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Novel antimicrobial and antitumor organic thermal stabilizers for rigid Poly (vinyl chloride)

Magdy W. Sabaa · Samira T. Rabie · Riham R. Mohamed

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Abstract Pyrazolodithiones of expected biological activity were examined as thermal stabilizers and co-stabilizers for rigid poly(vinyl chloride) (PVC) in air at 180 °C. Their high stabilizing efficiency were shown by their high thermal stability values (T_s), which is the time needed for the liberation of HCl gas, if compared with dibasic lead carbonate (DBLC) and calcium–zinc soap (Ca–Zn soap) reference stabilizers used industrially, with better extent of discoloration. Blending these derivatives with reference stabilizers in different ratios greatly lengthens the thermal stability value and improves the extent of discoloration of the PVC. The structure of the novel organic stabilizers was confirmed by elemental analysis, FTIR, Mass spectra, and ^{1}H-NMR. Thermogravimetric analyses confirmed the improved stability of PVC in the presence of the investigated organic stabilizers, compared to blank PVC and PVC stabilized with the reference stabilizers. Also, GPC measurements were done to investigate the changes occurred in the molecular masses of the degraded samples of PVC in presence of the newly synthesized stabilizers. The stabilizing efficiency of pyrazolodithiones is attributed to the replacement of the labile chlorine atoms on the PVC chains by a relatively more stable moiety of the organic stabilizer. The investigated stabilizers showed a good antimicrobial activity toward two kinds of bacteria, *Escherichia coli* and *Staphylococcus aureus*; and also toward two kinds of fungi, *Aspergillus flavus* and *Candida albicans*. They also exhibited antitumor activity against both liver and colon human cell lines.

Keywords PVC · Pyrazolodithiones · Stabilizer · Co-stabilizer · Antimicrobial · Antitumor

M. W. Sabaa (✉) · R. R. Mohamed
Department of Chemistry, Faculty of Science,
Cairo University, Giza, Egypt
e-mail: mwsabaa@hotmail.com

S. T. Rabie
Department of Photochemistry,
National Research Centre (NRC), Dokki, Giza, Egypt

Introduction

For many years, poly(vinyl chloride) (PVC) has been one of the most important technical polymers that have wide applicability specially in medicine due to its easy modification by various additives, such as polymeric modifiers, heat and light stabilizers, plasticizers, pigments, lubricants,…etc. A great disadvantage of PVC is its rather low thermal stability due to the various defect sites in the polymer chain [1]. It is well known that PVC splits off hydrogen chloride at high temperatures; during this process, polyene sequences are formed and the polymer is discolored. The dehydrochlorination process during the degradation of this polymer involves three primary steps [2]: the initiation of HCl loss, the rapid zip-elimination of HCl and simultaneously the formation of polyenes in the PVC chain, and termination of the zipping process. It is widely accepted that chain defects containing labile (reactive) allylic or tertiary chlorines play an important role in the initiation of the degradation. It has been reported that the initial rates of HCl loss by irregular allylic defects and regular repeat structures are of the same order of magnitude [3].

In the presence of oxygen, in addition to the dehydrochlorination, oxidation reactions can occur, which can also initiate chain scissions. It became possible to suppress these undesired degradation reactions by using thermal stabilizers. The mechanisms for the thermal degradation of

PVC and its prevention by commercial stabilizers have been discussed in detail in some reviews [4, 5].

Many of the most effective thermal stabilizers for PVC contain toxic heavy metals. Thus, it has been highly desirable to replace these additives with others that are at least as effective but are relatively safe and non-toxic, thus some organic stabilizers were prepared and reported to give high stabilizing efficiency [6–12]. Heterocyclic nitrogen containing compounds as triazines, triazoles, and pyrazolones have been investigated as both photo and thermal stabilizers for PVC [13, 14].

Many studies were performed to prepare antibacterial PVC. These trials were based on surface modification of PVC using different nanoparticles to prepare PVC/antibacterial composites.

Zirconium phosphate [15] and TiO_2/Ag^+ [16] were used for this purpose. Isothiocyanate nucleophilic substituted PVC was also used to obtain antibacterial PVC [17].

In the present study, novel biologically active heterocycles containing nitrogen and sulfur were prepared, characterized via several analyses, and had been investigated as thermal stabilizers and co-stabilizers for rigid PVC at 180 °C in air.

Materials and experimental techniques

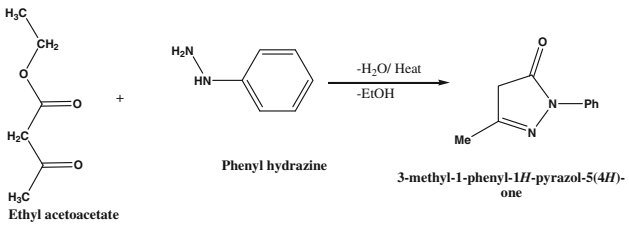
Materials

The commercial PVC (suspension) used in this study was additive free, with a *K* value 70 and supplied by Hüls Co, Germany, dibasic lead carbonate (DBLC) (Rolite lead) from the National Lead Co, Germany was also used. Ca–Zn Soap was obtained from "Lagor–S.P.A" Company, Italy. All chemicals used were of the analytical reagent grade (AR) and of highest purity available. Ethyl acetoacetate and Ethyl cyanoacetate of pure grade were used (BDH). CS_2 and organic solvents used included absolute ethyl alcohol were also purchased from (BDH).

