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PAPER

Synthesis, characterization and application of enrofloxacin complexes as thermal stabilizers for rigid poly(vinyl chloride)

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Synthesis and characterization of both binary Co(II)- (**1**), Ni(II)- (**2**) complexes with enrofloxacin drug ($\mathbf{HL_1}$) and ternary Co(II)- (**3**), Ni(II)- (**4**) complexes in presence of DL-alanine ($\mathbf{H_2L_2}$) are reported using physico-chemical techniques. The antimicrobial activity of these complexes has been screened against two *Gram-positive* and two *Gram-negative* bacteria. Antifungal activity against two different fungi has been evaluated and compared with reference drug. All the binary and ternary complexes showed remarkable potential antimicrobial activity higher than the recommended standard agents. Ni(II)-complexes exhibited higher potency as compared to the parent drug against bacterial and fungal strain. In addition, it was of interest to investigate the reported complexes as thermal stabilizers and co-stabilizers for rigid PVC in air at 180 °C. Their high stabilizing efficiency is detected by their high induction period values (T_s) compared with some of the common reference stabilizers used industrially, such as dibasic lead carbonate (DBLC) and calcium-zinc soap. Blending these complexes with some of the reference stabilizers in different ratios had a synergistic effect on both induction period as it gave better thermal stability and lower extent of discoloration. The stabilizing efficiency is attributed at least partially to the ability of the metal complex stabilizer to be incorporated in the polymeric chains, thus disrupting the chain degradation and replace the labile chlorine atoms on PVC chains by a relatively more s moiety of the inorganic stabilizer. Their amenability to use as a biomedical additives for PVC, has afforded them great potential for various medical applications.

Introduction

Drugs are compounds which specifically have designed structure in order to facilitate the interaction with specific receptors or organs. The development of biomaterials and drugs represents a critical aspect in biological and technical science. A synergistic interaction between biomaterial development and industry exists in which a unique material platform can be made and used for many applications of technology.

Quinolone drugs have recently received considerable recently owing to their current and wide potential applications in medical and biological fields.[1] Their potent activity is caused by the inhibition of DNA replication. In particular enrofloxacin ($\mathbf{HL_1}$), 1-cyclopropyl-7-(4-ethyl-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (Fig. 1) is the first developed drug for many veterinary applications, it was highly recommended as treatment for many infections of the urinary tract, skin and tissue as well as urethral and cervical gonococcal infections.[2] In addition, a broad spectrum of activity against a wide range of *Gram-negative* and *Gram-positive* bacteria has been reported.[3] The interaction of metal ions with enrofloxacin becomes an increasingly important field due to the antibacterial properties of the resulting complexes. The effect of binary and ternary complexes of Cu(II), Ni(II), Co(II) and Zn(II) with enrofloxacin in presence of phenanthroline has been studied potentiometry.[4] Several metal ion complexes with enrofloxacin are known. Interaction of the complexes with calf-thymus DNA was investigated and the antimicrobial activity of the complexes was evaluated.[5] Depending on the solvent quinolones can show a different complex formation acting as nono-,[6] bidentate-[7] or bridging ligands in polynuclear complexes,[7b,8] so that many different species can be obtained. Although several reports have described effect of metal ions on quinolones, it is of significance

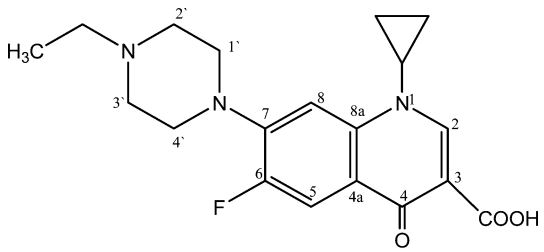

Fig. 1 The structure of 1-cyclopropyl-7-(4-ethyl-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.

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to explore the possible synergetic effects and provide insight into binding modes, therefore this work is initiated and conducted to investigate and elucidate the coordination sphere of the drug under study.

Poly(vinyl chloride) (PVC) is synthetic commodity polymer with many advantages for use but also some drawbacks. In spite of its enormous technical and economic importance, PVC possesses a number of problems, such as its low stability due to the influence of heat and light, which results in autocatalytic hydrogen chloride loss, discoloration, corrosion and change in the mechanical properties accompanied with decrease or increase in molecular weight as a result of chain scission or cross linking of the polymer molecules.[9–11]

Highly effective additives such as metallic soaps,[12–13] basic compounds[14] and organotin compounds[15] have been commercially used to increase thermal stabilization of PVC however; some of them are toxic and cause environmental problems, as most of them leave toxic residues during the degradation process. Therefore several coordination compounds have been prepared from aromatic compounds containing pendant functional groups that act as chelating groups for binding polyvalent metal ions.[16–18] Such complexes exhibit a higher thermal stability than the parent compounds. This has recently directed attention towards the use of inorganic stabilizers.[10,19,20]

