Examination of Aqueous Oxidized Cellulose Dispersions as a Potential Drug Carrier. I. Preparation and Characterization of Oxidized Cellulose-Phenylpropanolamine Complexes

Submitted: February 4, 2004; Accepted: October 8, 2004.

Lihua Zhu,[1] Vijay Kumar,[2] and Gilbert S. Banker[2]

[1]Current address: Hospira Inc., Pharmaceutical R&D, 275 North Field Drive AP4/D438, Lake Forest, IL 60045

[2]Division of Pharmaceutics, College of Pharmacy, The University of Iowa, Iowa City, IA 52242

ABSTRACT

Partially neutralized aqueous dispersions of oxidized cellulose (OC) (COOH content 24.2%; degree of neutralization [DN] 0.22-0.44; solid content 14.4% wt/wt), a biocompatible biodegradable polymer, were prepared and their use to entrap an amine drug was demonstrated. Phenylpropanolamine hydrochloride (PPA.HCl) was used as a model drug. OCA-PPA complexes were prepared by adding the drug solution to the OC dispersion. Light microscopy, powder x-ray diffractometry (PXRD), and Fourier-transform infrared (FT-IR) spectroscopy were used to characterize hydrated and dried OC and the OC-PPA complexes. Drug loading and drug-loading efficiency were calculated from high-performance liquid chromatography. Light microscopy revealed the partially neutralized OC to exist as swollen fibers in the dispersion. The degree of swelling increased with increasing DN of the OC. All dispersions, irrespective of DN, showed a pseudo-plastic flow. The drug loading (12.6%-26.7%) and drug-loading efficiency (30%-48%) increased linearly with increasing DN and drug concentration. The PXRD of the OC-PPA complexes showed no diffraction peaks due to PPA, suggesting that the drug exists in the amorphous state. The FT-IR spectra of the complexes revealed the presence of an ionic linkage between OC and PPA. In conclusion, the results show that the aqueous OC dispersions can be used to molecularly entrap amine drugs to produce an OC-drug complex linked via an ionic linkage.

KEYWORDS: oxidized cellulose, oxycellulose, oxidized cellulose dispersions, molecular scale drug entrapment, phenylpropanolamine hydrochloride, oxidized cellulose-phenylpropanolamine ionic complex

INTRODUCTION

Numerous synthetic and natural biodegradable polymers have been investigated as drug carriers in parenteral drug delivery.[1] Drugs can be incorporated in polymers by methods such as coacervation/phase separation, interfacial polymerization, organic phase separation, and physical mixing. Recently, ionic interaction between anionic polymers and amine drugs has also been used to enhance drug activities or produce sustained-release drug delivery systems.[2,3] In general, the selection of the incorporation method is often based on the physicochemical properties of the drug and the polymer as well as the drug delivery goals. Oxidized cellulose (OC, 6-carboxycellulose) containing 3% to 25% carboxyl content is a biocompatible and bioabsorbable polymer.[4] It is currently used in humans as a hemostatic agent and as a postsurgical adhesion barrier.[5] Studies also show that it possesses antibacterial,[6] antitumor,[7] and immunostimulant[8] properties. Owing to the presence of free carboxylic acid groups, OC is also useful as an immobilizing matrix for drugs, enzymes, and proteins.[9-14] The first application of OC, used in the form of a gauze or viscose fabric, as an immobilizing agent for antibiotics was reported by Dol'berg et al[9] and Yasnitskii and Dol'berg.[10] Recently, several chemotherapeutic agents, such as photrin,[11] spirobromine, and prospidine,[12] and a mixture of methotrexate and hydroxythiamine,[13] have been immobilized on OC. The immobilized methotrexate and hydroxythiamine demonstrated higher antitumor efficacy compared with a mixture of these drugs or each of the agents applied individually.

In this study, the preparation of the partially neutralized OC dispersions and their use to entrap amine drugs were investigated. Phenylpropanolamine hydrochloride (PPA.HCl) was used as a model drug. The goal was to develop a precise and reproducible method for the entrapment of an amine drug. The succeeding article reports the in vitro and in vivo drug release characteristics of the OC-PPA complexes.

MATERIALS AND METHODS

Materials

PPA.HCl was purchased from Aldrich Chemical Co (Milwaukee, WI). Ethylenediamine (anhydrous), 1-heptanesulfonic acid sodium salt and triethylamine were obtained from Fisher Scientific (Fair Lawn, NJ). Hydrochloric acid (1.0 N) and sodium phosphate monobasic were bought from Spectrum Quality Product, Inc (Gardena, CA). OC powder containing 24.2% (wt/wt) carboxyl content (molecular weight [MW] 6658) was prepared from cotton linters (grade 270; Southern

Corresponding Author: Vijay Kumar, Division of Pharmaceutics, College of Pharmacy, The University of Iowa, Iowa City, IA 52242. Tel: (319) 335-8836. Fax: (319) 335-9349. E-mail: vijay-kumar@uiowa.edu.