Preparation of *N*-phenyl pyrazolone

N-phenyl-5-pyrazolone was synthesized by the procedure originally developed by Vogel [18].

Equimolar amounts of ethyl acetoacetate were mixed with phenyl hydrazine, drops of glacial acetic acid were added, and the mixture was refluxed for 3 h with stirring from time to time. After it is been left to cool, 50 mL of petroleum ether (or diethyl ether) was added. The mixture was stirred until solidification, filtered with a pump, washed with diethyl ether, and dried in an air oven. Pale yellow crystals of *N*-Phenyl pyrazolone were obtained. (m.p. = 130 °C).



Preparation of pyrazolo-thienopyrimidine dithione (I)

N-Phenyl pyrazolone undergoes Gewald reaction [19], it reacts with sulfur and malononitrile in equimolar ratios under reflux for 3 h in presence of triethylamine (TEA) and absolute ethanol as solvent. The obtained dark yellow crystals were treated with petroleum ether 40–60 °C and benzene for purification. The pure yellow crystals of pyrazolothieno derivative (m.p. 234 °C) was obtained. The pyrazolothieno derivative was then treated with excess CS_2 and 10% KOH in absolute ethanol and then refluxed for

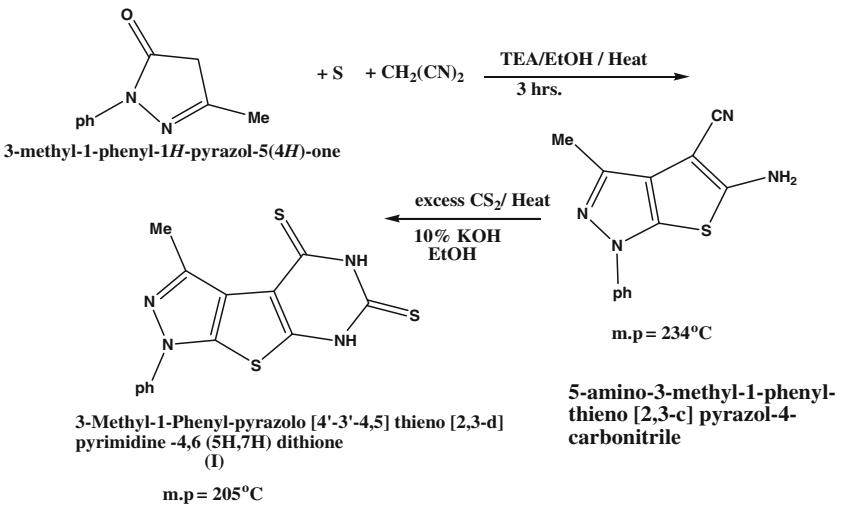


Table 1 Physical properties and analyses data for the prepared derivatives

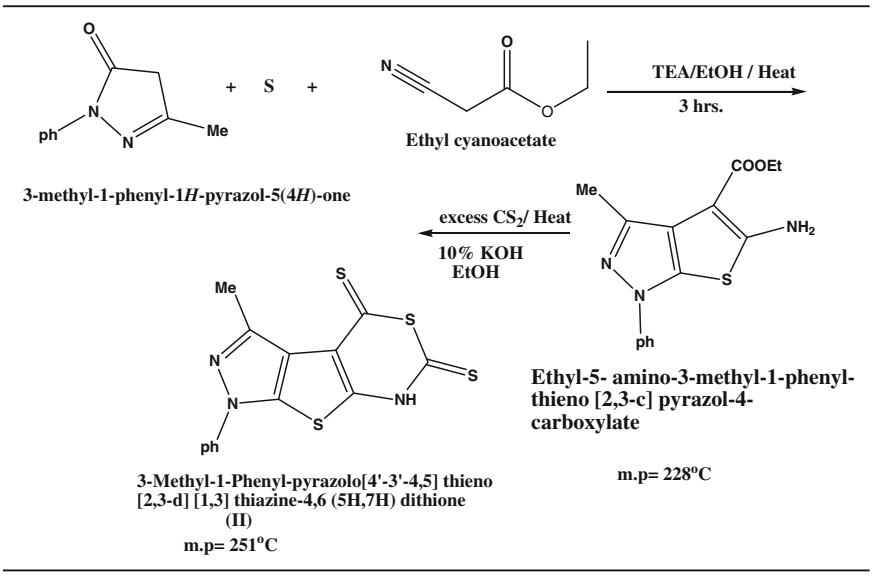
Material	Code name	Melting point/°C	Elemental analysis/%	
			Found	Calculated
3-Methyl-1-Phenyl-pyrazolo [4′-3′-4,5] thieno [2,3-*d*] pyrimidine-4,6 (5*H*,7*H*) dithione	I	205	C: 50.20, H: 3.00, N: 16.65, S: 29.09	C: 50.88, H: 3.05, N: 16.95, S: 29.11,
3-Methyl-1-Phenyl-pyrazolo [4′-3′-4,5] thieno [2,3-*d*] [1, 3] thiazine-4,6 (5*H*,7*H*) dithione	II	251	C: 48.20, H: 2.04, N: 11.90, S: 36.50	C: 48.39, H: 2.61, N: 12.09, S: 36.91

7 h. The dark orange powder obtained was crystallized from petroleum ether at 40–60 °C. The final pure product (I) was of melting point 205 °C.

