

Stability of Neodymium(III) Complexes of 4-Hydroxycoumarins with Anticoagulant Activity

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Abstract: Complexes of neodymium(III) with 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one, 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one and 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one were synthesized by mixing of water solutions of neodymium(III) nitrate and the respective ligands, in amounts equal to metal: ligand molar ratio of 1: 3. The complexes were characterized and identified by elemental analysis, conductivity, IR and ¹H NMR spectral data. DTA and TGA have been applied to confirm the compositions of the compounds. The formation of the complexes was studied by IR and ¹H-NMR-spectroscopy. The complexation reactions were studied in detail by spectrophotometric method. The stepwise formation of the complexes has been established in the pH region studied. The equilibrium constants for the complexes have been determined. The complexes of neodymium(III) with 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one, 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one and 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one were assayed for acute intraperitoneal and per oral toxicity and for influence on blood clotting time.

Key words: 4-hydroxycoumarins, neodymium(III) complexes, IR spectra, NMR spectra, stability constants, anticoagulant activity

INTRODUCTION

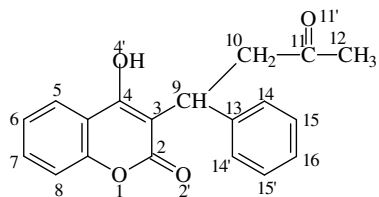
Coumarins are wide spread in nature, also the biological properties of different coumarins and their derivatives are well known-they include anticoagulant, antiproliferative, antimicrobial, spasmolytic, antitumor, antioxidant, etc. activities^[1-3]. A large number of structurally novel coumarin derivatives have ultimately been reported to show substantial cytotoxic and anti-HIV activity *in vitro* and *in vivo*^[4]. The different pharmacological values found for the coumarins could be related to presence and the positions of the hydroxyls in their structures.

Unfortunately, little is known about the complexing ability of neodymium(III) with coumarins. Earlier it has been shown that interesting metalorganic compounds of Mendiaxon, Warfarin, Coumachlor and Niffcoumar with lanthanides displayed antitumor activity against P3HR1, K-562 and THP-1 cell lines^[1-3]. The complexes had a strong cell proliferation inhibiting effects. One could expect that lanthanide complexes with selected active coumarin ligands should retain or even improve their biological activity similarly to other lanthanide complexes with hydroxycoumarin derivatives. It has been observed that Ce(III), La(III) and Nd(III) ions possessed cytotoxic activity and literature data showed the coumarins had also these properties^[5]. For that reason the synthesis of complexes

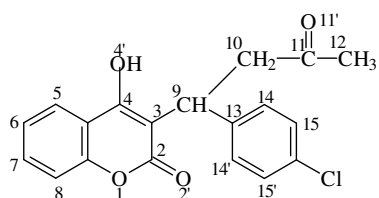
of Nd(III) was taken into consideration with cytotoxicity and anticoagulant activity screening, as well as with further pharmacological study. Recently we have reported the synthesis of Ce(III), La(III) and Nd(III) complexes with very strong cytotoxic effects^[6-9]. Here we show also an increased biological activity, especially anticoagulant one, for the Nd(III) complexes with 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one, 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one and 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one, Scheme (1).

Coumarins have been studied extensively for their complexation behavior with metal ions. A number of dioxouranium (VI) and titanium (IV) complexes with hydroxycoumarins e.g. 3-hydroxycoumarin and 7,8-dihydroxy-4-methylcoumarin etc. have been studied spectrophotometrically and it has been observed that depending upon pH, 1:1, 1:2 and 1:3 complexes are formed in solution^[10,11]. We recently reported spectrophotometrical study of lanthanide complexes with coumarins^[12,13]. In the present investigation complexation reactions of neodymium (III) with the above ligands have been studied. The obtained Nd(III) complexes with these coumarin ligands were characterized by elemental analysis, physicochemical methods, NMR and IR-spectroscopy. The complicated vibrational spectra of neodymium(III) complexes were

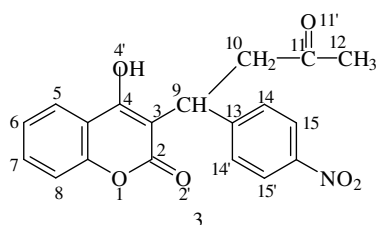
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1
4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one



2
4-Hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one



3
4-Hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one

Scheme 1: Structures of the ligands

interpreted on the basis of comparison with the vibrational spectra of the free ligands. The most sensitive to coordination modes of the ligands have been assigned and discussed.