Cellulose Inc, Chattanooga, TN) by treatment with a mixture of phosphoric acid–nitric acid–sodium nitrite at room temperature for 48 hours, according to the literature procedure.[15]

Determination of Ionization Characteristics

The ionization constant of OC was determined using a potentiometric method reported by Davis et al.[16] Briefly, 200 mg of OC was suspended in 50 mL of deionized water and dissolved by drop-wise addition of 0.1 N NaOH. Water was then added to bring the total volume to 100 mL. Titrations were then performed at 25°C using 0.1 N HCl as the titrant until a pH of 3.3 was reached. The solution was constantly stirred during titration. The pH of the solution was measured after each addition (0.1 mL) of titrant. The degree of ionization (DI), α, was calculated as follows: $\alpha = 1 - [H - (B - P)]/P$, where H is the total number of milliequivalents of acid used as titrant, B is the number of milliequivalents of base used to dissolve the polymer prior to titration, and P is the number of milliequivalents of carboxylic acid groups in the OC sample used in the titration. The carboxylic acid content in the sample (P) was determined by the *United States Pharmacopeia* (*USP*) method.[17] A plot of pH versus $\log[(1 - \alpha)/\alpha]$ was constructed from the titration data. When $\alpha = 1$, pH is equal to pK_a.

Preparation of Oxidized Cellulose Dispersions

To an accurately weighed amount of the OC powder (~7 g), appropriate volumes of water and 0.5 N NaOH solution, determined based on the degree of neutralization (DN) and the OC content desired in the dispersion, were added. The mixture was then homogenized to produce a uniform dispersion. The DN was the ratio of ionized carboxyl groups to the total carboxyl content per gram of the OC sample. The biggest OC dispersion batch size prepared was 50 g.

Rheologic Properties of Oxidized Cellulose Dispersions

The rheologic properties of the OC dispersions were measured using a controlled stress rheometer (Haake RS 100, Haake-Mess-Technik GmbH, Karlsruhe, Germany) with a 35/4° cone-plate system. The measurements were conducted in a controlled-shear rate mode, and the resulting shear stress was measured. The data of each rheogram were analyzed by the Martin equation ($\eta'\gamma = \tau^N$), where γ is the rate of shear and τ is the shearing stress.[18] The viscosity coefficients, η' and N, were obtained from the power regression analysis.

Powder X-Ray Diffraction Studies

The powder x-ray diffraction (PXRD) patterns of samples were obtained between 10° 2θ and 40° 2θ using a Siemens D5000 x-ray diffractometer (Siemens Energy and Automation Inc, Madison, WI), which irradiated the samples with monochromatic CuK_α x-rays (40 kV, 30 mA, α_1 = 1.54060 Å, α_2 = 1.54438 Å). The step width was kept at 0.02° 2θ, and the time constant was maintained at 0.4 seconds per step for all the samples. The collected data were processed by the Diffrac^{plus} x-ray diffraction software (Eva, Version 2.0, Siemens).

Morphological Studies

A Nikon Bio-Rad MRC1024 laser scanning confocal/optical microscope (Hercules, CA) was used to study the morphology of the hydrated OC and OC-PPA particles. Images were saved at a resolution of 512 × 512 pixels/in and processed in Adobe Photoshop 5.0 (Adobe Systems Incorporated, San Jose, CA).

Preparation of Oxidized Cellulose-Phenylpropanolamine Complexes

A solution of PPA.HCl in water (concentration, 0.5 or 1.4 M) and OC dispersion (DN 0-0.44; OC content 1-7 g) were mixed in a 1:1 (vol/wt) ratio in a centrifuge tube. The mixture was shaken by hand for 2 minutes and then was allowed to settle. After 10 to 30 minutes, the separation of supernatant and precipitate began to take place, depending on the DN of the OC dispersion. After 2 hours, the mixture was centrifuged in an ICE HN-S centrifuge (Damon Corp, Needham Heights, MA) at 7500 rpm for 15 minutes. The supernatant was removed. The solid that remained was washed with water (using twice the volume of the PPA solution used) and then oven dried at 40°C for 24 hours. The resulting solid was ground using a mortar and pestle and separated on a set of US standard mesh screens (no. 60, 80, 140, and 325 meshes). Powder fractions with a particle size range from 45 μm to 106 μm and from 180 μm to 250 μm were collected and used in the study.

