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International Journal of Pharmaceutics 213 (2001) 117–125

international journal of pharmaceutics

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Potential use of gelcasting hydroxyapatite porous ceramic as an implantable drug delivery system☆

D.J.A. Netz [a], P. Sepulveda [b], V.C. Pandolfelli [b], A.C.C. Spadaro [a], J.B. Alencastre [a], M.V.L.B. Bentley [a], J.M. Marchetti [a,*]

[a] *Departamento de Ciências Farmacêuticas da Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo. Av. do Café s/n. CEP 14.040-903 Ribeirão Preto, SP, Brazil*

[b] *Departamento de Engenharia de Materiais da Universidade Federal de São Carlos. Rod. Washington Luis, Km 235–905, 13565–905 São Carlos, SP, Brazil*

Received 11 August 1999; received in revised form 27 October 2000; accepted 27 October 2000

Abstract

Hydroxyapatite (HA) ceramic in a porous configuration is suggested as a drug release system. A new technique for the production of this material, based on the foaming of suspensions and in situ polymerization (gelcasting method), resulted in a material whose characteristics are likely to make it useful as an implantable drug delivery system. Three batches of HA ceramic with different porosities were characterized by X-ray diffraction and scanning electron microscopy (SEM). Pore size and shape as well as density were determined. In vitro experiments were performed in order to evaluate the dissolution behavior of cisplatin in the system. X-ray diffraction analysis showed that the final product consisted of a single phase, indicating that the sintering process had not affected the structure of the HA. Energy dispersive X-ray analysis (EDX) showed absence of impurities. Pore diameters were in the range 15–34 μm. SEM showed that the material presented a highly interconnected spheroidal porous network with open micropores and closed macropores. In vitro experiments showed significant differences in the release rate of cisplatin between three different porosities.

Keywords: Hydroxyapatite; Porous ceramic; Implantable drug delivery systems; Gelcasting

1. Introduction

New delivery systems have been developed to improve the therapeutic effect of several drugs in different pathologies (Batra et al., 1994; Hnatyszyn et al., 1994; Shenoy et al., 1997). Alongside the development of new delivery systems, biomaterial science has been shown to be of great interest for Pharmaceutical Technology. Clinical and commercial acceptance of biocompatible materials has been widespread (Ratner, 1993; Peppas and Langer, 1994). Low cost and ease of manufacture render ceramic materials

☆ Based on a preliminary presentation at the American Association of Pharmaceutical Scientists, New Orleans, LA, USA. Abstract in Pharm. Sci. (Suppl.), 1 (4), p. S-374, 1999.

* Corresponding author. Tel./Fax: +55-16-6024300.

0378-5173/01/$ - see front matter
PII: S0378-5173(00)00659-1

promising vehicles for drug delivery (Hnatyszyn et al., 1994).

Due to their biocompatibility, osteoconductive and ostheophilic nature, hydroxyapatite and other calcium phosphates have been studied aiming at their use as implantable materials (Shinto et al., 1992; Arita et al., 1995; Lemons, 1996; Osawa and Kasugai, 1996; Zafirau et al., 1996; Tampieri et al., 1997; Fanovich and Porto Lópes, 1998). The excellent biocompatibility and the porous structure of HA ceramics permit their utilization as an implantable system for the delivery of hormones (Zafirau et al., 1996; Shenoy et al., 1997), antibiotics (Calhoun and Mader, 1997), anti-cancer agents (Yapp et al., 1997; Itokazu et al., 1998), vaccines (Walduck et al., 1998) and anti-aids drugs (Dhillon et al., 1998).

Differents methods have been employed for the production of porous ceramics, namely, replication of polymers (Lange and Miller, 1987), use of hollow spheres (Verweig et al., 1985), foaming of sol–gel based systems (Klein and Woodman, 1996), GASAR processing (Wolla and Provenzano, 1995), incorporation of the fugitive phase (Komameni et al., 1995), and others.

The development of ceramics by gelcasting has been allied with traditional concepts of the production of ceramic materials with polymer chemistry (Omatete et al., 1991). In this method, a slurry of ceramic powder in a solution of organic monomers is placed in a mold. The monomer mixture is polymerized in situ to form gelled parts.

