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Kinetics of Disulphide–Disulphide Interchange Reactions in Polysulphide Polymers by Mass Spectrometry. I

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ABSTRACT

A novel analytical method using Electron Ionization Mass Spectrometry (EI/MS) was developed for studying the kinetics of disulphide–disulphide interchange reactions in polysulphide polymer blends. During the disulphide–disulphide interchange process, the concentration or reactants and products were measured from the observed peak heights of the respective dimer units. Reactions were studied in melt blends, over a temperature range of 207–220°C. An activation energy of 79.80 $\mathrm{KJmol^{-1}}$ and pre-exponential factor $\log A = 2.54$ was observed. The dominant relaxation process for the intermolecular disulphide–disulphide interchange reaction in the polymer blend, without the influence of solvents and other reagents, is assumed to follow an associative mechanism. The model polysulphide polymers A (PS1) and B (PS2), used in this study, were formulated from bis (2-chloroethyl) ether and bis (2-chloroethoxy) methane, respectively.

Key Words: Disulphide; Polysulphide; Kinetics; Mechanism Electron-ionization; Mass spectrometry; Disulphide–disulphide interchange.

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INTRODUCTION

Thiol functionalities, together with disulphides and other higher rank sulphur bridges structured into alkyl polysulphides, peptides, proteins, and synthetic polysulphide polymers, have been studied as a means of probing the influence of thiol–disulphide, disulphide–disulphide and other such interchange reactions.[1–4] For example, vulcanizates in natural rubber, copolymerization of methylmethacrylate onto wool, disulphide crosslinking of nylon and other systems containing thiol functionalities and polysulphide bridges, undergo thiol–disulphide interchange as the controlling stress relaxation mechanism.[5–8] Compounds capable of undergoing mixed disulphide interchange with disulphide bonds have been explored as therapeutic agents for the treatment of HIV infection. Cystamine and cysteamine were found to protect HIV infected human T lymphocytes from the effects of viral infection due to the disulphide–disulphide interchange process.[9] Because of the consequence of such interchange reactions on the physiochemical and biochemical properties of synthetic and biopolymers, various analytical methodologies have been explored in order to gain insights into the kinetics and mechanism of such reactions. Spectrophotometric and Nuclear Magnetic Resonance (NMR) methods, for example, enabled kinetic parameters, structure reactivity correlations and plausible reaction mechanism to be established. The kinetics of thioltransferase catalyzed glutathione/glutathione–disulphide interchange reaction was investigated by ^{1}H Nuclear Magnetic Resonance Spectroscopy, wherein the exchange broadened multiplet patterns and inversion magnetization transfer, enabled a measure of concentration of reactants and products in real time. In addition, rate constants and relevant kinetic parameters were established for various model compounds.[10] Dispersive X-ray techniques, and FTIR, were employed in the study of structural modifications of quartz surfaces by the thiol–disulphide interchange process.[11] Mass spectrometric techniques incorporating ionization processes such as Electrospray (ESI), Matrix Assisted Laser Desorption (MALDI), and Liquid Chromatography/Mass Spectrometry, have been applied in the study of thiol–disulphide interchange in various biochemical systems.[12–19] Electron Ionization (EI/MS), using the dimer analysis method, was applied to the study of ester–ester interchange reactions in polyesters.[20–22]

Unlike thiol–disulphide interchange, disulphide–disulphide interchange involves the intermolecular reaction of two different homopolymers to produce a randomized copolymer viz.:

$$\sim\sim\sim\ \mathrm{RSSR}\ \sim\sim\sim\ +\ \sim\sim\sim\ \mathrm{R^1SSR^1}\sim\sim\sim\ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}\ 2\ \sim\sim\sim\ \mathrm{R^1SSR}\sim\sim\sim \qquad (1)$$

This class of disulphide–disulphide interchange reaction particularly in the absence of catalysts and other reagents has not been extensively studied.[3–4] Although the integrity of number average molecular weight $\bar{M}n$ is maintained, this disulphide–disulphide interchange reaction lead to the randomization of structural units, with corresponding changes in the weight-average molecular weight $\bar{M}w$. Consequently, the kinetics of such reactions could also be followed by viscosity and melting point methods. Likewise, the resultant heterolinkages in the exchanged product open the way for following these reactions by Nuclear Magnetic Resonance (NMR), and Mass Spectrometry.

