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ScienceDirect
Ceramics International 35 (2009) 509–513
CERAMICS INTERNATIONAL
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Spray-dried hydroxyapatite-5-Fluorouracil granules as a chemotherapeutic delivery system

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Received 25 September 2007; received in revised form 11 November 2007; accepted 4 January 2008
Available online 10 April 2008

Abstract

This study describes the preparation and characterization of hydroxyapatite-5-Fluorouracil (5FU) granules, which are intended to be used as chemotherapeutic delivery matrices and bone regeneration templates. Suspensions of hydroxyapatite (Hap) nanoparticles in 5FU solution are spray dried as micro sized granules having donut type shape. Several spray drying temperatures are studied 80 °C being the optimized condition for obtaining granules composed by Hap and 5FU without secondary phases. The produced granules at 80 °C reveal a fast releasing rate of 5FU when soaked in buffer phosphate solution maintained at physiological temperature (37 °C), thereby indicating the potential application of the produced Hap matrices for drug delivery systems (DDSs).

Keywords: [A] Spray drying; [A] Drug delivery; [D] Hydroxyapatite; [D] 5-Fluorouracil

1. Introduction

Hydroxyapatite (Hap, $Ca_{10}(PO_4)_6(OH)_2$) is an appealing biomaterial for applications in drug delivery systems (DDSs) due not only to its chemical similarity to the bone inorganic phase but also to its ability for adsorbing and releasing several molecules with biological interest [1,2].

Different synthesis methods are already available for obtaining Hap particles with convenient morphological characteristics to be used in DDS [3]. Recent research on spray drying technique has revealed its manufacturing potentialities for producing powders aiming at different applications [4,5]. The preparation of porous Hap granules by spray drying aqueous suspensions of Hap nanoparticles showing adequate properties for subsequent loading with the drug 5-Fluorouracil (5FU) has been already reported [6,7]. It has been shown that hydroxyapatite nanosized particles (nHap) by themselves have inhibitory action towards different kinds of tumours [8,9]. Moreover recent studies revealed that a strong cooperation effect might be obtained upon their association with other anti-tumour medicines, namely with 5FU: the inhibition rate of the tumour increases with the combined therapy while the poisonous by-effect of the drug is reduced [8,9]. 5FU is an antineoplastic agent having a relatively short (10–20 min) plasma half-life and commonly used in the therapy of different solid tumour types due to its biopharmaceutical and pharmacological properties [10]. From the point of view of the DDS engineering the possibility of combining directly the chemotherapeutic agent 5FU with the supporting Hap material in a simple spray drying step has not been addressed so far. The advantage of such one step procedure for obtaining the 5FU loaded granules might rely on the shorter processing times and consequent reduction of 5FU toxicity issues due to prolonged drug exposure and manipulation. In order to improve the 5FU therapeutic efficacy and patients compliance, it is desirable to formulate adequate sustained release compositions so as to maximize therapeutic benefits and to reduce its unwanted side effects. Therefore local administration of 5FU through a convenient tailored DDS will benefit the toxicity reduction and effectiveness of chemotherapeutic drug.

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0272-8842/$34.00
doi:10.1016/j.ceramint.2008.01.012

The objective of the present work is to identify and study the effects of relevant experimental parameters including spray drying temperature and Hap precursor suspension formulation on the properties and releasing behaviour of a potential Hap based DDS using a selected model compound, 5-Fluorouracil (5FU), as a case study.

2. Experimental

2.1. Materials

Nanosized hydroxyapatite particles (nHap) were first synthesized by a precipitation method reported elsewhere [7]. 5-Fluorouracil (5FU) powder (99%) was purchased from Sigma. $NaH_2PO_4H_2O$ (99%) and $Na_2HPO_42H_2O$ (99.5%) reagents from Analyticals Carlo Erba and from Merck, respectively, were used to prepare a phosphate buffer saline (PBS) solution which was necessary for carrying out in vitro release experiments, at pH 7.4 and at 37 °C.

