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BCP ceramic microspheres as drug delivery carriers: synthesis, characterisation and doxycycline release

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Abstract Resorbable ceramics such as biphasic calcium phosphates (BCP) are ideal candidates as drug delivery systems. The BCP ceramic is based on the optimum balance of the most stable hydroxyapatite (HA) phase and more soluble tricalcium phosphate phase (TCP). Doxycycline is a broad-spectrum antibiotic used for the local treatment of periodontitis. The development of BCP microspheres and its release kinetics with doxycycline have been studied. The BCP ceramic powder were prepared by microwave processing and characterised by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) methods. The BCP microspheres were formed by liquid immiscibility effect using gelatin and paraffin oil. Difference in the morphology of the microspheres as a function of gelatin content has been observed. Scanning electron microscope indicated spherical and porous morphology of the microspheres. Drug incorporation was studied at varying pH and the pH 7 was found to be optimal for drug loading. Release pattern tend to depend on the morphology of BCP microspheres. An optimum release of 80% drug has been observed for BCP microsphere with HA:TCP = 65:35 ratio. The surface area measurement results also correlate with drug release obtained.

Introduction

Particulate-based systems for the delivery of drugs are claimed to have enhanced bioavailability, predictable therapeutic response, greater efficacy and safety and controlled and prolonged drug release profile [1, 2]. They are especially important in the case of poor drug distribution at the site of infection due to limited blood circulation to the surrounding skeletal tissue [3]. Ceramic particulate systems for the delivery of antibiotics, anticancer drugs and hormones are under development [4–7]. Doxycycline is a broad-spectrum antibiotic with activity against a wide range of gram positive and gram-negative organisms [8]. It is the drug of choice in the treatment of lyme disease, brucellosis and several rickettsial infections. It is also extensively used for the treatment of juvenile periodontitis. The formation of periodontal pockets has been the result of a localised pathogenic bacterial infection below the gum line [9]. Periodontal pockets are easily accessible from the oral cavity and are therefore a convenient site for treatment by a localised drug delivery system. The use of resorbable ceramics has caused enhanced interest with particular attention focused on biphasic calcium phosphate (BCP) ceramics. The BCP ceramic is based on the optimum balance of the most stable hydroxyapatite (HA) phase and more soluble tricalcium phosphate phase (TCP) [10]. The HA is known to bond with bone directly and thus can be used as a bone replacing material while the TCP (both α and β phases) is known to be a bone substituting material because as it dissolves gradually and a new bone will be formed where it is resorbed. The BCP allows its bioactivity and biodegradation to be controlled by varying the HA/TCP ratio. The bone ingrowth into BCP ceramics has been found to be rapid [11]. The microwave synthesis offers the advantages of rapid heating of the entire volume resulting in the mixing of the phases in the atomic levels that also influences the resorption rate of the BCP ceramics [12, 13]. In an earlier study we have shown the controlled release of doxycycline by BCP powder prepared by microwave

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processing as well as BCP sintered pellet systems [14]. As the behaviour of particulates in the body depends on their morphology and microstructure, the present study is focussed on the preparation of BCP microspheres its characterisation and doxycycline release profiles.

Materials and methods

High purity calcium hydroxide ($Ca(OH)_2$) and di-ammonium hydrogen ortho phosphate (DAP, $(NH4)_2HPO_4$) were used for the preparation of BCP powder (Sigma Aldrich Chemicals USA). Pharmaceutical grade, low viscosity paraffin oil, gelatin (bovine source, SD fine chemicals, India), and the antibacterial drug doxycycline hyclate (Periostat, Ranbaxy Pharmaceuticals, India) were procured locally. The BCP powder were synthesised by the microwave method as reported earlier [12]. Basically it involves the addition of DAP solution to a ($Ca(OH)_2$) suspension and subsequent microwave irradiation. The different BCP systems were prepared by varying the Ca/P molar ratio. The BCP systems consisting of 65HA:35TCP, 80HA:20TCP, 40HA:60TCP, 50HA:50TCP, 60HA:40TCP and 70HA:30TCP have been coded as BCP65, BCP80, BCP40, BCP50, BCP60 and BCP70 respectively.