Analysis of Phenylpropanolamine

The analysis of PPA was performed by high-performance liquid chromatography (HPLC) according to the *USP* procedure[19] except that sodium-1-heptanesulfonate, an ion-pair agent, was added to the mobile phase. The chromatograph used was a Shimadzu HPLC system equipped with a pump (model LC-6A), a system controller (model SCL-6A), an auto injector (model SIL-6A), an ultraviolet-visible (UV-Vis) spectrophotometric detector (model SPD-6AV), and a data processor/recorder (model C-R5A) (Shimadzu Corp, Kyoto, Japan). The separation was performed on a Zorbax Rx-C8 reversed-phase analytical column (5 μ; 4.6 mm × 250 mm, Mac-Mod Analytical Inc, Chadds Ford, PA) with an Alltech Hypersil BDS C8 guard column (5 μ, lot no. 5/120/4682,

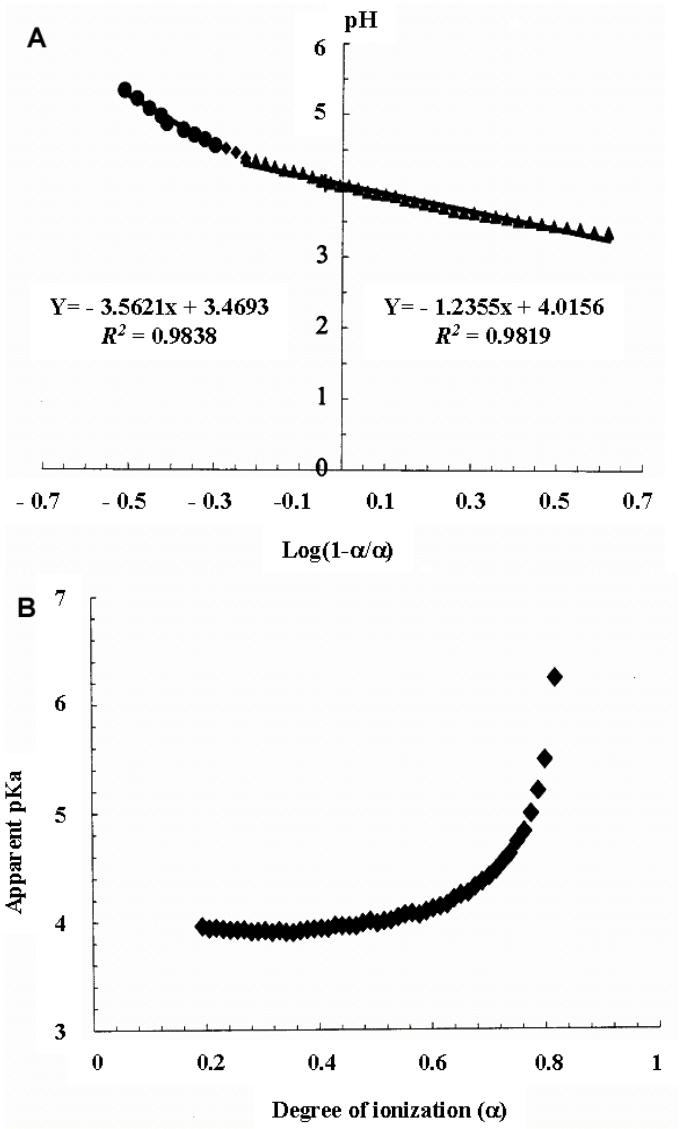

Figure 1. (A) Plot of pH vs log[(1 - α)/α] and (B) relationship between pK_a and degree of ionization (α).

Alltech Associate Inc, Deerfield, IL). The mobile phase consisted of a 6.5:10 (vol/vol) ratio of methanol:aqueous solution containing 0.202% (wt/vol) of sodium 1-heptanesulfonate, 0.278% (wt/vol) of sodium phosphate monobasic, and 0.01 M of triethyleneamine phosphate. The flow rate was set at 1 mL/min, and the effluent was detected at 254 nm.

Determination of Drug Loading

About 20 mg of the OC-PPA sample was accurately weighed and dispersed in 100 mL of 0.01 N HCl in a volumetric flask. The flask was shaken on a Wrist Action shaker (Burrell Corp, Pittsburgh, PA) for 0.5, 1, or 2 hours. The solution in the flask was brought up to 200 mL with water. The mixture was thoroughly mixed, filtered through a Gelman syringe filter unit (nylon acrodisc 0.45 μm), Cole-Parmer Instrument Company, Vernon Hills, IL, and then analyzed by HPLC. All samples were analyzed in triplicate. The optimal extraction time was determined to be 1 hour, based on the data obtained. The drug loading was calculated using the following Equations (1-3)

$$\% \text{ PPA Loading} = \frac{\text{Amount of PPA Extracted}}{\text{Weight of OC - PPA Sample}} \times 100 \quad (1)$$

or

$$\text{Millimoles of PPA/g of OC} = \frac{100 \times \text{Amount of PPA(g)}/151.2}{\text{Weight of OC - PPA Sample(g) - Amount of PPA(g)}} \quad (2)$$

The drug loading efficiency was calculated as follows:

$$\text{PPA Loading Efficiency} = \frac{\% \text{ PPA Loaded}}{\% \text{ Initial PPA Concentration in the Reaction Mixture}} \times 100 \quad (3)$$

