Examination of Aqueous Oxidized Cellulose Dispersions as a Potential Drug Carrier. II. In Vitro and In Vivo Evaluation of Phenylpropanolamine Release From Microparticles and Pellets

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ABSTRACT

The purpose of this research is to investigate the release of phenylpropanolamine from oxidized cellulose-phenylpropanolamine (OC-PPA) complexes prepared using aqueous OC dispersions (degree of neutralization, DN, 0-0.44) and phenylpropanolamine-hydrochloride (PPA.HCl) (concentration, 0.5 M or 1.4 M) in vitro and in vivo. The results showed a faster drug release from the OC-PPA complex made using the OC dispersion with a DN value of 0.22 than from those prepared using dispersions with DN values of 0.29 to 0.44. No significant difference existed between the release profiles of OC-PPA microparticles made using OC dispersions with DN values of 0.29 to 0.44. OC-PPA complexes that contained smaller size particles or higher drug levels, or that were processed by freeze drying released PPA faster. Compared with microparticles, the pellets of OC-PPA complexes released PPA more slowly initially. An increase in pH or ionic strength of the dissolution medium increased the release of PPA, which is attributable to increased polymer hydration and solubilization at higher pH and ionic strength conditions. The OC-PPA pellets implanted subcutaneously in rats released 100% of their PPA in 9 to 12 hours. A good correlation was found between the in vivo and in vitro release data. Tissue pathology results showed no significant inflammatory tissue reactions. In conclusion, the partially ionized aqueous OC dispersions have the potential to be used as an implantable biodegradable carrier for amine drugs.

KEYWORDS: oxidized cellulose, oxycellulose, aqueous oxidized cellulose dispersions, phenylpropanolamine hydrochloride, oxidized cellulose-phenylpropanolamine ionic complex

INTRODUCTION

Oxidized cellulose (OC; 6-carboxycellulose) is an important but relatively little used class of biodegradable polymers. It has been investigated as an immobilizing matrix for drugs, enzymes, and proteins. Various bioactive agents immobilized on OC gauze or viscose fabric include (1) antibiotics, such as sulfanilamide, kanamycin sulfate, lincomycin, and gentamycin[1-4]; (2) antiarrhythmic drugs, such as trimecaine and verapamil[5]; and (3) antitumor agents, such as photrin, spirobromine, and prospidine, and a mixture of methotrexate and hydroxythiamine.[6-8] These products showed either enhanced activities or sustained drug release. Studies show that OC also possesses antibacterial[9] and antitumor activities.[10]

Recently, anionic polysaccharides, such as sodium alginate, have been used as immobilizing matrices to produce sustained-release delivery systems.[11] Using phenylpropanolamine (PPA) as a model amine drug, it was found that partially neutralized aqueous OC dispersions are superior complexing agents compared with OC powder, resulting in both higher drug loading and drug loading efficiencies (see preceding article). In this article, we report the results of an in vitro and in vivo evaluation of the OC-PPA complexes. A good correlation was found between in vitro and in vivo release data. There was no inflammatory reaction following implantation of the complex in rats. The results suggest that the partially neutralized OC dispersions have the potential to be used as a biodegradable implantable sustained-release carrier for amine drugs.

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MATERIALS AND METHODS

Materials

Phenylpropanolamine hydrochloride (PPA.HCl), 1-heptanesulfonic acid sodium salt, and triethylamine were purchased from Fisher Scientific (Fair Lawn, NJ). The OC-PPA microparticles used in the study were prepared using partially neutralized aqueous OC dispersions and PPA.HCl, as described in the preceding article. The OC-PPA microparticles were dried in an oven at 40°C for 24 hours or by lyophilization at –60°C and 200 millitorr using a Freeze Mobile 6 drier (The Virtis Co Inc, Gardiner, NY). The dried product sufficient to make ~50 tablets for the release study was lightly ground using a mortar and pestle and sieved on a set of US standard mesh screens. Powder fractions with particles ranging in size from 45 to 106 μm and between 180 and 250 μm were collected and used in the study.

Bulk and Tap Densities

About 1 g of the sample was accurately weighed and put in a 10-mL graduated cylinder. The cylinder was lightly tapped to

ensure that no powder was sticking to the walls of the cylinder. The powder volume was recorded. The cylinder was then tapped on a hard surface from a distance of 1.5 inches, until a constant powder volume reading was obtained. The bulk and tap densities of the powder were calculated using the relationships: bulk density = sample mass/bulk volume, and tap density = sample mass/tapped volume.

Preparation of Pellets

The OC-PPA complexes dried in an oven and containing particles ranging in size from 180 to 250 μm were used in the study. Fifty pellets, each weighing 50 ± 0.5 mg, were prepared on a Carver Laboratory Press (model C, Fred S. Carver Inc, Menomonee Falls, WI) using a 1/8-inch die and standard concave punch set, a loading force of 600 pounds, and a dwell time of 10 seconds. After ejection from the die, the pellets were measured for thickness using a digital electronic caliper (Marathon Management Co Ltd, Richmond Hill, Ontario, Canada). The hardness of the pellets was measured on a Computest hardness tester (Vector Corp, Marion, Iowa).

High-Performance Liquid Chromatography Analysis of PPA

The analysis of PPA was performed by high-performance liquid chromatography (HPLC) according to the *United States Pharmacopeia (USP)* procedure,[12] with minor modifications as noted in the preceding article.