Polymers such as polyethylene, polyurethanes, and poly (vinyl chloride) (PVC) have been extensively used for the fabrication of hospital indoors. Presence and formation of a biofilm on the surface due to adhesion of bacteria to material exploited in contact of living tissues, organisms or micro-organisms is acting as a protective barrier against any drug, which slowdown the healing process and make it quite difficult and require either higher doses or more potent antibiotics.[21–27]

To prevent the secondary infection in the course of surgical operation, increasing numbers of attempts have been directed towards the development of antibacterial biomaterials that can prevent or reduce biofilm formation; therefore modifying the physico-chemical properties of the surface of biomaterials is regarded as an essential approach to reduce bacterial adhesion and biofilm formation by interference of some additives to the polymers.

The preparation of TiO_2 film on PVC surface by dip-coating with PVC–THF solution containing $P_{25}TiO_2$ (Ti-phosphorous) nano-crystals was reported.[28] This technique is attractive for obtaining highly photoactive and antibacterial TiO_2 film on thermolabile substrates such as poly(vinylchloride).

Pyrazolodithiones of expected biological activity were examined as thermal stabilizers and co-stabilizers for rigid poly (vinyl chloride) (PVC).[29] The investigated stabilizers showed a good antimicrobial activity towards two kinds of bacteria, *Escherichia coli* and *Staphylococcus aureus* and also towards two kinds of fungi, *Aspergillus flavus* and *Candida albicans*. They also exhibited antitumor activity in both liver and colon human cell lines. In the present work, the complexes under study can be used as biomedical additives for PVC used in the surgical or medical fields like strings of plastic surgery, *etc.*

Herein, the synthesis and characterization of novel Ni(II), Co(II) complexes with enrofloxacin (**HL_1**) in the absence or presence of DL-alanine (**H_2L_2**) is presented. The antimicrobial activity of the complexes is evaluated on four different microorganisms under identical conditions, where Ni(II) complexes exhibit remarkably higher inhibition comparing with the free drug and the remaining complexes. The present work was also aimed to investigate the metal complexes under study as thermal stabilizers for rigid PVC. Our results suggest that these complexes (Co–EFA) and (Ni–EFA) are suitable as biomedical additives for PVC for further biomedical uses. These compounds are available, easily prepared and possess variety of functional groups which can interact with the evolved HCl gas. Furthermore, by using them as co-stabilizers they can react with the metal chloride by-products resulting from the thermal degradation of PVC in the presence of metallic-containing stabilizers.

Results and discussion

Structural characterization of (1)–(4)

The metal to ligand ratio corresponds to binary complexes is 1 : 2 and 1 : 1 in case of ternary complexes. The observed and calculated elemental analysis percentages for (**1**) C 50.62 (50.67), H 5.31(5.33), N 9.34 (9.33); (**2**) C 51.71 (51.73), H 5.12 (5.22), N 9.51 (9.53); (**3**) C 47.12 (47.22), H 5.89 (5.90), N 11.42 (11.45); (**4**) C 43.15 (43.17), H 5.06 (5.07), N 10.49 (10.47), respectively.

The molar conductivity measurements of the complexes in DMF solution were in the range 17.10, 12.80 Ω^{-1} cm^2 mol^{-1} for (**1**), (**3**), respectively. These relatively low values indicated non-electrolytic nature of the complexes; whereas Ni complexes were ionic in nature and of the type 2 : 1 electrolytes,[30] their conductivities were 158, 165 Ω^{-1} cm^2 mol^{-1} for (**2**), (**4**), respectively.

The pyridone stretch $_{\nu}(C{=}O)_p$ in **HL_1** appeared at 1627 cm^{-1} and the asymmetric and symmetric stretching $_{\nu}(COO)_{carb}$ appeared at 1735 and 1383 cm^{-1}, respectively. The pyridone band is decreased in intensity and was shifted to higher frequency (1–4 cm^{-1}) in binary complexes and to lower frequency (2–4 cm^{-1}) in ternary complexes indicates its involvement in the chelation.[1a] The participation of the carboxylate O atom in the binary and ternary complexes is confirmed by disappearance of these bands in all the prepared complexes.[1a]