Results and Discussion

Ionization Characteristics of Oxidized Cellulose

OC used in this study can be considered as a polyacid because over 90% of the monomer units were glucuronic acid. Katchalsky and Spitnik[20] developed a generalized Henderson-Hasselbach equation to describe the potentiometric titration curve for polyacids, which is $pH = pK_a - n'\log[(1 - \alpha)/\alpha]$, where pK_a and n' are empirical constants and α is the degree of ionization of the polyacid. For a dilute solution in the range of α from 0.2 to 0.8, this relationship is linear. To determine n' and the pK_a for OC, a plot of pH versus $\log[(1 - \alpha)/\alpha]$ in the α range of 0.21-0.76 was constructed. Figure 1A shows that 2 linear lines can be drawn from the data. The 2 values for n' and pK_a each calculated were $n_1' = 3.6$; $pK_{a1} = 3.5$ and $n_2' = 1.2$; $pK_{a2} = 4.0$. As shown in Figure 1B, the apparent pK_a gradually increased with increasing α from 0.2 to 0.7. A similar finding was reported for OC prepared using NO_2.[21] However, when α was raised to 0.75, a sharp rise in the pK_a of OC occurred. This result was attributed to an increased negative charge density on the polymer, which adversely affects the ionization of the carboxylic acid groups, and consequently, causes an increase in pK_a. Similar titration curves have been reported for polyuronic acids, polygalactouronic acid, and others.[22] Kotz et al[23] reported a pK_a value of ~2.8 for OC determined in 1.0 M NaCl solution. The lower pK_a value, compared with the value determined in the present study, is due to the higher ionic strength of the solution.

Preparation and Characterization of Oxidized Cellulose Dispersions

Aqueous OC dispersions with a DN value ranging from 0.22 to 0.44 and a solid content of 14% (wt/wt) were prepared. Below a DN of 0.2, OC failed to produce a physically stable

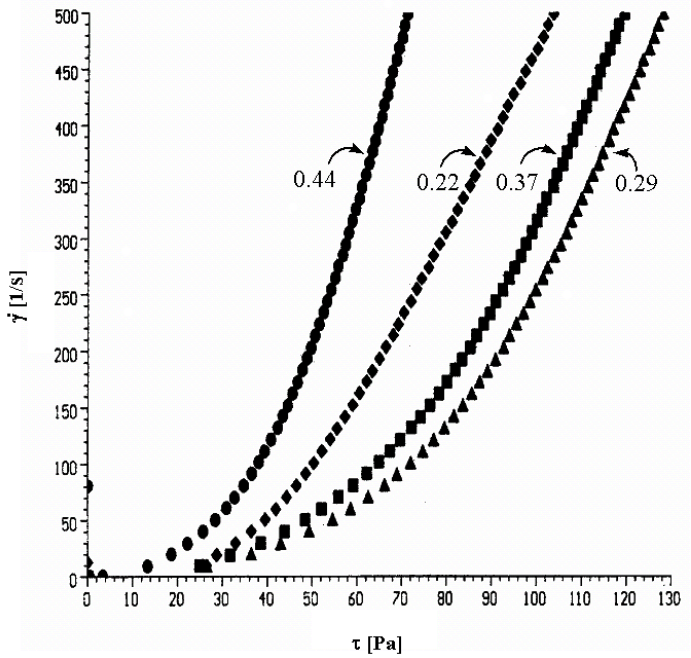

Figure 2. Rheograms of OC dispersions with different DN values at 25°C.

dispersion, while at a DN value of 0.5 or higher the dispersion resembled a colloidal sol. Above 14% wt/wt solid content, OC failed to produce uniform dispersions at the various DN values.

OC retains its fibrous structure in water (DN = 0) and swells when partially ionized (DN = 0.22-0.37). The degree of swelling of OC fibers appears to increase with increasing DN. This finding is attributed to an increased negative charge density on the polymer with increasing DN, which causes the OC chains to repel each other. This facilitates the accessibility of the hydroxyl and carboxyl groups to interact with water molecules, which, in turn, causes the OC chains to assume a swollen fibrous structure.

The rheograms of the OC dispersions (DN 0.22-0.44) are presented in Figure 2. The decrease in viscosity with increasing shearing rate suggests that these dispersions are pseudoplastic fluids. The values of η' and N obtained by fitting the data using the Martin equation[18] are listed in Table 1. The viscosity of the dispersion increased with increasing DN from 0.22 to 0.37. At a DN value of 0.44, however, the viscosity of the OC dispersion decreased. This decrease in the viscosity could be due to the conformational change of the OC chains. Cantoni et al[24] reported that OC exists in a fully extended ordered conformation under low ionic strength conditions, but at higher ionic strengths it assumes a compact, disordered conformation, leading to a drop in the intrinsic viscosity.

Table 1. Rheological Properties of Oxidized Cellulose Dispersions*

DN of OC	Viscosity Coefficient (η')	N
0.22	290	2.60
0.29	372	2.50
0.37	599	2.59
0.44	87	2.21

*DN indicates degree of neutralization; OC, oxidized cellulose.