An appropriate aqueous solution of gelatin content was prepared at 30 °C. Fine BCP65 powder were added to the above solution and the gelatin slurry with BCP powder was dispersed in light paraffin oil in an analytical flask by stirring with a glass paddle stirrer. The rpm of the stirrer and the time of stirring were optimised to form microspheres. The precipitated microspheres were washed in acetone followed by ethanol and dried in air. The gelatin bound beads were heated for an hour at 550 °C to burn off the gelatin and sintered at 950 °C in air to strengthen the microspheres. The microspheres were then thoroughly washed in distilled water to remove any unburned gelatin and dried in an oven. The microspheres formed with 4% (4 gm in 100 ml), 6% (6 gm in 100 ml) and 8% (8 gm in 100 ml) gelatin have been coded as 4BCP65MS, 6BCP65MS and 8BCP65MS respectively in the text. The same process was repeated for the other BCP powders and the microspheres were coded accordingly as shown above.

The synthesised BCP powder and microsphere samples were characterised by X-ray powder diffraction (XRD) method (Shimadzu XDD1 X-Ray diffractometer, reflection mode, Japan) using CuKα radiation. The functional groups present in the BCP powder were ascertained by Fourier transform infrared spectroscopy (FT-IR) method (Bruker, IFS66V FT-IR spectrometer, Germany). The FT-IR spectra were obtained in the region 400–4,000 cm^{-1} using KBr pellet technique with the spectral resolution of 4 cm^{-1}. The morphology of the BCP granules and microspheres were observed under a scanning electron microscopy (JEOL JSM 5410 & JSM 5300, Japan). A few milligram of dried BCP sample was deposited on a black adhesive tape, vacuum coated with gold film for 15 min and analyzed directly. The surface area, pore distribution and porosity of the microspheres was determined by the triple-point BET method (Sorptomatic 1990, USA) with nitrogen as the adsorbate gas and helium as an inert non-adsorbable carrier.

The effect of pH on the encapsulation of doxycycline into BCP microsphere was initially carried out in phosphate buffer saline (PBS) at room temperature to determine the suitable pH for loading of the maximum amount of the drug. Buffers of varying pH were prepared and 10 mg of the drug was dissolved in the pH solutions. The BCP microspheres were suspended in the above phosphate buffers for 3 h. The microspheres were then separated and the concentration of the doxycycline was measured using UV-VIS spectrophotometry (Varian Cary 5E UV-VIS-NIR Spectrophotometer, USA) with a 1-cm path length cuvette. Subsequently the loading was carried out at the optimal pH of the buffer. A typical UV visible spectrum of the doxycycline hyclate has four characteristic peaks in the UV region as shown in Fig. 1, and of the 4 peaks the very prominent peaks at 270 nm and 350 nm were monitored for the drug loading profile. The amount of drug absorbed by the BCP is calculated by finding the difference in doxycycline concentration in the loading buffer, before and after loading by spectrophotometrically using the equation:

$$\text{Percentage drug loading} = ((AB)/A) * 100 \quad (1)$$

where A and B represent the initial and final drug concentrations of the buffer solution, respectively.

To have maximum amount of drug loading, initially 10 mg of BCP microspheres were immersed in 10 ml of PBS containing 10 mg of doxycycline for 24 h. The microspheres were separated by centrifugation and dried at room temperature for 48 h. The amount of drug absorbed

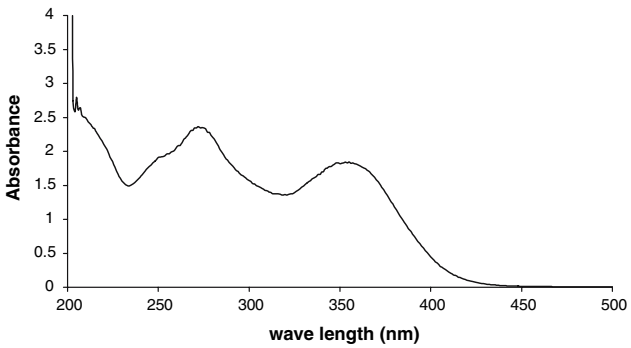

Fig. 1 Typical UV-Visible spectrum of doxycycline

was calculated spectrometrically and by varying the concentration of drug and the BCP microspheres the maximum amount of loading of the drug was obtained.

The in-vitro studies were carried out soaking the drug loaded microspheres in sodium phosphate buffer solution as the medium for release of drug at pH 7.4 and 37 °C. Triplicate samples of 10 mg of granules were suspended in 20 ml phosphate buffer in tubes. The tubes are placed in a bench top constant temperature water bath. At regular intervals of time, solutions are withdrawn and drug release profiles were obtained by the UV-Visible spectrophotometry as mentioned above. The doxycycline release from the specimen to the buffer was determined by measuring the absorbance values at the maximum observed at λ = 270 nm. The spectra were recorded for every 30 min until there was no change in the subsequent values.