Preparation of pyrazolo-thienothiazine dithione (II)

The same procedure of Gewald [19] was followed but this time with ethyl cyanoacetate instead of malonotrile.

Physical properties and analyses data for the prepared stabilizers were tabulated in Table 1.



Preparation of PVC samples

Samples of PVC for heat degradation were prepared by thoroughly mixing 1 g of PVC powder with 2 mass% of the stabilizer (or a mixed stabilizer) in a mortar and 0.2 g of the resulting powder was used in the investigation.

Method of evaluation of the stabilizing efficiency

Evaluation of the stabilizing efficiency of PVC in presence of the investigated thermal stabilizers was carried out by measuring the induction period (T_s) using Congo-red dye paper for determination of the evolved HCl gas at 180 °C, in air, that turns its color from red to blue once it meets the evolved HCl gas [20].

Thermogravimetric analysis

Thermogravimetric analysis (TGA) measurements were made with a Shimadzu TG-50 H thermal analyzer system. Samples were heated from 0 to 500 °C in a platinum pan at a heating rate of 10°C min^{-1} in a nitrogen atmosphere (30 mL min^{-1}).

Infrared spectroscopy, NMR, and mass spectroscopy

IR spectra were recorded on a Shimadzu FT-IR 8201 PC Spectrophotometer using KBr pellets. ^{1}H-NMR spectra were recorded with Jeol 270 MHz (Japan) in DMSO-d_6 as a solvent and the chemical shifts were recorded in ppm relative to (TMS) as an internal standard. Mass spectra were recorded on GCMS-QP 1000 ex spectra mass spectrometer operating at 70 eV. Elemental analyses were carried out by the micro-analytical unit at the National Research Centre, Giza, Egypt.

Molecular mass determination by GPC

The average molecular mass of PVC was determined using GPC–HPLC, Waters 600 System controller, 717 plus autosampler. Columns: Phenomenex Phenogel 5 μm 50 A, 300 × 7.8 mm; Detection: Waters model 2410 refractive index; ATTN = 16× Eluent: THF (100% by Vol.); Flow rate: 0.7 mL min^{-1}; Temperature: 50 °C; Injection volume: 25 μL.

Standards: Polystyrene (PS) 25,000; 13,000; 4,000; 2500; 200 g mol^{-1} (1.0% m v^{-1}). Cubic fit calibration curve by Waters Millennium 32 GPC System Software. Samples: dissolved in THF at an approximate 1.0% m v^{-1} concentration.

Biological activity of the prepared compounds

Antimicrobial activity Antimicrobial activity of the investigated samples was determined using a modified Kibry-Baur disk diffusion method [21] at Cairo University-Microanalytical Center. In brief, 100 μL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 108 cells mL^{-1} for bacteria or 105 cells mL^{-1} for fungi [22]. 100 μL of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disk diffusion method [23, 24].

Of the many media available, NCCLS recommends Mueller-Hinton agar due to its good batch-to-batch reproducibility results. Disk diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by researchers [25] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disk diffusion method for yeasts developed using approved standard method (M44-P) by NCCLS [26].

Plates were inoculated with filamentous fungi as *Aspergillus flavus* at 25 °C for 48 h; Gram (+) bacteria as *Staphylococcus aureus, Bacillus subtilis;* Gram (−) bacteria as *Escherichia coli*, *Pseudomonas aeuroginosa*. They were incubated at 35–37 °C for 24–48 h and yeast as *Candida albicans* incubated at 30 °C for 24–48 h and then the diameters of the inhibition zones were measured in millimeters.

Standard disk of *Tetracycline* (antibacterial agent), *Amphotericin B* (antifungal agent) served as positive controls for antimicrobial activity, but filter disks impregnated with 10 μL of solvent (distilled water, chloroform, DMSO) were used as a negative control.

The Agar used is Mueller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disk diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values.

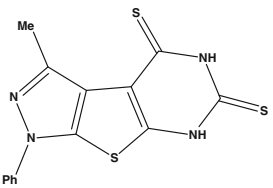
Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8 mm were impregnated with 10 μL of tested concentration of the stock solutions. When a filter paper disk impregnated with a tested chemical is placed on Agar, the investigated chemical will diffuse from the disk into the agar. This diffusion will place the chemical in the agar only around the disk. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration round the disk. If an organism is placed on the agar, it will not grow in the area around the disk if it is susceptible to the chemical. This area of no growth around the disk is known as a "Zone of inhibition" or "Clear zone". For the disk diffusion, the zone diameters were measured with slipping calipers of National Committee for Clinical Laboratory Standards [23].

Agar-based methods such as E-test and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods [27, 28].

Antitumor activity of the prepared materials Potential cytotoxicity of the two investigated compounds was determined at Bioassay-Cell Culture Lab, National research centre according to the methods of Mosmann [29] and Thabrew et al. [30].