IR spectrum of **H_2L_2** showed sharp bands at 1594 and 1410 cm^{-1} assigned to the asymmetric and symmetric stretching vibrations of the carboxylate moiety, respectively. Shifting to lower (58–64 cm^{-1}), (100–98 cm^{-1}) frequencies in (**3**) and (**4**), respectively confirming that **H_2L_2** coordinated to the metal ions *via* deprotonated carboxylate.[31] It is difficult to detect the coordination vibration band of NH_2 of **H_2L_2** due to presence of broad split bands between 3450 and 3150 cm^{-1}, which could be due to the presence of N–H stretching vibration of the piperazinyl moiety;[32,33] therefore the shift of in-plane bending, (NH_2) vibration from 1521 cm^{-1} to 1502–1517 cm^{-1} is used as a clue in complex formation.[34] In (**3**) and (**4**) several new bands at (534, 574 cm^{-1}) and (585, 590 cm^{-1}) can be assigned to the (M–O) stretching vibrations of carboxylate and pyridone groups, respectively.[25] In addition, bands at (540,528 cm^{-1}) and (420,415 cm^{-1}) regions are assigned to the $(M–O)_{carb}$ and $(M–N)_{amino}$ stretching vibration of the carboxylate and amino groups of (**3**) and (**4**), respectively.[35]

In cobalt(II) complexes the effective magnetic moments μ_{eff}, expressed in multiples of the Bohr Magneton μ_B, are calculated as 4.8 μ_B and 4.9 μ_B are calculated for (**1**) and (**3**), respectively, which indicate a high-spin octahedral configuration.[35b,36] These complexes displaced three bands at 15.675–15.380, 17,512–18.102

and 21,915–22.115 cm^{-1} assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(\nu_1)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(\nu_2)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)(\nu_3)$ transitions, respectively, indicating the octahedral geometry. Bands at 24.565–24.665 cm^{-1} could be attributed to charge transfer bands.[37] Values of μ_{eff} for nickel(II) complexes are reported to be 3.25 and 3.31 μ_B for (**2**) and (**4**), respectively indicating a high spin octahedral configuration.[35b,36] The electronic spectra give three bands at ν_1 = 16.085 cm^{-1}, ν_2 = 18.215 cm^{-1} and ν_3 = 21.380 cm^{-1}; these bands are assigned to $^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^4A_{2g} \rightarrow {}^3T_{1g}(P)$ transitions, respectively. Bands at 24.520–24.667 cm^{-1} were observed, which could be attributed to LMCT.[37]

Fig. 2 shows the UV-Vis absorption spectra of the binary and ternary complexes (2.5×10^{-4} M) in ethanol–water mixture. Three absorption peaks at 212, 276 and 324 nm are observed in $\mathbf{HL_1}$ with molar absorbance in range of (1.43–3.87)×10^5 L mol^{-1} cm^{-1}; this may be due to π–π* and n–π* transition within the ligand.[38] A relatively low bathochromic shift is observed in the binary complexes ($\Delta\lambda$ was in the range of 1–3 nm) and their molar absorbance was in the range of (1.102–3.918)×10^5 L mol^{-1} cm^{-1}. The coordination of the metal ions through the ketonic and carboxylic oxygen atoms in the conjugated system of enrofloxacin can be the reason for this red shift of the maximum absorption peaks. In ternary complexes all the absorption peaks are hypsochromic shifted in the range of (2–4 nm) decreasing intensity. Their molar absorbance was in the range of (1.88–6.54)×10^5 L mol^{-1} cm^{-1}. This hypsochromic shift can be interpreted as indication of a complex formation.

The complexes were studied by simultaneous thermogravimetric (TG) and differential thermal analysis (DTA).

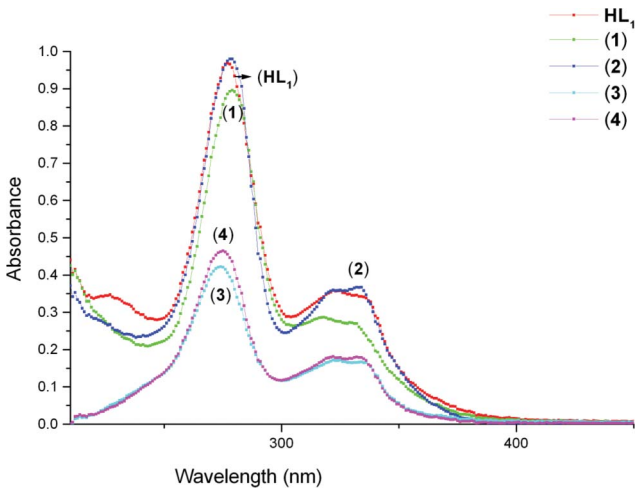


Fig. 2 UV-Vis absorption spectra (2.5×10^{-4} M) of the title ligand and complexes.