In-vitro solubility studies of BCP microspheres were carried out in phosphate buffer of pH 7.4 at 37 °C. Dissolution experiments were performed by using 0.1 g of the pure microspheres without drug loading in 50 ml of buffer of pH 7.4. Each tube containing the microspheres and buffer was mechanically shaken for 3 days and maintained in a constant temperature water bath. The change in the pH of the buffer solution containing the microspheres was recorded at regular intervals of time during the dissolution experiments. All experiments were performed in triplicate, by running three parallel independent measurements simultaneously. The weight loss was calculated by recording the initial and final weights of the microspheres. This weight loss indicates the percentage dissolution of the microspheres of various compositions.

Results and discussion

The XRD pattern of as synthesised BCP (BCP 65) and BCP heated to 900 °C is shown in Fig. 2. Although the diffraction pattern of as synthesised BCP show broad bands that corresponds to that of HA (JCPDS 9-432 & 9-169), the heated BCP sample consists of both the peaks of HA and β-TCP phases and without any impurities. Broad peaks around the characteristic peak regions indicate that the BCP is microcrystalline in nature.

The XRD patterns of the BCP80, BCP70, BCP65, BCP60, BCP50 and BCP40 samples are shown in Fig. 3. All the patterns look similar except with the difference in the relative intensities of the HA and TCP phases. The intensity of TCP peaks has been found to increase with the increasing amount of TCP phase.

The FT-IR spectra of the BCP80, BCP65 and BCP40 samples are shown in Fig. 4. The characteristic bands for HA are exhibited in all the spectra: 900–1,200 cm^{-1} for phosphate bending and stretching and 602, 633 and 3,571 cm^{-1} for librational and stretching modes of hydroxyl vibrations. The intensities of both the hydroxyl bands and the band at 962 cm^{-1} for phosphate can be used as an indication of the HA crystallinity. All the samples also show bands at 947, 986 and 1,122 cm^{-1} corresponding to TCP. As TCP is formed in all the samples, the hydroxyl band at 633 cm^{-1} was found to decrease with the increase in amount of TCP phase [12].

The Ca/P ratio of BCP80, BCP65 and BCP40 samples have been found to be equivalent to the Ca/P ratio present in the mineral constituent of bone. So, further characterisation and the formation of microspheres have been done only for the above BCP samples.

The XRD pattern of the BCP microspheres formed with various amounts of gelatin is shown in Figs. 5, 6, 7. The patterns look similar to that of the starting BCP powder as also shown in the same figure for comparison and consists of both the peaks of HA and β-TCP phases but without other impurities. This clearly indicates the stability of the microspheres even after heating at 950 °C for its formation.

The specific surface area of the microspheres determined using BET isotherm is listed in Table 1. The surface area has been found to vary between 26 and 87 m^2/gm depending on the gelatin concentration and BCP composition. The microspheres formed with 6% gelatin show larger surface area for all the BCP compositions. Similarly the BCP65 microspheres show larger surface area for the entire gelatin concentrations compared to other BCP ratios.

The SEM micrograph of the BCP65 powder is shown in Fig. 8a. The non-spherical and non-agglomerated morphology of the BCP65 powder are less than a micron in size. The SEM micrographs of the BCP65 microspheres are shown in Fig. 8b–d. Distinct difference in the morphology of the microspheres can be observed in the micrographs corresponding to a transition from irregular shape to spherical with smooth surfaces as a function of gelatin content. The 4BCP65MS microspheres have sharp corners and are irregularly shaped as shown in Fig 8b. The 6BCP65MS microspheres show considerable agglomeration and are uniformly spherical with a smooth surface as shown in Fig 8c. The 8BCP65MS microspheres seem to be highly agglomerated as shown in Fig 8d. Irregular morphology of the particulate is known to cause inflammatory reactions so rounded granules with smooth geometry are preferred as obtained in the case of the 6BCP65MS microspheres.