MATERIALS AND METHODS

Source substances for synthesis: The compounds used for preparing the solutions were Merck products, p.a. grade: $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one, 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one and 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one.

Synthesis of Nd(III) complexes: The sodium salts of 4-hydroxy-3-(3-oxo-1-phenyl-butyl)-2H-1-benzopyran-2-one, 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one and 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one were used for the preparation of metal complexes as ligands. The complexes were synthesized by mixing water solutions of neodymium(III) salt and the respective ligand, in amounts equal to metal: ligand molar ratio of 1: 3. The reaction mixtures were stirred with an electromagnetic stirrer at 25 °C for one hour. At the moment of mixing of the solutions, precipitates were obtained. The precipitates were filtered, washed several

times with water and dried in a desiccator to constant weight.

Analytical and spectroscopic measurements: The elemental analyses for C, H, Cl, N, Nd and H_2O were performed according to standard microanalytical procedures.

The experiments of DTA and TGA were carried out using a derivatograph produced by the firm MOM (Budapest). Samples with particle size below 0.25 mm were placed in platinum crucibles. The heating rate was 10 °C min^{-1} until 900 °C. The inert substance was Al_2O_3 .

The IR spectra (Nujol and KBr) were recorded on IR-spectrometer FTIR-8101M Shimadzu (3800-400 cm^{-1}) and on IR-spectrometer Perkin-Elmer GX Auto image system (700-200 cm^{-1}).

The ^1H NMR spectra were recorded at room temperature on Bruker WP 250 (250 MHz) spectrometer in DMSO-d_6 .

Stability constants: The complexation reactions were studied in detail by spectrophotometric method^[12,13]. The method was used for evaluating the values of ϵ_1 , ϵ_2 and ϵ_3 and calculating of the stability constants.

Pharmacology: In this study the complexes of neodymium(III) with coumarins were investigated for acute intraperitoneal and per oral toxicity and for influence on blood clotting time.

Acute intraperitoneal toxicity (i.p. LD_{50}) of the studied compounds was assessed by dissolving in saline (0.9% NaCl) with 1 drop of Tween 80, administered to mice via i.p. route. LD_{50} was evaluated of 4 different doses, each on 6 animals and calculated by the method of Litchfield-Wilcoxon, using a personal computer.

Acute per oral toxicity (p.o. LD_{50}) of the studied compounds was assessed by dissolving in saline (0.9% NaCl) with 1 drop of Tween 80, administered to mice via p.o. route. LD_{50} was evaluated of 4 different doses, each on 6 animals and calculated by the method of Litchfield-Wilcoxon, using a personal computer.

Index of absorption was calculated using data from i.p. and p.o. toxicity.

The influence of investigated compounds on blood clotting time was determined by the method of Moravitz. The investigation was performed on 40 male white mice, weighting 20 ± 2 g. The compounds were administered three days in doses of 1/10 of p.o. LD_{50} . On the fourth day (24h after the last administration) the clotting time was assessed after small incision of sublingual vein and measuring the clotting time of the second drop blood on clean glass.

RESULTS AND DISCUSSION

Characterization of Nd(III) complexes: The data of the elemental analysis of the complexes are in accordance with the presented empirical formulas (Table 1). The compositions of the complexes were

Table 1: Elemental analysis of Nd(III) complexes

Complex	m.p.	found/	Calculated		(%)	
	°C	C	H	Cl/N	H ₂ O	Nd
Nd(HW) ₃ .4H ₂ O	>300	59,70	4,57	-	6,67	12,61
		60,15	4,66		6,33	12,66
Nd(HC) ₃ .4H ₂ O	>300	55,01	4,09	8,89	6,06	11,29
		55,20	4,03	8,47	5,81	11,62
Nd(HN) ₃ .6H ₂ O	> 300	52,48	4.10	3.01	7.95	8.26
		52.29	4.13	3.21		10.83

HW = C₁₉H₁₅O₄.
 HC = C₁₉H₁₄ClO₄⁻
 HN = C₁₉H₁₄NO₆.