Gelcasting was developed originally to produce dense bodies and has been adapted by Smith (1994), for the manufacture of porous ceramics from foamed suspensions. The association of gelcasting with foaming followed by solidification by in situ polymerization forms an internal cross-linked network that transforms the foam in a strong gelled body. The gel retains the foamed structure in such a way that both macrostructures and microstructures are preserved. In addition, this method, by being very versatile, can be applied successfully to other ceramic powders, such as zirconia, calcined clay and alumina (Sepulveda, 1997).

The influence of a porous microarchitecture on the biological behavior and in vitro dissolution of porous calcium phosphate ceramics has been demonstrated (Liu, 1997). The permeability of the porous materials is a function of characteristics such as percent average pore diameter, shape and connectivity of the porous matrix. These properties are affected by the preparative conditions (Vasconcelos, 1997). The characterization of biomaterials includes measurement of their physico-chemical, mechanical, morphological and cytotoxical properties (Shields, 1991; Bras et al., 1995; Fabri et al., 1995; Lelièvre et al., 1996; Suchanek et al., 1997; Bigi et al., 1998).

Cisplatin (*cis*-diamminedichloroplatinum), an organic platinum coordination complex, has been used clinically against various types of tumors such as small-cell lung cancer, metastatic testicular tumors, metastatic ovarian tumor, pancreatic cancer and advanced bladder cancer. However, important renal and gastrointestinal side effects have been reported and limited its clinical use (Cvitkovic, 1998).

Implantable delivery systems, with sustained drug release may be an alternative cisplatin therapy, because it permits an increase of the dose at the site of the tissue tumor, decreasing the frequency or eliminating systemic side effects (Hagiwara et al., 1992; Uchida et al., 1992; Kitchell et al., 1995; Mestiri et al., 1995; Kong et al., 1997; Yapp et al., 1997).

In the present study, the characteristics of HA porous ceramic were analyzed by X-ray diffraction (XDR) to verify the nature of the crystalline phase, scanning electron microscopy (SEM) coupled to image and EDX analysis for the evaluation of microstructure in terms of pore morphology, size distribution and chemical composition and by mercury porosimetry measurements. In vitro experiments were performed in order to evaluate the drug release profile of the implantable system.

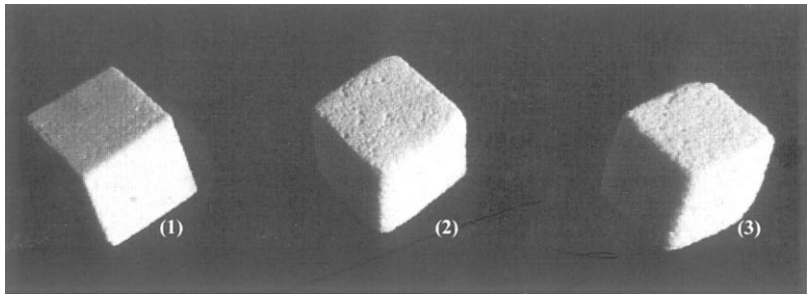

Fig. 1. Photograph of hydroxyapatite ceramic blocks (1.2 cm^3) produced by gelcasting method. Pore size as determined by image analysis were, (1) 15.43 μm; (2) 21.27 μm; and (3) 34.76 μm.

2. Experimental

2.1. Materials

Hydroxyapatite (HA) powder, Grade GA CS with 10–100 μm average particle size was obtained from Jesse Shirley Advanced Ceramics Limited (Staffordshire, UK). All other materials were of reagent grade. HA ceramic samples were prepared at Departamento de Engenharia de Materiais, Universidade Federal de São Carlos, Brazil.

2.2. Methods

Three batches of HA ceramics with different degrees of porosity were obtained using gelcasting (Sepulveda, 1997). Homogeneous suspensions of the HA powder in water were prepared by adding dispersing agents and monomer avoiding contact with oxygen; a surfactant was added in order to produce foam. Starting and catalytic substances promoted polymerization. The gelled body with a rubbery texture was obtained, dried and fired at 12 000°C, in order to improve and consolidate its mechanical resistance.

The crystallinity of the ceramic obtained was evaluated using an X-ray diffractometer (Siemens diffractometer D-500) with a Ni filter, radiation at 30 kV and 20 mA. Prior to analysis, samples were powdered to an average particle size of 0.043 mm.

