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Charge Transfer Interaction Between Styrene Maleic Anhydride With Dimethyl Sulphoxide: Characterization and Histopathology of The Product

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Styrene maleic anhydride co-polymer (SMA) belongs to a group of vinylic polymers. It follows the complex radical polymerization of vinyl aromatic styrene (donor) and maleic anhydride (acceptor). This co-polymer mixed with dimethyl sulphoxide in (1:2) ratio leads to its transformation, charge transfer interaction with dimethyl sulphoxide and form a polymer complex gel. It was found that the dissolution of co-polymer in dimethyl sulphoxide leads to its transformation on charge transfer interaction with dimethyl sulphoxide and subsequent Pummerer rearrangement. The detailed structural study of the polymer complex was done by using molecular spectroscopy. The co-polymer complex shows bio-medical property as it effects on the erythrocyte, Hb, TLC, DLC, platelets, *etc*.

Key Words: Charge transfer interaction, Co-polymer, Dimethyl sulphoxide, Histopathology, Polyelectrolytic complex.

INTRODUCTION

The copolymerization of styrene and maleic anhydride has been extremely studied over many years. Co-polymers of maleic anhydride with styrene, vinylic chloride and ethylene are available commercially[1]. Copolymerization of maleic anhydride (MA) and styrene provides a co-polymer with strong electron accepting ability. On the other hand, it is known that DMSO is not only widely used as a solvent but it can also act as an electron-donor during complex formation with different agents[2]. When this co-polymer is mixed with dimethyl sulphoxide, it forms a polyelectrolyte (intermediate) gel as an unstable complex[2,3]. To date less is known about the charge transfer interaction of DMSO with anhydride. Styrene maleic anhydride (SMA)-DMSO complex behaves as a pH lowering polymer as well as it has antibacterial activity against *E. coli*[4]. The ethereal oxygen of maleic anhydride is very reactive for pure DMSO, these ethereal oxygen also helps in the formation of SMA derivatives with amines[5], hydroxy compounds and aliphatic alcohols[6] by half esterification reaction, all were studied earlier. This SMA-DMSO complex has some bio-medical importance recently it has also been used in as a male contraceptive[7], nasal and optic drug.

In the present communication, the detailed structural study of co-polymer complex and its histopathology *viz.*, effect on erythrocyte, Hb, platelets and TLC, DLC are reported.

EXPERIMENTAL

Styrene (Aldrich) was washed with aqueous sodium hydroxide solution then distilled water followed by drying over anhydrous calcium chloride and finally distilled under vacuum for further use. Maleic anhydride was purified before use by recrystallization from anhydrous benzene and sublimation in vacuum. Benzoyl peroxide (BPO) of analytical grade was purified by recrystalization in chloroform and methanol mixture. Pure DMSO (spectroscopic grade) was used for the synthesis and the used solvent acetone was analytical grade. All the chemicals used were analytically pure and (S.D. Fine) grade.

An infrared spectrum of complex polymer was recorded on Perkin-Elmer spectrum RX1 FTIR in range of 4000-450 cm^{-1}, pellets were prepared by mixing of complex with KBr. ^1H NMR spectra of the polymer complex was recorded on 500 MHz-FT NMR spectrometer.

Hematological study: All the test were done on a digital Boehringer Mannheim Diagnostics, HC-555 Machine.

General procedure: Synthesis of co-polymer with different compositions, were carried out in a 250 mL round bottom flask with 100 mL of acetone as solvent and 0.1 g (0.4 mmol) of benzoyl peroxide as an initiator and it was mixed with 10 mL (0.086 mol) of styrene and 9.8 g (0.1 mol) of maleic anhydride. The mixture was stirred at room temperature for *ca.* 0.5 h till a homogenous solution was obtained. The mixture was then heated up to 80 ºC and stirred for 1 h. It was then cooled to room temperature. The cooled mixture was poured into a large volume of diethyl ether to precipitate the polymer product. These were obtained in white powder form after drying under vacuum at 50 ºC *in vacuo* overnight to constant weight. The yield was about 95 % and the calculated molecular weight was 22000 Daltons. The molecular weight of poly (maleic anhydride-co-styrene) was calculated from the single point viscosity of its acetone solution at 25 ºC using the following equation[8]:

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

Softening point measurements were carried out with a melting point apparatus (Buchi). The sample was placed into a capillary glass tube and its softening point was measured.

Thereafter, the co-polymer powder was mixed with DMSO in a 1:2 mole ratio, at room temperature and kept in desiccator with P_2O_5 for 10 d. DMSO was removed by vacuum evaporation. This resulted in the form of an adduct complex. Elemental analysis for carbon, hydrogen and sulfur of SMA-DMSO were conducted: C-31.34 %, H- 6.41 %, S-18.13 %.

