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Research paper

Substituted polyvinylalcohol as a drug carrier for β-carotene

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Abstract

The objective of this study was to evaluate the in vitro characteristics of polyvinylalcohol 10,000 (PVA10,000) and polyvinylalcohol 15,000 (PVA15,000) substituted with different alkyl chains (Iodododecane, Bromotetradecane) and crosslinked with Bis-chloro-ethoxy-ethane as an injectable drug carrier. β-carotene was used as a lipophilic model drug. Physical mixtures of the drug and the spray-dried polymers were prepared and the release of the drug from the mixtures was evaluated in vitro at pH 7.4. The results indicated that the substituted PVA10,000 and PVA15,000 provided faster drug release with respect to the pure drug at pH 7.4. In particular, among the substituted PVA used, PVA10,000 and PVA15,000 substituted with Iodododecane and crosslinked with Bis-chloro-ethoxy-ethane improved drug release.

Keywords: Substituted polyvinylalcohol 10,000; Substituted polyvinylalcohol 15,000; Spray-dryer; Controlled release; β-carotene

1. Introduction

Polymeric nanoparticles and microspheres have shown a certain degree of success for the delivery of poorly water soluble drugs to the systemic circulation [1–4]. The carrier systems containing a drug need to be retained in the blood stream for a long time for passive drug targeting. However, microspheres as well as other particulate systems tend to disappear from the circulation rapidly by suffering from uptake by phagocytic cells [5]. Many researchers have shown that the circulation time of the particulate system, e.g. microspheres, depends on the particle size (≤200 nm) [6,7] and the characteristics of amphiphilic polymers, such as mucoadhesive properties [8,9]. This work describes the use of polyvinylalcohol 10,000 (PVA10,000) and polyvinylalcohol 15,000 (PVA15,000) substituted with Iodododecane (IDD), Bromotetradecane (Mir) at 20% and crosslinked with Bis-chloro-ethoxy-ethane (Bis) at 50%. Polyvinylalcohol was selected because it is a biocompatible polymer and the presence of the hydroxy groups allows its crosslinkage by bifunctional crosslinking agents. We prepared physical mixtures of the substituted PVA and the drug and evaluated the influence of substituted polymers in increasing drug solubility by phase-solubility diagrams.

2. Materials and methods

2.1. Materials

PVA (Mw = 10,000 g/mole, 80% hydrolyzed; Mw = 15,000 g/mole, 89% hydrolyzed) was purchased from Sigma-Aldrich, 1-Iodododecane, 1-Bromotetradecane, 1,2-Bis(2-cloro-ethoxy)ethane, 1,8-Diazabicyclo[5.4.0]undec-7-ene and β-carotene were from Fluka and N-methylpyrrolidone was from Carlo Erba.

Other organic and inorganic chemicals were commercially available and used without further purification.

2.2. Synthesis of substituted PVA

PVA10,000 (4.19 g; 80 mmoles of monomer) and PVA15,000 (3.94 g; 80 mmoles) were dissolved in 30 ml of N-methylpyrrolidone, and the solution was supplemented with 1,8-Diazabicyclo [5.4.0] undec-7-ene (1.5–5) (8.52 g; 56 mmoles) and stirred at ambient temperature for 24 h. The polymers were separated from the solution by precipitation with diethyl ether. The polymers were dissolved in 30 ml of

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N-methylpyrrolidone and the solution was supplemented with 1-Iodododecane (4.74 g; 16 mmoles) or 1-Bromotetradecane (4.44 g; 16 mmoles) and stirred at ambient temperature for 3 h. Then 1,2-Bis(2-cloro-ethoxy)ethane (9.35 g; 50 mmoles) was added stirring overnight at ambient temperature. The substituted polymers were separated from the solution by precipitation with diethyl ether. The precipitates obtained were dissolved in 100 ml water and dialyzed against water. The dialyzed solutions were subsequently spray-dried and fine white powders were obtained.

2.3. ^{1}H-nuclear magnetic resonance (^{1}H-NMR)

The degree of substitution of the PVA was determined by ^{1}H-NMR using a Gemini 300 instrument (300 MHz) and recording the spectra in D_2O to assign non-exchangeable coupled protons.

2.4. Fourier transform infrared spectrometry (FTIR)

Infrared spectra of the substituted PVA were taken with a Jasco FT-IR-410 spectrophotometer. KBr disks were prepared by mixing the polymer and dry KBr in a weight ratio of 1:9 and subsequently compressing this physical mixture by a punch press working at 7 ton cm^{-2}.