In Vitro Drug Release

In vitro drug release studies were performed in water, NaCl solution (ionic strength 0.15 or 0.25), or phosphate buffer solutions (PBS) (pH 6.0 and 7.4; ionic strength 0.15 or 0.25). Fifty milligrams of the OC-PPA sample (microparticles or a pellet prepared using them) and 1 mL of the dissolution medium were placed in a pleated dialysis tubing (SnakeSkin; molecular weight cut-off [MWCT] 3500; surface area 4.2-4.8 cm^2; Pierce Chemical Co, Rockford, IL). After securely clamping the ends, the dialysis bag was placed in a flask containing 50 mL of the dissolution medium. The flask was shaken at 20 cycles per minute (cpm) at 37°C in a controlled-environment incubator shaker. At predetermined time intervals, 1 mL of the dissolution medium was removed. This was immediately replaced with an equal volume of the fresh dissolution medium. The removed dissolution sample was appropriately diluted with 0.01 N HCl and analyzed by HPLC.

In Vivo Drug Release

Male Sprague-Dawley rats, each weighing 280 to 300 g, were used in the study. The rats were anesthetized with an intraperitoneal (IP) dose of ketamine (40 mg/kg) and xylazine (5 mg/kg). They were then placed on a heating pad, and their hair was removed from the back (~3 × 4 cm^2), near the neck, using an electric clipper. The site of the incision was scrubbed using a surgical Betadine scrub (povidone-iodine, Purdue Frederick Co, Norwalk, CT) and 70% ethyl alcohol. The rats were then covered with a sterile Poly-Lined Towel (Allegiance Healthcare Corp, McGaw Park, IL). An incision was made through the skin using a scalpel to allow the pellet to be placed subcutaneously. The incision was closed with a sterile absorbable Vicryl (Polyglactin 910) surgical suture (Ethicon Inc, Somerville, NJ). The rats were put back in separate cages and monitored periodically until they regained consciousness.

The rats were euthanized by inhalation of carbon dioxide at predetermined time points. The remaining pellet was removed from the implantation site and placed in a glass vial. The surrounding tissues were repeatedly washed with water. The washings were collected in the same vial that contained the removed pellet. The collected sample was suspended in 0.01 N HCl, transferred into a 5-mL volumetric flask, and then the volume was brought to mark with HCl. The sample solution was further diluted to an appropriate volume, if necessary, for HPLC analysis. Before injection, the sample solution was filtered through a syringe filter unit (nylon, 0.45 μm). The amount of PPA remaining in the implant was calculated using the calibration curve method. The percentage of PPA released in vivo was calculated by subtracting the remaining PPA in the retrieved pellet and washings from the initial drug loading in the pellet.

Tissue Preparation for Histological Examination

Four rats were used for examination of tissue reactions to OC. Two of them were used as surgical controls and the other 2 were used for pellet implantation. The surrounding tissue at the implantation site was fully excised and then placed in 10% neutral buffered formalin for fixing. A representative section of the fixed tissue was selected for morphological evaluation. The tissue was embedded in paraffin and 4- to 5-μm sections were cut from the blocked tissue, followed by staining with hemotoxylin-eosin (H&E). The slides were examined under an Olympus BH-2 microscope (Olympus, Melville, NY); representative areas were photographed using a Sony 3CCD color video camera and were printed on a Sony UP-5200MD color video printer (Sony Corporation, New York, NY).

Results and Discussion

In Vitro Drug Release From Microparticles

Effect of the Degree of Neutralization of Oxidized Cellulose Dispersions and Drug Loading on Release

Figure 1 shows the percentage PPA released as a function of time from various OC-PPA complexes prepared using OC

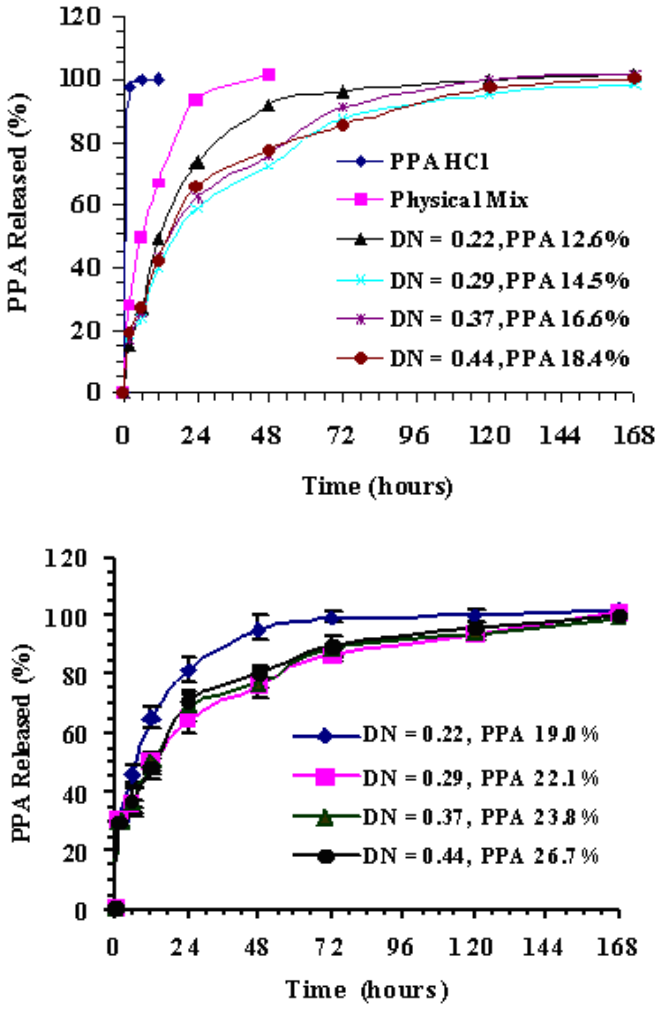

Figure 1. PPA release profiles in water at 37°C from the OC-PPA complexes prepared using aqueous OC dispersions (DN 0.22-0.44).