Histopathology: For histopathological investigations 30 male Charles foster stain of albino rat of about 175-200 g body weight were used for the study. 15 days quarantined (acclimatized) rats were kept in husbandry condition of 22-25 ºC room temperature, 50-70 % relative humidity and 12 h light and 12 h dark photoperiod. The animals were kept in identical environmental condition and were mentioned in pellet diet and water (24 h). The selected 24 male rats were divided into 4 groups of 6 rats, II, III, IV group served as treated, while Ist group served as control group.

For implantation of the complex, lower abdominal area was chosen, minor incision with the help of sharp sterile blade was given of the size 0.5 cm.The polymer complex was injected into the lumen. Different doses of complex as stated in (Table-1) were given and skin wound was stitched, dressed up neosprin antibiotic powder, all the rats were kept under strict watch till the healing was completed. Different hematological parameters were recorded in the control as well as the complex treated rats as follows: total leukocyte count (10^3/cumm), total erythrocyte count (RBC) (10^6 cumm), Hb g %, platelet count (10^3 cumm) (Table-1). Liver was removed for histological examination. All readings were obtained by standard methods of histopathology.

RESULTS AND DISCUSSION

The prepared complex gel is colourless having molecular formula $C_{14}H_{16}O_4S$. Co-polymer complex gel is highly soluble in large amount of DMSO, slightly soluble in alcohol, benzene, less soluble in toluene and insoluble in water.

It is well known that both St and Ma copolymerizes usually in an alternating way resulting in a poly (St-Ma) alternating polymer (SMA) with a highly regular structure. SMA is a type of important functional co-polymer as its anhydride group on backbone chain can react with other reagent, such as alcohol, amine, water DMSO, *etc*. to produce many derivatives. To ascertain the structure of the polymer complex, both FTIR, ^{1}H NMR, ^{13}C NMR, spectra have been recorded.

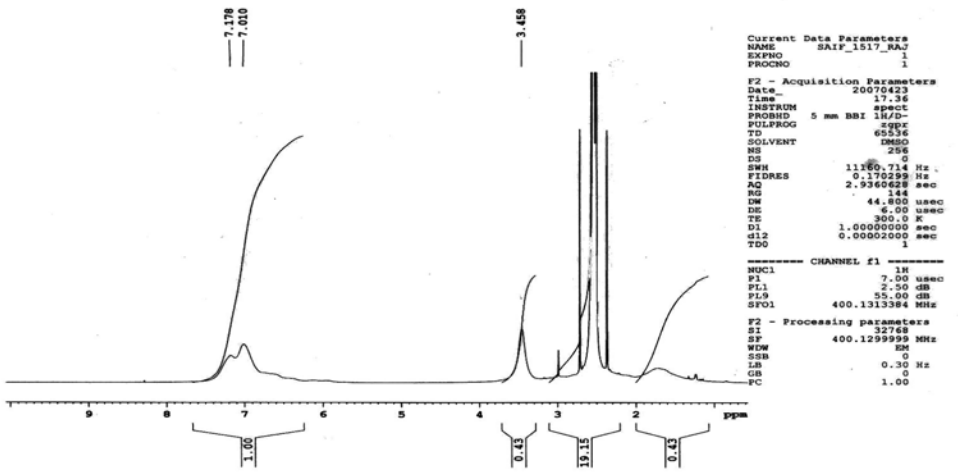
FTIR spectra was investigated for co-polymer complex. To obtain spectra with better signal to noise ratio, especially for weak bands, the measurements in the transition mode was also performed. Absorption peak at 1742 cm^{-1} which are characteristic absorption of C=O (*str.*), vibration of ester group. In the FTIR spectrum of the co-polymer complex the peak shows the higher conversion of anhydride to the ester group (1750-1735 cm^{-1}). After the reaction of the anhydride with DMSO, another peak at 1650 cm^{-1} clearly seen in the spectra which represent enolic form of the ester while peak at 1021 cm^{-1} due to C-O (*str.*) are also observed which are generally present in all the esters. In this case co-polymer chain becomes less rigid and it proved that the ester group is formed after the reaction. Peaks at 1461 cm^{-1} is indicative of C=C (*str.*) of the aromatic ring, 1561 cm^{-1} represents carboxylate ion, the absorption peak at 3020 cm^{-1} (=C-H *str.* for aromatic fragments). 1423, 762 cm^{-1} shows that (-CH_2) and mono substituted aromatic ring respectively.