The drug in-take by the microsphere depends on the pH conditions of the buffer solution. The amount of doxycycline loaded at various pHs for BCP microspheres are shown in Fig. 9. The drug was found to be stable at the various pHs studied. Although the amount of drug loading does not show any systematics either with gelatin concentration or BCP composition, higher amount of loading

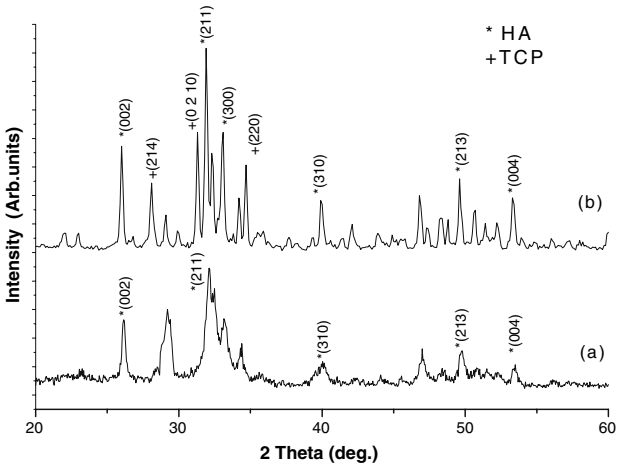

Fig. 2 XRD of pure HA (**a**) and BCP65 (**b**) powders

was shown by the BCP65 microspheres. Maximum amount of loading was found to be at physiological pH of around 7 and hence selected for release study.

The pH variation of the buffer solution with the BCP microspheres is shown in Fig. 10. The pH of the solution has been reported to decrease when TCP is immersed in it due to its dissolution with conversion to the HA phase [12]. So, the BCP samples show a decrease in pH value due to the dissolution of the TCP phase. The TCP phase present in the sample is converted to a stable HA phase as shown in Eq. 2.

$$4Ca_3(PO_4)_2 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 2Ca^{2+} + 2HPO_4^{2-}. \quad (2)$$

As the variation in pH depends on the amount of TCP present in the sample the BCP40 sample shows the maximum decrease when compared to BCP80 sample and BCP65 sample respectively. The slight rise in the pH in the BCP80 sample may be due to the presence of carbonate impurity [12]. Weight loss of the 6BCPMS microspheres of all the three types of BCP powder were observed during dissolution studies as shown in Fig. 11. Dissolution efficiency seems to depend on the amount of TCP phase. The BCP40MS microspheres show the maximum weight loss. The sample corresponds to maximum amount of TCP and explains the observed weight loss. Overall the BCP microspheres have been found to be resorbable.

The doxycycline release profiles of BCP65 microspheres are shown in Fig. 12. All the microspheres exhibit similar release profiles with an initial gradual increase reaching a maximum value and then nearly a constant release profile. A maximum amount of about 80% drug release was observed for the 6BCP65MS microspheres while both the 4BCP65MS and 8BCP65MS microspheres show about 60% drug release. The release rate for 4BCP65MS was found to be slower but the maximum amount of the drug release was noticed in the 8th hour. Both the 6BCP65MS and 8BCP65MS microspheres show maximum release at the 12th hour.

Figures 13 and 14 shows the in-vitro release profiles of BCP80 and BCP40 microspheres respectively. Although the profiles are similar to those obtained for the BCP65 microspheres, time taken to release the maximum amount of drug, duration of maximum release and overall release time is found to be less compared to BCP65 microspheres. The BCP80 release profile shows a consistent increase in all the three types of microspheres irrespective of the initial gelatin concentration. The variation in gelatin concentration leads to different loading efficiencies. The morphological features of the microsphere were found to correlate well with the in-vitro doxycycline release. The 6BCP65MS microspheres were found to be relatively spherical and

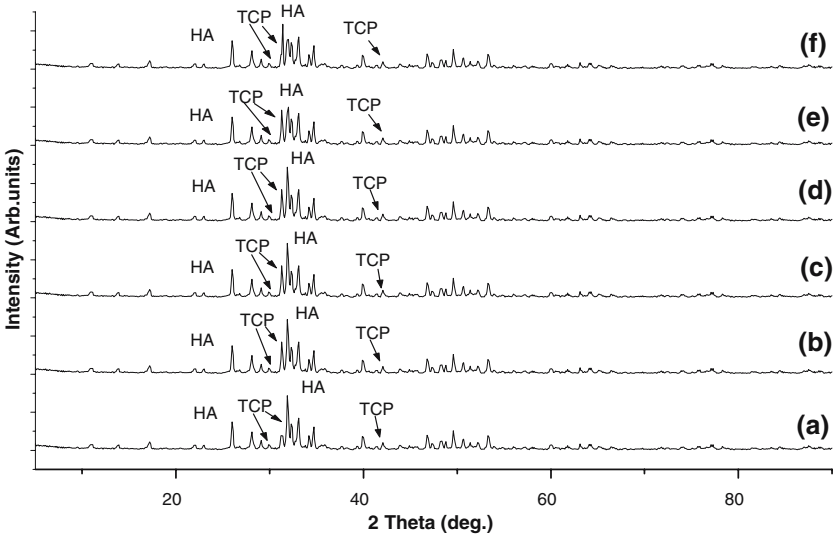

Fig. 3 XRD of BCP80 (**a**) BCP70 (**b**) BCP65 (**c**) BCP60 (**d**) BCP50 (**e**) and BCP40 (**f**) powders