Thermal decomposition of (**1**) is carried out in three successive decomposition steps within 50–650 °C. Hydrated water molecules are observed in the first step between 50–220 °C with estimated mass loss of 5.86% (calcd. 6.00%). This step is accompanied by two endothermic peaks at 54 and 166 °C. The second step within the range 221–429 °C can be assigned to the release of carbon monoxide with evolution of Cl_2 along with decomposition of $\mathbf{HL_1}$ and loss of $[C_3H_6]$ + $[C_2H_4N]$ with an estimated mass loss of 23.34% (calcd. 23.44%) (Fig. 3a). DTA shows one exothermic

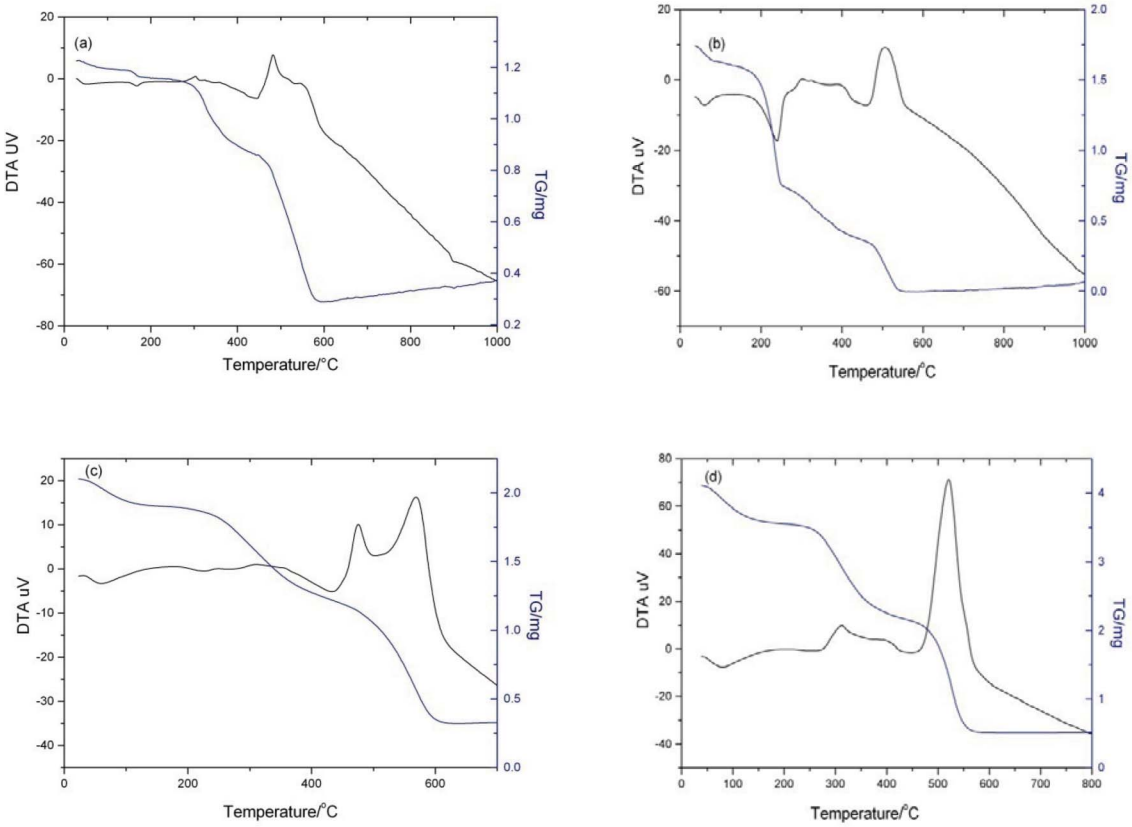


Fig. 3 Thermogravimetric analysis (TG/DTA) of (**1**)–(**4**) complexes.

signal at 301 °C. A third mass loss step 62.40% occurs in the range of 430–650 °C, due to the decomposition of the rest of organic ligand as $C_{31}H_{32}F_2N_5O_3$ (calcd. 62.22%) leaving CoO as a residue 8.40% (calcd. 8.34%). One strong exothermic peak is observed. (Fig. 3a)

The TG of (**2**) showed that the decomposition occurred in three steps within the temperature range 40–650 °C (Fig. 3b). The first step in the range 40–129 °C can be assigned to the liberation of $0.5Cl_2$ molecules accompanied with an endothermic effect at 60 °C with an estimated loss of 3.42% (calcd. 4.03%). The second decomposition step at 130–275 °C corresponds to loss of coordinated water, liberation of HCl and loss of carbon monoxide molecules and decomposition of the ligand as $2[C_6H_{10}N_2H_2]$ with a mass loss of 49.68% (calcd. 49.52%). This step is connected with strong endothermic effect at 237 °C. The last step is accompanied with decomposition of the remaining ligand as $[C_{21}H_{16}N_2F_2]$ in the range of 276–650 °C, with found loss of 38.95% (calcd. 37.88%) and formation of NiO as a final residue 7.95% (calcd. 8.57%). DTA represented an exothermic signal at 506 °C.