2.5. Dynamic light scattering (DLS)

The size of the substituted PVA in phosphate buffer solution (pH 7.4) was measured at different concentrations using an instrument (Brookhaven 90-Plus) equipped with a 50 mW He-Ne laser (532 nm) and thermostated at 37°C. The solutions of the polymers in pH 7.4 aqueous buffer were prepared at increasing concentrations up to 1 mg/ml. Measurements were carried out by fixing the scattering angle at 90°. Results were the combination of three 5 min runs for a total correlation function accumulation time of 15 min. The diffusion coefficient was evaluated from the time autocorrelation function, $g^2(\tau)$ using the forced single-exponential fit (Eq. (1)) [10]:

$$g^2(\tau) = Ae^{-2\Gamma\tau} + B \quad (1)$$

$$\Gamma = Dq^2 \quad (2)$$

$$q = (4\pi n/\lambda_0)\sin(\theta/2) \quad (3)$$

where τ is the delay time, both A and B are constant, D is the translational diffusion coefficient, q is the scattering vector, n is the refractive index of pure solvent, λ_0 is the wavelength of incident light in vacuo, and θ is the scattering angle. The hydrodynamic radius, R_H, was calculated using Stokes-Einstein equation:

$$R_H = k_B T/6\pi\eta D_0 \quad (4)$$

Where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.

2.6. Drug-polymer interaction studies

To evaluate the interactions between the drug and the polymers in solution, an excess of drug (10 mg) was added to 10 ml aqueous buffer (pH 7.4) containing the polymers at increasing concentrations up to 8 mg/ml. The suspensions were magnetically stirred at 37°C for 24 h and then filtrated to analyze the drug solution spectrophotometrically.

2.7. Preparation of drug-polymer physical mixtures

The physical mixtures were prepared by mixing drug and polymer in weight ratios of 1:1 in a mortar until homogeneity.

2.8. In vitro release studies

To detect the amount of free drug available from β-carotene-substituted PVA physical mixtures, an excess of physical mixture (100 mg) was suspended in 3 ml phosphate buffered saline (PBS; pH 7.4):ethanol 2.5:1 (v:v) mixture at 37°C. The suspension was placed in a donor cell separated by a dialysis membrane (Mw cut off = 10,000) from a receiving compartment containing 10 ml of the same PBS:ethanol mixture utilized to suspend the physical mixture, which was replaced after time intervals suitable to guarantee sink conditions throughout the runs. The drug was spectrophotometrically detected in the receiving phase over time, utilizing the polymers alone as blanks.

2.9. Statistical analysis

All the data are the arithmetic means of results from three experiments ± SD. Statistical data were analyzed using Student's *t*-test, with $P \leq 0.05$ as minimum level of significance.

3. Results and discussion

3.1. ^{1}H-NMR

On the basis of ^{1}H-NMR analysis (Fig. 1), the substitution degrees of IDD and Mir and the crosslinking degree of Bis were determined by comparing the signals at 0.95 ppm and at 0.97 ppm of the CH_3 of IDD (a) and Mir respectively and the signal at 3.85 ppm of Bis (b) with the signal at 1.95 ppm of the CH_3 of the PVA acyl group (c). The substitution degree was 17.8% for PVA10,000 and 16.7% for PVA15,000 in the presence of 20% of IDD and 16.5% for PVA10,000 and 16.3% for PVA15,000 in the presence of 20% of Mir. The crosslinking degree was 47.3% for PVA10,000 and 46.1% for PVA15,000 (moles of substituent per 100 moles of vinyl monomer).

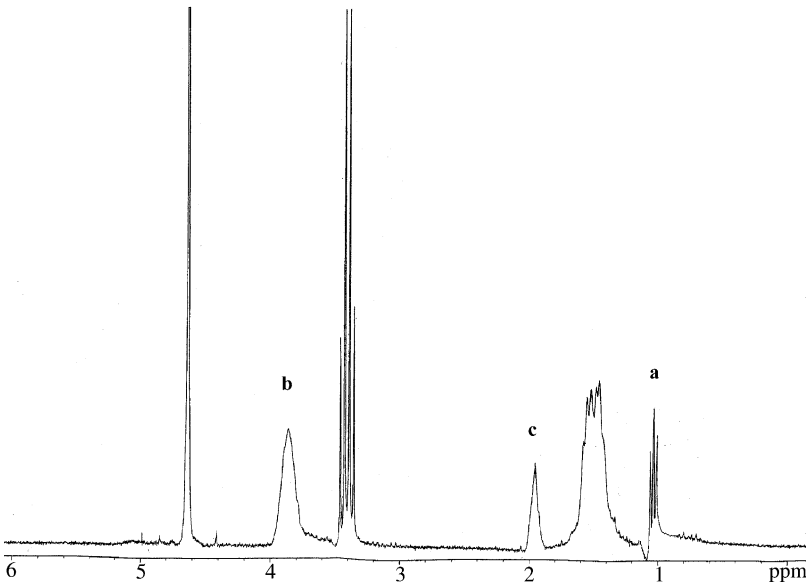

Fig. 1. ^{1}H-NMR spectrum of PVA10000-IDD20-Bis50.