Effect of the Degree of Neutralization of Oxidized Cellulose Dispersion on Drug Loading and Yield

Table 2 presents the drug loading, drug-loading efficiency, and yield of the OC-PPA complexes made using OC dispersions with DN values 0.0-0.44 and PPA.HCl solution in water (concentration 0.5 M or 1.4 M). As depicted in Figure 3, both drug loading and drug-loading efficiency linearly increased with increasing DN at both drug concentrations. The mol ratios of PPA to carboxyl groups in the products with 12.6% and 26.7% (wt/wt) PPA loadings were 1:5.6 and 1:2.2, respectively. Dol'berg et al[14] reacted OC powder containing 16.2% carboxyl groups with a 50-fold excess of aqueous kanamycin sulfate solution, corresponding to the mol ratio of 1.1-3.3:1 of kanamycin sulfate and carboxylic groups in OC, and found a maximum drug loading of 15% wt/wt or 0.3 mmol/g of OC. In the present study, the mol ratio of PPA to OC carboxyl groups corresponded to 0.6:1 and 1.8:1, and the resulting drug loadings were 12.6%-18.4%

Table 2. Drug Loading, Drug-Loading Efficiency, and Yields of Oxidized Cellulose-Phenylpropanolamine Complexes*

	0.5M PPA.HCl			1.4M PPA.HCl		
DN of OC	Drug-Loading Efficiency(SD‡)	Drug Loading (% wt/wt, SD‡)	Yield† (% wt/wt, SD‡)	Drug-Loading Efficiency (SD‡)	Drug-Loading (% wt/wt, SD‡)	Yield (% wt/wt, SD‡)
0	22.1	7.9	-	-	-	-
0.22	31.3 (1.0)	12.6 (0.4)	89 (2)	29.3 (0.6)	19.0 (0.4)	96 (1)
0.29	36.0 (1.5)	14.5 (0.6)	84 (1)	34.0 (0.6)	22.1 (0.4)	93 (1)
0.37	41.2 (2.0)	16.6 (0.8)	76 (3)	36.6 (1.2)	23.8 (0.8)	89 (4)
0.44	45.6 (1.2)	18.4 (0.5)	66 (2)	41.1 (0.6)	26.7 (0.4)	76 (2)

*PPA.HCL indicates phenylpropanolamine hydrochloride; DN, degree of neutralization; and OC, oxidized cellulose.

†Yield = (weight of product/weight of OC powder) × 100

‡ n = 3.

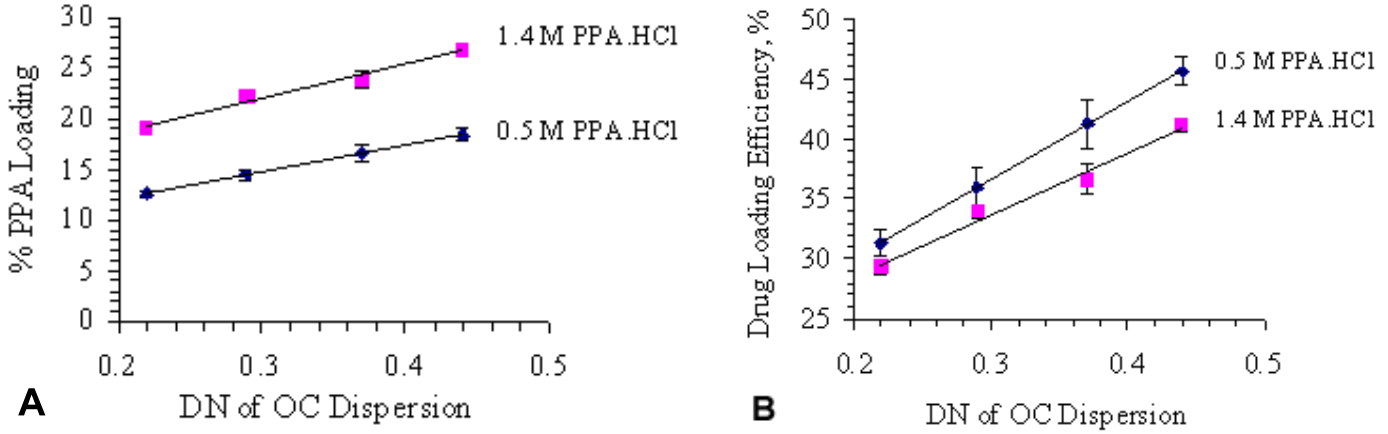

Figure 3. Effect of DN on (A) drug loading and (B) drug-loading efficiency.

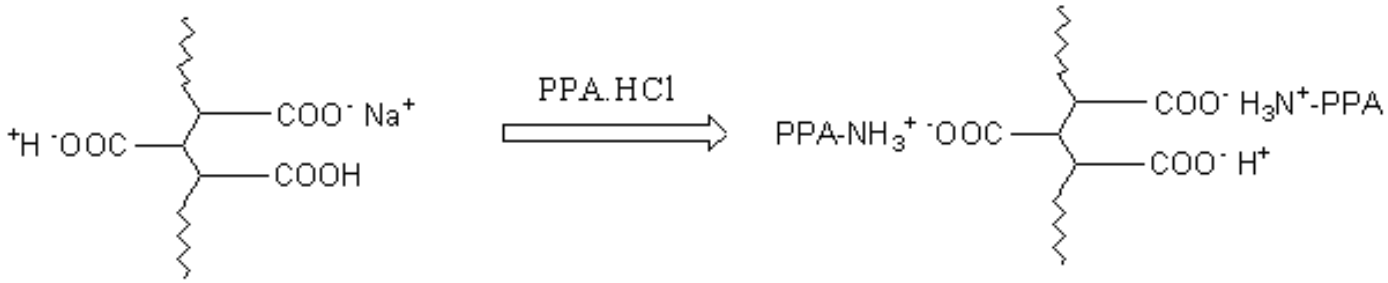

Figure 4. Ionic interaction between OC and PPA.HCl.