Results and discussion

Characterization of the investigated stabilizers



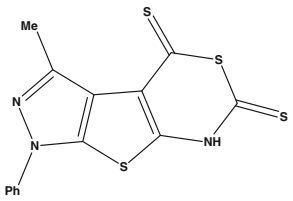
3-Methyl-1-phenyl-pyrazolo [4'-3'-4,5] thieno [2,3-d] pyrimidine -4,6 (*5H,7H*) dithione (I)

IR analyses for compound (I), Fig. 1a, showed a single band for the free (–NH) stretching at 3418 cm^{-1},also the (C–H) stretching for CH_3– group appears at 2916 cm^{-1}.

Aromatic C–C stretching has two bands appear both at 1557 at 1492 cm^{-1}, Aromatic C–H stretch band appears at 3054 cm^{-1}, –C=N– (st.) appears at 1414 cm^{-1}, while –C=S (st.) appears at 1085 cm^{-1} and C–N (st) appears at 1324 cm^{-1}.

^{1}H-NMR (DMSO): $\delta = 1.22$ ppm. (s, 3H, Me); $\delta = 7.28$ ppm (m, 5H, aromatic protons); $\delta = 7.3$ ppm

(s, 1H, –NH); $\delta = 5.16$ ppm (s, 1H, –NH). MS *m/z*: 330 (M^+).



3-Methyl-1-phenyl-pyrazolo[4'-3'-4,5] thieno [2,3-d] [1,3] thiazine-4,6(5H,7H)dithione (II)

IR analyses for compound (II), Fig. 1b, showed a single band for the free (–NH) stretching at 3398 cm^{-1}, also the (C–H) stretching for CH_3– group appears at 2852 cm^{-1}. Aromatic C–C stretching has two bands appear both at 1560 and 1491 cm^{-1}, Aromatic C–H stretch band appears at 3050 cm^{-1}, –C=N– (st.) appears at 1417 cm^{-1}, while –C=S (st.) appears at 1105 cm^{-1} and C–N (st) appears at 1321 cm^{-1}.

^{1}H-NMR (DMSO): $\delta = 1.20$ ppm. (s, 3H, Me); $\delta = 7.42$ ppm (m, 5H, aromatic protons); $\delta = 7.2$ ppm (s, 1H, –NH). MS *m/z*: 346 (M^+).

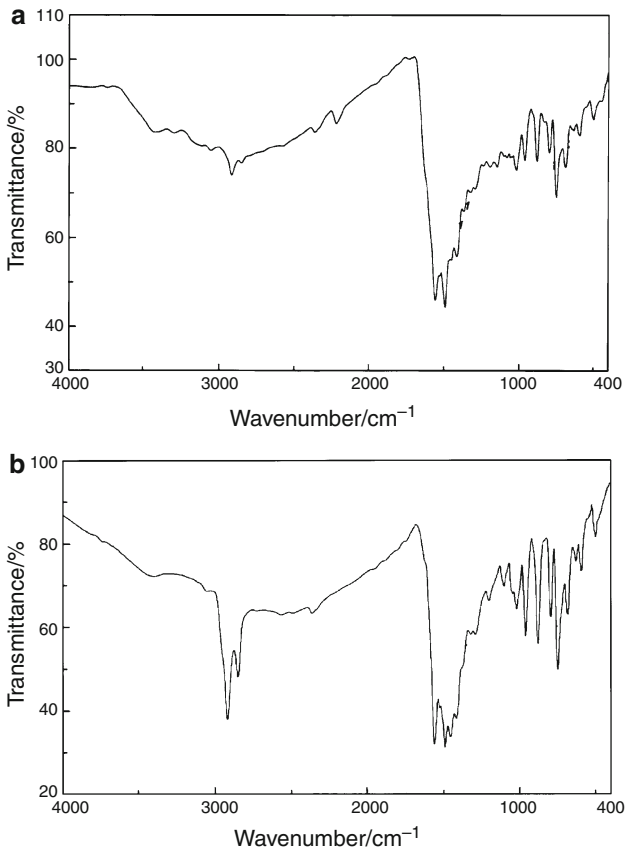


Fig. 1 IR chart of **a** Pyrazolo-thienopyrimidine dithione (I), **b** Pyrazolo-thienothiazine dithione (II)

Pyrazolodithione derivatives as thermal stabilizers for rigid PVC

Results of the thermal stability of rigid PVC degraded in air at 180 °C in presence of stabilizers I and II (by using *Congo-red dye paper method*) are shown in Table 2. The results for the unstabilized blank as well as those for samples stabilized by DBLC and Ca–Zn soap, used as reference stabilizers, are also given for comparison.

The results reveal that the investigated organic stabilizers exhibit a greater stabilizing efficiency than the two used commercial stabilizers, as it is shown by the higher thermal stability values (T_s). The thermal stability values for the investigated stabilizers are almost three to four times higher than the values obtained for the reference stabilizers. Moreover, the results reveal that the presence of either –NH or –S– group in the pyrimidine ring—the only difference in the structure of the two stabilizers—plays an important role in the efficiency of the stabilizer as denoted by the difference in the thermal stability values of the two investigated organic stabilizers (stabilizer II > I).