The SEM studies were performed using a Leica-Stereoscan 440 scanning electron microscope. The analyses of individual samples were performed after gold coating, using a Spulter Balzers SCD-050 Coater, at magnifications of $\times 400$ and $\times 2000$, at 20 kV.

Image analysis was performed using a Leica-Stereoscan 400 scanning electron microscope attached to a Leica Quantimet 600-F microanalyzer.

The qualitative chemical composition of the samples was obtained using a SEM/EDX combination. The scanning electron microscope was attached to an Oxford-Link XLII Analyzer, using a voltage of 20 kV for 100s (BSE systems-back scattered electrons). Samples were coated with gold–palladium prior to examination.

Porosity measurements were carried out using a 9320 Micrometrics Porosizer, under the following conditions — low pressure for 360–3.6 μm pore diameter and high pressure, for 6–0.003 μm pore diameter.

Density was calculated from the weight and volume of the samples, obtained by direct measurement using a Mitutoyo paquimeter. Relative values were calculated using as reference a HA theoretical density of 3.16 g cm^{-3}, using the following equation,

$$d = \frac{\text{weight (g)/volume (cm}^3\text{)}}{3.16} \times 100$$

Table 1
Weight and volume measurements of the samples

Sample	Weight (g)[a]	Volume (cm^{-3})[a]
1	1.14 ± 0.048	1.30 ± 0.076
2	0.75 ± 0.029	1.33 ± 0.087
3	0.57 ± 0.028	1.46 ± 0.079

[a] Values are averages of ten measurements of the samples.

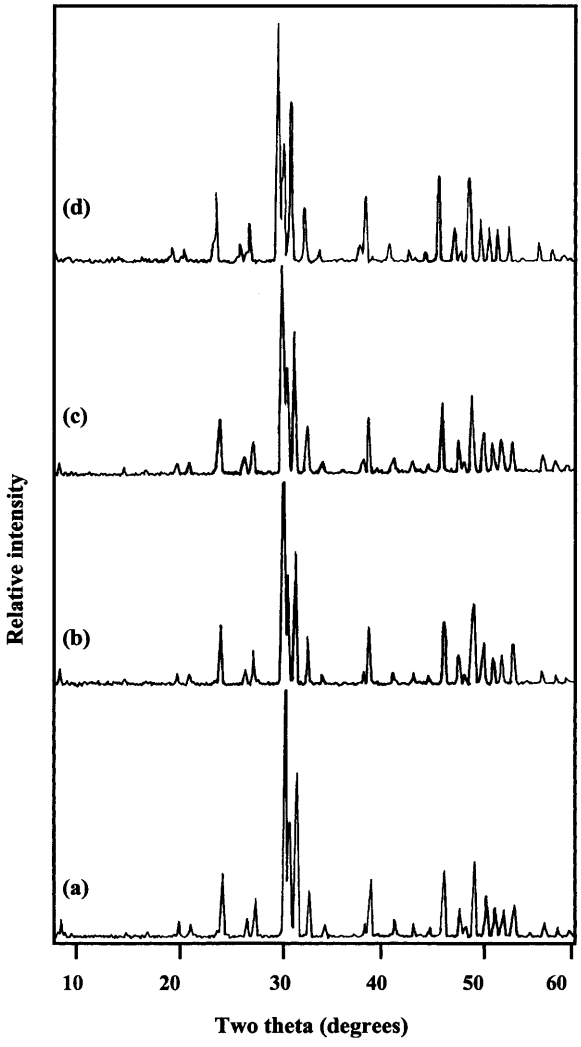

Fig. 2. X-ray diffraction analysis of hydroxyapatite porous ceramic produced by gelcasting, (a) HA starting powder; (b) sample 1; (c) sample 2; and (d) sample 3.

Drug incorporation in the HA ceramic samples, a cylindrical orifice was made in the center of each sample of porous HA ceramic and 10 mg of cisplatin were placed in the cavity, which was sealed with photo resin.

The dissolution profiles of individual samples were determined using 100 ml of isotonic phosphate-buffered, pH 7.2. The experiment was carried out during 168 h, at 37°C and 100 rpm.