This study seeks to define the kinetics of disulphide–disulphide interchange reactions in polysulphide polymer melt blends, using Electron ionization mass spectrometry (EI/MS). The model polysulphide polymers A (PS1) and B (PS2) used in this study were formulated from bis (2-chloroethyl) ether, and bis (2-chloroethoxy) methane. By controlling the sulphur rank, degree of polymerization, and removing all thiol end-groups, it was assumed that the dominant interchange process would proceed by way of the disulphide linkages along the respective polymer chains. While this novel mass spectrometric analytical procedure offers the advantage of studying these interchange reactions in polymer blends free of thiol functionalities, catalytic reagents and solvents, thermal stability and molecular weights were critical to the overall success. The model polysulphide polymers A (PS1) and B (PS2) used in this study were thermally stable up to 250°C in a nitrogen atmosphere; molecular weights were 4,200 and 4,350, respectively. Reactions were carried out in melt blends over the temperature range of 207–219°C and mass spectra were acquired at a source temperature of 180°C.

THEORY

Disulphide Equivalents "D"

Disulphide equivalents which defines the average number of disulphide linkages in a given polysulphide polymer, is directly related to the degree of polymerization $\bar{P}$. Thus during disulphide–disulphide interchange reaction between two different polysulphide polymers A and B, any variation in disulphide equivalent will be related to $\bar{P}$ as follows:

$$\frac{\bar{M}_A/m_a}{\bar{M}_B/m_b} = \frac{m_a\bar{P}_A}{m_b\bar{P}_B} \tag{2}$$

Where $\bar{M}_A$ and $\bar{M}_B$ are the molecular weights of reactants A and B, while m_a and m_b are the molecular weights of repeat units a and b, respectively.

$$\mathrm{A(PS1)} = \sim [\mathrm{CH_2CH_2OCH_2CH_2SS}]_a \sim \tag{3}$$

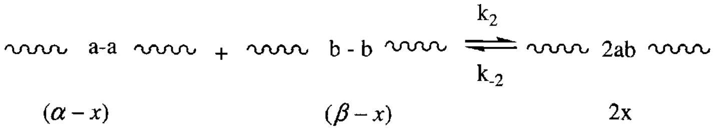
$$\mathrm{B(PSI)} = \sim [\mathrm{CH_2CH_2OCH_2OCH_2CH_2SS}]_b \sim \tag{4}$$

For an intermolecular disulphide–disulphide interchange process, maximum randomization is attained when $D_A/D_B = 1$ in the homopolymers before reaction. Initially, therefore, if $P_A \neq P_B$, then D_A/D_B would be less than 1 and would vary during the interchange reaction.

Kinetic Expression

During the disulphide–disulphide interchange process, the statistical distribution of structural units would be in the ratio of aa:bb:2ab, as 1:1:2 for complete randomization.

Therefore, the kinetic expression derived for an opposing bimolecular reaction is as follows:



$$\sim\sim\text{a-a}\sim\sim + \sim\sim\text{b - b}\sim\sim \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \sim\sim 2\text{ab}\sim\sim \qquad (5)$$

$$(\alpha - x) \qquad (\beta - x) \qquad 2x$$

$$dx/dt = k_2(\alpha - x)^2 - \mathrm{k}_{-2}(2\mathrm{x})^2 \qquad (6)$$

$$\int_0^{\mathrm{t}} dt = \int_0^{\mathrm{x}} \frac{dx}{k_2(\alpha - x)^2 - k_{-2}(2x)^2} \qquad (7)$$

As t tends to $\mathrm{T}_{\text{infinity}}$, x tends to x equilibrium. Thus the equilibrium constant K is expressed as:

$$K = \frac{k_2}{k_{-2}} = \frac{4x_e^2}{(\alpha - x_e)^2} \qquad (8)$$

Upon substitution for K in Eq. 7, the following expression is defined:

$$t = \frac{1}{k_{-2}} \int \frac{dx}{K(\alpha - x)^2 - 4x^2} \qquad (9)$$

Integration by partial fractions gives the final expression for an opposing bimolecular reaction:

$$t = \frac{1}{8k_{-2}} \left(\frac{1}{x_e} - \frac{1}{\alpha}\right) \ln\left[\frac{1 + x/x_e - 2x/\alpha}{1 - x/x_e}\right]$$

