Containing Cubane as a Crosslinking Agent

Mehrdad Mahkam

Chemistry Department, Faculty of Science, Azarbaijan University of Tarbiat Moallem, Tabriz, Iran
E-mail: mmahkam@yahoo.com Fax: + 98 411 4439469

Summary: Two anti-inflammatory drugs, indomethacin and aspirin together with cubane-1, 4-dicarboxylic acid (CDA) were covalently linked with 2-hydroxyethyl methacrylate (HEMA). The drug-linked HEMA (indomethacin-linked HEMA) is abbreviated as HI, aspirin-linked HEMA as HA and cubane-1, 4-dicarboxylic acid (CDA) linked to two HEMA group is the cross-linking agent (CA). A difunctional spacer group was introduced between the drugs and acrylic moiety of the monomer through a hydrolyzable ester linkage. Free radical cross-linking polymerization of the monomers with drug effect was carried out in dioxane solution at various CA ratios, using AIBN as initiator in the temperature range 60-70°C. The compositions of the cross-linked three-dimensional polymers were determined by FT-IR spectroscopy. The glass transition temperature (Tg) of the network polymers was determined calorimetrically. The hydrolysis of drug-polymer conjugates was carried out in cellophane membrane dialysis bags containing an aqueous buffer solution (pH 8 and pH 1) at 37°C. Detection of the hydrolysis product by UV spectroscopy shows that the drugs were released by hydrolysis of the ester bond located between the drug and spacer group.

Keywords: controlled-release; cross-linked polymer; cubane; hydrolysis; polydrug

Introduction

The combination of versatility and tailored molecules has relatively easily made acrylic and methacrylic esters prime candidates for diverse applications. The preparation of macromolecular prodrugs, in which active substances are linked to polymeric matrices by covalent bonds that can be hydrolyzed in body fluids is recognized as an effective way to prolong pharmacological activity.[1]

The use of acrylic or methacrylic derivatives covalently linked with drugs was reviewed by Dumitriu.[2] Many researchers continued on in this field thereafter.[3-8]

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Different synthetic routes have been employed in the preparation of polymers that contain drug pendent substituents. Firstly, the active agent is converted to a polymerizable derivative that is subsequently polymerized to generate the macromolecular combination. Bioactive agents have also been chemically bound to preformed synthetic or naturally occurring polymers by allowing them, or one of their derivatives, to react with the polymer's functional groups.[9-12]

An alternative to the direct drug-polymer linkage is the incorporation of a spacer group between the drug and polymer chain that, in general, is constituted by oxyalkyl segments. The use of suitable spacer arms can increase the mobility of drugs on the polymer chain and enhance the sensitivity of polymer-drug conjugates to undergo chemical or enzymatic hydrolysis.[13-14] The facility with which the drug can be converted to polymerizable derivatives depends to a large extent on their functionality. Drugs that contain reactive functional groups such as carboxyl or hydroxyl groups can be converted to a wide variety of polymerizable derivatives.

The majority of the drug monomers that have been synthesized and subsequently polymerized are acrylic type derivatives of pharmaceutically active compounds.[15-16] The ibuprofen (propionic acid derivative) is a non-steroid anti-inflammatory drug (NSAIDs) and is widely used for the treatment of rheumatoid arthritis. In recent years the polymeric prodrugs of ibuprofen in which the drug is attached covalently to a polymer backbone have been developed.[17]

In this article, we report the synthesis and hydrolytic behaviour of network methacrylic type polymers containing aspirin and indomethacin. The methacryloyloxyethyl esters of aspirin (HA), indomethacin (HI) and cubane-1, 4-dicarboxylic acids were prepared as polymerizable drugs and cross-linking agent (CA) respectively. Free radical cross-linking polymerization of the monomers with the various CA ratios produced drug pendent network polymers, which have two hydrolyzable bonds on each side of the spacer group. The obtained network polymers were hydrolyzed in aqueous buffer solutions at physiological conditions. Influences of different factors, such as cross-linking and neighbouring effect of side groups were studied.

Experimental Section

Materials and Measurements

2-hydroxyethyl methacrylate (HEMA) was purchased from Aldrich Chemical Co. Azobisisobutyronitrile (AIBN) was obtained from Aldrich Chemical Company, and crystallized twice from methanol.

Cubane-1, 4-dicarboxylic acid was prepared according to the Chapman method.[18,19] ^{1}H NMR and ^{13}C NMR spectra were recorded on an 80 MHz Bruker AC 80 spectrometer in DMSO d_6 and $CDCl_3$ solvents. The IR spectra were recorded on a Shimadzu FT IR-4300 spectrophotometer. The DSC curves were obtained on an STA 625 calorimeter at heating and cooling rates of 10°C/min under N_2. The amount of released drugs was determined by a 2100 Shimadzu UV spectrophotometer at the absorption maximum of the free drug in aqueous alkali using a 1cm quartz cell.