2.2. Preparation of the apatite based 5FU delivery system

A 5FU aqueous solution with a concentration of 250 mg/L was used for dispersing nanosized Hap particles (∼1 wt.%). The obtained Hap suspension was used to feed a laboratory spray dryer (Buchi B-191) under specific operating conditions, namely the inlet temperature of the drying air (T_{in}), the inlet flow of the hot air (F_{in}) and the inlet flow of the feeding suspension (P_{in}), both flows expressed a percentage of the allowed maximum values. These operating conditions were selected after preliminary tests for identifying optimum experimental spray drier settings. The resulting spray-dried powders were collected from the cyclone and characterized. Table 1 summarizes the experimental conditions used during spray drying.

2.3. Drug release evaluation

The spray-dried powders produced at 80 °C (SD80) were suspended in a phosphate buffer saline solution (pH 7.4) and maintained at 37 °C during 60 min. After predefined time intervals solution aliquots (2.5 mL) were withdrawn for monitoring the released 5FU. The evaluation of the 5FU concentration in the releasing medium was carried out by Ultraviolet (UV) spectroscopy at the wavelength of 265 nm in triplicate essays. The amount of 5FU existing in SD80 samples was accessed by dissolving SD80 powders in an 1 M HCl solution and subsequent evaluation of 5FU concentration by UV spectroscopy.

Table 1
Spray drying conditions

Sample	T_{in} (°C)	F_{in} (%)	P_{in} (%)
SD80	80	70	10
SD120	120	60	20
SD180	180	60	20

2.4. Powders characterization

The morphology of the spray-dried powders obtained at different temperature (SD80, SD120 and SD180) was analyzed by scanning electron microscopy (SEM) (Hitachi S-4100). The crystalline phases were identified by X-ray diffraction (XRD) analysis (Rigaku PMG-VH, Cu Kα = 15405 Å). Fourier transform infrared spectroscopy (FTIR) (Mattson galaxy 3020) in transmittance mode was used to identify the particle functional groups in the range of 400–4000 cm^{-1}, using KBr pellets. The thermal behaviour of 5FU was characterized by thermogravimetric and differential thermal analysis (TG-DTA) from room temperature up to 1000 °C, using a 10 °C/min heating rate (Setaram Labsys 1600 under N_2). The presence of 5FU in spray-dried powders was analyzed by confocal microscope (Leica TCS SP5, Leica Microsystems, Wetzlar).

3. Results and discussion

3.1. Characterization of the spray drying powder

The X-ray diffraction patterns of the spray-dried powders are shown in Fig. 1. Comparing them with those corresponding to the original nHap and to 5FU powder one observes that the diffraction peaks exhibited by SD80 and SD120 (Fig. 1c and d) reveal the presence of both Hap and 5FU crystalline phases. This indicates that the two operating temperatures of 80 and 120 °C enable the precipitation of 5FU from the sprayed droplets of the original suspension. The obtained powders are consequently a mixture of nHap and 5FU. However, when the operating temperature is increased up to 180 °C, different crystalline phases are obtained: according to the XRD results presented in Fig. 1 bifosfamite ($NH_4H_2PO_4$), ammonium oxide $(NH_4)_2O$ and fluoroapatite ($Ca_5(PO_4)3F$) are detected in the spray-dried product. This result suggests that the higher spray drying temperature (180 °C) induced chemical modifications on the nHap structure and the decomposition of 5FU molecules which are thereby reflected by the different chemical compounds