Cobalt ternary complex (**3**) thermally decomposed in three decomposition steps (Fig. 3c). The first step in the range 40–180 °C can be assigned to liberation of coordinated water with endothermic effect at 60 °C, estimated mass loss 9.66% (calcd. 9.60%). The second mass loss step in the range 181–375 °C is accounted for the decomposition of both ligands molecules as liberation of $0.5N_2$ and carbon monoxide and loss of $[C_6H_{10}N_2H_2]$ with an estimated percent of 26.05 (calcd. 26.30%), this decomposition followed by endothermic signal at 296 °C. The last step occurred with the range of 375–660 °C due to loss of the remaining ligands as $C_{15}H_{15}NO_3F$ with loss of 49.45% (calcd. 49.37%), this loss is accompanied with two successive exothermic effects at 475 and 568 °C. Cobalt oxide is remained as a residue 14.84% (calcd. 14.73%).

In case of nickel ternary complex (**4**), liberation of 2HCl might be occurred in the first step connected with endothermic process at 80 °C within the temperature range 40–154 °C with mass loss of 12.59% (calcd. 11.94%) (Fig. 3d). The coordinated water is eliminated in the second step accompanied with loss of carbon monoxide and $[C_6H_{10}N_2H_2]$ between 155–383 °C with an estimated loss of 32.88% (calcd. 33.36%). DTA represented two exothermic signals at 312, 356 °C. In the last step the decomposition of the remaining ligands took place in the range of 384–650 °C as $C_{14}H_{13}N_2O_2F$, 41.50% (calcd. 42.52%) leaving NiO as a residue 13.03% (calcd. 12.18%). DTA shows strong exothermic signal at 520 °C (Fig. 3d). The decomposition of the complexes is discussed successfully according to the recent reported decomposition route.[39]

The proposed structures of the binary Co(II) (**1**), Ni(II) (**2**) and ternary Co(II) (**3**), Ni(II) (**4**) complexes with enrofloxacin (**HL**$_1$) and DL-alanine (**H$_2$L$_2$**) complexes are confirmed by the elementary analysis, vibrational spectroscopy, molar conductivity, magnetic properties, UV-Vis, and simultaneous TG-DTA analysis. IR spectra revealed that **HL**$_1$ drug behaves as a deprotonated bidentate ligand coordinated to the metal ions *via* pyridone oxygen and carboxylate oxygen. DL-alanine is a uninegative bidentate ligand coordinated to the metal ions *via* the deprotonated carboxylate-O and amino-N. According to the molar conductivity data, it is found that the complexes are non-electrolytes while Ni complexes are of the type 2 : 1 electrolyte. On the basis of the above observations and from the magnetic measurements, the proposed structure of prepared complexes is six-coordinated with distorted octahedral geometry (Fig. 4).

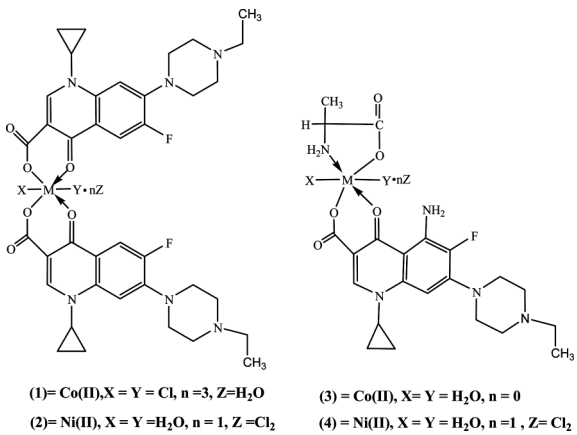


Fig. 4 The structures of the complexes (**1**)–(**4**).

Studies of antibacterial and antifungal properties

In evaluating the antimicrobial activity of the mentioned complexes, more than one test organism have been used in order to increase the chance of identify and observe the biological efficiency of the tested materials. The studies were carried out on the bacteria *Staphylococcus aureus* (G^+), *Escherichia coli* (G^-) and the fungi *Aspergillus flavus* and *Candida albicans* using the assay plates disc method on appropriate nutrient medium.

