μm. The lower pattern is the reference pattern for hydroxyapatite, obtained from the ICPDS data base.

hyde solution (month 4) and embedded in paraffin. The sections (5 μm) were stained with 'haemaluin eosine' and inspected histologically.

3. Results and discussion

3.1. Encapsulation mechanism

In this study a new encapsulation mechanism for drugs in hydroxyapatite microcarriers was explored, being a combination of drug adsorption onto the hydroxyapatite surface and subsequent drug incorporation by hydroxyapatite crystal growth. Preliminary experiments had demonstrated that BSA cannot be incorporated into single hydroxyapatite crystal needles, but only in radially grown hydroxyapatite whiskers. Since for the formation of radially grown hydroxyapatite whiskers, conditons must be used that are unfavourable for drug incorporation, a two-step procedure was applied. In the first step primary hydroxyapatite particles were formed at 100°C; in the second step these primary particles were applied as seeds for further crystal growth at room temperature in the presence of BSA. The BSA adsorbed on the hydroxyapatite surfaces. Although the BSA at the surface of the hydroxyapatite crystals did not prevent crystal growth, the hydroxyapatite crystal growth rate appeared to be reduced. The BSA adsorbed is incorporated into the growing three-dimensional network of the radially crystallizing hydroxyapatite.

3.2. Formation of primary hydroxyapatite particles

Particles in the form of radially grown whiskers were prepared from aqueous solutions of $CaCl_2$, KH_2PO_4/K_2HPO_4 and KOH in boiling H_2O at constant pH. The process conditions were chosen to render highly crystalline hydroxyapatite particles as was demonstrated by the narrow lines in the resulting X-ray diffraction patterns (Fig. 1). The whisker type 'star-shaped' particles obtained cannot be prepared at room temperature. Moreover, at 100°C there is only a limited range of process conditions leading to this type of hydroxyapatite particles with radially grown needles.

Table 1
Experimental conditions and particle size for the hydroxyapatite batches prepared at 100°C

Seeds (100°C)	Conc. [a] (%)	Addition rate of reactants (ml/h)	Particle diameter (μm)
Batch I	100	50	10 –150
Batch II	100	50	10 –150
Batch III	200	50	10 – 15
Batch IV	100	500	2 – 5
Batch V	150	500	0.5– 2
Batch VI	100	50	5 – 40

[a] Concentrations of the feed stock solutions as given in section 2 are defined as 100%.

Since the hydroxyapatite particles were meant to be used as seeds in the next BSA incorporation step, the preferred size of the primary particles is < 1 μm. Depending on the conditions applied, primary particles had diameters up to 150 μm with whisker needle lengths ranging from several μm up to 50 μm and thicknesses ranging from 0.1 to 0.4 μm (Table 1). When relatively large batches were prepared at 100°C (cooling overnight and subsequent reheating), the particle size obtained was approx. 10–150 μm (batches I and II). Doubling the concentrations of the feed stock solutions at the same dosing speed resulted in smaller particles with diameters of 10–15 μm (batch III). A 10-fold increase in the dosing speed of the reactants led to the desired size for the primary particles of approx. 2 μm (batches IV and V). However, in batch V, the critical point of the combined parameters concentration and dosing speed was somewhat exceeded, resulting in smaller and less regular particles.

After preparation of a batch of primary particles at 100°C, the particles should not be dried and resuspended, but used directly for drug loading.

3.3. Drug loading by crystal growth

The crystal growth rate of hydroxyapatite at room temperature in the presence of BSA can be assumed to be affected by the crystal surface area available for growth, the concentrations of Ca^{2+}

and phosphate, pH, BSA concentration and the BSA/hydroxyapatite ratio. According to Inskeep and Silvertooth (1988), the growth rate of hydroxyapatite crystals is linearly proportional to the growth surface and the concentrations of Ca^{2+} and PO_4^{3-}. This refers to hydroxyapatite growth surfaces without the presence of an adsorbed protein; in the case of star-shaped particles with growing particles and BSA present, obviously the effective growth surface is smaller. The maximum concentrations of Ca^{2+} and phosphate during growth were chosen conservatively to prevent spontaneous nucleation.

The incorporation of BSA during the crystal growth of hydroxyapatite is a process that needs to be optimized, since conditions favourable for BSA adsorption are not necessarily favourable for crystal growth. A BSA loading experiment is illustrated in Fig. 2. Calcium chloride and phosphate solution were added to the primary hydroxyapatite particles. Additional 1% BSA solution was supplied periodically in order to keep the BSA/hydroxyapatite mass ratio within limited ranges (see Table 2). The BSA concentrations in the reaction vessel ranged from 0.2 to 0.9 g/l, depending on the amount of hydroxyapatite present. Fig. 3 shows an SEM micrograph of the resulting BSA-loaded hydroxyapatite particle.