The FTIR spectrum of blank PVC (Fig. 2a) was shown for comparison. FTIR analyses of thermally degraded PVC in presence of both the investigated stabilizers either pyrazolopyrimidine derivative (I) or pyrazolothiazene derivative (II) were done, after its purification from the unreacted stabilizers by extensive washing with hot methanol, Fig. 2b, c. These spectra revealed the presence of two bands at 1557 and 1492 cm^{-1}, while the blank PVC does

Table 2 Thermal stability values of the investigated compounds as thermal stabilizers and co-stabilizers for rigid PVC

Material	T_s/min
PVC	2
DBLC	9
Ca–Zn soap	8
I	30
II	38
I + DBLC (25%:75%)	45
I + DBLC (50%:50%)	48
I + DBLC (75%:25%)	42
I + Ca–Zn soap (25%:75%)	58
I + Ca–Zn soap (50%:50%)	62
I + Ca–Zn soap (75%:25%)	60
II + DBLC (25%:75%)	45
II + DBLC (50%:50%)	58
II + DBLC (75%:25%)	43
II + Ca–Zn soap (25%:75%)	41
II + Ca–Zn soap (50%:50%)	50
II + Ca–Zn soap (75%:25%)	39

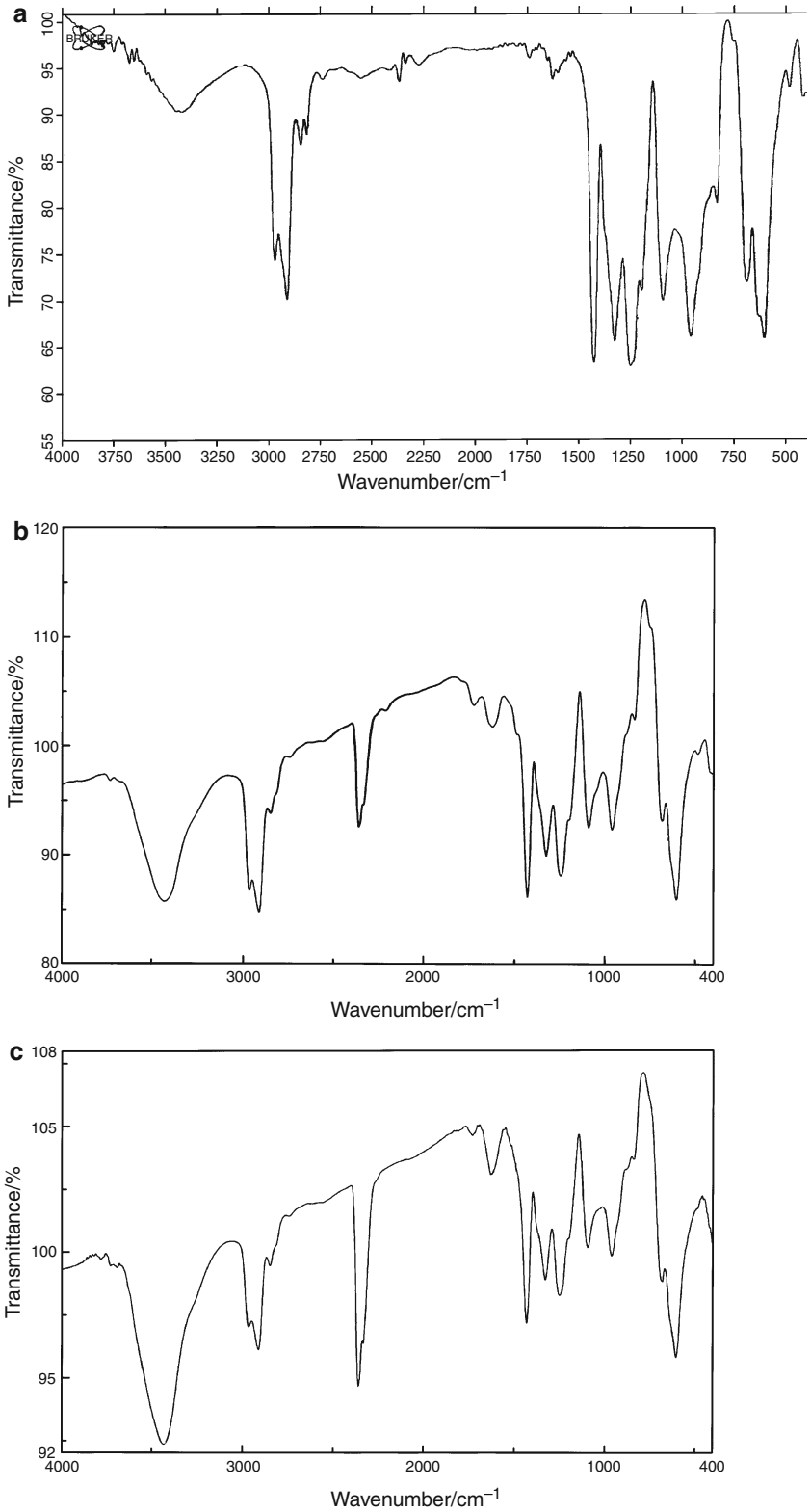


Fig. 2 IR chart of **a** Blank PVC, degraded PVC stabilized with **b** Pyrazolo-thienopyrimidine dithione stabilizer (I), and **c** Pyrazolo-thienothiazine dithione stabilizer (II) after 45 min of degradation

not have these two bands which indicates the presence of the aromatic ring of the stabilizer at 45 min degradations in the degraded polymer.