Table 2: Results of the DTA and TGA

Compound	Therm.effect	T, °C	Δm, % (Δm _{calcul.})
C ₁₉ H ₁₆ O ₄	Endo	190	0.73
	Exo	523; 605; 765	61.8; 77.9; 89.7
C ₁₉ H ₁₅ O ₄ Na	Exo	200	2.5
	Endo	260; 340; 505	8.2; 20.0; 55.3
	Exo	588	64.7
	Endo	760	84.7
Nd(HW) ₃ .4H ₂ O	Endo	220	6.00 (6.35)
	Exo	580	60.0
	Endo	900	86.0
C ₁₉ H ₁₅ NO ₆	Endo	240; 465	1.6; 30.7
	Exo	540; 620; 738	42.0; 58.0; 80.6
C ₁₉ H ₁₄ Na NO ₆	Endo	170	10.2
	Exo	300	40.9
	Endo	450	47.7
	Exo	480	56.8
	Endo	630	65.9
Nd(HN) ₃ .6H ₂ O	Endo	105; 220	4.00; 8.00
	Exo	350; 500	25.9; 46.0
	Endo	750	75.3

Table 3: Results of the IR spectra

Compound	V _{OH/H₂O}	V _{C=O} ^k	V _{C=O} ^l	V _{C=C}	V _{Ar}	V _{C-C}	V _{C-O}	V _{C-O-C}
C ₁₉ H ₁₆ O ₄	3272	1680	1617	1570	1492	1277-1243	1191	1075
C ₁₉ H ₁₅ NaO ₄	3400	1721	1668	1597	1516	1265-1223	1163	1092
Nd(HW) ₃ .4H ₂ O	3394	1695	1651	1580	1505	1280-1245	1179	1108
C ₁₉ H ₁₅ ClO ₄	3286	1679	1615	1569	1492	1277-1243	1190	1075
C ₁₉ H ₁₄ NaClO ₄	3400	1722	1668	1598	1522	1266-1223	1159	1087
Nd(HC) ₃ .4H ₂ O	3378	1693	1651	1598	1514	1282-1229	1180	1092
C ₁₉ H ₁₅ NO ₆	3295	1686	1619	1572	1512	1249-1212	1173	1101
C ₁₉ H ₁₄ NaNO ₆	3364	1715	1653	1595	1516	1267-1226	1163	1076
Nd(HN) ₃ .6H ₂ O	3392	1697	1650	1598	1514	1281-1229	1179	1111

Table 4: ¹H-NMR spectra (250 MHz, DMSO-d₆), δ(ppm)

Compound	H ₅ ,H ₈	H ₉	2H ₁₀	3H ₁₂	H _{14,15,16}
C ₁₉ H ₁₅ NaO ₄	7,00-7,50 m	4,90 t	3,25d	2,05 s	7,50-7,80m
Nd(HW) ₃ .4H ₂ O	6,95-7,20m	6,85bs	3,45bs	1,85bs	7,40-7,70m
C ₁₉ H ₁₄ NaClO ₄	6,95-7,30m	4,90t	3,27d	2,05s	7,55-7,80d
Nd(HC) ₃ .4H ₂ O	7,35-7,65m	6,85bs	3,45bs	1,90s	7,15-7,60d
C ₁₉ H ₁₅ NO ₆	7,36-7,66 m	4.18 s	3.47	1.94	7.83-8.12 dd
C ₁₉ H ₁₄ NaNO ₆	6,95-7,40 m	5,05 t	3,40 d	3,05 s	7,60-8,10 dd
Nd(HN) ₃ .6H ₂ O	7,20-7,60 m	6,80 s	3,50 b s	1,90 s	7,50-7,90 dd

Table 5: pH-intervals for the complexes of Nd (III) with coumarins

Complex	ML ²⁺	ML ₂ ⁺	ML ₃
Nd(HW) ₃ .4H ₂ O	2.5-5.0	5.5-6.5	7.0-8.0
Nd(HN) ₃ .6H ₂ O	3.0-4.0	5.0-6.0	6.5-7.5
	3.0-4.5	5.0-6.0	6.5-7.5

Table 6: Molar absorptivities of the Nd(III) complexes with coumarins, ε.10⁴ l.mol⁻¹.cm⁻¹

Complex	ε ₁ (ML ²⁺)	ε ₂ (ML ₂ ⁺)	ε ₃ (ML ₃)
Nd(HW) ₃ .4H ₂ O	1.625	2.250	3.350
Nd(HN) ₃ .6H ₂ O	1.675	3.525	4.850
	2.275	3.075	4.375

Table 7: Compositions and stepwise stability constants of the complexes

Complex	Composition	lgK1	lgK2	lgK3	
Nd(HW) ₃ .4H ₂ O	Nd(HC) ₃ .4H ₂ O	ML ²⁺ , ML ₂ ⁺ , ML ₃	6.69	3.34	1.55
Nd(HN) ₃ .6H ₂ O		ML ²⁺ , ML ₂ ⁺ , ML ₃	6.84	3.54	1.77
		ML ²⁺ , ML ₂ ⁺ , ML ₃	6.20	3.46	2.58

confirmed by DTA and TGA (Table 2). At the beginning of the DTA-curves of the complexes there are clearly manifested endothermic effects, corresponding to the elimination of molecules of water per molecule of Nd(III) complex. The amount of this weight loss, determined also by Karl Fisher analysis, is correlated with the intensity of these endothermic effects and with the respective decreases in the mass. On heating the complexes the decomposition step corresponds to the loss of molecules of the ligand, which is in agreement with the proposed compositions. Exothermal effects dominate in the thermograms of the complexes, resulting from the decomposition of the organic matter. A further weight loss recorded up to 900 °C indicates the formation of thermally stable oxides.