For statistical equilibrium: $\alpha = 2x_e$

$$\therefore \frac{k_2}{k_{-2}} = K = \frac{4x_e^2}{(2x_e - x_e)^2} = 4 \qquad (11)$$

Substituting for χ_5 in equation 10, gives the expression:

$$t = [2.303/8k_{-2}\alpha|]\log[\alpha/(\alpha - 2x)|] \qquad (12)$$

Rate constants are established from plots of $1/\alpha \log \alpha/(\alpha - 2x)$ as a function of time, while activation energies (E) and pre-exponential factors (log A) are determined from plots of log k_{-2} against 10^3 K/T as defined by the Arhenius equation.

$$k_{-2} = Ae^{-Ea/RT} \qquad (13)$$

Instrumentation

A VG-ZAB-HF mass spectrometer interfaced to the OPUS Data System, and an HP1100 gas chromatograph were employed in this study. A secondary oven was constructed within the main oven of the gas chromatograph in order to accommodate the Pyrex glass reactor and to enable sampling of the reaction mixture without causing temperature fluctuations, during the sample withdrawal phase.

EXPERIMENTAL

Preparation of Polysulphide Polymers

The polysulphide polymers (A) PS1 and (B) PS2, were synthesized by condensation of (a) bis (2-chloroethyl) ether to give PS1, and (b) bis (2-chloroethoxy) methane to give PS2.

To the reaction mixture containing 4.8 mols of sodium disulphide, 20 mls of sodium alkylnaphthalene sulphonate, 17 mls of sodium hydroxide (50%); and 117 mls of 25% magnesium hexahydrate, was added 4 moles of dichloride monomer over a period of 60 min. Maintained at 80°C, the solution was continuously stirred for another 60 min, then allowed to settle. After decanting the supernatant liquor, the polymer latex was washed with hot water until a negative lead acetate test was confirmed. This high molecular weight product was then converted to lower molecular weight thiol terminated fractions by treating with sodium hydrosulphide dihydrate and sodium sulphite at 85°C for 60 min. The solution was adjusted to pH6 using acetic acid then washed with hot water and dried at 100°C under vacuum. Molecular weight was determined using the silver nitrate titration method. In order to maintain the integrity of disulphide linkages, the product was reacted with acetylchloride to remove all thiol end groups. Finally, excess acetyl chloride, HCl by product and water was removed under vacuo at 100°C.

Results:

$$\mathrm{A(PS1)} = \sim [\mathrm{SCH_2H_2OCH_2CH_2}]_n \sim \quad \bar{M}W = 4200$$

$$\mathrm{B(PS2)} = \sim [\mathrm{SCH_2CH_2OCH_2OCH_2CH_2S}]_n \sim \quad \bar{M}W = 4350$$

Procedure

For kinetic studies, the glass reaction vessel housed in the HP1100 gas chromatograph, continuously purged with a stream of dry nitrogen gas, was equilibrated to the desired reaction temperature. About 1 gram of polymer blend consisting of 1 part A to 1.18 parts B, previously homogenized under nitrogen at 50°C, was transferred to the reaction vessel and allowed to react. About 100 μg of sample was withdrawn at predetermined time intervals and analyzed by direct probe EI/MS. Changes in concentration of reactants and products were determined from a measure of the ion current observed for the respective dimer units at m/z 272, 302, and 332. The mass spectrometer source temperature was maintained at 180°C while the water-cooled direct probe was allowed to stabilize to the source temperature. The resolving power of the mass spectrometer was maintained at 2000 (10% valley definition), and the ionization energy was maintained at 60 ev. Data acquisition was at a scan rate of 5 seconds/decade in mass, over a mass range of 80 to 800 daltons. The analysis time was 125 seconds (25 scans) per sample point. These experimental conditions were maintained for the entire study.