Samples from the dissolution medium were withdrawn at predetermined times over a 168-h period and the platinum concentration determined by atomic absorption at 265.9 nm in an atomic absorption spectrophotometer type AA-680 G, (Shimadzu). An equal volume of the medium was added immediately to maintain a constant volume. All experiments were repeated five times. Mathematical models were employed to study the release profile of the systems.

3. Results and discussion

3.1. Samples of HA porous ceramic

Fig. 1 shows ceramic samples of different porosities produced by the method previously described. Values corresponding to weight (g) and volume (cm^3) of the samples are shown in the Table 1.

3.2. X-ray diffraction analysis

X-ray diffraction patterns are shown in Fig. 2. The diffraction angles and the relative amplitude of each diffraction peak were identified from the diffractograms. The figure shows that the three samples presented only one phase (pure hydroxyapatite).

X-ray diffraction patterns presenting peaks characterizing a crystalline phase (Sorrell, 1991), are useful to identify each phase by comparison with peaks presented by standards. Phase composition is one the most important parameters of HA ceramics, because it determines their biocompatibility. In the present study, the pattern of the three samples with different porosities coincides with the standard diffraction pattern of pure HA (starting powder). At temperatures above 12 000°C, HA can become unstable and may eliminate OH groups forming decomposition products and additional phases like α and β-TCP, tetracalciumphosphate and CaO (Suchanek et al., 1997; Tampieri et al., 1997). No such additional phases were founded in the diffractograms, indicating that the sintering process had not changed HA's composition.

3.3. Scanning electron microscopy (SEM)

Fig. 3 shows SEM photomicrographs of HA porous ceramics, indicating that they present open micropores and closed macropores.

SEM provided information about pore size and shape. SEM showed that the porous ceramic structure obtained, consisted of a highly interconnected spheroidal porous network with open micropores and closed macropores, ranging between 15 and 34 μm according to the specimen's density. Such analysis of the ceramic architecture is significant, because it is related to mechanical, osteoconductive, dissolution and permeability properties. Interconnected and open pores are very important to allow the flow of substances (Arita et al., 1995; Liu, 1997; Sepulveda, 1997).

3.4. Image analysis

Fig. 4 shows the histograms of pore diameters of HA ceramics, determined by image analysis.

Image analysis was performed in order to determine pore size distribution. Three HA porous ceramics presented significant differences (15.43, 21.27, 34.76 μm, respectively). Structures with dif-

