A Novel Synthesis and Characterization of Poly[4-imino(N-4-ethylbenzoate)benzene p-styrenesulphonate] and the Investigation on Polymer Ability for Drug Release

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ABSTRACT

The present paper describes the release of benzocaine from polymer carrier. The chemically controlled release method has been applied in which the benzocaine (drug) with active group (NH₂) links up with the spacer group (p-hydroxybenzaldehyde) by imine bond formation. The resulting schiff base reacts with the monomer p-styrenesulphonyl chloride. The obtained monomer is polymerized by AIBN as an initiator at the temperature range 60–70 °C. The obtained polymer was identified by IR and NMR spectroscopies. Gel permeation chromatography (GPC) was used for determination of average molecular weight of drug pendant polymer. The hydrolysis of drug polymer conjugates was carried out in cellophane membrane dialysis bag containing aqueous buffer solution (pH 1.2) at 37 °C. Detection of hydrolysis solution by UV spectroscopy showed that the drug can be released by hydrolysis of imine bond between the drug and spacer group.

Key Words: controlled drug release, synthesis, polymeric prodrug, benzocaine

INTRODUCTION

One of the most important methods to increase the therapeutic effects and decrease the side effects of drug is by controlled drug release. Therefore, it has attracted great interest in recent years and application of polymers has been also extended in this field [1]. Polymers are the most important and dominant biomaterials, because they can be designed and prepared in a wide range of compositions and properties, from hard and hydrophobic systems to highly hydrophilic and soft biomaterials when they are in contact with the physiological fluids [2]. Polymers may be used as carriers for pharmaceutical agents [3]. Therefore, the drug is released in certain parts of the body in a required dose and desirable specific rate which itself consists of various techniques [4].

Synthetic polymers used in biomedical applications are making a significant contribution to the progress currently being achieved in health care and in...
this regard the combination of pharmacologically active compounds with polymers via chemical reactions has been attracting an increasing degree of attention during recent years.

The major objectives in such studies are aimed at prolonging the duration of drug activity by controlling the release of drug. Bioactive agents have also been chemically bonded to preformed synthetic or naturally occurring polymers by allowing them, or one of their derivatives, to react with the polymers, functional groups [6—9]. An alternative to direct drug-polymer linkage is the incorporation of a spacer group between the drug and polymer chain. The use of suitable spacer arms can increase the mobility of drug on the polymer chain and enhance the sensitivity of conjugates to undergo chemical or enzymatic hydrolysis [10, 11].

The facility with which the drug can be converted to polymerizable derivatives depends to a large extent on their functionality. The value of the hydrolysis rate constant depends on the strength and chemical nature of the agent-polymer chemical bonds, the polymer structure and the surrounding condition [12, 13]. Recently hydrolyses of synthetic macromolecules with linked benzocaine has been studied [14—17].

In this work, chemically controlled release method has been applied in which the benzocaine (as drug) with active group (–NH₂) links up with the spacer group (p-hydroxy benzaldehyde) by imine bond formation. The resulting Schiff base reacts with the monomer p-styrenesulphonyl chloride, the obtained monomer is polymerized by AIBN as an initiator. Finally hydrolysis reaction was carried out in buffer solution (pH=1.2) at 37 °C. Scheme 1 depicts the reactions followed in this work.

**EXPERIMENTAL**

**Materials**
Benzocaine (obtained from Tehran Daru Pharmaceutical Co.); 4-vinylbenzenesulphonic acid sodium salt, p-hydroxybenzaldehyde (Fluka AG); cellophane membrane dialysis bag (Sigma); triethylamine (TEA), 2,2-azobisisobutyronitrile (AIBN), N,N-dimethyl formamide (DMF), buffer solution with pH=1.2 (HCl=0.08 mol/L, NaCl=0.0342 mol/L) all obtained from Merck.

