

Note

Study on characterization and degree of esterification of styrene maleic anhydride by some medicines

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Styrene-co-maleic anhydride has been used for the preparation of chemically and diffusionally controlled polymeric prodrugs poly(styrene-co-maleic anhydride) conjugates with phenol based medicinal natural products like 2-methoxy-4-(2-propenyl)-phenol (eugenol) and synthetic *o*-hydroxy benzoic acid (salicylic acid) etc. in acetone and dioxane. The hydrolytic and binding behavior of the phenol based medicine was compared with the degree of esterification. 2-Methoxy-4-(2-propenyl)-phenol and *o*-hydroxy benzoic acid have low binding with styrene-co-maleic anhydride while other has good binding with styrene-co-maleic anhydride.

Keywords: Esterification, polymeric prodrug, polymer conjugation, styrene-co-maleic anhydride, hydrolyzing reaction

The efficiency of therapeutics is often diminished by their insolubility, instability, low bioavailability, non-specificity and systemic toxicity. A considerable number of strategies have been developed in order to obtain a drug delivery system for an effective therapy. Attachment to suitable polymers significantly alters the properties and pharmacokinetics of drugs. Polymer conjugation, largely investigated with both protein and low molecular mass drugs, represents a promising method to improve the physicochemical and biopharmaceutical properties of drugs^{1,2}. Polymer conjugation can endow derivatives with increased water-solubility and chemical stability, improved pharmacokinetics and distribution profile, reduced side effects, and sometimes properties targeted at the disease site either by active or passive mechanism³. Styrene-co-maleic anhydride copolymer belongs to a group of vinylic polymers. It has no teratogenic activity and no acute or chronic toxic effects⁴. The reaction of anhydride group containing copolymers with phenothiazine derivatives⁵, conjugation with

disaccharides, amines^{6,7} and doxorubicine⁸ (polymeric micellar) were studied earlier. Styrene-co-maleic anhydride has been used for preparation of chemically and diffusionally controlled polymeric prodrug of pyrazole, acriflavin⁹, barbituric acid, dopamine¹⁰ and ampicilline¹¹. There are also examples of attachment of some antimicrobial agents such as 4-hydroxybenzoic acid¹² and 4-aminophenol¹³ to styrene-co-maleic anhydride. Recently the potential use of styrene maleic anhydride and ethanol as enteric coating material has been reported.

In this article, we describe the supporting of phenol based medicinal compounds such as (i) 2-methoxy-4-(2-propenyl)-phenol (eugenol), which is obtained from oil of clove and used as local antiseptic and dental analgesic (ii) N-acetyl-*p*-aminophenol (paracetamol) and (iii) *o*-hydroxybenzoic acid (salicylic acid) which are analgesic, to conjugate with maleic anhydride co-polymers. Styrene-co-maleic anhydride derivatives were used in order to determine the effect of polymer solubilities, and microstructures on the yield of coupling and hydrolyzing reactions.

Results and Discussion

Styrene-co-maleic anhydride copolymer was synthesized in order to study the reaction between anhydride groups of copolymer and phenol based compounds. This copolymer (P1) is suitable for spectroscopic examination of the reaction and for solution properties. The copolymer (P1) was soluble in dioxane and acetone but insoluble in aromatic solvents and chloroform. This polymer was treated under the experimental conditions without any phenolic compounds as a blank experiment. This enabled us to determine the effect of reaction conditions on anhydride groups. The reactions of phenolic medicinal compounds such as *o*-hydroxybenzoic acid, 2-methoxy-4-(2-propenyl)-phenol and N-acetyl-*p*-amino-phenol with SMA (P1) was carried out under anhydrous conditions and the results are summarized in **Table I**. The mole ratio of the hydroxyl group to the anhydride group was taken as 1:1. The reaction yield varied between 24-90% and was dependent on temperature and the type of solvent. Thus the yield increased from 24% to 90% in dioxane if the temperature was increased from 25°C to 80°C (**1c**).

However, for compound C the reaction yields of polymer P1 was somewhat higher than those of compounds A and B. This difference is probably due to the local concentration of anhydride of the polymer which favours reaction with compound C with higher reaction rate rather than with A and B. Besides the formed ester structure, the effect of the random coil formation in solution and the chemical reactivity of neighboring anhydride group in reacted polymer **1c** would be decreased in styrene-co-maleic anhydride polymers. Temperature and % yield of the products, **1a, 1b, 1c** are shown in **Table I**. P1, **1a, 1b, 1c** were insoluble in water, ether, toluene and chloroform. The copolymer P1 and its respective derivatives **1a, 1b, 1c** were studied with FTIR.