The mode of bonding of the ligands to Nd(III) was elucidated by recording the IR spectra of the complexes as compared with these of the free ligands (Table 3).

IR spectrum of the complex of 4-hydroxy-3-(3-oxo-1-phenyl-butyl)-2H-1-benzopyran-2-one: The bands appear in the IR spectrum of sodium salt of 4-hydroxy-3-(3-oxo-1-phenyl-butyl)-2H-1-benzopyran-2-one at 3400, 1721, 1668, 1597, 1516, 1265-1223, 1163, 1092 cm⁻¹. A band at 1721 cm⁻¹ can be attributed to the stretching vibrations of the keto-carbonyl group of the side chain; a band at 1668 cm⁻¹ corresponds to the carbonyl group of the lacton ring and a band at 1597 cm⁻¹ can be related to the stretching vibrations of the conjugated olefinic system. The vibrations at 1516 cm⁻¹ correspond to the aromatic system.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300-3400 cm⁻¹ in the spectra of the complexes. The weak band observed at 3272 cm⁻¹ in the spectrum of the free ligand is missing in the spectra of the complexes.

The ν_{C=O} band at 1721 cm⁻¹ exhibits a shift of 20-30 cm⁻¹ to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O group in coordination.

IR spectrum of the complex of 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one: The bands appear in the IR spectrum of sodium salt of 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one at 3400, 1722, 1668, 1598, 1522, 1266-1223, 1159, 1087 cm⁻¹. A band at 1722 cm⁻¹ can be attributed to the stretching vibrations of the keto-carbonyl group of the side chain; a band at 1668 cm⁻¹ corresponds to the carbonyl group of the lacton ring and a band at 1598 cm⁻¹ can be related to the stretching

vibrations of the conjugated olefinic system. The vibrations at 1522 cm⁻¹ correspond to the aromatic system.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300-3400 cm⁻¹ in the spectra of the complexes. The weak band observed at 3286 cm⁻¹ in the spectra of the free ligand is missing in the spectra of the complexes.

The ν_{C=O} band at 1722 cm⁻¹ exhibits a shift of 30 cm⁻¹ to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O group in coordination.

The C-C and C-O stretch and the C-O-C band are all shifted to higher frequency (1282-1229, 1180 and 1092 cm⁻¹) in the complexes. Similar frequency shifts are observed for the other complexes and are attributed to complexation of the lanthanide ion with the carbonyl oxygen^[14].

IR spectrum of the complexes of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one:

The bands appear in the IR spectrum of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one sodium salt at 3364, 1715, 1653, 1595, 1516, 1267-1226, 1163, 1076 cm⁻¹. A band at 1715 cm⁻¹ can be attributed to the stretching vibrations of the keto-carbonyl group of the side chain; a band at 1653 cm⁻¹ corresponds to the carbonyl group of the lacton ring and a band at 1595 cm⁻¹ can be related to the stretching vibrations of the conjugated C=C system. The vibrations at 1516 cm⁻¹ correspond to the aromatic system.

A broad band, characteristic of ν_{OH} of coordinated water was observed in the range 3300-3400 cm⁻¹ in the spectra of the complexes. The weak band observed at 3295 cm⁻¹ in the spectra of the free ligand (4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one) is missing in the spectra of the complexes.

The ν_{C=O} band at 1715 cm⁻¹ exhibits a shift of 20 cm⁻¹ to lower wavenumber values on complexation which may be taken as evidence for the participation of the C=O group in coordination. The C-C and C-O stretch and the C-O-C band are all shifted to higher frequency (1281-1229, 1179 and 1111 cm⁻¹) in the complex.

IR-spectra of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one, 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one sodium salt and of the neodymium(III) complex with 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one were recorded on solid state in Nujol in the range 700-220 cm⁻¹.