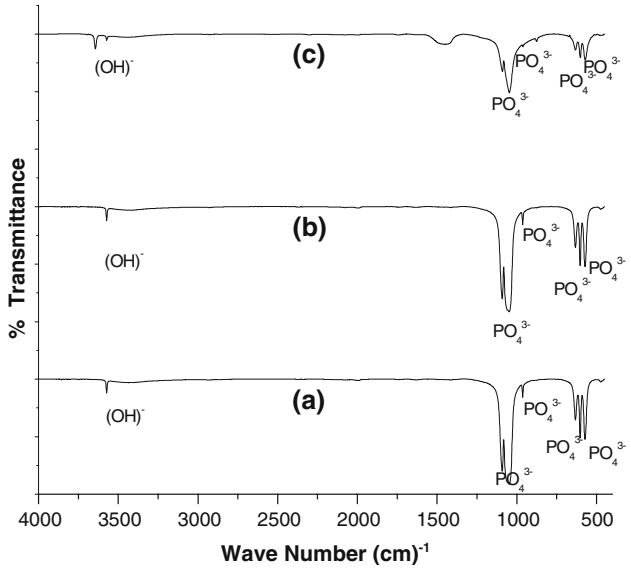

Fig. 4 FTIR spectra of BCP80 (**a**) BCP65 (**b**) and BCP40 (**c**) powders

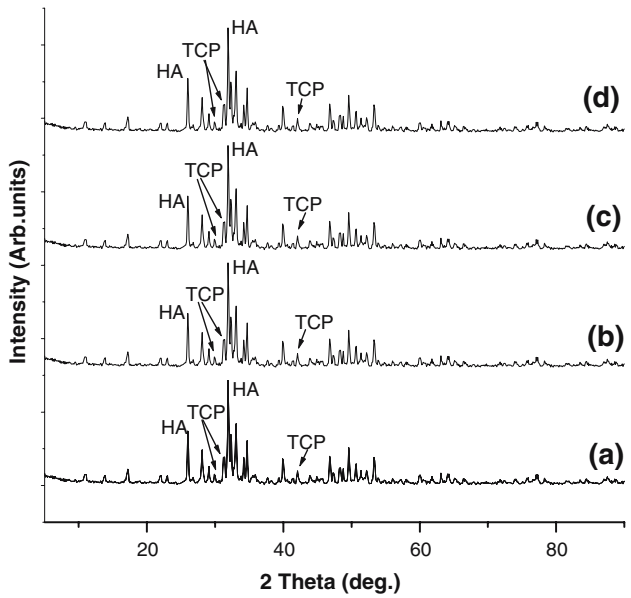

Fig. 5 XRD of BCP80 powder (**a**) 4BCP80 (**b**) 6BCP80 (**c**) and 8BCP80 (**d**) microspheres

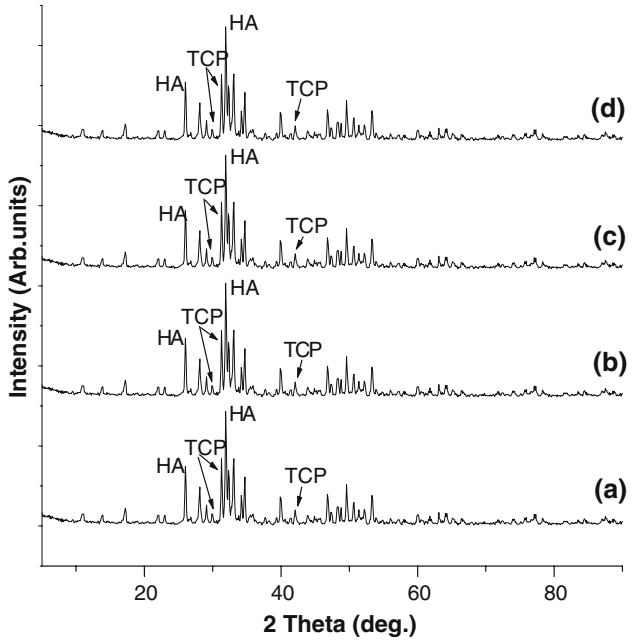

Fig. 6 XRD of BCP65 powder (**a**) 4BCP65 (**b**) 6BCP65 (**c**) and 8BCP65 (**d**) microspheres