Table 3. Determination of the PPA Loading in Products Prepared From the OC Dispersions (DN 0.22 and 0.44) and PPA.HCl Solutions (0.5 M and 1.4 M)*

Batch No.	Particle Size (μm)	% PPA Loading	
		OC (DN = 0.22) + PPA.HCl (0.5M)	OC (DN = 0.44) + PPA.HCl (1.4M)
1	<106	13.0	26.1
	180-250	12.4	27.1
2	<106	12.4	26.3
	106-180	12.6	26.5
3	106-180	12.9	26.5
	180-250	12.1	26.7

*PPA indicates phenylpropanolamine; OC, oxidized cellulose; DN, degree of neutralization; and PPA.HCL, phenylpropanolamine hydrochloride.

and 19.0%-26.7%, respectively. These results show that the use of OC as a dispersion leads to a higher drug loading.

The yield of the OC-PPA complex decreased with increasing DN of OC (Table 2). This result is to be expected because with increasing ionization of the COOH groups the solubility of OC progressively increases. At a DN of ≥0.37, OC-PPA complexes with yields of ≥76% were obtained.

The initial pH values of the OC dispersions with DN values of 0.22, 0.29, 0.37, and 0.44 were 2.9, 3.0, 3.2, and 3.3, respectively. The pH values of the 0.5 M PPA.HCl and 1.4 M PPA.HCl solutions, prior to being added to the OC dispersion, were 4.6 and 4.2, respectively. After addition of 0.5 M PPA.HCl, the pH of the each dispersion dropped by 0.4 units. With 1.4 M PPA, the pH drop was 0.6 units. Since the pK_a of OC is 3.5-4.0 and the pH of the OC dispersion, before and after adding the PPA solution, ranged between 2.9 and 3.3, the decrease in pH upon addition of PPA.HCl is due to further ionization of the OC carboxylic acid groups. Figure 4 illustrates the possible interaction mechanism between OC and PPA.HCl.

Effect of Phenylpropanolamine Concentration on Drug Loading

The OC dispersion with a DN of 0.37 was used to further study the effect of PPA.HCl concentration on drug loading. A series of drug solutions ranging in concentration from 0.5 M to 1.8 M were employed. Both drug loading and yield increased linearly with an increasing concentration of PPA.HCl. The results also show that, at a constant DN, the solution with a higher drug concentration produces the complex with a higher drug loading and higher yield.

Reproducibility of Phenylpropanolamine Loading

The reproducibility of the drug-loading method was tested by preparing the OC-PPA complex using the OC dispersion with a DN of 0.22 and 0.44 and a PPA.HCl concentration of 0.5 M or 1.4 M. The results are presented in Table 3. The close drug-loading values obtained for different batches of a product or different particle size fractions of a batch of a product

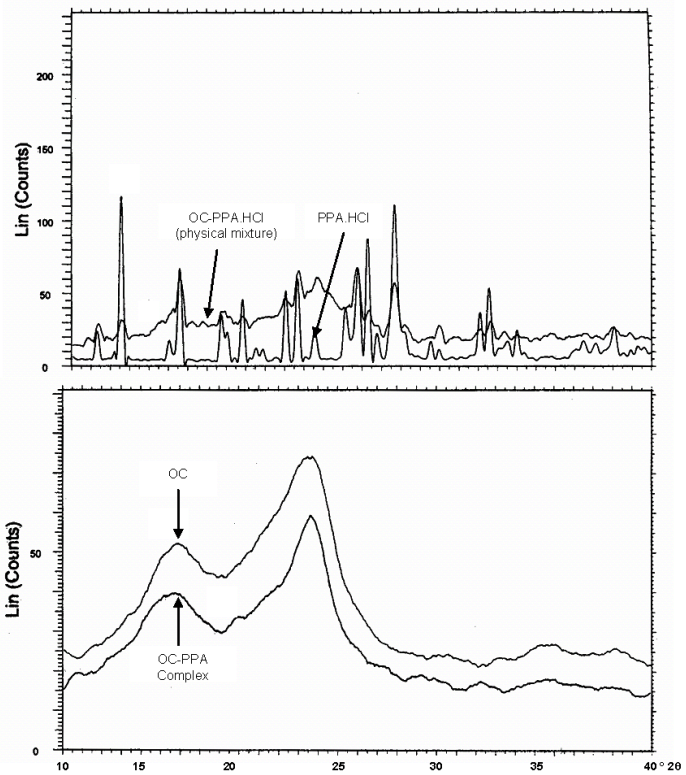

Figure 5. PXRD patterns of OC-PPA complex and physical mixture.

indicate that the method is highly reproducible. The reproducibility and consistency of the method between different batches and different particle size fractions within a batch are highly desirable features in controlled-release process methodology and product design.