Consequently, the polymer complex was ultimately characterized by spectral analysis using 500 MHz FT-NMR spectrometer. The ^{1}H NMR spectra recorded

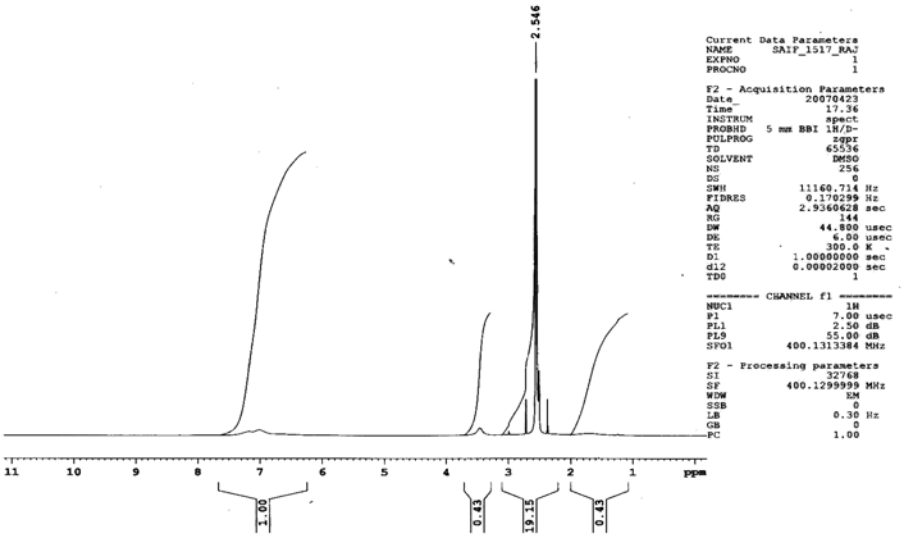
immediately after mixing of co-polymer in DMSO-d_6, are characterized by the following broad signal at δ, the chemical shift of phenyl proton is about δ 7.010, 7.178 ppm and that of the -CH and -CH_2 groups on the backbone chains are at δ 3.48 and δ 2.546 ppm, respectively (Fig. 1A,B).The mole fraction of styrene in the co-polymer was obtained by comparing the integration intensity I, of phenyl proton with that of the total proton[9].

$$\chi = \frac{2\,\mathrm{I\ phenyl}}{5\,\mathrm{I\ total} - 6\,\mathrm{I\ phenyl}} = 0.49$$

This value is consistent with the alternating structure reported in the literature. Beside the ^{13}C NMR spectrum of the polymer gel complex showed peaks at 204 ppm due to carbonyl carbon of maleic anhydride, peak at 125, 126, 128, 129, 137, 139 ppm due to aromatic carbon of styrene and 13, 21, 33, 34, 44 ppm due to aliphatic carbon, respectively. Energy dispersive X-ray spectroscopy has been carried out to determine the amount of sulfur relating to the amount of DMSO in the complex. The value of 10.6 is shown to be consistent with various concentration of the SMA in DMSO. According to the **Scheme-I** several steps are involved in the mechanism of the co-polymer reaction with DMSO. The first step is the charge transfer interaction between carboxylic oxygen as electron acceptor and sulfoxide group of DMSO as an electron donor, thereafter an ionic intermediate in which an unstable counter ion close to each other is formed. The latter undergoes tautomeric transformation by proton transfer from methyl group of DMSO to the carboxylic oxygen, as shown in the **Scheme-I**. It is known that simple dialkyl sulphoxide in the presence of strong neucliophilic agent such as anhydrides of carboxylic acids can posses Pummerer type rearrangement[10].

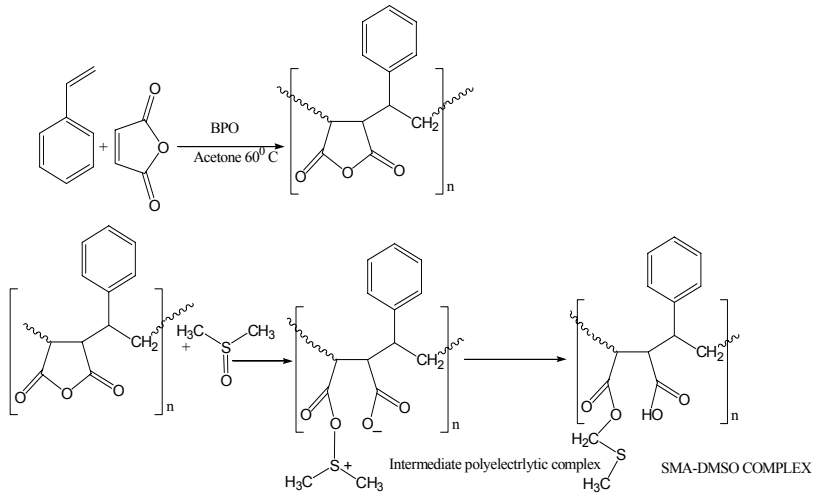


(A)



(B)

Fig. 1A,B. ^{1}H NMR spectra of polymer complex



Scheme-I: Mechanism of the adduct formation

Histopathological study has revealed that the total leucocytes count increased 7.6-2.1 % at low dose, 13.6-7.8 % at mid dose and 17.0-10.5 % at high dose in mid term and final reading was recorded in treated group rats in comparison to control dose. While red blood cell count also increased in treated group rats below control group rats. RBC increased 9.6-5.5 % in low dose, 10.3-12.2 % at mid dose and 14.8 % at high dose in mid term, hemoglobin also increased 6.3-6.7 % at low dose 14.64-10.5 % at high dose, pellet count and differential leucocytes count are also increased in comparison to control dose of control group rats (Table-1).