RESULTS AND DISCUSSION

Disulphide Equivalence

Disulphide equivalence for the polysulphide polymers A (PS1) and B (PS2), were determined from the observed average molecular weights:

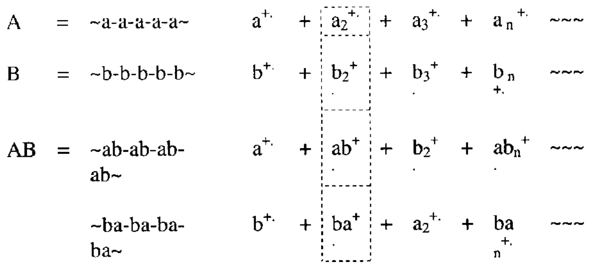
$$\bar{M}_A = 4200 \quad \bar{D}_A = 30.88$$

$$\bar{M}_B = 4350 \quad \bar{D}_B = 26.20$$

For the interchange reaction to equilibrium, the integrity of disulphide equivalence where $\bar{D}_A = \bar{D}_B$ in the polymer blend before reaction is desirable in order to facilitate complete randomization of structural units to yield an alternating copolymer. Therefore, by melt blending the two homopolymers, 1 part A to 1.18 parts B, $\bar{D}_A/\bar{D}_B = 1$, the reaction product at equilibrium should give the presumptive alternating copolymer $\sim$ababab$\sim$.

Mass Spectrometry

The analytical procedure developed for defining the qualitative and quantitative nature of reactants and products during disulphide–disulphide interchange reaction is based on the behavior of these polymers when subjected to EI/MS conditions. Typically, these polymers will undergo retropolymerization in the gas phase producing monomers, dimers, trimers, and other higher mass product ions in the following manner:



(10)

Before melt blending of polymers A and B, the mass spectra observed, Figs. 1 and 2, show the dominance of $RSS^{+\cdot}$, m/z 136; $RSSRSS^{+\cdot}$, m/z 272; $R^1SS^{+\cdot}$, m/z 166; and $R^1SSR^1SS^{+\cdot}$, m/z 332, where the base peaks are observed at m/z 136 and m/z 166, respectively. For the melt blend of polymers A + B, where the integrity of

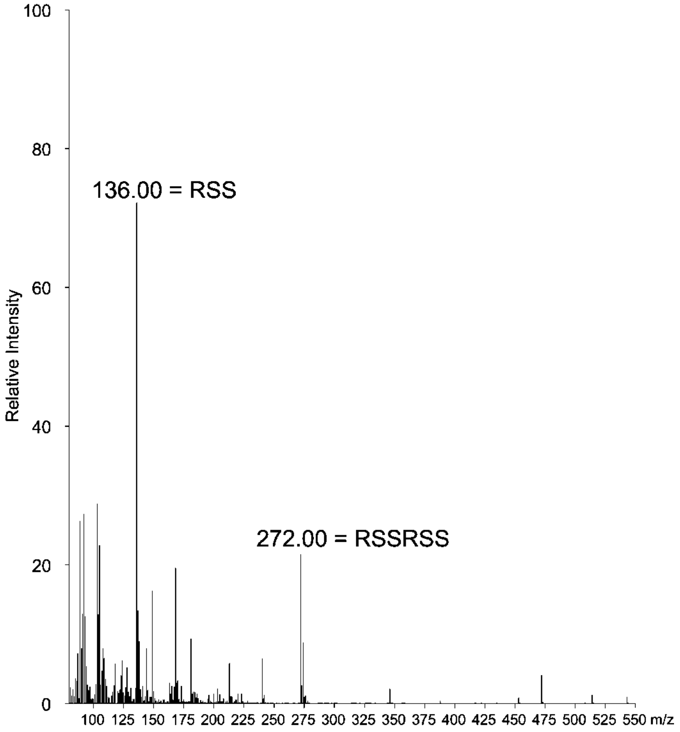


Figure 1. 60 ev mass spectrum of the homopolymer A (PS1) at probe temperature of 180°C.