Physicochemical Characterization of Oxidized Cellulose-Phenylpropanolamine Complexes

The PXRD patterns of PPA.HCl, OC, OC:PPA.HCl (physical mixture) and an OC-PPA complex are reproduced in Figure 5. The presence of strong diffraction peaks for PPA.HCl indicates it is a highly crystalline material. OC is a low-crystallinity polymer, which is indicated by the lack of sharp diffraction peaks.[15] The PXRD pattern of the OC-PPA complex, containing 14.5% drug, appears similar to that of the starting OC material, while that of the physical mixture of OC and PPA (concentration 14.5%) shows peaks predominantly owing to PPA. These results indicate that the entrapment of PPA by OC occurs at a molecular level.

The FT-IR spectra of PPA.HCl, OC, OC:PPA.HCl (physical mixture), and OC-PPA complex prepared using the OC dispersion with DN = 0.29 are shown in Figure 6. The FT-IR spectra of OC and its sodium salt have been previously reported.[24,25] The characteristic carbonyl stretching vibration band in the spectra of these polymers appears at 1730 cm^{-1} and 1640 cm^{-1}, respectively. The moisture adsorbed on cellulose

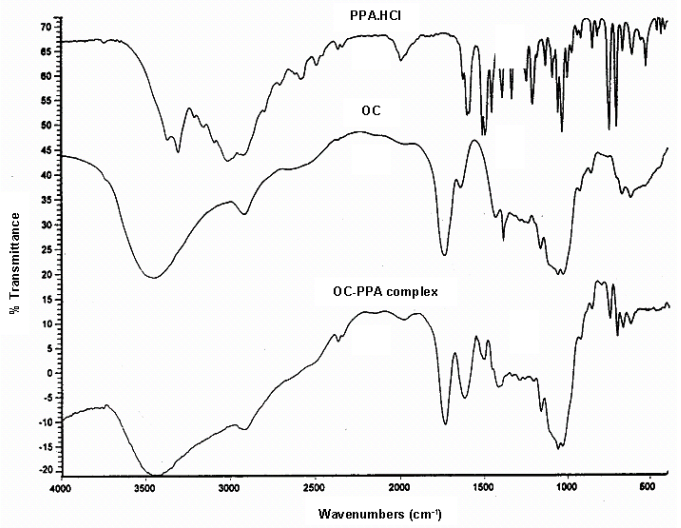

Figure 6. FT-IR spectra of OC, free drug and OC-PPA complex.

has also been shown to appear at 1640 cm^{-1}. The OC-PPA complex showed a significantly reduced intensity of the 1737 cm^{-1} peak, while the 1640 cm^{-1} peak increased in intensity, compared with the respective peaks in the spectrum of OC. The peaks appearing at 705 and 748, 1505, and 1992 cm^{-1} in the spectrum of the complex are owing to PPA. These results show that the OC forms an ionic complex with PPA. Recently, Takka[26] studied the interaction between propranolol hydrochloride and anionic polymers (Eudragit S 100, Eudragit L 100-55, and sodium carboxymethylcellulose) and concluded that the formation of anionic polymer-propranolol hydrochloride complexes involves ionic interaction and hydrogen bonding. In the present study, however, no evidence for the presence of hydrogen bonding between OC and PPA could be drawn from the infrared spectra shown in Figure 6.

The particles of the complexes made using the OC dispersions with DN values of 0.29 and 0.37 possess similar morphologies. They appear to be more swollen compared with the complexes made using the OC suspension (DN = 0) and the OC dispersion with a DN of 0.22. However, compared with the particles in the dispersion containing no PPA, the OC-PPA particles are less swollen. This is because the OC-PPA complexes are less hydrophilic than OC.

CONCLUSION

Results show that the partially neutralized OC dispersions readily form ionic complexes with primary amines. The hydrated OC provides ready access for drug molecules into the polymer network, allowing the preparation of the OC-amine drug complex with a high drug-loading efficiency. The ionized carboxyl groups on the OC polymer chains serve as interaction sites, causing the drug to be molecularly entrapped at these sites.

REFERENCES

1. Langer RS, Wise DL, eds. *Medical Applications of Controlled Release*. Boca Raton, FL: CRC Press Inc; 1984.

2. Giunchedi P, Gavini E, Moretti MD, Pirisino G. Evaluation of alginate compressed matrices as prolonged drug delivery systems. *AAPS PharmSciTech*. 2000;1:E19.

3. Fernandez M, Alvarez-Fuentes J, Iruin A, Holgado MA. In vitro evaluation of a morphine polymeric complex: flowability behavior and dissolution study. *AAPS PharmSciTech*. 2004;5:E39.