TABLE-1
HAEMATOLOGICAL PARAMETERS (MEAN, ± SD) OF SMA-DMSO COMPLEX TREATED RATS

Parameter		Group-I			Group-II			Group-III			Group-IV		
		Initial	Mid term	Final	Initial	Mid term	Final	Initial	Mid term	Final	Initial	Mid term	Final
TLC		6.30	5.88	5.98	6.00	6.33	6.11	5.91	6.68	6.45	6.10	6.88	6.61
$\times 10^3$ cumm		0.79	0.91	0.63	0.93	0.96	0.59	0.57	0.82	0.68	0.69	1.16	1.10
RBC		6.34	5.60	5.76	6.27	6.14	6.10	6.04	6.18	6.50	6.19	6.43	6.68
$\times 10^6$ cumm		1.00	0.57	0.62	0.79	0.50	0.78	0.67	0.62	0.52	0.60	0.82	0.53
Hb.		14.36	12.63	13.80	12.56	13.43	14.73	12.46	13.58	14.66	13.25	14.48	15.26
g (%)		1.47	1.88	1.92	1.39	1.62	1.64	1.04	1.35	1.50	1.51	1.24	0.95
Plt.		619	612	622.83	619	612	622.83	638.5	674.83	67.10	582.83	705	694.5
$\times 10^3$ cumm		89.18	86.40	74.14	89.18	86.40	74.14	84.52	90.14	67.15	65.58	165.8	70.74
DLC (%)	P	21.83	21.33	22.16	24.66	26.00	22.50	19.33	22.50	24.33	18.83	23.00	24.66
		4.26	4.17	6.61	3.88	7.40	6.25	6.25	4.17	2.20	3.48	5.60	8.14
	L	79.33	75.33	73.66	72.50	69.66	73.16	77.5	74.5	71.66	77.83	75.5	72.16
		6.74	5.71	7.86	5.52	6.77	5.74	3.61	7.14	3.71	3.71	6.71	8.32
	M	1.50	2.0	1.5	1.5	1.5	2.0	2.0	1.66	2.50	2.16	1.50	1.66
		0.54	0.89	0.40	0.40	0.83	0.48	1.09	0.81	1.04	0.75	1.04	1.03
	E	0.83	0.83	0.50	0.50	0.66	0.66	0.66	0.83	0.10	0.50	0.83	0.83
		0.75	0.75	0.54	0.54	0.81	0.51	0.51	0.75	1.89	0.54	0.75	0.75
	B	0.50	0.50	0.33	0.50	0.50	0.66	0.66	0.50	0.50	0.66	0.50	1.66
		0.54	0.54	0.50	0.50	0.54	0.51	0.51	0.54	0.54	0.51	0.54	1.51

P = Neutrophils, L = Lymphocytes, M = Monocytes, E = Eosinophiles, B = Basophiles

Dose schedule-			
	Group-I	Control	0.03 mL DMSO
	Group-II	Low Dose	0.5 mg SMA + 0.03 mL DMSO
	Group-III	Mid Dose	2.0 mg SMA + 0.03 mL DMSO
	Group-IV	High Dose	4.0 mg SMA + 0.03 mL DMSO

Conclusion

For the SMA-DMSO interaction is more efficient in pure DMSO and probably further transformation of charge-transfer complexes takes place. The sulphur moiety of pure DMSO is highly reactive. When styrene maleic anhydride (SMA) is mixed with this particular form of DMSO the sulphur moiety of DMSO interacts with the etheric oxygen of the maleic anhydride moiety of the SMA thereby leading to the formation of an intermediate unstable complex of SMA and DMSO. The carbonyl oxygen of SMA being resonance stabilized is not affected. FTIR studies proved that reality of the reaction between co-polymer (SMA) and DMSO (**Scheme-I**). As it follows from above **Scheme-I**, the reaction results in the increase of the local concentration of COO^- group in unit of transformed polymer, Hence, the relative intensity at 1644 cm^{-1} increases and the intensities of the peaks at 1777 and 1709 cm^{-1} due to C=O (*str.*) decrease. It is presumed that the results obtained fully agree with the mechanism.

In histological study, the polymer complex has one exposure level, NOEL (No observable adverse effect level) the high dose that did not cause any clinical adverse effect.

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