The pH of the solution is an important parameter controlling crystal growth (Table 2). A decrease from pH 8.4 to 7.4 decreases the growth rate in the presence of BSA by about a factor of 2, whereas a factor of 5 is the theoretical ratio of

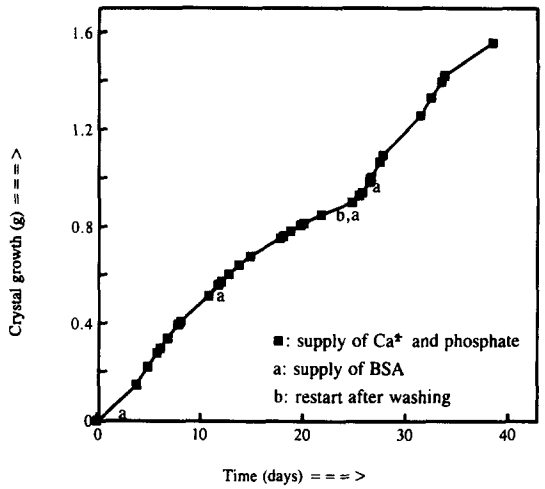

Fig. 2. Growth of hydroxyapatite particles (1.055 g of seeds from batch VI) in the presence of BSA.

growth rates for hydroxyapatite without BSA present (Inskeep and Silvertooth, 1988).

In order to determine what fraction of the BSA was fully incorporated into the hydroxyapatite micocarrier particles, the particles were exposed to a desorption treatment. Acidic proteins like BSA (p*I* 4.7) are known to be eluted from hydroxyapatite material by 0.05–0.15 M phosphate solution (K_2HPO_4/KH_2PO_4, pH 7.4; Gurbunoff, 1984). The desorption treatment consisted of thorough washing of the particles with 0.15 M phosphate solution, suspension in 1 M KOH for hydrolysis of possible residual BSA, followed by neutralization and desorption with

Table 2
Crystal growth experiments in the presence of BSA near 22°C

Batch of BSA loaded microcarriers	Primary hydroxyapatite particles used in the crystal growth experiments			BSA/HAP ratio [a] (%)	pH	Time (days)	Total mass increase (%)	Mass increase per day (%/day)
	Batch	Amount (g)	Particle size (μm)					
II/g [c]	II	6.2	10 –150	33–15	7.4–8.4 [b]	67	128	1.9
III/g	III	5.1	10 – 15	25–18	7.4	32	67	2.1
IV/g	IV	4.7	2 – 5	25–15	8.4	6	62	10.3
V/g	V	1.6	0.5– 2	32–16	7.4	24	99	4.1
VI/g	VI	1.1	5 – 40	37–24	7.4	39	148	3.8

[a] Weight percentage of BSA with respect to the total weight of hydroxyapatite.
[b] pH of growth solution was varied (7.4–8.4). During most of the reaction time the pH was 8.2.
[c] Extension x/g refers to the fact that primary hydroxyapatite microcarriers are further grown in the presence of BSA.

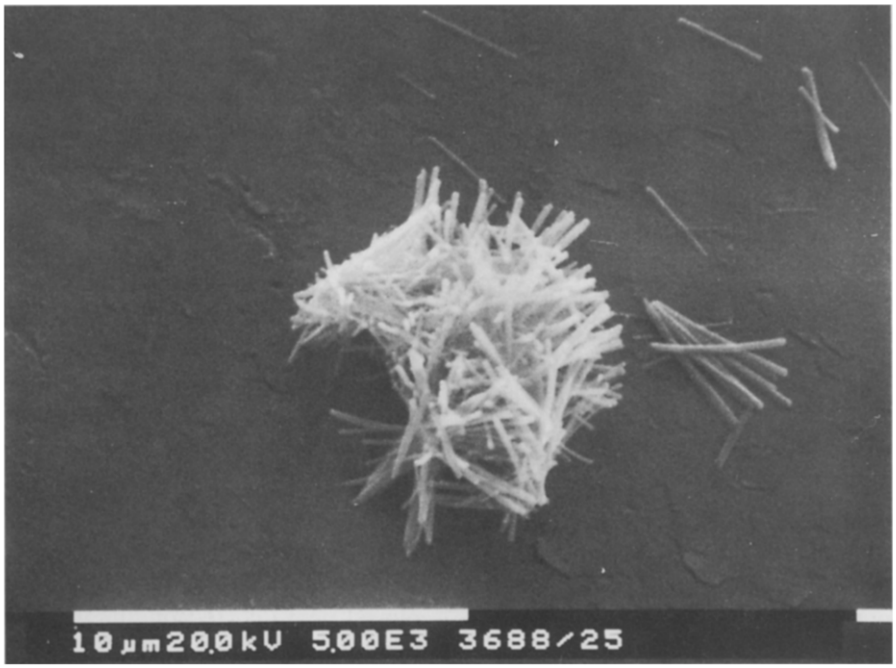

Fig. 3. An SEM micrograph of a hydroxyapatite microcarrier loaded with BSA (magnification 1800×). The particle has the appearance of a radially grown star-like array of crystal needles.