3.2. FTIR

Fig. 2 shows the FT-IR spectra of the samples under study. The FTIR spectrum of PVA10,000 reveal characteristic absorption bands at 3437, 2921 cm^{-1}, which represent the presence of the OH group and CH_2 group. The presence of a peak at 1733 cm^{-1} (due to CH_3-C = O group denoting the presence of acetyl group) confirms that PVA is a partially deacetylated product.

The FTIR spectra of PVA10,000-IDD20-Bis50, PVA10,000-Mir20-Bis50, PVA15,000-IDD20-Bis50 and PVA15,000-Mir20-Bis50 show the absorption assigned to the ether bound at 1093 cm^{-1} and the two characteristic peaks relative to CH deformation of CH_3 of IDD and Mir at 1375 and 1376 cm^{-1} for substituted PVA10,000 and at 1330 and 1377 cm^{-1} for substituted PVA15,000.

3.3. DLS

Fig. 3 shows the diameter of PVA10,000, PVA15,000 and substituted PVA solubilized in PBS (pH 7.4) at 37°C and shows that the PVA is always smaller than the substituted PVA, suggesting the ability of substituted polymers to swell in PBS. The sizes decreased for PVA10,000-IDD20-Bis50 and PVA15,000-IDD20-Bis50 and increased for PVA10,000-Mir20-Bis50 and PVA15,000-Mir20-Bis50, due to the length of the substituent chain: Bromotetradecane ≥ Iodododecane.

3.4. Drug polymer interaction studies

The phase-solubility diagrams of β-carotene in the presence of the substituted PVA revealed an increase in drug solubility (Fig. 4). This is probably due to hydrophobic interactions between the drug and the substituted PVA resulting from compenetration of acyl chains (Iodododecane and Bromotetradecane) with the drug. The enhanced drug solubility expressed by the slope of the linear trend of the diagrams (Table 1) was more evident in the presence of PVA10,000-IDD20-Bis50 than PVA10,000-Mir20-Bis50, PVA15,000-IDD20B-is50 and PVA15,000-Mir20-Bis50, respectively. This indicated a greater tendency of the PVA10,000-IDD20-Bis50 than other substituted polymers to interact with the drug in solution.

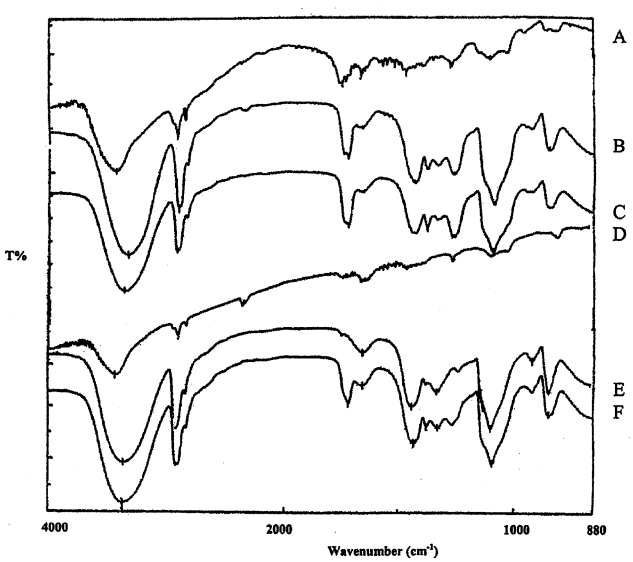

Fig. 2. FTIR-spectra of PVA10,000 (A); PVA10,000-IDD20-Bis50 (B); PVA10,000-Mir20-Bis50 (C); PVA15,000 (D); PVA15,000-IDD20-Bis50 (E); and PVA15,000-Mir20-Bis50 (F).