Infrared Spectroscopy

The absorption bands at 3002 cm^{-1} (C-H stretching for aromatic fragments) and 1602 cm^{-1} are indicative of the phenyl group (C=C str.) while a strong absorption peak at 1779 cm^{-1} is characteristic of asymmetrical C=O coupled (str.) vibration of anhydride group. These are characteristic bands of SMA(P1). As expected, the higher the conversion of anhydride to ester groups after the reaction with hydroxyl containing compounds, the lower the

1750 and 1735 cm^{-1} . It indicates that conversion of the anhydride ring to the ester group. Other peaks at $1021, 1185\text{ cm}^{-1}$ due to C-O (str.) are present in all the esters, those at 1730 and 1185 cm^{-1} show the presence of ester carbonyl and acyclic C-O. In this case the copolymer chain is less rigid. In all of the reaction products **1a-c** peaks due to unreacted anhydride groups ($1855\text{ cm}^{-1}, 1779\text{ cm}^{-1}$) in the FTIR spectrum of **1a-c** are clearly seen. The FTIR spectrum of **1c** shows a hydrogen bonded N-H stretching band at 3325 cm^{-1} , amide C=O stretching at 1650 cm^{-1} , as also NH bending and CN stretching at 1560 cm^{-1} and 1310 cm^{-1} respectively.

For determination of the degree of esterification, two absorption bands were chosen, the peak at 1602 cm^{-1} characteristic of the styrene residue, which does not vary with the reaction, and the peak at 1779 cm^{-1} corresponding to the maleic anhydride residue. The conversion ratio p was defined as:

$$p = (1 - A_t) / A_0 \times 100$$

A_0 = Initial ratio between absorbance at 1779 cm^{-1} and 1602 cm^{-1} .

A_t = Ratio between absorbance at 1779 cm^{-1} and 1602 cm^{-1} at reaction time t .

Table II gives the degree of esterification of the copolymer with *o*-hydroxy benzoic acid *versus* reaction time. The esterification reaction was followed by observing the 1779 cm^{-1} and 1602 cm^{-1} peaks which correspond to C-O-C stretching absorbance of maleic anhydride and C=C vinyl stretching of the styrene respectively. There is a decrease in intensity of the 1779 cm^{-1} band from the initial stage of the copolymer esterified with *o*-hydroxybenzoic acid up to 72 hr of reaction time. By considering the kinetics of esterification reaction, *o*-hydroxybenzoic acid and N-acetyl *p*-aminophenol are found to have big difference in the conversion at 4 hr of reaction time. In the case of *o*-hydroxybenzoic acid, actually there is no conversion to the ester at this time, while with 2-methoxy-4-(2propenyl)-phenol

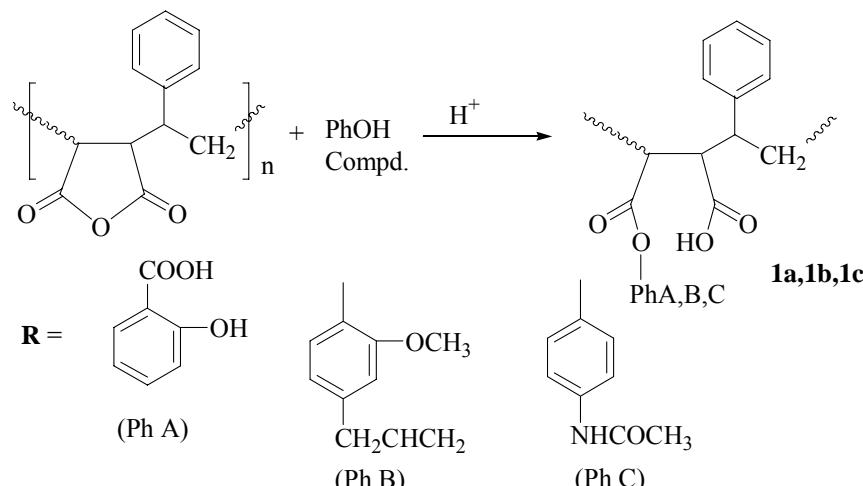
Table I — End percent yield of SMA and SMA-phenolic compounds

Derivatives	Solvent	Temp (°C)	% Yield
P1(SMA)	Toluene	80	90
1a	Acetone	80	70
1a	Dioxane	80	76
1b	Acetone	80	80
1b	Dioxane	80	53
1c	Dioxane	80	90
1c	Acetone	40	80
1c	Dioxane	25	24

residual anhydride absorption intensities of peaks at

Table II — Absorbance values for the esterification reaction with *o*-hydroxy benzoic acid comparison percentage (p) calculation.