dispersions with DN values of 0.22 to 0.44. The PPA content in the products ranged from 12.6% to 26.7%. Also included with these plots are the dissolution profiles of free PPA and of a physical mixture of OC and PPA (composition: 83.4% and 16.6%, respectively). As is evident from Figure 1, both free drug and drug in the physical mixture dissolved rapidly, whereas the complexes showed a significantly slower PPA release. The OC-PPA complexes made from OC dispersions with DN values of 0.29 to 0.44 had very similar control release profiles ($P > .05$) (Table 1). The OC dispersion with a DN value of 0.22 released PPA faster.

At 3 hours of release, all OC-PPA particles appeared hydrated. However, only those made using the OC dispersion with a DN value between 0.29 and 0.44 and that contained the PPA content from 12.6% to 18.4% converted into a uniform gelatinous mass. The pH of each hydrated mass was measured and ranged from 2.5 to 2.9. The inability of the OC-PPA complex with a DN value of 0.22 to convert into a uniform gel was attributed to the lower pH environment of the hydrated mass, which also explains why PPA was released faster from the complex (Figure 1).

Table 1. Time to Release 50% PPA and the Amount of PPA Released at 6 Hours From Various OC-PPA Complexes*

OC-PPA Complex		PPA	
DN of OC	PPA Content (%)	Released at 6 Hours % (SD)§	$t_{0.5}$ (hours)
0.22	12.6†	26.4 (1.8)	12.0
0.22	19.0‡	45.8 (3.1)	7.5
0.29	14.5†	23.9 (1.4)	16.5
0.29	22.0‡	35.9 (1.2)	12.0
0.37	16.6†	25.8 (1.9)	16.0
0.37	23.8‡	36.3 (5.1)	12.1
0.44	18.4†	26.5 (1.3)	15.0
0.44	26.7‡	36.9 (4.2)	13.5

*PPA indicates phenylpropanolamine; OC, oxidized cellulose; DN, degree of neutralization; and HCL, hydrochloride.
†Prepared using 0.5 M PPA.HCl.
‡Prepared using 1.4 M PPA.HCl.
§Values shown are SD, n = 3.

Two levels of drug loading at each DN value of OC were used to study the effect of drug loading on drug release. Since all particles appeared hydrated within 6 hours, the initial drug release was compared at 6 hours. The results presented in Table 1 show that, for the products made using the same OC dispersion (ie, with the same DN value), the higher the drug loading, the greater the percentages of drug release at 6 hours. This finding indicated that the extent of initial drug release was faster when the drug loading was higher. This may be due to the larger amount of drug bound on the particle surface being dissolved more rapidly after hydration, which would be expected because PPA in the protonated form is highly soluble in water.

The results in Table 1 also show that the effect of the 2 different PPA loading levels on the percentage release of PPA at 6 hours tended to decrease as the DN levels increased. For example, at a DN of 0.22, the difference in percentage release at 6 hours for the products at a 12.6% PPA loading and at 19.0% PPA loading differed by 19.4% (26.4% vs 45.8%). For the complexes made using the OC dispersions with DN values of 0.29, 0.37, and 0.44, the percentage release differed by 12.0%, 10.5%, and 10.4%, respectively. From a comparison of the $t_{0.5}$ values of each pair of samples with the same DN value, the product containing a lower percentage of PPA loading had a higher $t_{0.5}$ value than the product having a higher drug loading.

Effect of Particle Size on Drug Release

Powder fractions ranging in size from 45 µm to 106 µm and from 180 µm to 250 µm were used in the study. The results presented in Figure 2 show a faster drug release profile from smaller size particles ($P < .05$) (Figure 2). This should be expected since hydration would occur more rapidly as particle size decreased and surface area increased.

