DETERMINATION OF HEPARIN USING TERBIUM-DANOFLOXACIN AS A LUMINESCENT PROBE

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A new spectrofluorimetric method is described for the determination of trace amounts of heparin (Hep) using terbium-danofloxacin (Tb^{3+}-Dano) as a fluorescent probe. Hep can enhance the fluorescence intensity of the Tb^{3+}-Dano complex at 545 nm and the enhanced fluorescence intensity of the Tb^{3+} ion is proportional to the concentration of Hep. Optimum conditions for the determination of Hep are: pH 7.2, $[Tb^{3+}] = 4.0 \cdot 10^{-5}$ mol/l, and [Dano] $=2.5 \cdot 10^{-5}$ mol/l. A dynamic range for the determination of Hep is 0.1–1.5 μg/ml with a detection limit (S/N = 3) of 24.62 ng/ml. The proposed method is simple, practical, and relatively free from interferences.

Keywords: *heparin, danofloxacin, spectrofluorimetry, sensitized luminescence, terbium.*

ОПРЕДЕЛЕНИЕ ГЕПАРИНА С ИСПОЛЬЗОВАНИЕМ ТЕРБИЙ-ДАНОФЛОКСАЦИНА В КАЧЕСТВЕ ФЛУОРЕСЦЕНТНОГО ЗОНДА

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Предложен новый спектрофлуориметрический метод определения следовых количеств гепарина (Hep) с использованием тербий-данофлоксацина (Tb^{3+}-Dano) в качестве флуоресцентного зонда. В присутствии гепарина происходит усиление флуоресценции комплекса Tb^{3+}-Dano на длине волны 545 нм, при этом интенсивность флуоресценции иона Tb^{3+} пропорциональна концентрации гепарина. Установлены оптимальные условия определения гепарина: pH 7.2, $[Tb^{3+}] = 4.0 \cdot 10^{-5}$ моль/л, [Dano] $= 2.5 \cdot 10^{-5}$ моль/л. Динамический диапазон определения гепарина составляет 0.1—1.5 мкг/мл при пределе обнаружения (S/N = 3) 24.62 нг/мл. Предложенный метод отличается простотой, практичностью и относительной устойчивостью к помехам.

Ключевые слова: *гепарин, данофлоксацин, спектрофлуориметрия, сенсибилизированная люминесценция, тербий.*

Introduction. Heparin (Hep) is a naturally occurring anticoagulant produced by basophiles and mast cells, with an average molecular weight of 15000 Da. It consists of repeating units of uronic/glucuronic acid and glucosamine residues. The most common disaccharide unit is composed of a 2-O-sulfated iduronic acid and 6-O-sulfated N-sulfated glucosamine. Hep is widely used as an injectable anticoagulant. Hep is negatively charged in an aqueous solution with the average charge of –70 [1]. It can also be used as an anticoagulant in various experimental and medical devices such as test tubes and renal dialysis machines. Pharmaceutical grade Hep is derived from mucosal tissues of slaughtered meat of animals such as porcine (pig) intestine or bovine (cow) lung. It acts as an anticoagulant, preventing the formation of clots and extension of existing clots within the

blood stream. It is one of the oldest drugs with widespread clinical applications. Hep and its derivatives have a variety of biological activities including antilipemic, antithrombotic, immunoregulatory, antiphlogistic, and antianaphylactic actions [2]. So, the Hep level in the patient's blood needs to be carefully and accurately monitored during surgery and recovery processes. The reported methods for the Hep determination are focused on: flow injection analysis [3], ion-channel sensors [4], resonance Rayleigh scattering spectra [5, 6], capillary chromatography [7], high-performance liquid chromatography [8], surface plasmon resonance sensor analysis [9], rotating electrode potentiometry [10], piezoelectric quartz crystal sensor [11], protamine titration using a membrane electrode [12], and extracorporeal membrane oxygenation [13]. Up to now, some researchers reported the detection of Hep by using rare earth fluorescent probes such as Tb^{3+} [14, 15] or Eu^{3+} [16]. But there was no report on the determination of Hep using Tb^{3+}-Danofloxacin (Dano) as a fluorescent probe. Dano, an antibacterial agent containing the α-carbonyl carboxylic acid configuration, is an ideal ligand for Tb^{3+}. In this work, Dano is chosen as the ligand of Tb^{3+} and the possibility to enhance the Tb^{3+} fluorescence sensitized by using Hep as a co-ligand has been investigated. Experimental results showed that the characteristic peak of Tb^{3+} at 545 nm can be enhanced, with the fluorescence intensity being proportional to the concentration of Hep. Therefore, a new highly sensitive method for the spectrofluorimetric determination of Hep was developed and validated.