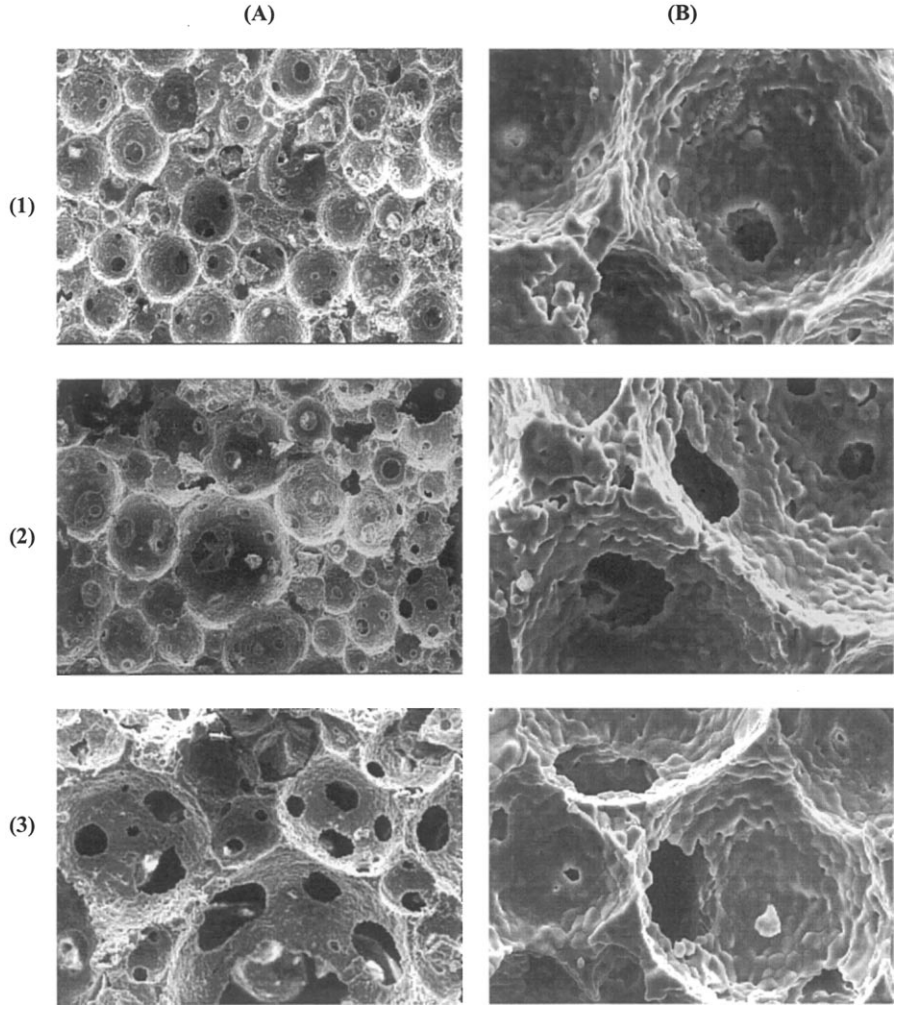

Fig. 3. Scanning electron micrograph of HA porous ceramics with porosities of respectively, (1) 58.48%; (2) 76.29%; (3) 82.63%. Magnifications were × 400 (series A) and × 2000 (series B).

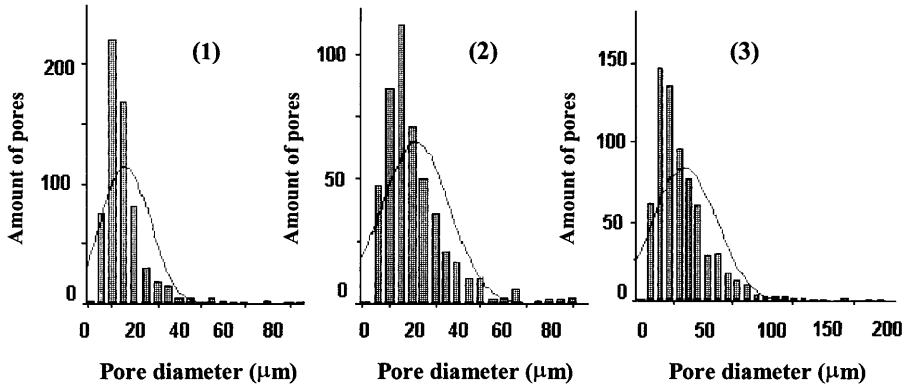

Fig. 4. Image analysis of mean porous size diameter, (1) 15.43 μm; (2) 21.27 μm; (3) 34.76 μm. Total amount of pores analyzed were 700, 480 and 631, respectively.

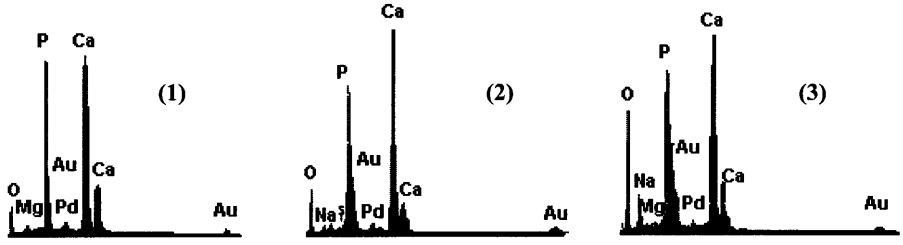

Fig. 5. EDX analysis of HA porous ceramics.

ferent pore size can lead to the products having different permeabilities, indicating that the materials have potential applications as drug delivery systems, when sustained rates of release are desired.

3.5. Energy dispersive X-ray analysis (EDX)

Fig. 5 illustrates EDX analysis of HA porous ceramics demonstrating that they are formed essentially by Ca, P and O.

EDX analysis of the results of SEM demonstrated that no impurities were present in the samples. Traces of Mg, Si, and Na, present in the samples, were also found in the HA powder used as starting material.

3.6. Porosity and density

Porosity values obtained by the mercury intrusion method and density values, calculated arithmetically, are presented in Table 2.