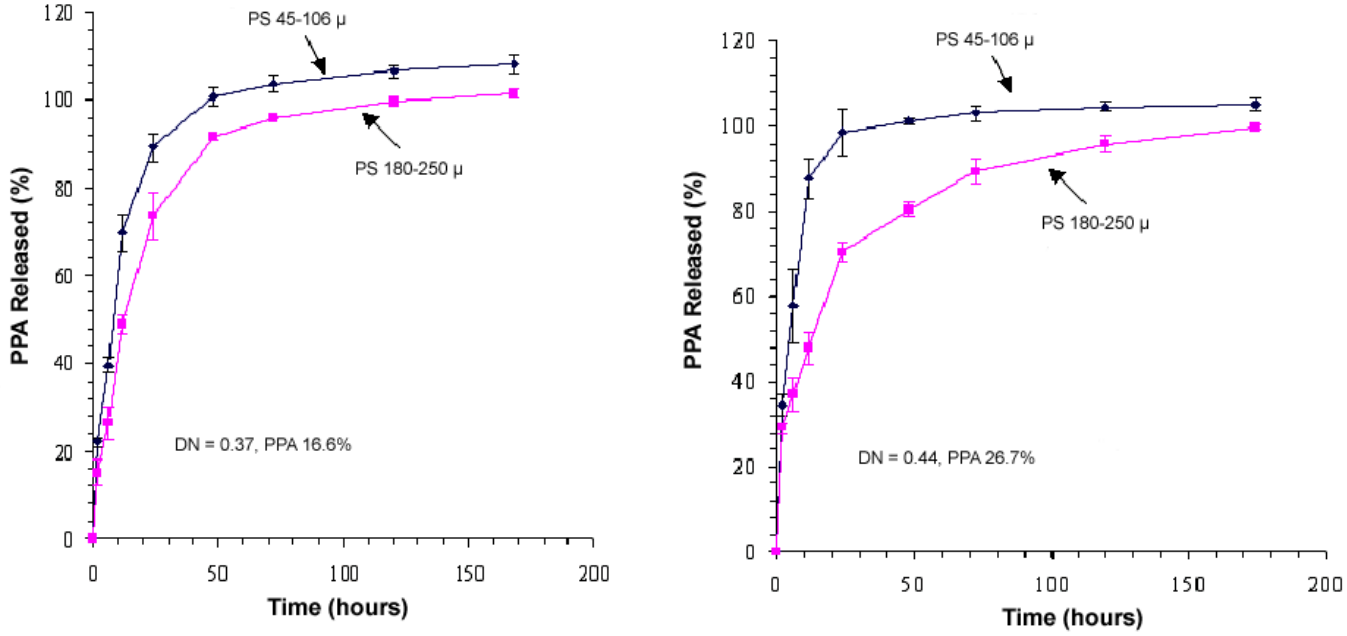

Figure 2. PPA release profiles from the OC-PPA complexes as a function of particle size (PS).

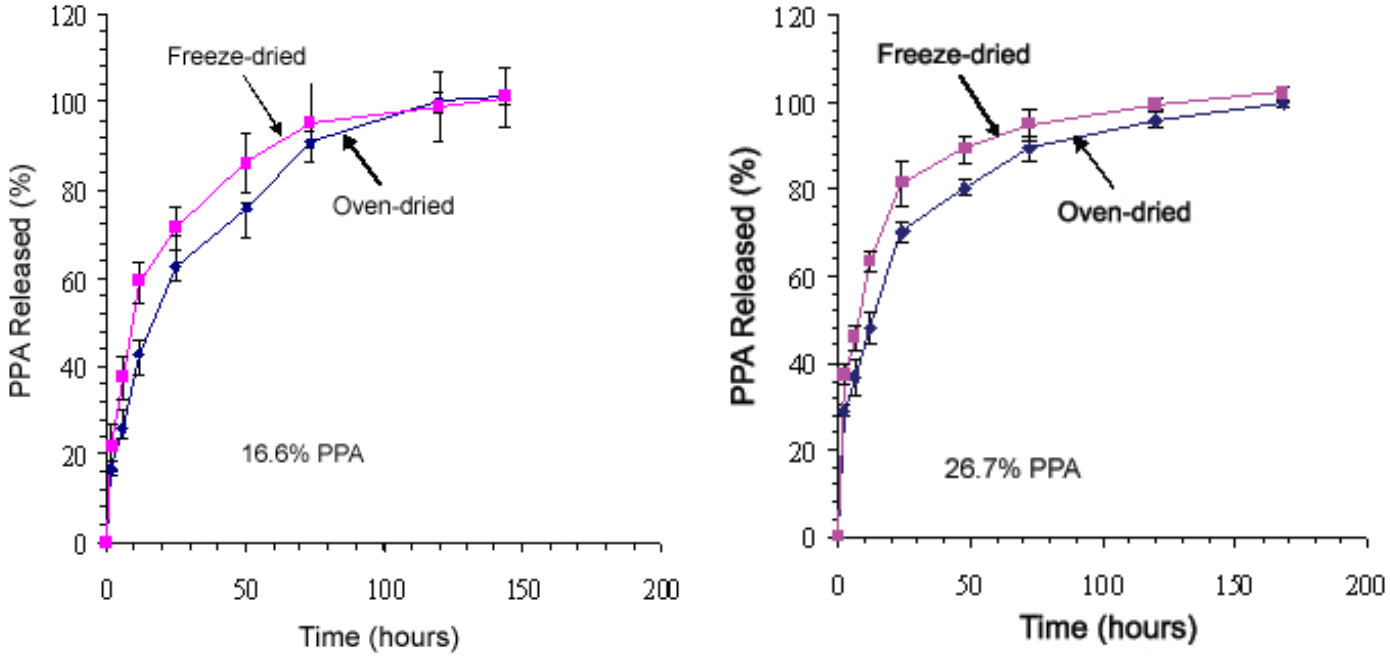

Figure 3. PPA release profiles from oven-dried and freeze-dried OCA-PPA complexes.

Effect of Drying Method on Drug Release

The effect of the drying method on the PPA release rate was compared using OC-PPA complexes (particle size 180-250 µm) containing 16.6% and 26.7% PPA. The drug release was faster from the products prepared by freeze drying than from the oven-dried products ($P < .05$) (Figure 3). The freeze-dried powders were less dense compared with those prepared by oven drying (Table 2), suggesting that more void space existed in the freeze-dried matrix, which facilitated matrix hydration and subsequent drug release.