Table 3
Composition of samples grown in the presence of BSA near 22°C

Batch of BSA loaded microcarriers	Particle size of seeds (µm)	pH	Composition (wt%) before desorption		Composition (wt%) after desorption		Percentage of BSA included
			H_2O	BSA	H_2O	BSA	
II/g	10 –150	7.4–8.4	1.3	5.7	1.3	2.6	45%
III/g	10 – 15	7.4	1.3	7.1	1.4	2.1	29%
IV/g	2 – 5	8.4	2.7	10.7	3.0	0.9	8%
V/g	0.5– 2	7.4	2.8	7.8	3.1	0.9	12%
VI/g	5 – 40	7.4	1.8	10.9	1.9	3.0	28%

Table 4
HP-SEC analysis of the BSA content of hydroxyapatite microcarrier batches

Batch	Condition of BSA present	BSA contents (from LOI, wt% of total)	BSA released (from HP-SEC, wt% of total)
VI/g desorption treated	only included	3.0	2.5% monomer 1.2% dimer
VI/g	included + adsorbed	10.9	5.5% monomer 2.7% dimer
IV/g	mainly adsorbed	10.7	8.7% monomer 5.0% dimer

The BSA batch originally had a monomer/dimer ratio of 95:5.

0.15 M phosphate solution once more. Finally, the particles were thoroughly washed with water. To determine the mass of BSA incorporated in the hydroxyapatite particles, the mass lost upon ignition was determined and corrected for the water contents. The water content was approximated by the mass loss on ignition for reference samples (without included BSA). The amount of BSA adsorbed on the hydroxyapatite microcarriers varied between 6 and 11 wt%; the amount of BSA incorporated into the hydroxyapatite microcarriers was only 1–3 wt% (Table 3). The main factors that could influence the amounts of BSA adsorbed and incorporated are probably the pH of the growth medium and the hydroxyapatite growth rate. No clear relation exists between the total amount of BSA adsorbed and the fraction incorporated. At high pH the adsorption of BSA onto hydroxyapatite is known to be lower in extent than at low pH (Hlady and Füredi-Milhofer, 1979). However, for the batch grown at pH 8.4 (batch IV/g) relatively large amounts of BSA were adsorbed, but only 8% of the adsorbed BSA was incorporated into the particle.

The release of BSA from BSA-loaded hydroxyapatite microcarriers is expected to take place via two processes: (a) BSA desorption and (b) hydroxyapatite dissolution. In water the BSA release from desorption treated hydroxyapatite microcarriers after 3 weeks is less than 0.01%.

In order to release the BSA incorporated inside the hydroxyapatite microcarrier the hydroxyapatite must dissolve. This can be achieved by incubating the hydroxyapatite microcarriers in EDTA buffer at low pH. Removing free Ca^{2+} from the solution by chelation with EDTA and adding protons causes the reversal of the reaction (Eq. 1): dissolving of the hydroxyapatite. The dissolution medium was assayed for BSA.

After incubating a suspension of desorption treated batch V/g for 8 days in EDTA buffer pH 5.0, the suspension had become completely clear, indicating the dissolution of the hydroxyapatite. A sample was filtered and analysed by size exclusion chromatography (Fig. 4). An HP-SEC diagram of a freshly prepared BSA standard solution shows two peaks, one major peak for the BSA monomer and a minor peak (< 5%) for the BSA

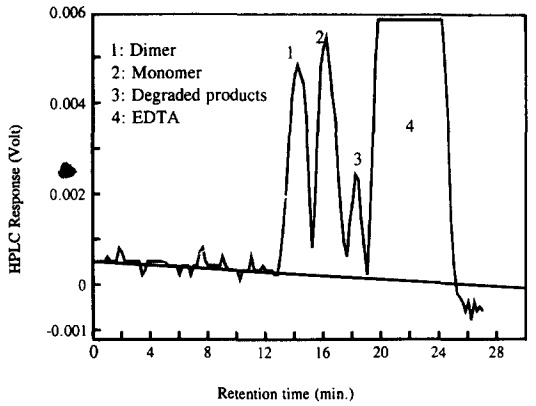

Fig. 4. Size exclusion chromatogram for the quantification of BSA. The samples were obtained by complete dissolution in EDTA-solution of BSA loaded hydroxyapatite microcarriers of batch VI/g (0.50 g/l).

dimer. The chromatogram of the sample displayed peaks corresponding with monomeric and dimeric BSA and probably degraded products. The dimer/monomer ratio of the sample had increased considerably, also indicating destabilization of the BSA incorporated. Adding the monomeric and dimeric contributions, the maximum release of batch V/b is 3.7%, which is of the same order of magnitude as the BSA content estimated using the LOI method (3.0%).