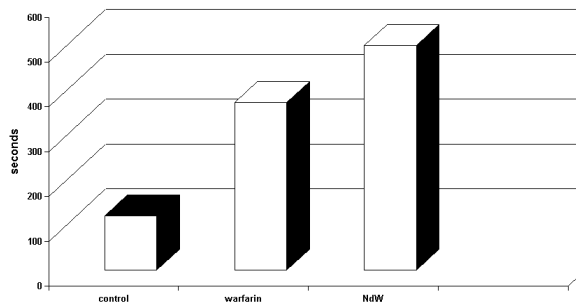


Fig. 1: The influence of Nd(III) complex of 4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one (Warfarin) on coagulation time after administration of 1/10 of LD50 p.o.

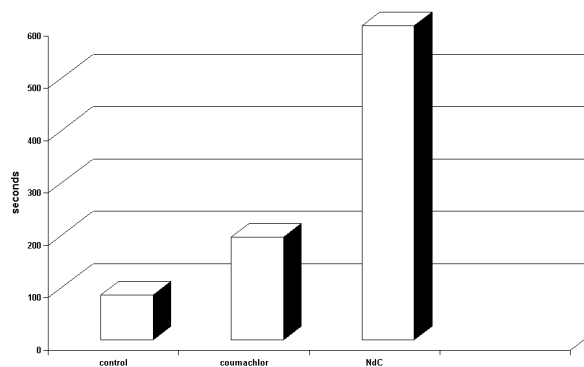


Fig. 2: The influence of Nd(III) complex of 4-Hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one (Coumachlor) on coagulation time after administration of 1/10 of LD50 p.o.

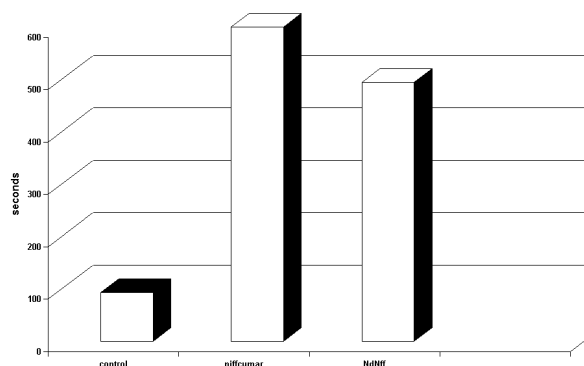


Fig. 3: The influence of Nd(III) complex of 4-Hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one (Niffcumar) on coagulation time after administration of 1/10 of LD50 p.o.

The spectrum of the complex showed new bands, in comparison with that of the free ligand, at about 460 and 300 cm^{-1} and they were assigned to metal-oxygen stretching vibrations.

Metal ion coordination with ligands by means of oxygen atom of C=O group was shown owing to data of $^1\text{H-NMR}$ spectra. Proton spectra of the compounds

recorded in DMSO-d_6 , confirmed the formation of the complexes (Table 4).

The changes of chemical shifts of the $^1\text{H NMR}$ spectra were observed in the complexes and they were attributed to coordination of the ligands to Nd(III). The chemical shifts of the H_9 and H_{10} protons vary in the complexes, because of the shift properties of Nd(III). It was an evidence that in the Nd(III) complexes with 1, 2 and 3, Scheme (1) there were strong positive shift effects for H_9 and a weaker ones for H_{10} . Chemical shifts of H_9 and H_{10} protons in the complexes were attributed to coordination of the ligands to Nd(III).

Spectrophotometrical study: A families of curves were obtained at $\lambda_{\text{max}} = 316 \text{ nm}$ with variations in ligand concentration and pH but at fixed concentration of the metal ion. The curves indicated that at constant pH the absorbance increases if the ligand concentration is raised.

pH-absorbance curves have been interpreted in two ways: logarithmic method of analysis and corresponding solutions method^[12,13], confirming the existence of 1:1, 1:2 and 1:3 complexes in different pH regions (Table 5).

Stability constants of the complexes: The stability constants K_1 , K_2 and K_3 of the each three complexes were calculated. The dissociation constants of the ligands were determined pH-metrically. The values of molar absorptivities of the each three complexes were obtained (Table 6). The values of stepwise stability constants of the each three complexes in the different pH regions were presented (Table 7).

CONCLUSION

The stepwise formation of the complexes of Nd(III) depending upon the concentration of ligand and pH has been established. The liberation of one proton in different pH ranges indicates the stepwise formation of protonated complexes.

All the complexes formed are mononuclear, vis. NdR^{2+} , NdR_2^+ and NdR_3 .

Analysis of the obtained data from blood clotting time showed that the complexes of neodymium (III) with 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one and 4-hydroxy-3-[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one have the greatest effect on clotting time, which is statistically significant, compared to control, Fig. 1-3.

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