The results are included in Fig. 5, the inhibition zone diameters were measured after 48 h at 37 °C of incubation all the mentioned complexes. On comparing the biological activity of enrofloxacin and its metal complexes with the standard (*tetracycline*, antibacterial) and (*Amphotericin B*, antifungal), the following results are obtained: The results revealed that **HL**$_1$ drug exhibited a greater antibacterial effect than the standard tetracycline on the tested

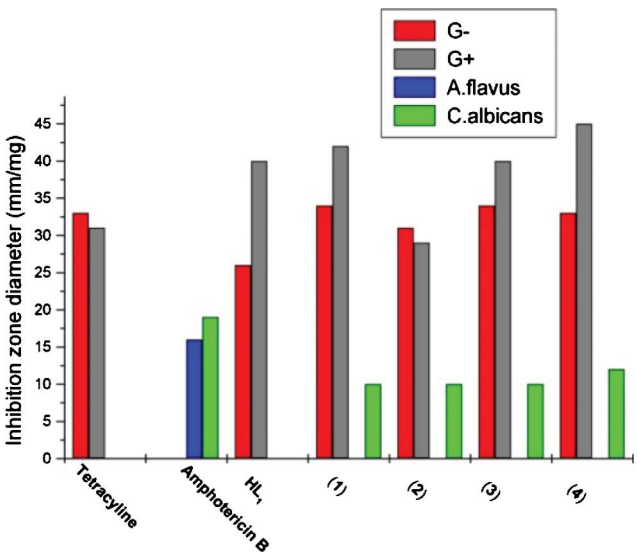


Fig. 5 Biological activity of the tested samples against *S. aureus* (G^+), *E. coli* (G^-), *A. flavus*, *C. albicans*.

Table 1 Induction period (T_s) (min) of (**1**)–(**4**) complexes as thermal stabilizers for rigid PVC compared to PVC and some reference stabilizers

Material	T_s (min)
PVC Blank	2
Dibasic lead carbonate (DBLC)	9
Ca–Zn Soap	8
(1)	20
(**2**)	18
(**3**)	25
(4)	22

microorganisms. (Fig. 5), the prepared complexes represented higher capability in inhibiting the growth of bacterial than the tested drug and the standard tetracycline. By examining the antifungal activity of the complexes, some activity is recorded. Although the drug itself does not represent any activity Ni(II) ternary complex displayed higher antifungal activity comparing with the other complexes under study.

Metal complexes as thermal stabilizer for rigid PVC

Results of the thermal stability of rigid PVC degraded in air at 180 °C in presence of binary and ternary complexes (*by using Congo-red dye paper method*) are shown in Table 1. The results for the non-stabilized 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 revealed that the investigated stabilizers exhibited a greater stabilizing efficiency than the two used commercial stabilizers, as it is shown by their larger thermal stability values (T_s), The thermal stability values for the binary complexes are almost two times larger than the values obtained for the reference stabilizers and the thermal stability values for the ternary complexes are almost about three times larger than the values obtained for the reference stabilizers.

In each case, the cobalt complex gave a better T_s value than that of the nickel complex. These results enhanced us to examine the thermal stability of another drug like sparfloxacin of the same family (Fig. 6) and some of its binary and Ni(II) ternary complexes using the same procedure condition. The preparation of binary and ternary sparfloxacin complexes is published elsewhere.[40]

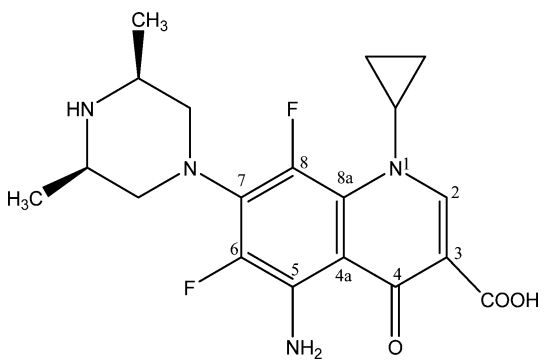


Fig. 6 The structure of sparfloxacin.

By comparing the thermal stability of rigid PVC in presence of sparfloxacin complexes, we found a higher stabilizing efficiency by recording higher T_s value than the other commercial stabilizers. (Table 2).

Table 2 Induction period (T_s) (min) of the sparfloxacin binary and Ni ternary complexes as thermal stabilizers for rigid PVC, compared to PVC and some reference stabilizers

Material	T_s (min)
PVC Blank	2
Dibasic lead carbonate (DBLC)	9
Ca–Zn soap	8
N–sparfloxacin	18
Cu–sparfloxacin	11
Ni–sparfloxacin (ternary)	25

Ni(II)–sparfloxacin complex is almost two times larger than the values obtained for the reference stabilizers, while the thermal stability values for Ni(II)-ternary complex are about three times larger than the values obtained for the reference stabilizers, this might be due to the presence of more $-NH_2$ and $-CH_3$ groups in the ternary complexes, which can trap more chloride atoms to form chloride salts which accelerate further degradation. We observed the minimum recorded T_s value in case of Cu(II)–sparfloxacin complex, as the $CuCl_2$ by product can itself act as lewis acid and accelerate further degradation of PVC.

The elemental analysis of the degradative sample of PVC in presence of the investigated stabilizers (**3**) and (**4**) showed (N = 11.4%), (F = 3.0%) and (Co = 8.5%) or (Ni = 9%), respectively at 35 mins. degradation. This implies that these stabilizers are chemically bonded to the degraded polymeric chains during the stabilization process.