Density plays an important role in the determination of the structure of porous ceramics sintered from foams, and can offer information about pore size and distribution, mechanical strength, permeability and presence of structural faults (Sepulveda, 1997). A close relationship between these data was established. Low porosity and small pore size coincided with a high level of densification. Porosity characterization is based usually on the presence of open-pores and is designated as apparent porosity. This property is most important because it is related to properties

Table 2
Values corresponding of the porosity (%) and density (%) of HA porous ceramic samples

Sample	Porosity (%)	Density (%)
1	58.48	27.49
2	76.29	17.74
3	82.63	12.38

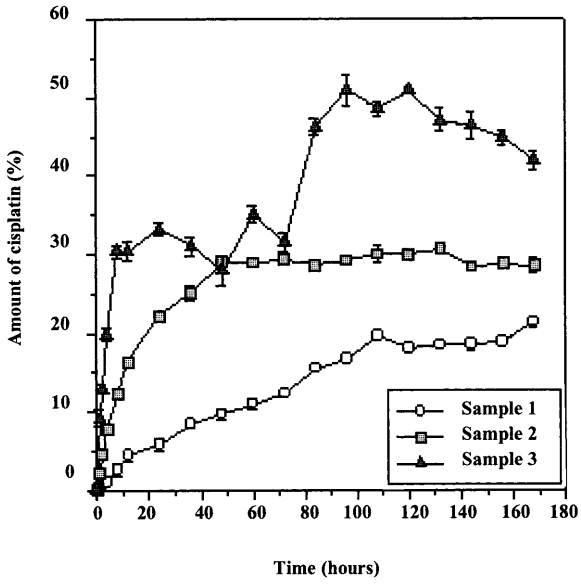

Fig. 6. In vitro release profiles of cisplatin from the samples 1, 2 and 3, produced by gelcasting method. Each point and bar represent the mean ± S.D. of five samples.

such as permeability and surface area. Using the mercury intrusion method, three different porosities were demonstrated (58, 76 and 82%). These values suggest that the pores are, essentially, open structures accessible to mercury penetration.

3.7. In vitro drug release

Results shown in Fig. 6 indicate that the final amount of cisplatin released from samples 1, 2 and 3, were respectively 21.6, 28.35 and 41.85%.

Fig. 6 shows that cisplatin release from samples of porous ceramics of HA is related to their pore size and shape. Samples 1 and 2, that possessing average pore size between 15.43 and 21.27 μm, respectively, showed uniform behavior, with a lower percentage of cisplatin release (21.6 and 28.35%, respectively). Sample 3 presented a higher (41.85%), but irregular percent cisplatin release. The analysis of the results of SEM demonstrated the microstructure of sample 3 to be irregular, presenting some pores very much larger than others. This suggests that microstructure can affect release behavior. The total amount of cisplatin released from all samples after 168 h, was lower than 50%.

The data on the kinetic parameters indicated that the Higuchi model characterized the in vitro release profile of the systems studied, because it presented the highest correlation coefficients for all samples analyzed. These results can be seen on Table 3.

4. Conclusion

Structures with different pore sizes produce materials of varying permeabilities, having potential application as drug delivery systems. HA ceramics display characteristics that are important potentially for systems used for drug release. The Gelcasting method provides samples with different porosities. For 58.48 and 76.29% porosities, the results of drug release were more adequate because of the system's regular microstructure. Samples with higher porosity presented an irregular structure, that could be interfering with drug release. Therefore, we suggest that hydroxyapatite porous ceramics would be useful as drug delivery systems only for matrices with porosities below 78.29%. The results showed that variations in the porous network including porosity fraction and pore size, may provide different drug delivery behavior and that the control of these parameters

Table 3
Values corresponding to linear correlation coefficient (r), obtained from the in vitro cisplatin release profile

Sample	Linear regression (concentration × time)	Higuchi model (concentration × $\sqrt{}$time)	First order kinetics (log concentration × time)
1	0.9677	0.9893	0.8228
2	0.9481	0.9940	0.8244
3	0.9257	0.9865	0.9098

is very important if a sustained release behavior is to be obtained. However, they still need to be defined better.

Acknowledgements

The financial support of FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) is acknowledged gratefully.

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