**Instruments**
Infra-red spectra were taken on a shimadzu 435-U-04 spectrophotometer using KBr pellet. ¹H NMR spectra were recorded on a JEOL FT-NMR 90 MHz spectrophotometer using CDCl₃ and (CD₆)₂CO as solvent. Ultra-violet spectra were taken on a shmadzu UV-265 spectrophotometer. Molecular weight of polymers was determined with a Waters 150 GPC analysis instrument (mobile phase: THF; flow: 1.0 mL/min and column temperature: 30 °C).

**p-Styrenesulphonyl Chloride Synthesis**
p-Styrenesulphonyl chloride was synthesized as follows [13]; p-styrenesulphonyl chloride was prepared by the reaction of 5 g (0.024 mol) of 4-vinylbenzenesulphonic acid sodium salt with 7.5 g (0.036 mol) of PCl₃ [13].

**Monomer Synthesis**
Preparation of p-Imino(N-4-ethylbenzoate)phenol (I)
Schiff base (I) was prepared by heating a mixture of 4.05 g (0.0245 mol) benzocaine and 3 g (0.0245 mol) p-hydroxybenzaldehyde at 120 °C in oil bath for 15 min. The resulting powder was purified with solving it in CH₂Cl₂ at 40 °C and then cooling it in a refrigerator. The resulting crystals were separated and dried in vacuum. The yield was 75.8 % and melting point was 174–176 °C. IR and ¹H NMR spectra confirmed the structure of product (I).

Preparation of 4-Imino(A-4-ethylbenzonate)benzene p-Styrenesulphonate (II)
Monomer (II) was prepared by the reaction of Schiff base (I) and p-styrenesulphonyl chloride. Mixture of 4.74 g (0.0245 mol) of Schiff base (I), 3 mL triethylamine and 70 mL of acetone was poured in a three-necked flask equipped with a reflux condenser, dropping funnel, thermometer and a magentic stirrer. The solution was cooled to 0 °C and the prepared p-styrenesulphonyl chloride (3.57 g 0.0176 mol) dissolved in 20 mL of acetone was added to the reaction
\[
\begin{align*}
\text{Scheme I}
\end{align*}
\]
mixture.

The system was maintained in this condition for 6 h. Finally, the formed precipitate was separated, washed with acetone and dried. IR spectrum, solubility in water, chloroform and also melting point (245–252 °C) confirmed that the precipitate was triethylamine hydrochloride salt.

Acetone was evaporated from the remaining solution under vacuum and the residue was dissolved in chloroform. Then the amount of 25 mL of ether and water were added to the solution and the organic phase was washed with 5% aqueous NaOH and water, and finally it was dried with MgSO₄. The solvent was allowed to evaporate completely.

The product was washed with ethanol in order to purify it from initial materials like Schiff base, then separated and dried under vacuum. The yield was 71.2 %, based in initial salt. IR and ¹H NMR spectra confirmed the structure of product (II).

**Polymer Synthesis**

The prepared monomer (II) was polymerized in dried DMF at 60–70 °C in pyrex glass ampoules sealed off under high vacuum. Azobisisobutyronitrile (AIBN), was used as initiator ([I]=0.01 M). The sealed ampoules were immersed in a water bath held at the required temperature of polymerization. After the desired time (48 h), the ampoules were removed from the bath and the mixture was poured into a large amount of appropriate non-solvent (methanol). The precipitated polymer was collected and dried in vacuum [14]. The melting point of polymer obtained is 130 °C and the average molecular weight of polymeric prodrug from GPC is 33246. IR and H NMR spectra confirmed that the product obtained was poly[4-imino(N-4-ethylbenzoate)benzene p-styrenesulphonate] (III).

**Drug Release Study**

Drug release is in fact a hydrolysis reaction, which involves the break of imine bond in acid buffer (pH 1.2). The powdered polymer (35 mg) was poured in 1 mL of aqueous buffered solution (hydrochloric acid buffer pH 1.2) at 37 °C. The mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 99 mL of the same buffer solution maintained at 37 °C.