IR Absorbance cm^{-1}	A_0	A_t 2 hr	A_t 4 hr	A_t 6 hr	A_t 8 hr	A_t 10 hr	A_t 24 hr
1779	2.696	1.028	1.848	1.183	1.103	0.726	0.467
1602	0.466	0.141	0.311	0.207	0.245	0.212	0.184
1779/1602(ratio)	5.79	5.791	5.792	5.715	4.502	3.425	2.538
A_t / A_0	-	1.00	1.00	0.987	0.778	0.591	0.438
$p = (1 - A_t / A_0) \times 100$	-	0	0	1	22	41	56



Scheme I

Table III — Percent yield of hydrolysed product of derivatives of SMA-phenolic compounds

Phenolic Compounds	Derivative	Time (hr)	Temp (°C)	% Yield
<i>o</i> -Hydroxy benzoic acid (<i>salicylic acid</i>)	1a	24	80	9
	1a	48	80	12
	1a	72	80	15
2-methoxy-4-(2-propenyl)phenol (<i>eugenol</i>)	1b	72	40	4
	1b	96	40	2
N-acetyl- <i>p</i> -aminophenol (<i>Paracetamol</i>)	1c	24	25	20
	1c	24	80	90
	1c	36	80	95
	1c	48	80	98

39% of the ester has been already formed. A similar situation occurs at 8 hr. With *o*-hydroxybenzoic acid only 22% of the ester had formed and with N-acetyl-*p*-aminophenol 54% of the ester was generated.

Hydrolysis

The products of **1a**, **1b**, **1c** were hydrolyzed with water in the presence of the acid catalyst. After the hydrolysis reaction the products were examined with FTIR and the amounts of the hydrolyzed products were analysed, the hydrolysis rate was affected by the time, the temperature and the type of compound (A,B,C) and the main polymer (P1). For the compound **1b** an appreciable amount of hydrolysis was achieved at RT. The hydrolysis of SMA showed rather different behavior. Even after 96 hr at 40°C yield of hydrolysis of **1b** was very low (2%) while the hydrolysis of **1c** was about 98% complete in 48 hr at 80°C. This was probably due to the fact that P1 coupled with 2-methoxy-4-(2-propenyl)-phenol was not swollen by water and 2-methoxy-4-(2-propenyl)-phenol molecules chemically trapped inside the precipitated polymer could not interact with water,

while N-acetyl-*p*-aminophenol molecules are chemically bonded only to the active anhydride site of styrene-co-maleic anhydride on the surfaces where water molecules can easily diffuse.

Experimental Section

Chemicals were purified by suitable methods. The structures of the desired products were assigned by analysing FTIR and ¹H NMR spectral data. FTIR spectra were recorded on Jasco FTIR-5300 spectrophotometer as KBr pellets, ¹H and ¹³C NMR spectra were obtained in (CD₃)₂CO solution, on a Bruker AC-200 MHz spectrometer.

Synthesis

A dry 250 mL round bottom flask was charged with 10 mL (0.086 mole) of styrene, 9.8 g (0.1 mole) of maleic anhydride, 30 mL of toluene and 0.1 g of benzoyl peroxide (dispensed using a plastic spoon). The flask was stirred until most of the solid material had dissolved (may still be somewhat cloudy) and a boiling chip was added. The reaction mixture was refluxed at 80°C using

a thermo well for 25 min till a precipitate formed. It was then cooled to RT, 10 mL of methanol was added and the entire mass stirred with a glass rod. The product was collected by vacuum filtration, and washed with methanol, the boiling chip was removed and the product allowed to dry. The solubility of the polymer was tested in toluene, acetone, ethanol, dioxane, chloroform and water was treated. The polymer was spread on a watch glass and was left in a drier at 50°C *in vacuo* overnight. The product obtained was purified. The yield (P1) was about 90% and the molecular weight of **P1** was 22000 Dalton. The maleic anhydride group was analyzed by the morpholine method. Molecular weight of poly styrene-co-maleic anhydride was calculated from single point viscosity of its acetone solution at 25°C using the following equation¹⁴.

$$[\eta] = 8.69 \times 10^{-5} M_n^{0.74} \quad \eta = \frac{\eta_{sp}}{1 + 0.28 \chi \eta_{sp}}$$

Synthesis of styrene-co-maleic anhydride with phenol based compounds

A round bottom two necked flask equipped with a magnetic stirrer, a thermometer and a reflux condenser was charged with 2 g of copolymer P1(SMA) (0.01mole anhydride group), 0.01 of mole phenol based compound (*o*-hydroxybenzoic acid), 50 mL of solvent (acetone) and a few drops of concentrated HCl, the yield was 70% (**1a**).