disulphide equivalence $D_A = D_B$ is maintained, the mass spectrum observed at T_o min, Fig. 3, exhibits a base peak at m/z 166 due to $R^1SS^{+\cdot}$, while the dimer product ions $RSSRSS^{+\cdot}$ and $R^1SSR^1SS^{+\cdot}$ are observed at 20% and 30% relative intensities. However, at $T_{infinity}$ and a constant temperature of 207°C, the characteristics of the mass spectrum changed, revealing the presence of the equilibrium product $R^1SSRSS^{+\cdot}$ at m/z 302 as shown in Fig. 4. These results, while providing the identity of the respective monomers and dimers, for reactants and products, are fundamental to the experimental design necessary for following these intermolecular interchange reactions to equilibrium. Accordingly, changes in concentration of reactants and products during the disulphide–disulphide interchange process, were readily followed by measuring the observed ion currents of a_2, b_2 and ab (dimer units), as a function of time. Here, it is assumed that the observed ion current for the respective dimer units I_{a2}, I_{b2}, and I_{ab}, are proportional to the concentration of A, B, and AB in the reaction mixture. However, to compensate for differences in ionization efficiencies, due to physicochemical properties affecting sensitivity to ionization, relative sensitivity coefficients "(S)" were computed. The mass spectrum Fig. 4, observed for the equilibrium product at $T_{inifinity}$, and constant temperature of 207°C, shows I_{a2}, 32.2%; I_{b2}, 48.5%; and I_{ab}, 55%. Since the reaction to equilibrium is expressed

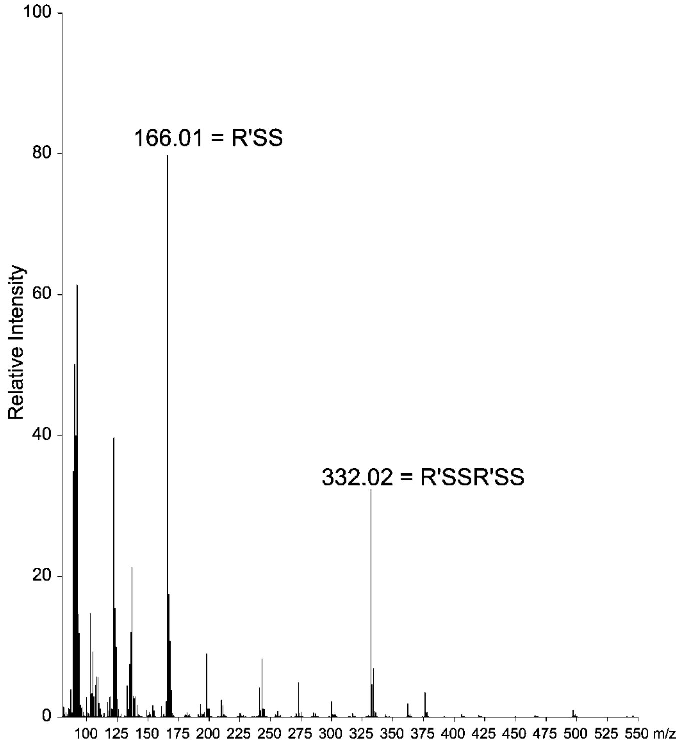


Figure 2. 60 ev mass spectrum of the homopolymer B (PS2) at probe temperature of 180°C.

stoichiometrically as:

$$\sim \text{aa} \sim + \sim \text{bb} \sim \rightleftharpoons \sim 2\text{ab} \sim$$

Then: $I_{a2}S_A + I_{b2}S_B = 2\,I_{ab}S_{AB}$ by setting $S_{B=}1$, S_A and S_{AB} were calculated to be 1.5, and 0.88 respectively.

Kinetic Analysis

Rate constants for the disulphide–disulphide interchange reaction, Table 1, and Fig. 5, were established from graphs of $1/\alpha \log \alpha/(\alpha - 2x)$ as a function of time in accordance with the kinetic expression-12, derived for an opposing bimolecular reaction. Slopes of these curves gave the rate constant k_{-2}, while k_2 was determined from Eq. 11. The observed activation energy of 79.80 KJ mol^{-1}, and frequency factor $\log A = 2.54$; were determined from the graph of $\log k - {}_2$ against 10^3 K/T, Fig. 6, in accordance with the Arhenius Eq. 13.