Figure 2b, c revealed the presence of –NH band of stabilizer (I) at 3418 cm^{-1} in Fig. 2b and that of stabilizer (II) at 3398 cm^{-1} in Fig. 2c, while these two bands are not

Table 3 Extent of discoloration of compounds I and II as thermal stabilizers and co-stabilizers for rigid PVC in air, at 180 °C

Type of stabilizer	Color at 0 min	Color at 15 min	Color at 25 min	Color at 35 min	Color at 45 min
Blank PV	White	Light brown	Dark brown	Black	Black
DBLC	White	Light brown	Brown	Dark brown	Dark brown
Ca–Zn soap	White	Orange	Light brown	Dark brown	Dark brown
I	Cream	Light yellow	Yellow	Dark yellow	Dark yellow
II	White	White	White	Yellow	Yellow
I + DBLC (50%:50%)	White	White	White	White	White
I + Ca–Zn soap (50%:50%)	White	Cream	White	Cream	White
II + DBLC (50%:50%)	White	White	White	White	White
II + Ca–Zn soap (50%:50%)	White	Cream	White	Cream	White

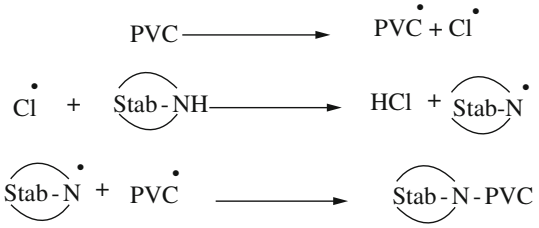
existed in IR of PVC, Fig. 2a, this may be due to the hydrogen atom abstraction from the –NH– group of the stabilizer either I or II by the Cl atom detached from the PVC chains—as a result of degradation—which is followed by blocking the PVC macroradical by the stabilizer(s) radical.

The results of the elemental analyses of the degraded samples of PVC in presence of compound (I) and (II) supported this explanation.

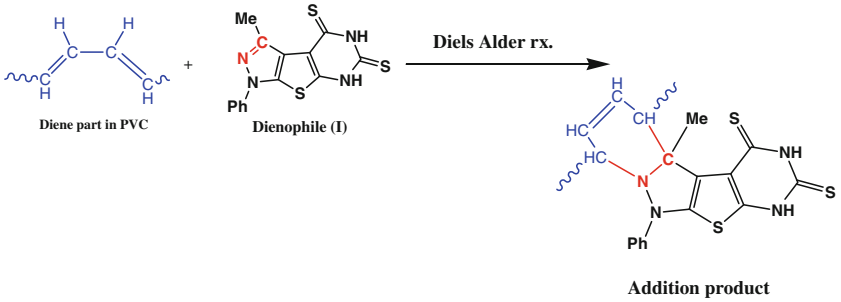
Elemental analysis of the degraded sample of PVC in presence of compound (I) showed (% N = 0.5 and % S = 1) at 35 min degradation, while the elemental analysis of the degraded sample of PVC in presence of compound (II) showed (% N = 0.3 and % S = 1.3) at the same period of degradation.

This implies that these thermal stabilizers are chemically bonded to the degraded polymeric chains during the stabilization process and that their stabilizing efficiency are at least partly due to the ability of these organic stabilizers to be incorporated in the polymeric chains, thus disrupting the chain degradation.

reference stabilizers (Table 3). These results reveal the improvement of the discoloration of the degraded PVC samples in presence of the investigated stabilizers as they retard the high discoloration rate of PVC arising from the great conjugation of –C=C– bonds resulting from the dehydrochlorination process of PVC.



In Table 3, Ca–Zn degraded mixture with both stabilizers (I) and (II) give oscillating color as a function of time; this may be due to Diels–alder reaction between the –N=C– bond (dienophile) in both stabilizers I or II with the conjugated system in PVC (diene), which interrupts in one moment the conjugation giving rise to a white color after the dark color (cream) of the conjugated form.



Another experimental proof for the high stabilizing efficiency of the investigated stabilizer, as compared with the reference stabilizers, is shown from the lower rate of discoloration of sample stabilized with the investigated stabilizers at different intervals of time relative to the blank sample and PVC samples stabilized with any of the

Elucidation of the molecular mass by GPC

GPC measurements were carried out for both PVC before and after 30 min of thermal degradation. Results are both

Table 4 GPC measurements of degraded PVC samples

Sample	Degradation time/min	M_w/ g mol^{-1} × 10^4	M_n/ g mol^{-1} × 10^4	PD
PVC blank	0	25.40	9.137	2.779
PVC	30	18.95	4.226	4.485
PVC + I	30	20.85	6.425	3.246
PVC + II	30	22.81	7.908	2.885

tabulated in Table 4 and illustrated in Fig. 3a, d. They showed the values of M_w, M_n, and the polydisperity (PD).