Another experimental proof for the high stabilizing efficiency of the investigated stabilizers, as compared with the reference stabilizers, is shown from the lower rate of discoloration of sample stabilized with the two of the investigated stabilizers – as an example of both binary complex (Co–EFA) and ternary complex (Ni–EFA) at different intervals of degradation time relative to the blank sample and samples stabilized with either of the two reference stabilizers (Table 3).

These results revealed 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 a high number of conjugated –C═C– bonds, which resulting from the dehydro-chlorination process of PVC.

It is well known that during the autocatalytic stage of thermal degradation process of PVC, occurrence of free radicals took place[41] so we had to use a stabilizer which is radical trapper like the investigated stabilizers which have multifunctional groups which can act as radical scavengers for the radicals resulting from the degradation process of PVC.

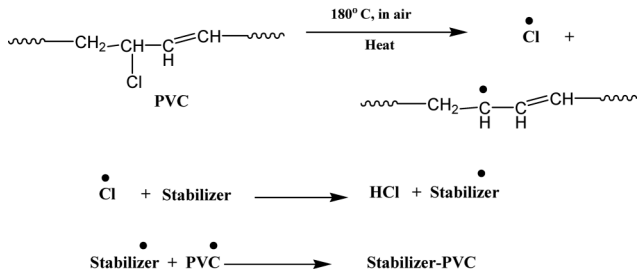
From the aforementioned experimental findings, we suggest a free radical mechanism for the stabilizing efficiency of the investigated stabilizers during the thermal degradation of PVC. The proposed mechanism is outlined by the following equations (Scheme 1).

Effects of mixed stabilizers on thermal stabilization efficiency in rigid PVC

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 metal complexes were examined as co-stabilizers with the two

Table 3 Extent of discoloration of thermally degraded rigid PVC at 180 °C, in air, in presence of 2 wt.% of the investigated stabilizers as a function of degradation time

Type of stabilizer	Colour at 0 min	Colour at 15 min	Colour at 25 min	Colour at 35 min	Colour at 45 min
Blank PVC	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
Co–EFA	White	White	White	Yellow	Yellow
Ni–EFA	White	White	White	Yellow	Yellow



Scheme 1 General free radical mechanism of the investigated stabilizers during the thermal stabilization of PVC.

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 wt.% based on the polymer weight. The results reveal a true synergism resulting from the combination of the organic stabilizer with each of the reference stabilizers, irrespective to the class of which the reference stabilizer belongs to. Maximum synergism was attained at the ration 50% : 50% of both the investigated stabilizers with each of the reference stabilizers (Table 4).

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 or 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.[42–46]

Conclusions

Rationally, designing novel compounds for a desired application is pragmatically very difficult, because it is quite a challenge to predict the chemical properties of the compounds based on their structures. PVC presents an exciting opportunity to create new organic/inorganic hybrid compounds that exhibit highly functional chemistries. For this purpose we report the synthesis and characterization of Co(II) and Ni(II) binary and ternary complexes with enrofloxacin $\mathbf{HL_1}$ and DL-alanine ($\mathbf{H_2L_2}$).

Antimicrobial and antifungal activities were evaluated; it has been observed that the metal complexes exhibited a higher antimicrobial and antifungal activity comparing with the ligand and the standard references. Demonstrating the value of the prepared complexes for their antimicrobial and antifungal activity is of beneficial interest in many biomedical applications.

The reported complexes were examined as both stabilizers and co-stabilizers for rigid PVC based on their greater induction period values relative to those of the reference stabilizers and the extent of discoloration is much improved too. Mixing the parent complexes with the two used reference stabilizers in different proportions greatly improves the induction period and also improves the extent of discoloration. The maximum synergism was obtained in the weight ratio (50% : 50%) of the stabilizer and any of the reference stabilizer. Our study show how novel complexes are being explored to improve the thermal stability of PVC which might increase its potential technical applications, moreover the prepared complexes can be utilized as biomedical additives for

Table 4 Induction period (T_s) of mixed investigated compounds with reference stabilizers