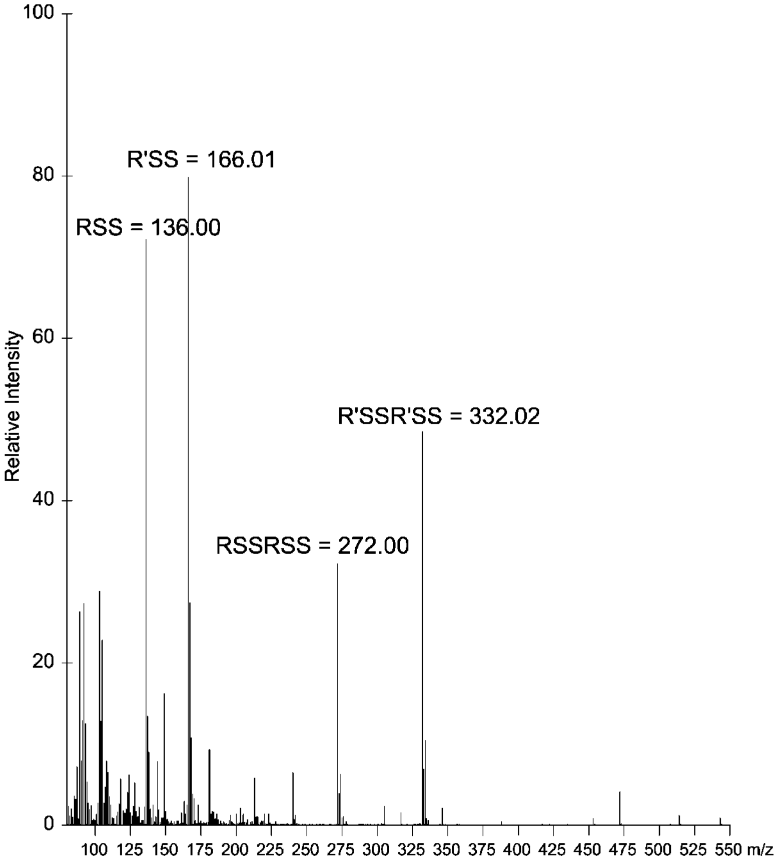


Figure 3. 60 ev mass spectrum of polymer blend 1 part A to 1.18 parts B at T_o before reaction.

Given that the reaction to equilibrium is expressed stoichiometrically as:

$\sim aa \sim + \sim bb \rightleftharpoons \sim 2ab \sim$

and assuming that the randomization of structural units is a statistical process, then a-a, b-b, and a-b units can be produced in the following way:

If the probability of selecting an "a" unit = P_a

and the probability of selecting a "b" unit = P_b

Then: $P_a = P_b = 1/2$

Therefore: $P_{aa} = P_a \times P_a = 1/4$

$P_{bb} = P_b \times P_b = 1/4$

$P_{ab} = P_a \times P_b + P_b \times P_a = 1/2$

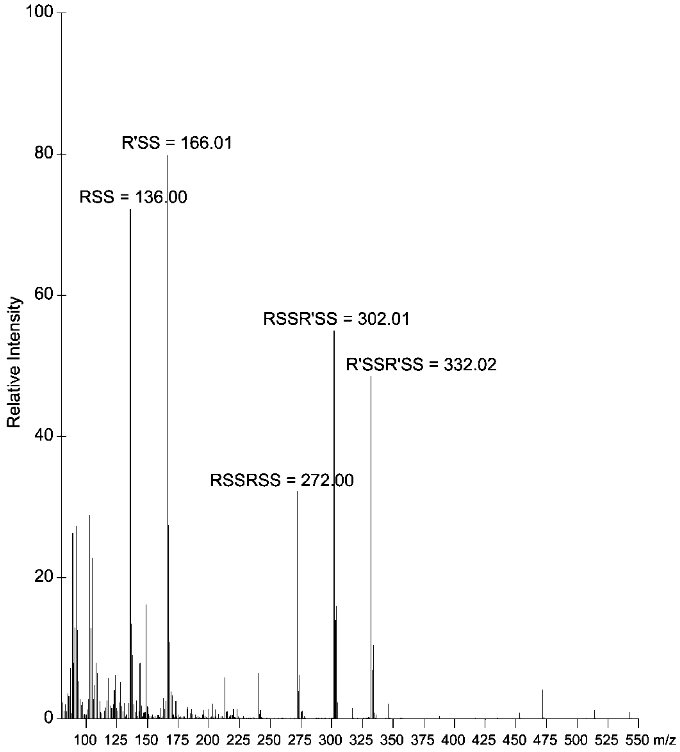


Figure 4. 60 ev mass spectrum of polymer blend, 1 part A to 1.18 parts B at equilibrium, T_{infinity}.