Comparison of Drug Release From Microparticles and Pellets

The OC-PPA complex (PPA loading 16.6%, particle size 180-250 µm) prepared using the OC dispersion with a DN value of 0.37 was used in the study. Pellets, each weighing ~50 mg, with an average thickness of 5.2 mm and an average hardness value of 4.0 kp, were made according to the procedure described in the experimental section. The results are depicted in Figure 4. Except for the initial time points, the release of PPA was similar from both microparticles and pellets. The initial slower

Table 2. Effect of Drying Methods on Bulk and Tap Densities*

OC-PPA Complex		Drying	ρ_{bulk}	ρ_{Tap}
DN of OC	PPA Content (%)	Method	(g/mL)	(g/mL)
0.37	16.6†	Freeze	0.28	0.48
0.37	16.6†	Oven	0.59	0.74
0.44	26.7‡	Freeze	0.36	0.56
0.44	26.7‡	Oven	0.59	0.69

*OC-PPA indicates oxidized cellulose-phenylpropanolamine; DN, degree of neutralization; and HCL, hydrochloride.
†Prepared using the 1:1 (wt/vol) ratio of OC dispersion (DN = 0.37) and 0.5 M PPA•HCl.
‡Prepared using the 1:1 (wt/vol) ratio of OC dispersion (DN = 0.44) and 1.4 M PPA•HCl.

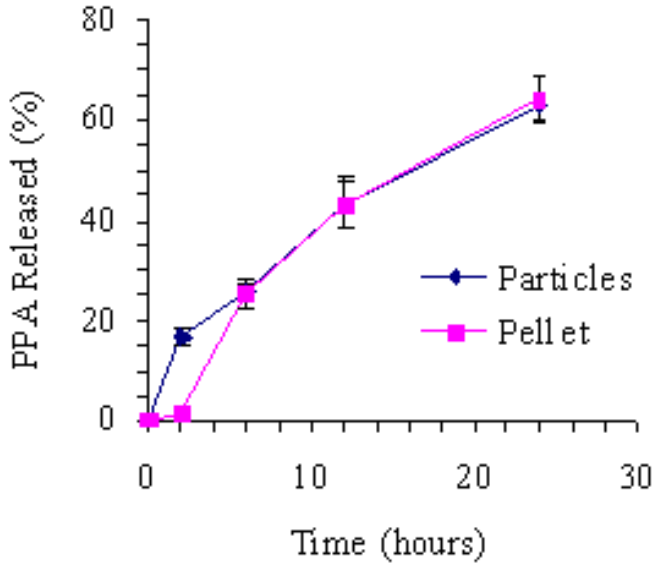

Figure 4. Comparison of PPA release profiles from OC-PPA microparticles and pellets containing 16.6% PPA.

release of PPA from the pellet compared with microparticles was primarily owing to the slower hydration of the pellet. Upon hydration, the pellet converted into a gelatinous mass, which probably served as a barrier and led to the slower PPA release.

Effect of Media on Drug Release From Pellets

The release studies were performed in pH 6.0 and 7.4 normal saline (ionic strength 0.15 or 0.25) and PBS (ionic strength 0.15 or 0.25). The results are shown in Figure 5. The release of PPA was substantially faster in solutions with an ionic strength of 0.25 than in solutions with an ionic strength of 0.15. The percentage drug release at 6 hours and the time for 50% drug release ($t_{0.5}$) at the 4 pH-ionic strength conditions are listed in Table 3. Since the sodium salt of OC is soluble in the dissolution medium, it is plausible that the exposure of OC to more highly concentrated salt solutions results in an increased interaction between sodium and carboxylate ions, which, in turn, causes the OC to more rapidly hydrolyze and solubilize. Thus, drug release would be expected to be faster

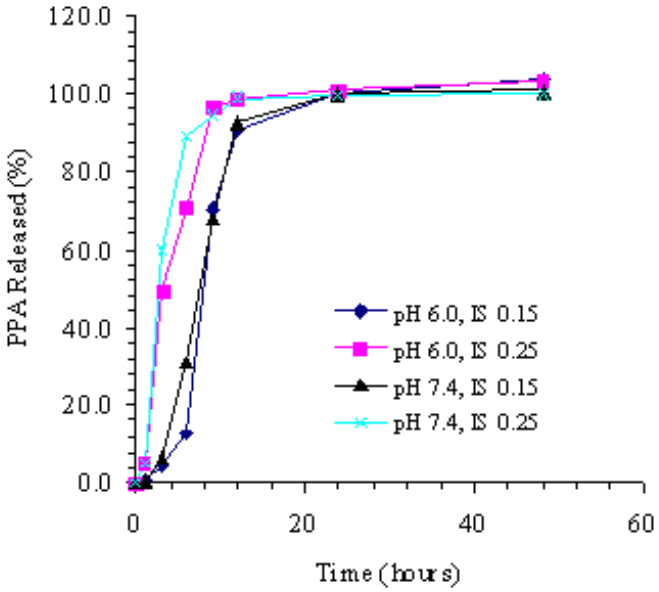

Figure 5. Release of PPA from OC-PPA pellets in pH 6.0 and pH 7.4 PBS at 2 ionic strengths (IS).