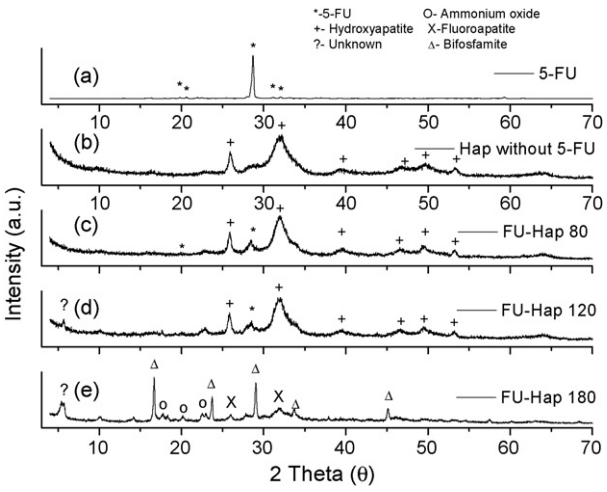

Fig. 1. X-ray patterns of pure 5FU (a); Hap nanoparticles without 5FU (b); spray-dried Hap-FU granules at 80 °C (c); 120 °C (d); 180 °C (e).

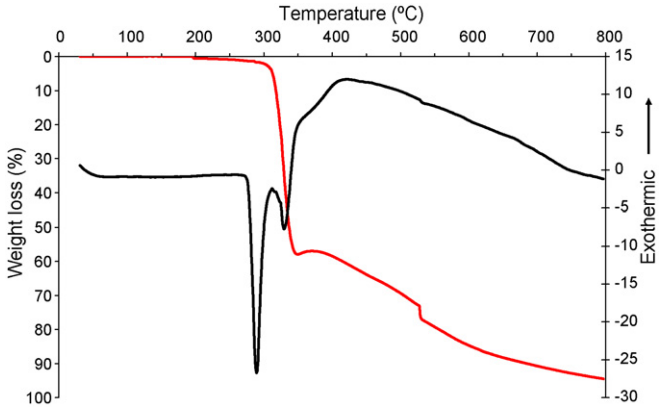

Fig. 2. Thermogravimetric and differential thermal analyses of 5FU.

obtained at that temperature. The suggested decomposition of 5FU at the used operating temperature could not be anticipated from the obtained thermograms of this compound shown in Fig. 2.

As observed a very steep and strong weight loss (∼60%) starting at ∼300 °C characterizes the thermal behaviour of 5FU powder. After that the weight loss proceeds with the temperature increase but at a much lower rate. At 750 °C 95% of the drug mass is already lost. The differential thermal analysis (DTA) curve of 5FU indicates the first abrupt weigh loss to be accompanied by two sharp endothermic peaks while the subsequent weight loss is framed by a broad exothermic band. The interpretation of this behaviour is that the two endothermic peaks are associated to 5FU decomposition reactions whose products are mostly lost at ∼300 °C. The residual products of 5FU decomposition are burned later, at higher temperature, thus producing the observed broad exothermic effect. In conclusion the thermal behaviour of 5FU does not foresee its decomposition at temperatures far below 300 °C. However, for explaining the XRD results of the sample SD180 one has to assume that at 180 °C the integrity of 5FU molecule is not preserved anymore. Moreover taking into account the small peak at low 2θ present in the XRD pattern of the sample SD120 which was assigned to unknown second phase, the 5FU chemical modification is believed to be already incipient at 120 °C. Studies aiming at the clarification of 5FU reactions in presence of nHap are now in progress.

The SEM images of the spray-dried materials obtained at different temperatures (80, 120 and 180 °C) are shown in Fig. 3a–c. As observed the operating temperature of 80 °C favoured a very regular donut type morphology (Fig. 3a) which is quite analogous to that obtained when spray drying aqueous suspensions of nHap [7,10]. At 120 °C basically the same morphology was dominant though minor amounts of scattered rod like particles were detected too (Fig. 3b). This rod like morphology may perhaps correspond to the unknown compound previously referred in SD120 XRD results. Concerning the material spray dried at 180 °C a mixture of different morphologies is clearly evidenced in SEM image (Fig. 3c) reflecting naturally the presence of the different crystalline phases identified by XRD analysis. It may be thus concluded that the operating temperature of 80 °C enables the preparation of homogeneous microspheres of Hap and 5FU mixture with a rather uniform shape and size. For this type of granules the confocal microscopy image of Fig. 3d presents