Therefore, for a statistical equilibrium:

$$K = \frac{1/2 \times 1/2}{1/4 \times 1/4} = 4$$

This is consistent for the equilibrium constant defined for an opposing bimolecular reaction expressed by Eq. (11).

Table 1. Observed rate constants within the temperature range of 480 to 492°K for the disulphide–disulphide interchange reaction between polymers A and B.

$T°_K$	$l/T°_K$	k_{-2} (dm^3 mol^{-1} sec^{-1}	log $_{-2}$	$k_2$dm^3 mol^{-1} sec^{-1}	log k_2
480	2.083×10^{-3}	8.208×10^{-7}	-6.0858	3.2832×10^{-6}	-5.5479
484	2.066×10^{-3}	9.487×10^{-7}	-6.0229	3.7948×10^{-6}	-5.4202
488	2.049×10^{-3}	11.299×10^{-7}	-5.9473	4.5156×10^{-6}	-5.3449
492	2.032×10^{-3}	13.325×10^{-7}	-5.8754	5.3300×10^{-6}	-5.2733

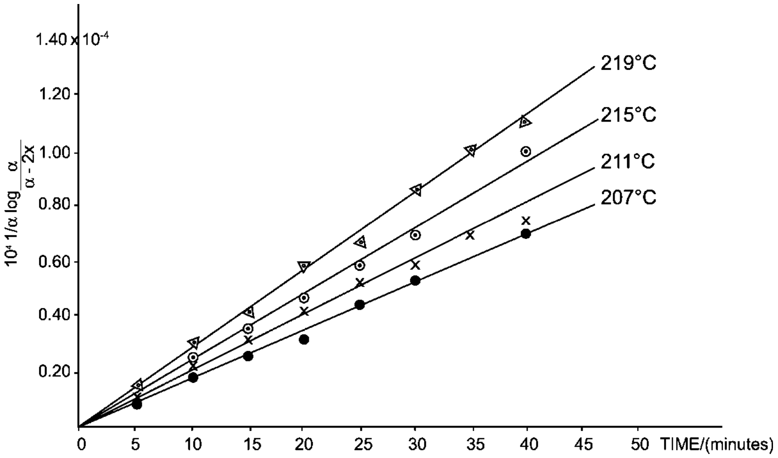


Figure 5. Graph of $1/\alpha \log \alpha/(\alpha - 2x)$ as a function of time for the disulphide–disulphide interchange reaction between polymers A and B.

In general, most reactions are performed in a homogeneous liquid phase environment in the presence of suitable solvents which does not enter into the reaction stoichiometry. Of importance, however, are the complex reactions between solvent/solute molecules resulting in modifications to free energies and overall reactivity. The extent to which

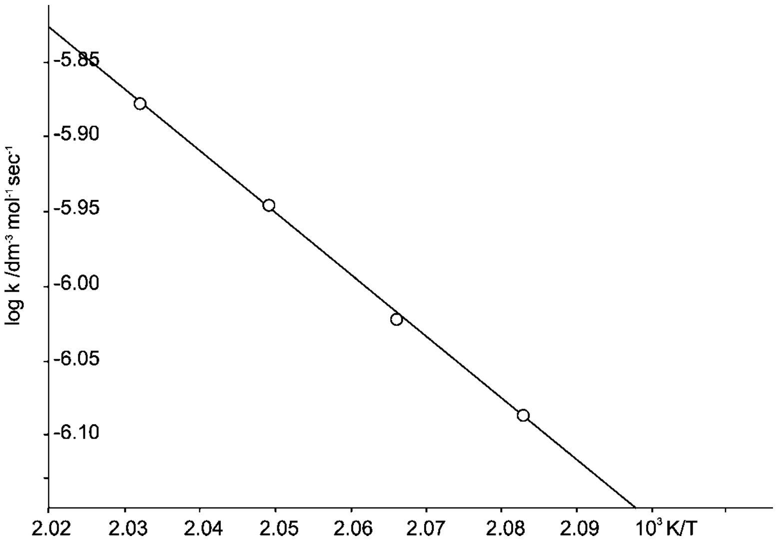


Figure 6. Graph of $\log k_{-2}$ against 10^3 K/T for the reaction between the polysulphide polymers A and B.