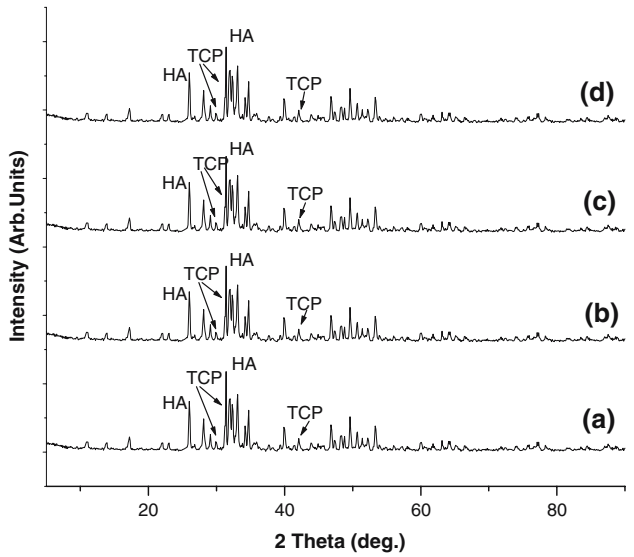

Fig .7 XRD of BCP40 powder (**a**) 4BCP40 (**b**) 6BCP40 (**c**) and 8BCP40 (**d**) microspheres

with a smoother surface. The uniform size of the particles leads to efficient loading. The observed release profile has been attributed to the surface bound drug [15]. Hence, more amount of drug could have been absorbed on the 6BCP65MS microsphere, which results in the maximum amount of drug released.

The behaviour of a particulate in the body depends on its morphology and porosity. The open pore content in the microsphere is dependent on the gelatin content in starting BCP/gelatin mixture. Although the 8BCP65MS microspheres were found to be porous (porosity = 0.384), only 60% of the drug was released, but at the 12th hour as that of 6BCP65MS. This indicated that the size of the pores (open pore size = 200–250 Å) may be larger so as to not retain the doxycycline. The 4BCP65MS microspheres were found to be larger but with less porosity (0.138) and small size pores (180–220 Å) which results in lesser amount of drug absorption and release at a shorter interval of time compared to other microspheres. The results thus suggest

Table 1 Surface area and drug release percentages of BCP microspheres

Gelatin Concentration (%)	BCP80MS		BCP65MS		BCP40MS	
	BET Area (m^2/gm)	% Drug release	BET Area (m^2/gm)	% Drug release	BET Area (m^2/gm)	% Drug release
4	26	22	54	65	41	36
6	35	26	87	83	49	46
8	29	24	75	68	43	38

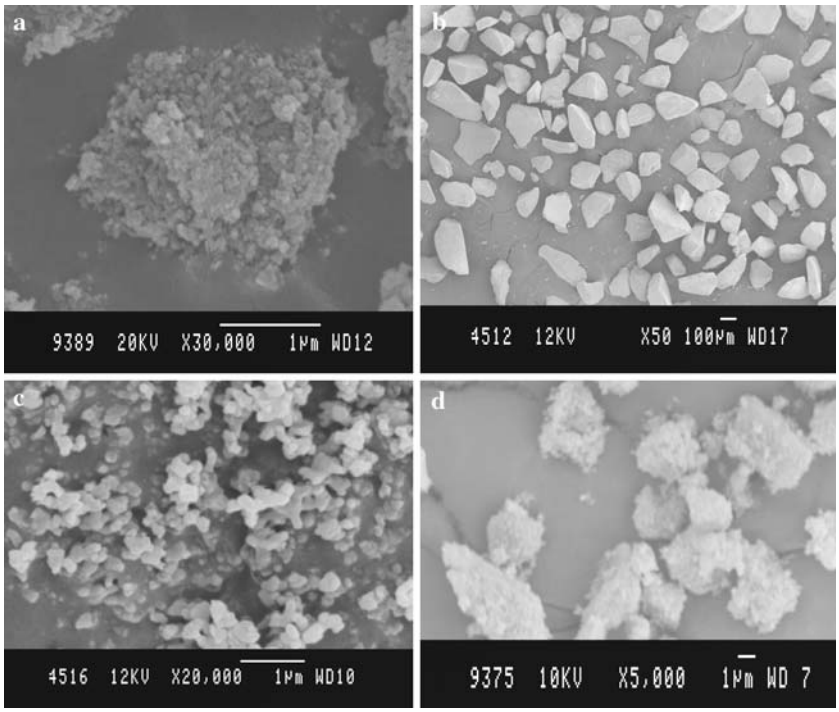

Fig. 8 SEM micrographs of BCP65 powder (**a**), 4BCP65MS (**b**), 6BCP65MS (**c**) and 8BCP65MS (**d**) microspheres