Table 3. PPA Release Data From OC-PPA Pellets Under Different Dissolution Conditions*

pH of the Solution	Ionic Strength	% PPA Released at 6 Hours (SD)†	$t_{0.5}$ (hours)
7.4	0.25	89.0 (4.5)	2.6
6.0	0.25	70.3 (7.9)	3.1
7.4	0.15	30.7 (8.4)	7.5
6.0	0.15	17.6 (1.8)	7.9

*PPA indicates phenylpropanolamine; OC, oxidized cellulose; DN, degree of neutralization; and HCL, hydrochloride.
†Values shown are SD, n = 3.

in media with a higher ionic strength. Similar results were also reported for the morphine release from the Eudragit-morphine complex[13] and for the o-pivaloylpropranolol release from a cation exchange polystyrene sulfonic acid resin.[14]

The mean effect of pH or ionic strength (IS) on the percentage PPA released at 6 hours as well as $t_{0.5}$ was analyzed by a 2-factor-2-level experimental analysis and the results are presented in Table 4. These results indicate that the effect of the pH of the dissolution media was less than the effect of ionic strength on percentage drug release at 6 hours. A higher pH value should favor the solubilization of the OC polymer (pK_a 3.6-4.0) and decrease the solubility of PPA (pK_a 9.4). The results show that the drug release increased with an increase in pH, indicating that the effect due to the solubilization of the OC polymer is larger than the effect due to the reduced solubility of PPA with increasing pH. These results suggest that the hydration of the OC matrix and its solubilization are the controlling factors for drug release from this system with PPA.

Table 4. Estimated Effects and Coefficients of PPA Released at 6 Hours*

Term	Effect	Coefficient
Constant	51.90	-
pH	15.90	7.95
IS	55.50	27.75
pH*IS	2.80	1.40

*PPA indicates phenylpropanolamine; and IS, ionic strength.

In Vivo Drug Release

Pellets of the OC-PPA complex (containing 16.6% PPA) made using the OC dispersion with a DN value of 0.37 were subcutaneously implanted in rats. After 3, 6, 9, or 12 hours of implantation, the incision was opened and the remaining pellet or gelatinous mass was removed. Representative photographs of pellets in their implantation site are shown in Figure 6. These photographs show the incision before implantation, and pellets at 3, 6, and 12 hours postimplantation. It can be seen that the pellet was hydrated at 3 hours, and its size was substantially larger than the original pellet. The pellet was fully hydrated and became a gelatinous mass at 6 hours, and at 12 hours the volume of the remaining gelatinous fluid was considerably reduced compared with at 6 hours.

The percentage drug released was calculated by subtracting the remaining PPA in the implantation site at the specified time point from the initial PPA loading in the pellet. The in vivo release profile shown in Figure 7 indicates that the drug was completely released within 9 to 12 hours. A good correlation was observed between the in vivo results and the in vitro release data determined in pH 7.4 PBS (ionic strength 0.25). This correlation implies that the in vitro condition used in this study can be applied to estimate the in vivo drug release for a very soluble drug from an OC-based subcutaneous implant. As observed by the in vivo testing, hydration also appeared to be the controlling factor for drug release in rat tissue from the pellets.

Tissue Reaction After Implantation

Representative photomicrographs of the tissue samples from a control rat after a surgical incision and from a rat bearing a pellet implantation for 12 hours are compared in Figures 8 and 9, respectively. In both the control and pellet-implanted rats, there was some tissue reaction. No rats showed any sign of an acute inflammatory reaction. A lymphocytic reaction, however, was noted in the surrounding tissue. Based on a comparison of the density of lymphocytes present in the surrounding tissue, pellet implantation did not appear to cause a significant inflammatory response compared with the surgery control. These histological results clearly suggest that OC may be biocompatible as a subcutaneous implant.