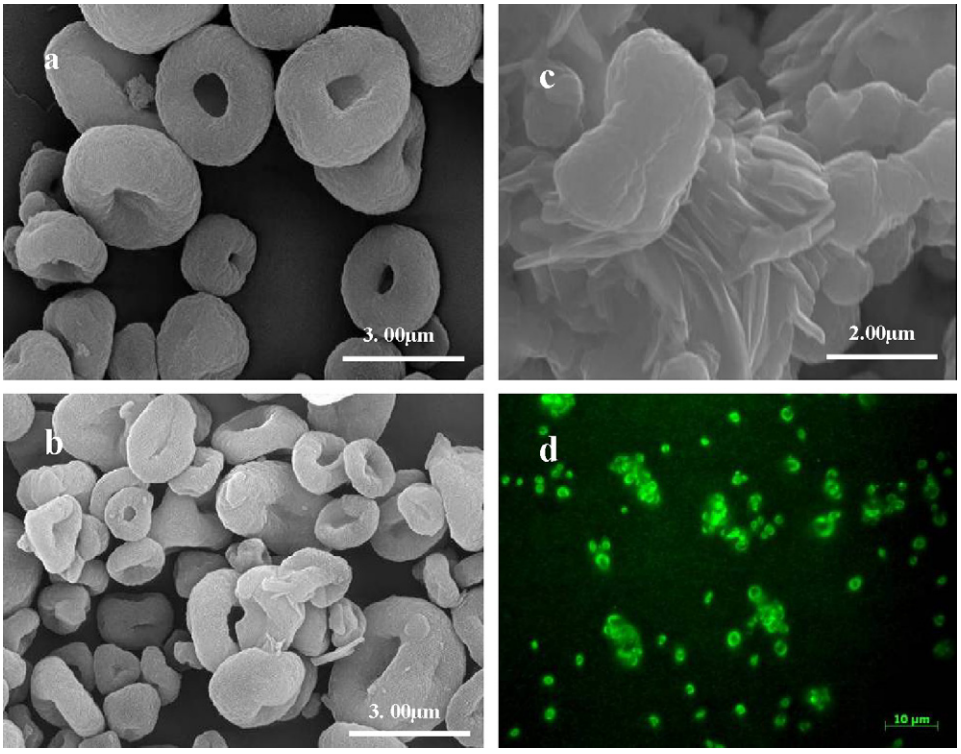

Fig. 3. SEM image of spray-dried powders at (a) 80 °C, (b) 120 °C, (c) 180 °C and (d) confocal image of SD80.

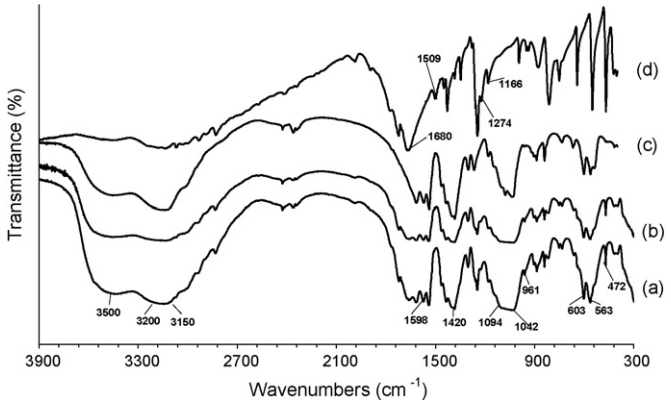

Fig. 4. FT-IR spectrum of (a) SD80; (b) SD120; (c) SD180; (d) pure 5FU.