The results of GPC measurements indicate the low decrease in the values of molecular masses of PVC samples by using the pyrazolodithione derivatives as thermal stabilizers. The results of GPC measurements show the decrease in M_w value of blank PVC sample from 2.540×10^5 to 1.895×10^5 upon 30 min of thermal degradation with a % decrease in M_w value of 25.39. After the same time of degradation, the PVC sample stabilized with compound I, the % decrease in M_w is 17.91, while this decrease % reaches only 10.15 by using the thermal stabilizer II. This may be due to the good stabilizing effect of investigated compounds I and II that decreases the extent of chain scission of PVC. The solubility test of thermally degraded PVC exhibits the absence of gel formation. This indicates the absence of cross linking during degradation. As an evidence for the high efficiency for these investigated stabilizers, that they can decrease the chain scission and prevent the gel formation, so they can preserve both the mechanical and physical properties of the polymer.

Thermal stabilization efficiency of rigid PVC in presence of mixed stabilizers

It was of interest to investigate the effect of mixing the investigated stabilizers with those used in industry on both the stabilization efficiency and the extent of discoloration. For this purpose, the investigated stabilizers were examined as co-stabilizers with the two used commercial stabilizers. Mixing was done in the range of 0–100% of the investigated stabilizers relative to each of the reference stabilizers. The total mixed-stabilizers concentration was kept constant at 2 mass% based on the polymer mass. The results reveal a true synergism resulting from the combination of the organic stabilizer with each of the reference stabilizers, Tables 2, 3 and Figs. 4, 5, irrespective to the class of which the reference stabilizer belongs to. Thus, it seems that the different mechanisms by which both the investigated and the reference stabilizers work are beyond the obtained synergistic effect. DBLC reference stabilizer works by absorption and neutralization mechanism of HCl evolved by PVC, while Ca–Zn soap reference stabilizer works by displacement mechanism of active, labile substituent groups; such as the chlorine atom attached to a tertiary carbon or in α-position to a tertiary carbon, or allylic chlorine. The investigated stabilizers work by a different mechanism; they block the odd electron sites created on the PVC chain, thus disrupting the radical chain degradation.

These results are in accordance with many data presented in the literature for cases where conventional stabilizers are mixed with organic stabilizers [6–14, 31, 32].

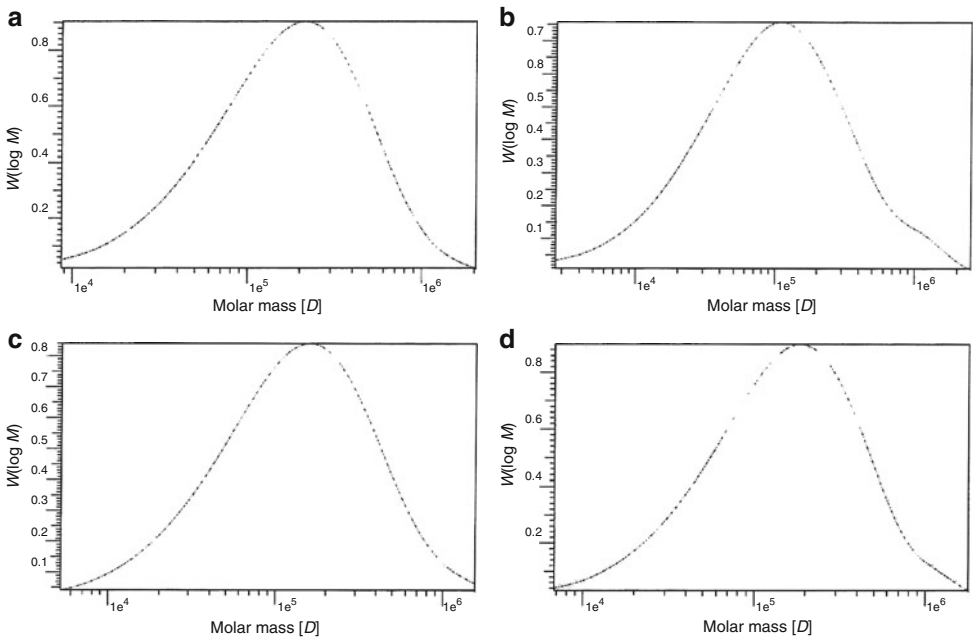


Fig. 3 GPC chromatogram of **a** blank PVC (0 min degradation), **b** degraded PVC (30 min degradation), **c** PVC + I (30 min degradation), and **d** PVC + II (30 min degradation)

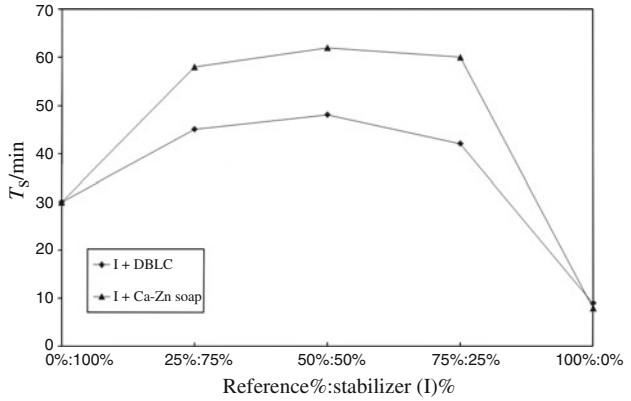


Fig. 4 Composition curve of the T_s values of Pyrazolo-thienopyrimidine dithione (I) with both the reference stabilizers in different ratios