Type of stabilizer	0%:100% Ni-EFA:DBLC	25%:75% Ni-EFA:DBLC	50%:50% Ni-EFA:DBLC	75%:25% Ni-EFA:DBLC	100%:0% Ni-EFA:DBLC
(**4**) (Ni–EFA)	9	36	49	40	22
Type of stabilizer	0%:100% Ni-EFA:Ca–Zn soap	25%:75% Ni-EFA:Ca–Zn soap	50%:50% Ni-EFA:Ca–Zn soap	75%:25% Ni-EFA:Ca–Zn soap	100%:0% Ni-EFA:Ca–Zn soap
(**4**) (Ni–EFA)	8	40	45	39	22
Type of stabilizer	0%:100% Co-EFA:DBLC	25%:75% Co-EFA:DBLC	50%:50% Co-EFA:DBLC	75%:25% Co-EFA:DBLC	100%:0% Co-EFA:DBLC
(**3**) (Co-EFA)	9	57	65	53	25
Type of stabilizer	0%:100% Co-EFA:Ca–Zn soap	25%:75% Co-EFA:Ca–Zn soap	50%:50% Co-EFA:Ca–Zn soap	75%:25% Co-EFA:Ca–Zn soap	100%:0% Co-EFA:Ca–Zn soap
(**3**) (Co–EFA)	8	53	**60**	55	25

PVC which expand its applications in biomedical and clinical field. Exploiting these complexes by their interaction with PVC should lead to the increased utility of the inorganic complexes with a glance at future trends and challenges in biotechnology and industrial field.

Experimental

General procedures and materials

All chemicals used were of the analytical reagent grade (AR) and of highest purity available. They included enrofloxacin, DL-alanine, sparfloxacin (Sigma), $CoCl_2 \cdot 6H_2O$ and $NiCl_2 \cdot 6H_2O$ (BDH), were used. Absolute ethyl alcohol, diethyl ether (Adwic), Yeast extract and agar (Sigma) were also used. De-ionized water collected from all glass equipment was used in all preparations. The commercial PVC (suspension) used in this study was additive free, with a K-value 70 (Hüls) dibasic lead carbonate (DBLC) (Rolite lead) (National Lead, West Germany), Ca–Zn soap (Lagor-S.P.A., Italy).

FTIR spectra were obtained from dispersions in KBr using a Perkin-Elmer FT-IR type 1650 spectrophotometer. The spectra were collected in the range from 200 to 4000 cm^{-1} with a resolution of 2 cm^{-1}. UV-Vis spectrophotometric measurements were carried out using automated spectrophotometer UV-Vis Thermo Fischer Scientific Model Evolution 60 ranged from 200 to 900 nm. Molar conductivity was measured on a ELICO(CM82T) conductivity bridge. The molar magnetic susceptibility was measured on powdered samples using the Faraday method. The diamagnetic corrections were made by Pascal's constant and $Hg[Co(SCN)_4]$ was used as a calibrant. The solid reflectance spectra were performed on a Shimadzu 3101pc spectrophotometer. Thermal analyses of the complexes were carried out using a Shimadzu TGA-50H and DTA-50H thermogravimetric analyzer in a dynamic nitrogen atmosphere (flow rate 20 ml min^{-1}) with a heating rate of 10 °C min^{-1}. The percentage weight loss was measured from ambient temperature to 1000 °C, highly sintered α-Al_2O_3 was used as reference.

Antimicrobial activity

Antimicrobial activity of the tested samples was determined using a modified Kirby–Bauer disc diffusion method.[47,48] 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.[49] 100 µl of microbial suspension was spread onto agar plates. Plates inoculated with filamentous fungi as *Aspergillus flavus*, *Candida albicans* at 25 °C for 48 h; *Gram-positive* bacteria as *Staphylococcus aureus* and *Gram-negative* bacteria as *Escherichia coli*; were incubated at 35–37 °C for 24–48 h and yeast as *Candida albicans* incubated at 30 °C for 24–48 h and the diameters of the inhibition zones were measured in millimetres.[50] Standard discs of tetracycline (antibacterial agent), Amphotericin B (antifungal agent) served as positive controls, while filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control.

Synthesis of (1)–(4)

The reported complexes were prepared according to the following procedure by addition of a hot water-ethanolic solution (60 °C) of the metal chlorides for Co(II), Ni(II) (25 ml, 0.1 mmol) to a hot ethanolic solution of $\mathbf{HL_1}$ (25 ml, 0.2 mmol in case of binary or 0.1 mmol in case of ternary complexes) and DL-alanine ($\mathbf{H_2L_2}$) (0.1 mmol). The resulting mixture was stirred under reflux for 2 h and left to cool, whereby the complexes precipitated as fine powders. The solid complexes were filtered, washed with ethanol, then with diethyl ether, and dried in a vacuum desiccator over anhydrous calcium chloride where the solid complexes were obtained as a solid powder; several trials are in process in order to obtain suitable crystals. The formed complexes showed similar melting points (~260 °C, 263, 267 and 269 °C) for (**1**), (**2**), (**3**) and (**4**), respectively.

Preparation of PVC samples

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

Evaluation of stabilizing efficiency

Evaluation of the stabilizing efficiency of the investigated thermal stabilizers was carried out by using *Congo Red* dye paper to measure the thermal stability values (T_s), *i.e.*, the time elapsed for the detection of HCl gas evolved at 180 °C, in air.[51]

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