solvation can influence the free energy of solute molecules in equilibrium, will affect the free energy, ΔG°, of the reaction and consequently, the equilibrium constant K. Because of the similarities in physicochemical properties, melt blends of polysulphide polymers, A(PSI) and B(PS2), will behave as an ideal solution, wherein the energies of interaction between solvent/solute is the same as solute/solvent, thereby satisfying Raoult's Law. For this reaction system, i.e., melt blends of equivalent amount of A(PS1) and B(PS2), the solvent/solute: solute/solvent will enter into the reaction stoichiometry described by equation 1 and will affect the reaction to equilibrium. The overall extent of these interactions while temperature dependent will not affect the equilibrium constant K. Accordingly, it is assumed that the equilibrium constant K will be independent of temperature, as the enthalpy change for the equilibrium process would be zero; i.e., $Ef = Er = 0$. Where *Ef* and Er are the activation energies for the forward and reverse reactions. This would be consistent for an associative mechanism where the bonds being formed and those being broken are similar. Because of the complexity of this macromolecular reaction system, formation of the activated complex leading to exchanged products would be accompanied by a large decrease in entropy, as reflected by the observed frequency factor $\log A = 2.54$.

CONCLUSION

This simplified kinetic treatment of disulphide–disulphide interchange reaction to equilibrium, assumes that the reactant polysulphide polymers are compatible; the integrity of homogeneity in the polymer blend is maintained during reaction; reactivity of disulphide linkages along a polymer chain are equivalent and independent of chain length: exchange reactions are random; only one reaction along a polymer chain is permitted at any one time; only those reactions leading to the formation of copolymer products are important; other inherent competing reactions due to intramolecular disulphide–disulphide reactions, and intermolecular reaction between like homopolymer chains; are assumed to have limited influence on the intermolecular interchange reactions leading to the formation of randomized copolymers. Given that these polysulphide polymers were thermally stable up to 250°C under nitrogen atmosphere, (TGA results not included), and that the kinetic studies were conducted within the temperature range of 207–219°C, the likelihood of thermally generated reactive species which could compromise the dominant interchange reaction mechanism was negligible. The mass spectrometric "Dimer Analysis" method developed for following the progress of the presumptive disulphide–disulphide interchange reaction enabled both qualitative and quantitative determinations of reactants and products in real time. The advantage of direct probe EI/MS analysis, is the ability to analyze melt blends of polymer without the need for solvents as a vehicle. However, this analytical procedure has limitations, in that higher molecular weight polymers requiring much higher temperatures for volatilization, would undergo thermal degradation and fractionation; thereby influencing the integrity of the interchange process. The kinetic parameters observed, i.e., activation energy $79.80\,\mathrm{KJ\,mol^{-1}}$, and pre-exponential factor $\log A = 2.54$, gives a measure of the complexity of this reaction system. The assumption that intermolecular disulphide–disulphide interactions are equivalent and independent of chain length, best describe these random molecular motions in a convoluted environment. Because of solution dynamics of these melt blends, where solvent/solute and solute/solvent interactions are operative, (Raoult's Law), random molecular collisions will promote the formation of cybotactic regions extending beyond the inner solvation shell, thereby affecting the properties of functional groups, (S -S), along the polymer chain. Therefore, due to these solvation forces, the density and reactivity of the association complex formed by intermolecular disulphide–disulphide interaction, will be enhanced, promoting only one interchange reaction at any one time. Absence of these assumptions, the reactivity of (S -S) groups will be affected in different ways due to their geometric environment, and would compromise the desired randomization process as well as the kinetics. The influence of pendant groups, and other structural variations of monomer units on the interchange mechanism are being reviewed for future studies. This method of analysis using other ionization techniques such as Electrospray (ES) and Matrix Assisted Laser Desorption Ionization (MALDI), will be applied to probing the integrity of disulphide bridges in peptides, and protein digests.

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