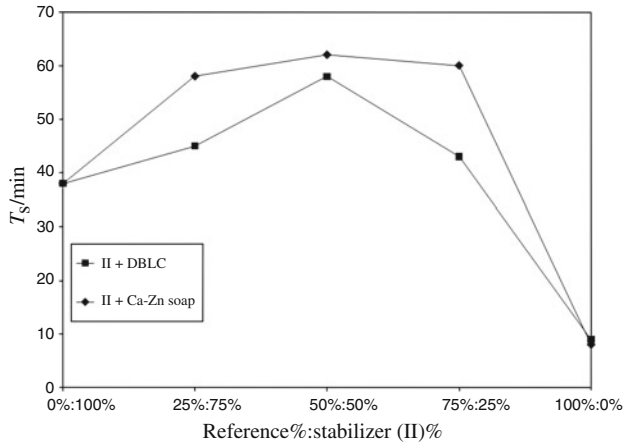


Fig. 5 Composition curve of the T_s values of Pyrazolo-thienothiazine dithione (II) with both the reference stabilizers in different ratios

The maximum synergistic effect is attained when the ratio of the organic stabilizer to each of the two reference stabilizers is (50%:50%), as seen in the composition curves for the investigated stabilizers with the two reference thermal stabilizers.

Table 5 Thermogravimetric data for PVC blank and PVC stabilized with the investigated stabilizers

T/°C	PVC	PVC + I	PVC + II
IDT	180 °C	220 °C	270 °C
T/°C	Mass loss % of PVC	Mass loss % of PVC + I	Mass loss % of PVC + II
200	5	0	0
250	11	10	0
280	32	20	5
300	44	25	10
350	61	30	20
400	62	32	20

Thermogravimetric analysis

Thermogravimetric data for PVC non-stabilized and PVC stabilized with the investigated stabilizers are shown in Table 5. The investigated stabilizers improved the thermal stability of PVC, as the initial decomposition temperature (IDT) of PVC stabilized with I was recorded as 220 °C, while PVC stabilized with II was recorded as 270 °C, where the IDT of the unstabilized PVC was found to be 180 °C.

At 300 °C, the unstabilized PVC lost 44% of its mass, while PVC stabilized with I lost only 25% of its mass and PVC stabilized with II lost only 10%, whereas at 350 °C, the unstabilized PVC lost 61% of its mass, while PVC stabilized with I lost 30% of its mass and PVC stabilized with II lost 20%. From the aforementioned results, it was clear that both stabilizer I and II had increased the thermal stability of the virgin PVC. Stabilizer (II) is more thermally stable than stabilizer (I).

Antimicrobial activity for investigated stabilizers for rigid PVC

Blank PVC doesn't exhibit high antibacterial or antifungal activity; while compound (I) exhibited an antibacterial

Table 6 Antimicrobial activity for the investigated compounds

Sample	Inhibition zone diameter/mm sample^{-1}			
	Escherichia coli (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus* (fungus)	*Candida albicans* (fungus)
Standard				
Tetracycline antibacterial agent	32	27	–	–
Amphotericin B antifungal agent	–	–	20	18
Blank PVC	11	14	2	3
I	17	17	12	13
II	17	16	12	12

Table 7 Cytotoxicity activity of the investigated compounds

Sample	$LC_{50}/\mu g\ mL^{-1}$	
	HePG2	HCT116
Adrinamycin	8.6	16.5
(I)	28	28
(II)	24	22

LC_{50} Lethal concentration of the sample which causes death of 50% of cells in 48 h

activity of 53.12% of the reference antibacterial agent. This activity was against two kinds of bacteria, *E. coli* (G^-) and *S. aureus* (G^+).

Also, it has antifungal activity as shown from Table 6. This activity reaches to 60 and 72.22% of the reference antifungal agents. This activity was against two kinds of Fungi, *A. flavus* and *C. albicans*.

Compound (II), on the other hand, showed an antibacterial activity reaches to 53.12 and 59.26% of the reference antibacterial agents against the two kinds of bacteria. Also, it showed antifungal activity that reaches 60 and 66% of the reference antibacterial agents.

Both compounds (I) and (II) exhibited relatively higher antifungal activities than antibacterial activities.

Antitumor activity for investigated stabilizers for rigid PVC

Evaluation of the anticancer activity of the investigated compounds (I) and (II) was performed at Bioassay Cell Culture Lab, NRC, the investigated compounds were evaluated for cytotoxicity in the human liver carcinoma cell line (HePG2) and human tumor colon cell line (HCT116). Different concentrations of the investigated compounds were added to the cell monolayer of tumor. Table 7 indicates the high cytotoxic activity of the investigated compounds, it was found that they exhibited very good antitumor activities compared to the standard antitumor drug used (Adrinamycin) due to the presence of either –NH or –S– group in the investigated stabilizers.

Conclusions

Pyrazolodithione derivatives exhibit a greater stabilizing efficiency than the two used commercial stabilizers, as it is shown by their higher thermal stability values (T_s). TGA studies showed that the investigated stabilizers improved the thermal stability of PVC. GPC studies were as an evidence for the high efficiency for these investigated stabilizers, that they can decrease the chain scission and prevent the gel formation, so they can preserve both the mechanical and physical properties of the polymer. Pyrazolodithione derivatives exhibited relatively higher antifungal activities than antibacterial activities. It was also found that pyrazolodithione derivatives exhibited very good antitumor activities compared to the standard antitumor drug used (Adrinamycin).

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