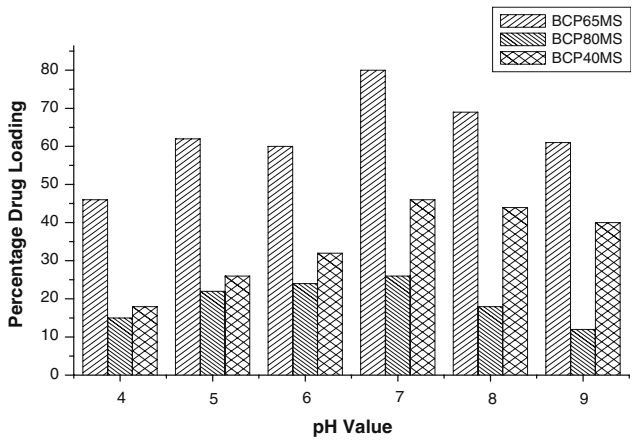

Fig. 9 Amount of drug loaded at various pHs of BCP80, BCP65 and BCP40 microspheres

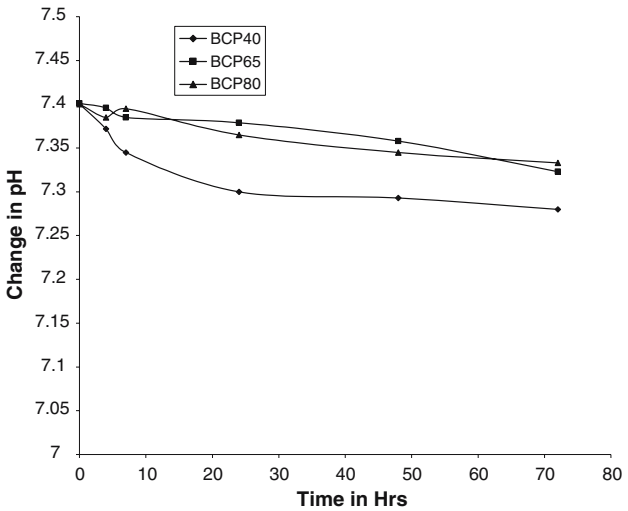

Fig. 10 pH variation of the buffer containing BCP microspheres as a function of time

optimum morphology, porosity (0.411), size and presence of micro pores (250–300 Å) in 6BCP65MS leading to the observed release profile.

The above results correlated with the surface area measurements as indicated in Table 1. The 6BCP65MS microspheres have the highest surface area, which leads to