The external buffer solution was continuously stirred and 9 mL samples were removed at selected intervals and 9 mL of buffer was replaced. The quantity of hydrolyzate benzocaine was analyzed by means of a UV spectrophotometer and determined

<table>
<thead>
<tr>
<th>No</th>
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<th>Concentration of released drug (10⁻³ g/L)</th>
<th>Time (min)</th>
<th>Released drug (%)</th>
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<td>330</td>
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from the calibration curve obtained previously under the same conditions (Table 1).

RESULTS AND DISCUSSION

First, p-styrenesulphonyl chloride was synthesized. IR spectrum shows stretching vibrations of S=O bond at 1375 cm\(^{-1}\) and 1160 cm\(^{-1}\). The stretching vibrations of aromatic –CH at 3100 cm\(^{-1}\) and 1490 cm\(^{-1}\) have been observed.

In IR spectrum of p-hydroxy benzaldehyde there are stretching vibrations of OH at 3168 cm\(^{-1}\) and 3400 cm\(^{-1}\), aromatic C=C at 1475–1600 cm\(^{-1}\) and 2930–3000 cm\(^{-1}\) and aromatic C=O peak at 1700 cm\(^{-1}\).

In benzocaine IR spectrum there are stretching vibrations of NH\(_2\) at 3320 cm\(^{-1}\) and 3400 cm\(^{-1}\), aromatic and aliphatic C–H at 3050–3200 cm\(^{-1}\) and 2650–3000 cm\(^{-1}\) and aromatic C=C at 1475–1600 cm\(^{-1}\), and C=O peak at 1680 cm\(^{-1}\).

In ¹H NMR spectrum of benzocaine there are peaks at 1.3 ppm (t, 3, CH\(_3\)); 4.3 ppm (q, 2, CH\(_2\)); 3.8 ppm (s, 2, NH\(_2\)); 6.5–7.9 ppm (2d, 4, benzene ring).

In schiff base (I) spectrum, stretching vibrations of OH at 3400 cm\(^{-1}\), HC=N at 1608 cm\(^{-1}\), aromatic and aliphatic C–H at 3200 cm\(^{-1}\) and 2850–3000 cm\(^{-1}\), and C=O peak at 1720 cm\(^{-1}\) are observed, but stretching vibrations of NH\(_2\) has been eliminated. There are also aromatic C=C at 1436 cm\(^{-1}\) and 1568 cm\(^{-1}\).

In ¹H NMR spectrum (acetone d\(_6\)) of prepared schiff base there are peaks at 1.3 ppm (t, 3, CH\(_3\)); 4.3 ppm (q, 2, CH\(_2\)); 8.4 ppm (s, 1, CH=N); 9.2 ppm (s, 1, OH); 6.9–7.9 ppm (2d, 4, benzene ring).

In monomer (II) spectrum, stretching vibrations of aromatic and aliphatic C–H at 3120 cm\(^{-1}\) and 2970 cm\(^{-1}\) are observed, but the OH peak has been eliminated and stretching vibrations of the S=O bond at 1370 cm\(^{-1}\) and 1175 cm\(^{-1}\) have appeared instead. The C=O peak at 1700 cm\(^{-1}\), CH=N at 1630 cm\(^{-1}\) and aromatic C=C at 1590 cm\(^{-1}\) and 1500 cm\(^{-1}\) are produced as well.

In ¹H NMR spectrum (acetone d\(_6\)) of prepared monomer there are peaks at 1.3 ppm (t, 3, CH\(_3\)); 4.3 ppm (q, 2, CH\(_2\)); 8.56 ppm (s, 1, CH=N); 5.4–6.1 (q, 2, CH\(_2\)=CH–); 6.7–7 ppm (q, 1, CH\(_2\)=CH–); 7.2–8 ppm (m, 12, benzene rings).