Synthesis of Monomers (HA and HI)

These monomers were prepared according to the procedures reported in the literature.[5,8] A solution of 6.2 g (30 mmol) of N, N-dicyclohexylcarbodimide (DCC) in 64 ml of methylene chloride was added dropwise at –20°C to a solution (30 mmol) of aspirin or indomethacin in 0.37 g (3 mmol) of 4-(dimethylamino) pyridine (DMAP) dissolved in 64 ml of CH_2Cl_2. The mixture was kept at –20°C while a solution of 3.9g (30 mmol) of HEMA in 40ml of CH_2Cl_2 was added dropwise over 30 min. The reaction mixture was slowly returned to room temperature and the reaction continued for 3h. The precipitate was filtered and the organic layer was sequentially extracted with 10 % wt of $NaHCO_3$ (three times), 2N HCl (twice), water (once) and finally a saturated solution of NaCl. The extracted solution was dried over $MgSO_4$ and the solvent was removed by a rotavapor. An oily residue was obtained which was separated by column chromatography, using n-hexane:ethyl acetate (4:1) as eluent to give about 40% yield.

Cubane-1, 4-Bis (methacryloyloxyethyl) carboxylate (CA)

A mixture of 1.92 g (10 mmol) cubane-1, 4-dicarboxylic acid and 25 ml thionyl chloride was refluxed on a steam bath for 2 h. The excess thionyl chloride was azeotropically removed with

dry benzene under reduced pressure. A mixture of 1.5 g (6.55 mmol) of cubane-1, 4-dicarbonyl chlorides in 50 ml Et_2O was heated to reflux and treated with a solution of 1.7 g (13.1 mmol) of 2-hydroxyethyl methacrylate and 1.9 g (15.7 mmol) of N, N-dimethyl aniline in 10 ml Et_2O. After 2 h stirring at reflux temperature, the reaction mixture was filtered and washed with a 10% H_2SO_4 solution and then with saturated $NaHCO_3$ solution. The ether layer was dried with Na_2SO_4 and evaporated. The residual solid was recrystallized from n-hexane to give the CA in 75% yield: mp 77.2±0.2; IR (KBr) : 3003, 1712, 1633, 1166 cm^{-1}; ^1H NMR ($CDCl_3$,) 6.11 (q, 2H, trans-H), 5.59 (t, 2H, Cis-H), 4.37 (s, 8H, -OCH_2CH_2O-), 4.24 [(s, 6H, CH (cubane))], 1.94 (q, 6H, C=C-CH_3); 1H NMR (DMSO-d_6,) 6.01 (s, 2H), 5.68 (t, 2H), 4.32 (s, 8H), 4.16 (s, 6H), 1.87 (s, 6H); ^{13}C NMR (DMSO-d_6,) 170, 166.2, 135.5, 125.7, 61.99, 61.55, 54.98, 46.11, 17.64 ppm.

Polymerization: General Procedure

Specific mol percent of CA (2, 4 and 6%) and monomers (HA and HI) were polymerized at 60-70°C in a thermostatic water bath, using 2,2-azobisisobutyronitrile (AIBN) as initiator ([I] = 0.02 M), and dried dioxane as solvent ([M] = 1.0 M). All experiments were carried out in Pyrex glass ampoules sealed under vacuum. After the specified time (24-48 h), the precipitated network polymer was collected, washed with methanol and dried in vacuum.

Method of Hydrolysis

The powdered polymer (20mg) was poured into 5ml of aqueous buffered solution (phosphate buffer pH 8, hydrochloric acid buffer pH 1) at 37°C. This solution was transferred into a cellophane membrane (dialysis bag), and then the bag was sealed and placed in a flask containing 25 ml of the same buffer solution maintained at 37°C. The external buffer solution was continuously stirred and then 3 ml samples were taken out at selected time intervals and replenished by the same amount of a fresh buffer solution. The sample of hydrolyzate was analysed by UV spectrophotometer and the quantity of drug was determined using a standard calibration curve obtained under the same condition.[5-8]

Results and Discussion

In the preparation of polymerizable monomers of pharmaceutically active compounds, it is necessary to create synthetic conditions mild enough to allow attachment with no adverse effect on the physiological activity of the drug.[20] The cubane system is not inherently toxic and most cubanes are biologically innocuous. Because the cubane frame is rigid, substituents have precise spatial relationship to each other. The distance across the cube (the body diagonal) is almost the same as that between the para position of the benzene ring. Each cubane unit adds 4.15 Å to the length of the molecule.[19]

Evidence suggests that the limited efficiency of polymeric prodrugs was a reflection of the limited loading and a very slow hydrolysis of the drug from the polymer backbone. The experimental results indicate that by incorporating a spacer group between the drug and the polymer chain and by increasing or decreasing the amount of cross-linking agent, this problem can be overcome. Therefore, by using this method, one should be able regulate the amount of hydrolysis.

Characterization data for the synthesized monomers and CA were obtained through a variety of techniques including IR and NMR spectroscopy. IR spectra confirmed existence of the ester (1710-1730 cm^{-1}) and the vinyl (1630-1640 cm^{-1}) linkages. In the ^{1}H NMR spectrum, cubane protons were observed at 4.2 ppm and in the ^{13}C NMR spectrum, the carbons of cubane appeared in the range 45-55 ppm.