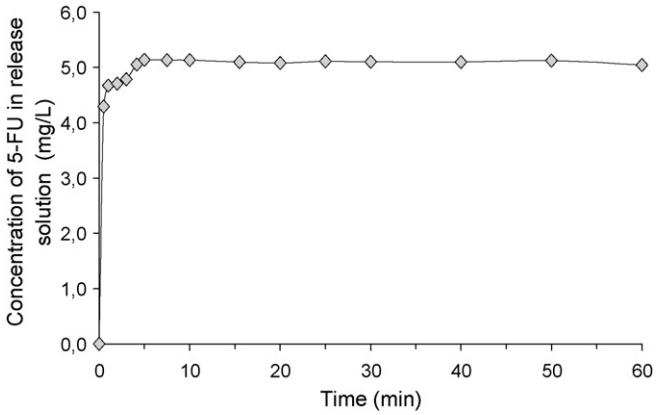

Fig. 6. 5FU release profile from the Hap-5FU spray-dried granules obtained at 80 °C.

further evidences of 5FU presence thereby indicating the adequacy of the spray drying process at 80 °C for producing Hap granules loaded with 5FU.

Supplementary data supporting the coexistence of Hap and 5FU in SD80 samples are supplied by FTIR spectroscopy. As shown in Fig. 4 the characteristic bands of Hap are maintained in the granules spectrum as inferred from the bands at 472, 563, 603, 961, 1032–1072 and 1094 cm^{-1} belonging to PO_4^{3-} vibration mode [11]. The broad band extending from 3800 to 3400 cm^{-1} is ascribed to adsorbed water molecules [12]. Characteristic peaks of 5FU are also detected as is the case of the broad band around 1500–1680 cm^{-1} corresponding to C=C, C=N and C=O vibrations (see Fig. 5 for chemical structure of 5FU molecule) and of the strong absorption bands at 1274 and 1166 cm^{-1} assigned to C–O and C–N vibration, respectively [13]. Additional bands observed at 1421 and 1598 cm^{-1} are attributed to citrate group (COO^-) [14] and at 3150 cm^{-1} [15] to the functional group NH_4, both groups derived from the particular precipitated medium where nHap were originated [7].

A preliminary analysis of the ability of SD80 granules to act as carriers for the 5FU, a chemotherapeutic compound used in the treatment of the cancer disease, was carried out. To the best of our knowledge this is the first time that these Hap granules are used for delivery studies of 5-Fluorouracil. The in vitro release profile of SD80 in a phosphate buffer solution is shown in Fig. 6. As observed after a releasing time of 5 min, the amount of released drug stabilizes and reaches the final value of 0.035 mg 5FU/g of granules. This value corresponds approximately to the amount of 5FU initially loaded in the granules by the spray drying process as accessed experimentally. The analysis of the granule morphology (SEM) performed after a releasing period of 1 h did not reveal any significant change as compared to that of the freshly prepared granules. A rapid release of 5FU was also reported by Wang et al. but using a different carrier material [16]. Taking into account the short time (5 min) required for the total release of the loaded drug and the confocal microscopy results, it may be assumed that 5FU exists as a solid phase in rather accessible regions of the granule, indicating that the precipitation of 5FU during spray drying probably occurred in such a way so as to become incorporated in the outer layer of the granule. In this way, the interaction with neighbouring solution molecules is sufficient for solubilising the drug from its transporting vehicle, i.e. the spray-dried granule. Some aspects remain, however, to be further investigated, namely the biological activity of the 5FU when released from the matrices developed in this work (ongoing studies).

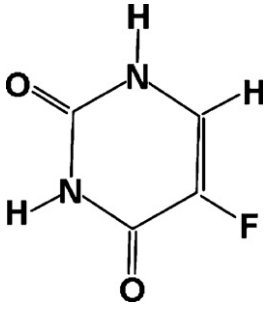

Fig. 5. Chemical structure of 5FU.

4. Conclusion

This study describes the preparation and initial characterization of Hap-FU microgranules, which are intended to be used as chemotherapeutic delivery matrices and bone regeneration templates. The proposed methodology enabled the preparation of homogeneous microgranules with a uniform size, where the bulk properties of the ceramic were maintained, indicating that the 5FU did not induce any modifications in the structure of the hydroxyapatite. Preliminary studies on the release of the therapeutic 5FU were also performed. The strategy used to release 5FU of the granule suggests that it is possible to release the 5FU in a rapid way of the Hap granules.

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