4. Ashton WH, Moser, CE**,** inventors. Oxidized cellulose product and method for preparation of the same. US patent 3 364 200. January 16, 1968.

5. Johnson & Johnson. *Surgical Absorbable Hemostate, PC-0506*; Somerville, NJ: Johnson & Johnson Patient Care Inc; 1989. Ethicon. *Interceed Absorbable Adhesion Barrier, AP0635*. Somerville, NJ: Ethicon Inc, Johnson & Johnson Patient Company; 1992.

6. Dineen P. Antibacterial activity of oxidized regenerated cellulose. *Surg Gynecol Obstet.* 1976;142:481-486.

7. Tokunaga YKT, Naruse T. Antitumor effect of oxycellulose as a hemostatic during operation. *Cancer Biother Radiopharm*. 1998;13:437-445.

8. Otterlei M, Erpvik G, Skjak-Braek G, Smidsord O, inventors. Diequatorially bonded b-1,4 polyuronates and use of same for cytokine stimulation. US patent 5 169 840. December 8, 1992.

9. Dol'berg EB, Yasnitskii BG, Shuteeva LN, Kovalev IP. Reaction of oxidized cellulose with medicinal compounds. II. Reaction of oxidized cellulose with isonicotinic acid hydrazide. *Zh Prikl Khim (Leningrad)*. 1973;46:21-23.

10. Yasnitskii BG, Dol'berg EG. Bactericidal hemostatic. USSR patent 389:794. July 11, 1973.

11. Kaputskii FN, Bychkovskii PM, Yurkshtovich TL, Nedorezov VL. Study of photrin sorption by monocarboxycellulose. *Colloid J*. 1995;57:41-45.

12. Bychkovskii PM, Kaputakii FN, Yurkshtovich TL. Sorption of antitumor drugs by cellulose oxidized by nitrogen (IV) oxide. *Ser Khim Navuk*. 1993;3:41-45.

13. Zimatkina TTY, Zimatkin S, Kaputsky F. Antitumor activity of hydroxythiamine and methotrexate immobilized on monocarboxycellulose. *Pol J Pharmacol*. 1996;48:163-169.

14. Dol'berg EB, Shuteeva LN, Yasnitskii BG, Obolentseva GV, Khadzha YaI, Furmanov YuA. Certain aspects of interaction of oxidized cellulose with pharmaceutical compounds. III. Synthesis and biological properties of the product of interaction of oxidized cellulose with kanamycin sulfate. *Khim Farm Zh.* 1974;8:23-26.

15. Kumar V, Yang T. HNO_3/H_3PO_4-$NaNO_2$ mediated oxidation of cellulose - preparation characterization of bioabsorbable oxidized cellulose in high yields and with different levels of oxidation. *Carbohydr Polym.* 2002;48:403-412.

16. Davis MB, Peck GE, Banker GS. Preparation and stability of aqueous-based enteric polymer dispersions. *Drug Dev Ind Pharm*. 1986;12:1410-1418.

17. United States Pharmacopeial Convention. Oxidized cellulose and oxidized-regenerated cellulose. In: *United States Pharmacopeia*/*National Formulary* (*USP* 23/*NF* 18). Rockville, MD: United States Pharmacopeial Convention Inc; 1995:318-319.

18. Martin A, Swarbrick J, Cammarata A. Rheology. In: *Physical Pharmacy*. 3rd ed. Philadelphia, PA: Lea & Febiger; 1983:525-526.

19. United States Pharmacopeial Convention. Phenylpropanolamine Hydrochloride Extended Release Capsules. In: *United States Pharmacopeia*/*National Formulary* (*USP* 23/*NF* 18). Rockville, MD: United States Pharmacopeial Convention Inc; 1995:2114-2115.

20. Katchalsky A, Spitnik P. Potentiometric titrations of polymethacrylic acid. *J Polym Sci [B]*. 1947;2:342.

21. Chowdhury FH, Neale SM. Acid behavior of carboxylic derivatives of cellulose. Part II. oxycellulose. *J Polym Sci Part A*. 1963;1:2893-2904.

22. Cesaro A, Delben F, Paoletti S. Thermodynamics of the proton dissociation of natural polyuronic acids. *Int J Biol Macromol*. 1990;12:170-176.

23. Kotz J, Phillipp B, Nehls I, Heinze TH, Klemm D. *Acta Polym*. 1990;41:333-337.

24. Cantoni C, Zennaro F, Bertocchi C, Mariotti P, Rizo R. C6-oxidized cellulose: ion interactions with mono- and divalent cations. *Biopolym*. 1998;45:157-163.

25. Friedlander BI. *An Infrared Spectroscopic Study of the Oxidation of Cellulose* [dissertation]. Toronto, Canada: The University of Toronto Department of Chemical Engineering; 1966.

26. Takka S. Propranolol hydrochloride-anionic polymer binding interaction. *Farmaco*. 2003;58:1051-1056.