Experimental. ***Materials and methods***. Analytical-grade ethanol, hydrochloric acid (HCl), methanol, 2-propanol, acetonitrile, tris-(hydroxymethyl) aminomethane (Tris) and other buffers were obtained from Merck (Darmstadt, Germany), terbium (III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$) from Acros Organics (Geel, Belgium), Dano powder from Jamedat Afagh Pharmaceutical Company (Tehran, Iran), and Hep powder from Caspian Tamin Pharmaceutical Co. (Rasht, Iran). Double distilled water prepared using a Millipore-Q-plus water purification system (Millipore, Bedford, MA, USA) was used in this study.

A $1.0 \cdot 10^{-2}$ mol/l terbium (III) solution was prepared by dissolving the appropriate amount of $TbCl_3 \cdot 6H_2O$ in double distilled water and stored in a polyethylene container to avoid memory effects of terbium adsorbed on glass vessels. A stock solution of 500 μg/ml Hep was prepared by dissolving in deionized water and stored at 4 °C. A stock solution ($1.0 \cdot 10^{-2}$ mol/l) of Dano was prepared in double distilled water and a 0.1 mol/l Tris buffer solution was prepared by dissolving the desired amount of Tris–base in 100 ml of water and adjusting the pH to 7.2 with HCl.

Apparatus. The acquisition of fluorescence spectra and intensity measurements were performed on a Jasco FP-750 spectrofluorimeter (Kyoto, Japan) equipped with a 150 W xenon lamp, using a 10 mm quartz cell and the monochromator bandwidth of 5 nm. The excitation wavelength was set at 347 nm and the fluorescence intensity was measured at 545 nm. All measurements were performed at 25 °C; temperature was controlled using a Peltier thermostated cell holder (Jasco, Japan). The pH of solutions was measured with a Metrohm 654 pH meter (Herisau, Switzerland). A Cary-100 UV-Visible spectrophotometer (Varian, USA) was used for UV spectrum scanning and absorbance mesurements.

Sample preparation. Working solutions of Hep (250 ng/ml), terbium ($4 \cdot 10^{-4}$ mol/l), and Dano ($2.5 \cdot 10^{-4}$ mol/l) were obtained by appropriate dilution from the stock solutions.

Experimental procedure. The solutions were added to 10-ml calibrated flasks in the following order: 1 ml of the $4 \cdot 10^{-4}$ mol/l Tb^{3+} solution, 1 ml of $2.5 \cdot 10^{-4}$ mol/l Dano solution, suitable aliquots of the Hep solution and 0.5 ml of 0.1 mol/l buffer (pH 7.2). The mixtures were diluted to the mark with double distilled water and allowed to stay for 5 min at room temperature. The fluorescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ = 347 nm/545 nm. The Hep-enhanced fluorescence intensity of Tb^{3+}-Dano was represented as $\Delta F\% = 100(F - F_0)/F_0$ where F and F_0 were the fluorescence intensities of the systems with Hep and without Hep, respectively.