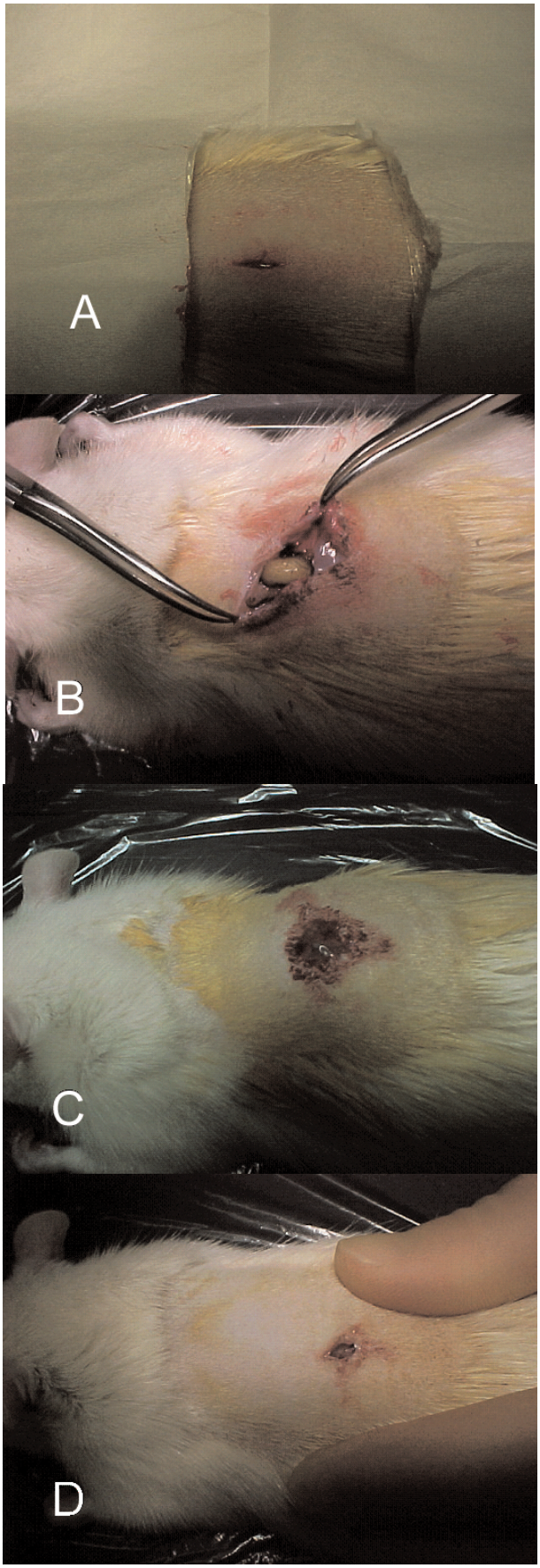

Figure 6. Photographs showing surgical incision (A) and the remaining pellets at 3 hours (B), 6 hours (C), and 12 hours (D) postimplantation in rats.

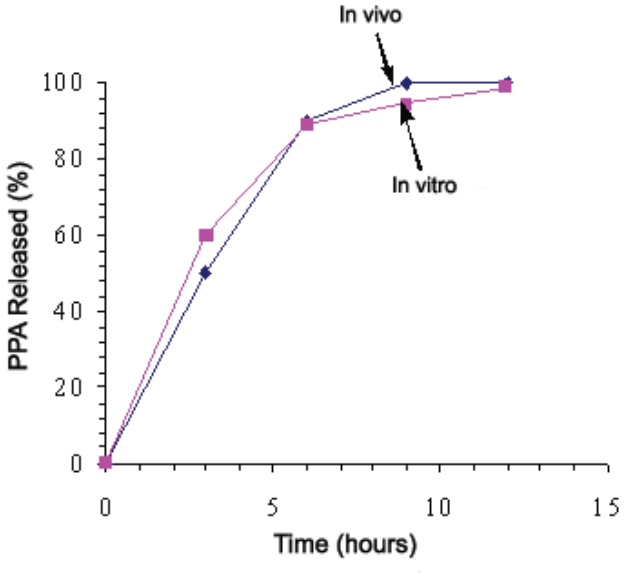

Figure 7. Correlation between in vitro and in vivo drug release data obtained from OC-PPA pellets containing 16.6% PPA.

Conclusion

The OC-PPA complexes prepared from partially neutralized OC dispersions and PPA.HCl release drug slowly. A higher drug loading, smaller particle size, and drying of the complex by freeze drying, all caused the drug to be released faster. An increase in pH or ionic strength of the dissolution medium also led to faster release of drug from the complex. This occurs because of increased polymer hydration and solubilization at higher pH and ionic strength conditions. These results show that drug complexes of OC, prepared using the partially neutralized dispersion, may have potential for implantable drug delivery.

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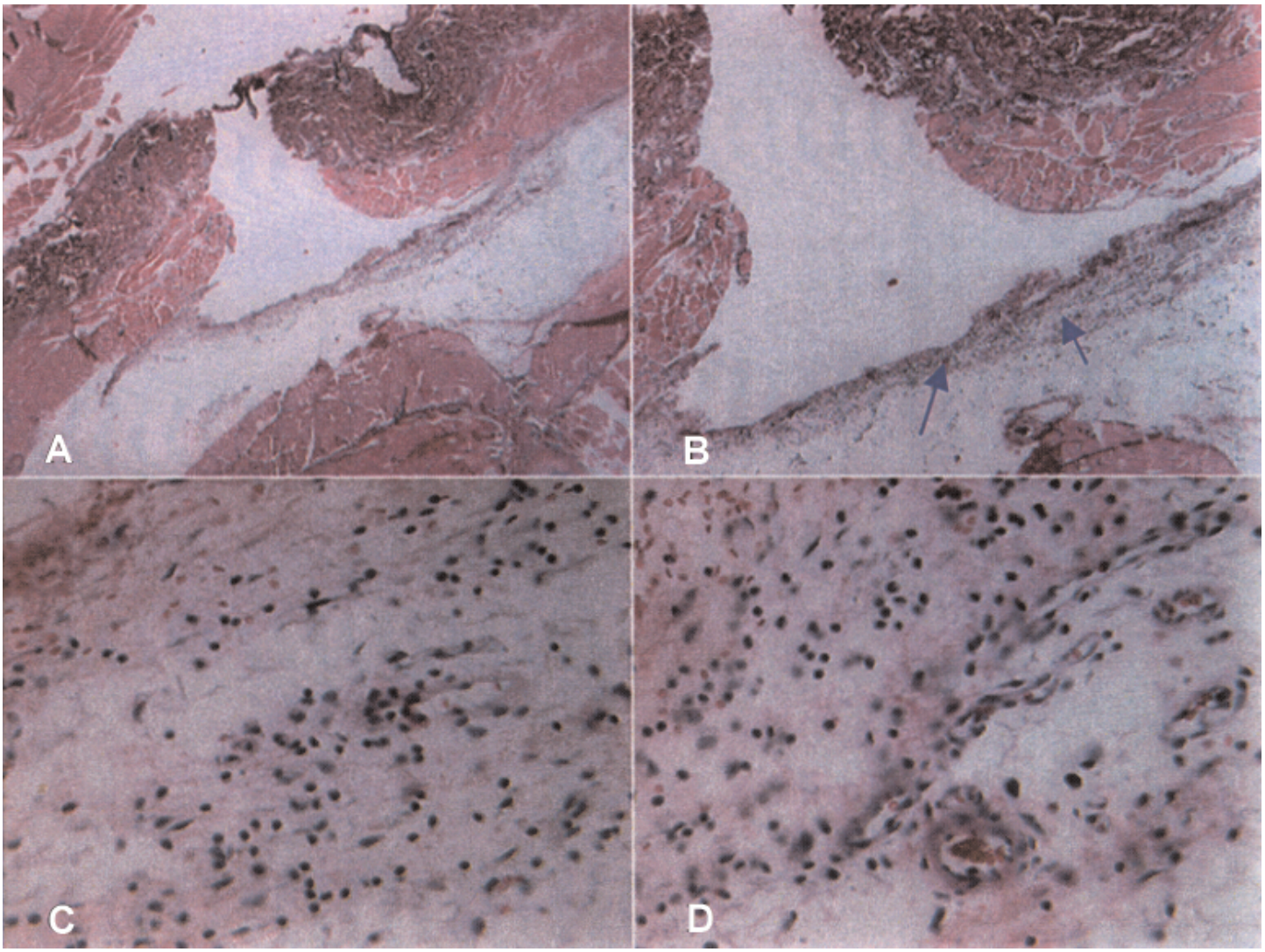