In vitro release testing of hydroxyapatite microcarrier preparations yielded release times for BSA ranging from several hours up to several weeks. From the loss on ignition data before and after desorption, it was deduced that batch IV had only 8% of its BSA contents truly incorporated into the particle. Release of BSA from this batch was only a matter of hours (Fig. 5), although complete dissolution of the hydroxyapatite matrix took many days.

As can be expected from Eq. 1, the dissolution of hydroxyapatite particles in EDTA is strongly pH dependent. This is reflected in the release rate of BSA from the desorption treated hydroxyapatite particles of batch VI/g (Fig. 6). After about 3–5 days the solution at pH 5.9 became clear. At pH 6.9 even after several weeks the solution was still turbid. The existence of a lag time for release can partly be attributed to the vigorous desorption treatment to which the hy-

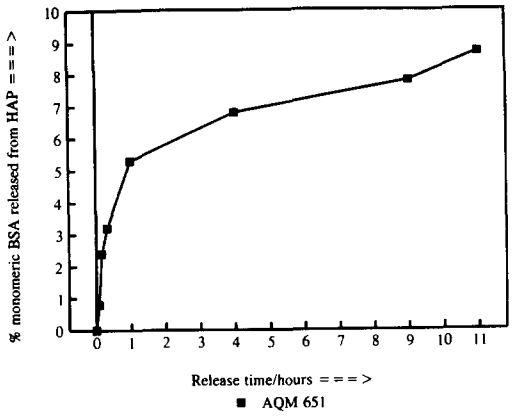

Fig. 5. In vitro release of BSA from batch IV (which contains mainly adsorbed BSA) in EDTA buffer pH 5.9.

droxyapatite sample had been subjected before release testing.

3.4. *In vivo experiment*

A sample from batch I was used for pilot in vivo experiments.

Global inspection after 1 and 4 months of the sural muscles of rabbits, which had been injected with an aqueous hydroxyapatite suspension (without BSA), showed that the site of injection could no longer be detected. Apparently, all hydroxyapatite particles had been resorbed. Visual inspection did not reveal severe inflammations. However, additional histological inspection of slices (thickness 5 μm) cut from the injected muscles showed a site suffering from necrosis and inflammation, 1 month after the injection. No deviations were found in the placebo groups and 4 months after the injection with the hydroxyapatite suspension.

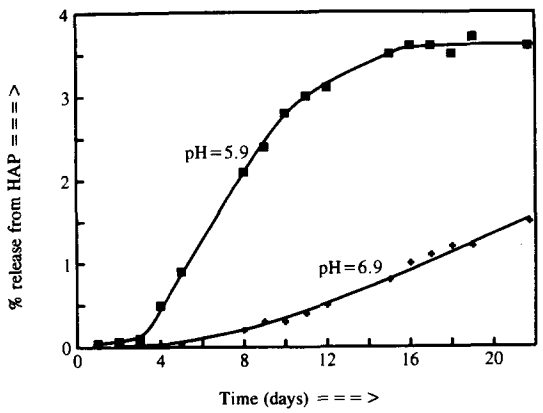

Fig. 6. In vitro release of BSA from BSA-loaded hydroxyapatite microcarriers of batch VI (treated to contain only incorporated BSA) in EDTA buffer as a function of pH.

4. Conclusions

The experimental work described in this paper has demonstrated that it is in principle possible to incorporate protein drugs into hydroxyapatite microcarriers by controlled crystallization under aqueous conditions at room temperature. The BSA incorporated could not be released without dissolving the hydroxyapatite particles. Preliminary in vivo experiments in rabbits indicated the complete resorption of hydroxyapatite particles in less than 1 month. Histological examinations provided encouraging results. To simulate the assumed biocontrolled release due to biological resorption of the hydroxyapatite, BSA-loaded hydroxyapatite microcarriers were dissolution tested in EDTA solution, resulting in prolonged release of BSA. Both the dissolution of the hydroxyapatite and the BSA rate of release were found to be pH dependent.

Before one can conclude that aqueous hydroxyapatite suspensions have potential as drug delivery systems in the area of biocontrolled drug release, several problems must be resolved. The main drawbacks encountered are the low amounts of protein drug that can be incorporated, slow manufacturing and low efficiency of drug incorporation. Other problems are related to drug stability, handling, storage and dosing requirements such as syringability. Nevertheless, this study may trigger further interest in the application of dispersable ceramic materials as drug delivery systems.

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