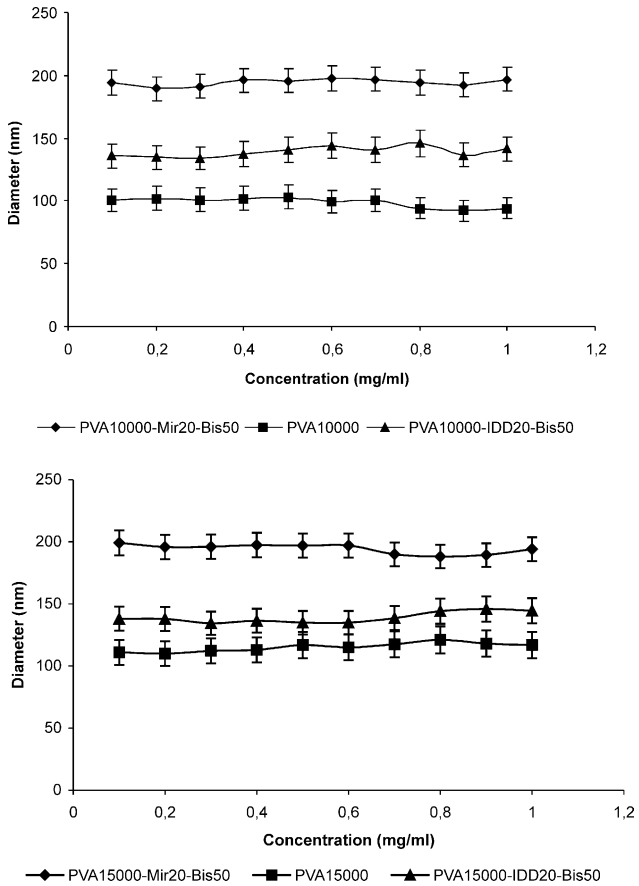

Fig. 3. DLS data of PVA and substituted PVA. All the data are the average of three determinations ± SD.

3.5. In vitro release studies

Free drug availability, expressed as fractional release over time, was higher from physical mixtures than the pure drug at pH analyzed (Fig. 5). This may be attributed to the presence of hydrophobic chains (IDD and Mir) attached to the PVA. It may be responsible for enhancing the affinity of the polymers for the drug, consequently allowing faster drug release. In particular, the release of β-carotene from the physical mixtures was in the order: PVA10,000-IDD20-Bis50 > PVA15,000-IDD20-Bis50 > PVA10,000-Mir20-Bis50 > PVA15,000-Mir20-Bis50.

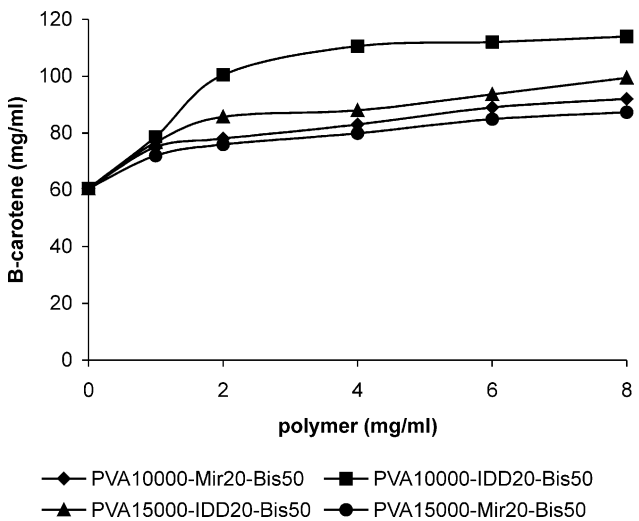

Fig. 4. Phase-solubility diagrams of β-carotene in the presence of the substituted PVA. All the data are the average of three determinations.

Table 1
Slopes of the linear trends of the phase-solubility diagrams of β-carotene in the presence of the substituted PVA at 37°C in aqueous buffer (pH 7.4)

Polymer type	Slope
PVA10,000-IDD20-Bis50	1.92 ± 0.07
PVA10,000-Mir20-Bis50	0.76 ± 0.02
PVA15,000-IDD20-Bis50	0.83 ± 0.05
PVA15,000-Mir20-Bis50	0.35 ± 0.04

Each value represents the mean of three experiments ± SD.

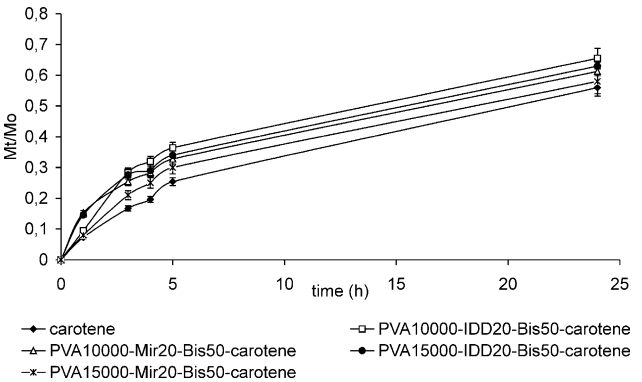

Fig. 5. In vitro release profiles of β-carotene in the presence of the substituted PVA. All the data are the average of three determinations ± SD.

4. Conclusions

PVA10,000 and PVA15,000 substituted with Iodododecane and Bromotetradecane and crosslinked with Bis-chloro-ethoxy-ethane represent an injectable drug carrier.

Physical mixtures of these polymers with β-carotene provide systems characterized by increased availability of free drug in solution with respect to the pure drug. The physical mixtures of β-carotene and substituted PVA provided faster drug release than the pure drug at pH 7.4. Among the substituted PVA analyzed, PVA10,000-IDD20-Bis50 improved drug release.

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