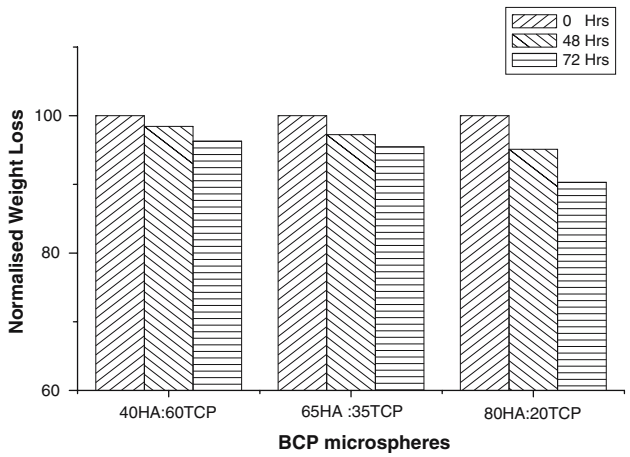

Fig. 11 Weight loss of BCP microspheres in buffer after 72 h

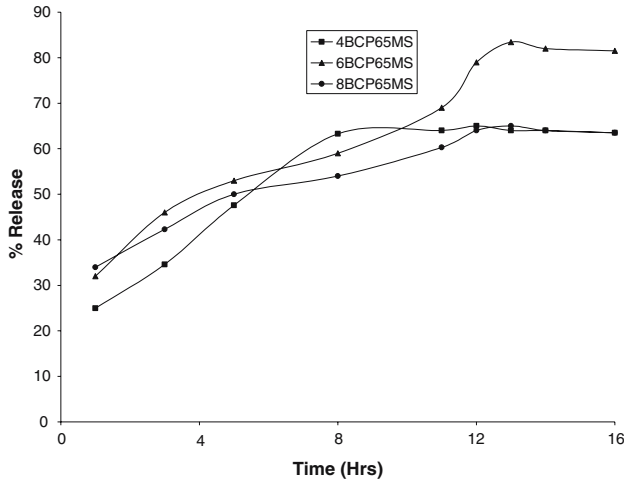

Fig. 12 In-vitro release profile of drug of BCP65 microspheres

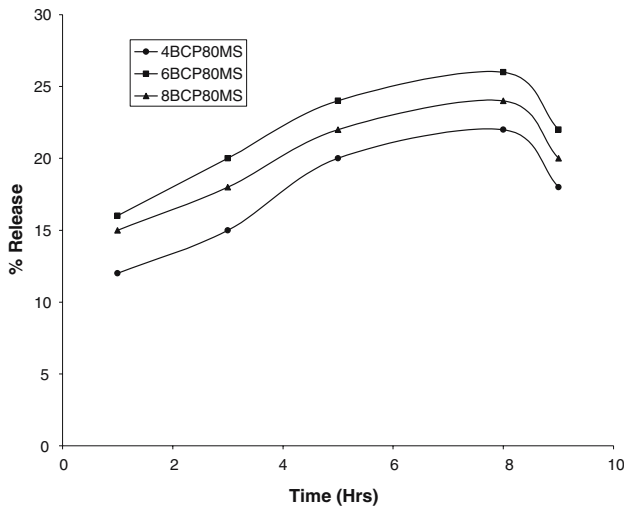

Fig.13 In-vitro release profile of drug of BCP80 microspheres

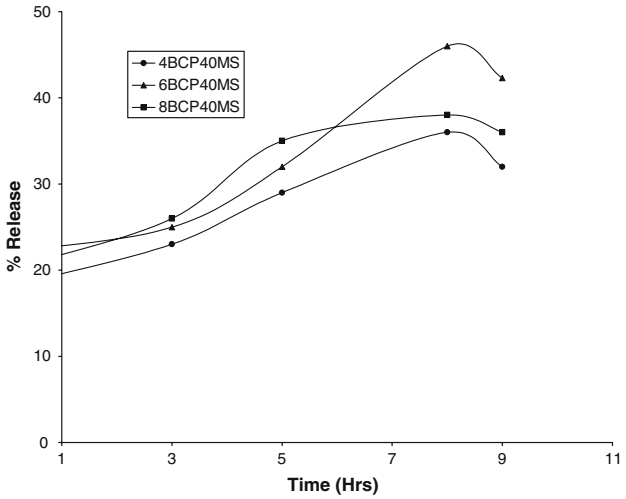

Fig. 14 In-vitro release profile of drug of BCP40 microspheres

more loading and ultimately better release characteristics. The release profiles usually reveal burst of antibiotics at short intervals followed by a longer period of continuous release [15]. The drug burst is due to the porosity present in the matrix or liberation of surface bound drug. The constant release of the drug however can be controlled by appropriate selection of the microsphere by optimising and fabrication methods. In many therapeutic delivery systems the rate of release should be relatively constant or of zero order dependence i.e. rate of release is independent of time [16].

The initial increase in drug release in BCP40MS is attributed to the presence of more amounts of TCP. An increase in the amount of TCP leads to more efficient dissolution and faster release. However, drug loading and release may not be sufficient in this case to achieve therapeutic results. The maximum drug release is much lower (26%) in BCP80MS and (46%) in BCP40MS than that obtained for the BCP65MS microspheres (83%). The surface area measurements as listed in Table 1 were also found to correlate with the drug release profiles obtained. The microspheres formed with 6% gelatin have shown larger surface area in both the BCP80 and BCP40 systems (Table 1). This explains the maximum amount of doxycycline release observed in both the cases. The results thus seem to indicate that the BCP65 system with HA:TCP = 65:35 ratio and microspheres formed with 6% gelatin seems to be more appropriate for ceramic based antibiotic delivery systems.

Conclusions

BCP microspheres with optimum pore content and morphology were prepared. Drug loading around pH 7 leads to higher amount of loading. Maximum drug release was

observed in the case of microspheres formed using 6% gelatin. The morphological features of the microsphere were found to correlate well with the in-vitro doxycycline release. The surface area measurements (maximum in the case of 6% gelatin) also correspond with the drug release profiles obtained. An optimum release of upto 80% has been shown by the BCP65 microspheres.

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