Figure 8. Photographs of tissue reaction for surgical control rat. Photographs (A) and (B) show the biopsy cavity, and photographs (C) and (D) show a mild inflammatory cell infiltrate composed primarily of lymphocytes.

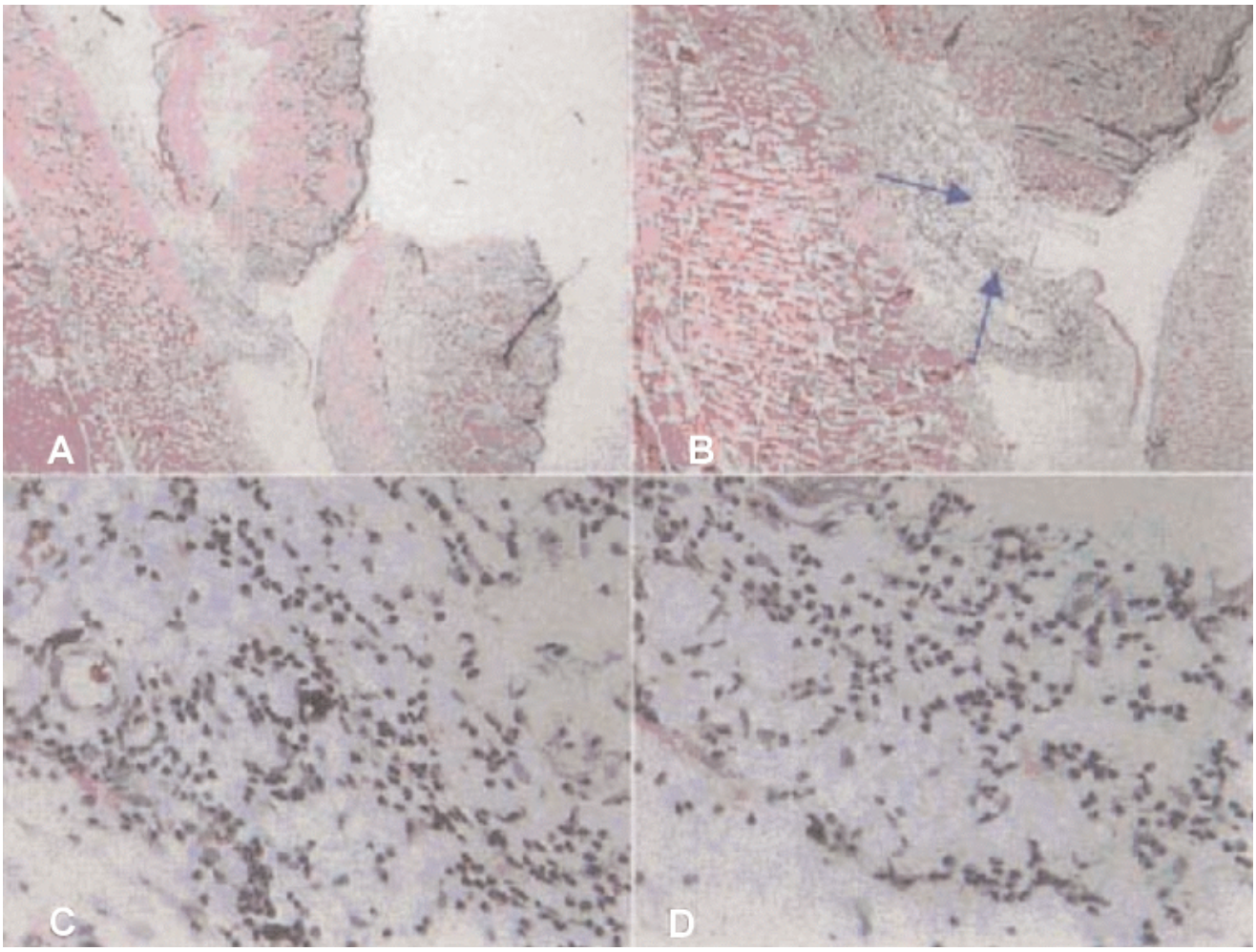

Figure 9. Photographs of tissue reaction after pellet implantation for 12 hours in the rat. Photographs (A) and (B) show the implantation cavity, and photographs (C) and (D) show a mild inflammatory cell infiltrate composed primarily of lymphocytes.

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