The observed peaks at 2.01 ppm and 7.2 ppm are pertained to the solvents (acetone and chloroform) and peak at 2.8 ppm is pertained to impurity in solvent. The formation of the insoluble salt as a by-product confirms the proceeding of reaction. The spectrum shows stretching vibrations of C–H bond in CH\(_2\) and CH\(_3\) groups at 2930 cm\(^{-1}\), bending vibration of CH\(_3\) groups at 1390 cm\(^{-1}\) and CH\(_2\) groups at 1470 cm\(^{-1}\), the observation of the intensive peak at 2590 cm\(^{-1}\), confirming the insoluble salt as triethylamine hydrochloride.

Polymer Synthesis

IR spectrum of synthesized polymer (III) shows stretching vibrations of aliphatic C–H at 2897 cm\(^{-1}\) and aromatic C–H at 3408 cm\(^{-1}\), C=O at 1700 cm\(^{-1}\), aromatic C=C at 1491 cm\(^{-1}\) and S=O at 1366 cm\(^{-1}\) and 1166 cm\(^{-1}\). Stretching vibrations of aliphatic –CH\(_2\) peak at 1448 cm\(^{-1}\) identify the breaking of double bond and formation of polymer chain.

In ¹H NMR spectrum (CDCl\(_3\)) of synthesized polymer there are peaks at 1.26 ppm (CH\(_3\)); 4.28 ppm (CH\(_2\)=N); 7–8.1 ppm (benzene rings). All of the peaks at 0.8–3.47 ppm pertain to the polymer chain, because the observed peaks at 2.01 ppm and 7.2 ppm are pertained to the solvents (acetone and chloroform) and peak at 2.8 ppm is pertained to impurity in solvent. The formation of the insoluble salt as a by-product confirms the proceeding of reaction. The spectrum shows stretching vibrations of C–H bond in CH\(_2\) and CH\(_3\) groups at 2930 cm\(^{-1}\), bending vibration of CH\(_3\) groups at 1390 cm\(^{-1}\) and CH\(_2\) groups at 1470 cm\(^{-1}\), the observation of the intensive peak at 2590 cm\(^{-1}\), confirming the insoluble salt as triethylamine hydrochloride.

Figure 1. UV Spectrum of released benzocaine from polymer.
reaction was carried out from free radical polymerization and molecule is chiral and hydrogens are diastropic.

**Drug Release Tests**

*It has been widely demonstrated that the side chain hydrolysis of drug pendant polymers depends on the strength and chemical nature of the polymer structure and the surrounding condition. The hydrolysis of a linkage is also dependent on its distance from the polymer backbone [12].*

The length and hydrophilicity of the spacer unit between the drug and polymer chain can effect the release rate. In order to study the possible applications of this type of polymer as a pharmacologically active compound, we have studied from a chemical point of view the hydrolysis of the synthesized polymer in buffer solution with pH=1.2 at 37 °C.

The results are obtained on the basis of UV curves observation. The released drug concentration is proportional to measured absorption that is shown in Figure 1 at 37 °C. The λmax of benzocaine is at the wavelength of 226.1 nm. Quantitative measurements have been carried out using the standard curve (Figure 2). The obtained results are quoted in Table 1.

In Figures 3 and 4 the concentration and percentage of drug released as a function of time are shown.

**CONCLUSION**

*p-Imino(N-ethylbenzoate)phenol* (monomer I as a shiff base) was synthesized from reaction between a mixture of benzocaine and *p*-hydroxybenzaldehyde. 4-Imino(N-4-ethylbenzoate)benzene-*p*-styrenesulphonate (monomer II) was prepared by the reaction of monomer I and *p*-styrenesulphonyl chloride.

The monomer II was polymerized with AIBN. The melting point of polymer was 130 °C. The average molecular weight of obtained polymer was 33246. The hydrolysis of drug-polymer was carried in cellophane membrane dialysis bag containing aqueous buffer solution (pH=1.2) at 37 °C. Detection of
hydrolysis solution showed that the drug can be released by hydrolyzed of imine bond between the drug and spacer group.

REFERENCES