2 g P1 (SMA) (0.01 mole anhydride group), 0.01 mole phenol based compounds (2-methoxy-4-(2-propenyl)-phenol, 50 mL solvent (acetone) and a few drops of concentrated HCl. The yield was 80% (**1b**).

2 g P1 (SMA) (0.01mole anhydride group), 0.01 mole phenol based compounds (N-acetyl-*p*-amino Phenol), 50 mL solvent (acetone) and a few drops of concentrated HCl. The yield was 80% (**1c**).

The corresponding mixture was heated upto a selected temperature (**Table I**) and stirred for 24 hr. The products (**1a,1b** and **1c** in **Scheme I**) were precipitated with toluene, collected by filtration and washed with alcohol, water and ether, and dried in vacuum (**Scheme I**).

Determination of esterification degree

The conversion of the reaction was calculated according to Lindt¹⁵. It is described in the Result and Discussion section (**Table II**).

Hydrolysis

0.5 g of derived polymer was put into 25 mL of water and the *pH* was adjust upto 3.0 by dropwise

addition of dilute HCl. The mixture was left to stand at 25, 40 and 80°C for a period ranging from 5 to 72 hr, the released compounds were extracted with ether and dried. The residue, *i.e.* *o*-hydroxybenzoic acid, 2-methoxy-4-(2-propenyl)-phenol and N-acetyl-*p*-aminophenol was weighed to determine the extent of hydrolysis (**Table III**).

Conclusion

Phenol based medicinal compounds such as *o*-hydroxybenzoic acid, 2-methoxy-4-(2-propenyl)-phenol and N-acetyl-*p*-aminophenol could be coupled with styrene-co-maleic anhydride copolymers. The binding reaction yield was generally higher for styrene-co-maleic anhydride copolymers for the paracetamol **1c**. It shows high percent yield of (SMA-N-acetyl-*p*-amino phenol) and its percent yield after the hydrolysis was also high which means N-acetyl-*p*-aminophenol (paracetamol) has good binding with styrene-co-maleic anhydride. Hence N-acetyl-*p*-aminophenol can be loaded on styrene maleic anhydride.

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References

- 1 Lu K R, Shiah J G, Sakuma S, Kopeckova P & Kopecek J, *J Control Rel*, 78, **1992**, 165.
- 2 Christie R J & Grainger D W, *Drug Del Rev*, 55, **2003**, 421.
- 3 Garnett M C, *Adv Drug Del Rev*, 53, **2001**, 171.
- 4 Winek C L & Burgun J J, *Clinical Toxicol*, 10, **1977**, 255.
- 5 Zovko M, Barbaric M, Zorc B & Hafner A, *Acta Pharma*, 55, **2005**, 169.
- 6 Felicity H, Prinal H & Cameron A, *The AAPS Journal*, 9, **2007**, 235.
- 7 Henry S M, Mohamed E H, Sayed E & Christopher M, *J Biomacro-molecules*, 7, **2006**, 2407.
- 8 Greish K, Sawa T, Fang J, Akaike T & Maeda H, *J Control Rel*, 97, **2004**, 219.
- 9 Patel H, Raval D A & Madamwar S R, *Die-Angewandt Makromolekulare Chemie*, 263, **1999**, 25.
- 10 Kalcic I, Zorc B & Butula I, *Int J Pharma*, 136, **1996**, 31.
- 11 Patel J S, Patel S V, Talpada N P & Patel H A, *Angew Makromol Chem*, 271, **1999**, 24.
- 12 Jeong J H, Byoun Y S & Lee Y S, *J Ind Eng Chem*, 7, **2001**, 310.
- 13 Jeong J H, Byoun Y S & Lee Y S, *Func Polym*, 50, **2002**, 257.
- 14 Atici O G, Ahmet A & Rahimian R, *Turk J Chem*, 25, **2001**, 259.
- 15 Martinz F & Neculqueo G M, *Bol Soc Chil Quim*, **2001**, 46, 124.