Identification of Network Polymeric Prodrugs

The resulting network polymers swell and become soft if they are exposed to solvents such as H_2O and most organic solvents without dissolving. The FT-IR spectra, absorption of ester carbonyl bond appeared in region of 1730-1740 cm^{-1}.

Thermal Behaviour

The thermal behaviour of a polymer is important in relation to its properties for controlling the release rate in order to have a suitable drug dosage form. Differential scanning calorimetry (DSC) and thermal gravimetry (TG) for the network polymers are summarized in Table 1. The difference is probably related to the introduction of cross-linking, which would decrease the

flexibility of the chains and the ability of the chains to undergo segmental motion, which would increase the Tg values.

Drug Release by Hydrolysis of Polymeric Prodrugs

It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug-polymer chemical bonds, the structure of the polymer and the surrounding condition. The hydrolysis of a linkage depends not only on its distance from the polymer backbone, but the length and hydrophilicity of the spacer unit between the drug and the polymer chain can also affect the release rate.[1]

Table 1. Thermogravimetric of the prodrugs.

Polymer	Tg(°C)
PCHA-2%	265
PCHA-4%	270
PCHA-6%	295
PCHI-2%	220
PCHI-4%	245
PCHI-6%	260

We have studied the hydrolysis behaviour of drug-polymer adduct under physiological conditions (aqueous phosphate or hydrochloric acid buffers, at 37°C). Although the polymers were not soluble in water, they were dispersed in a buffer solution and the hydrolysis occurred as for a heterogeneous system. The degree of hydrolysis of the network polymers containing drugs as a function of time is shown in Figure 1.

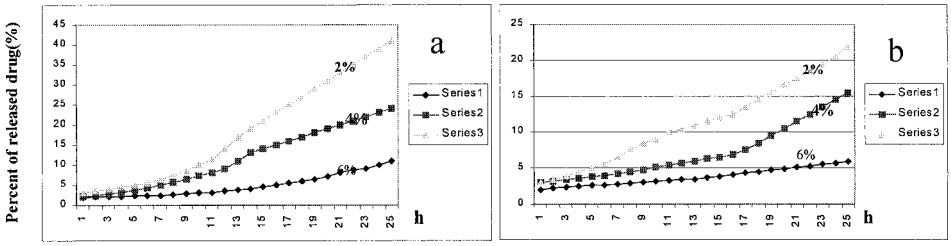
The concentration of the released indomethacin and aspirin at selected time intervals was determined with an UV spectrophotometer at absorption maximum region. The order of hydrolysis in this series was as follows, PCHA-2% > PCHA-4% > PCHA-6% and PCHI-2% > PCHI-4% > PCHI-6%

It appears that with increased cross-linking, diffusion of the hydrolyzing agents in the polymer network is reduced and the hydrolysis rate is slower.

Two hydrolyzable sites (diester) are present in the resulting polymers. It is noticeable that the formation of the hydroxyethyl ester indomethacin and aspirin are possible, but the intermediate acts as a second carrier of the drugs, which in turn can be hydrolyzed to the free drug during the

reaction. Therefore, it is difficult to determine the exact hydrolysis sequence at the hydrolysis sites.

Release of Indomethacin from polymeric carriers as a function of time at 37°C. a) pH 8 ; b) pH 1.



Release of Aspirin from polymeric carriers as a function of time at 37°C. a) pH 8 ; b) pH 1.

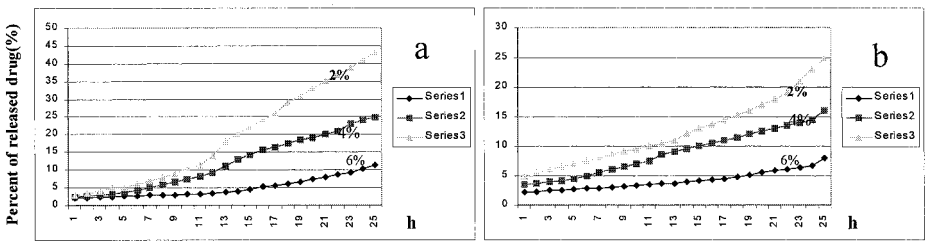


Fig. 1. Release of indomethacin and asperin.

Conclusion

The resulting polymer-drug conjugates possess a considerable amount of pharmaceutically active components, which are hydrolyzed slowly under physiological conditions. As the main purpose of polymeric prodrugs is the achievement of controlled drug release or slow release, application of these polymers as a drug delivery system are expected after in vivo examinations. Therefore by placing hydrophilic spacer groups and regulating the cross-linking degree, we should improve the hydrolysis rate.

Cubane-1, 4-bis (methacryloyloxyethyl) carboxylate (CA) is a versatile cross-linking agent for synthesis of new cross-linking polymers with many purposes.[17]

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