Results and discussion. ***Excitation and emission spectra.*** The excitation and emission spectra of Tb^{3+}, Hep, Tb^{3+}-Hep, Tb^{3+}-Dano, and Tb^{3+}-Dano-Hep are shown in Fig. 1. As seen from curves 1, 2 and 3, the single Tb^{3+} ion solution, Tb^{3+}-Hep solution, and Hep solution have small or no peaks. According to the figure (curves 1 and 4),

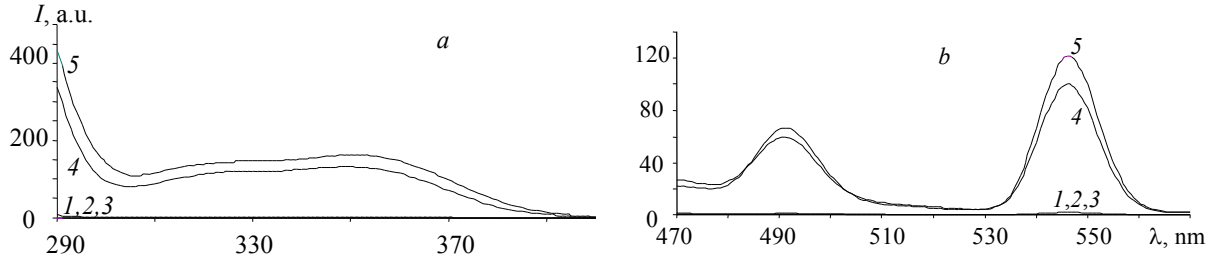

Fig. 1. Excitation (a) and emission (b) spectra of Tb^{3+} (1), Hep (2), Tb^{3+}-Hep (3), Tb^{3+}-Dano (4), and Tb^{3+}-Dano-Hep (5). Experimental conditions: $[Tb^{3+}] = 4 \cdot 10^{-5}$ mol/l, [Dano] = $2.5 \cdot 10^{-5}$ mol/l, [Hep] = 0.25 μg/ml, pH 7.2, $\lambda_{ex}/\lambda_{em}$ = 347 nm / 545 nm.

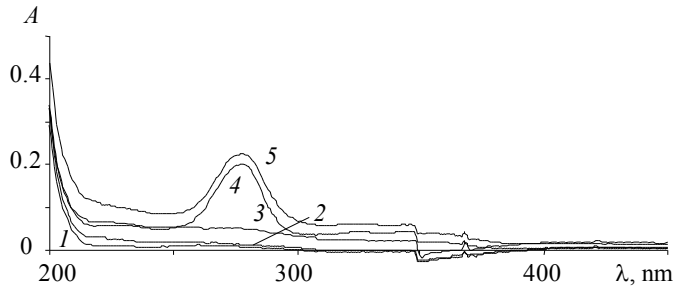

Fig. 2. Absorption spectra of Tb^{3+} (1), Hep (2), Tb^{3+}-Hep (3), Tb^{3+}-Dano (4), Tb^{3+}-Dano-Hep (5); $[Tb^{3+}] = 4 \cdot 10^{-5}$ mol/l, [Dano] = $2.5 \cdot 10^{-5}$ mol/l, [Hep] = 1 μg/ml, pH 7.2.

after the addition of Dano into the Tb^{3+} ion solution, Dano can form a binary complex with the Tb^{3+} ion. Two little characteristic peaks of the Tb^{3+} ions appear at 490 nm and 545 nm; they belong to the $^5D_4$–$^7F_6$ and $^5D_4$–$^7F_5$ transitions of Tb^{3+}, respectively. Comparing the curves 4 and 5, it can be seen that the characteristic peak of Tb^{3+} at 545 nm remarkably enhanced after the addition of Hep, which shows the formation of the stable ternary complex of Hep–Tb^{3+}-Dano.

The absorption spectra of Tb^{3+}, Hep, Tb^{3+}-Hep, Tb^{3+}-Dano and Tb^{3+}-Dano-Hep are shown in Fig. 2. The curves 1, 2 and 3 have few or no peaks; the comparison of curves 4 and 5 shows the absorbance enhancement from 0.199 to 0.225 which indicates the formation of the stable ternary complex of Hep–Tb^{3+}-Dano.

Optimization of experimental conditions. *Effect of pH and the choice of buffer solution.* Fluorescence intensities of a series of the 0.10 mol/l Tris-HCl buffer solutions in the pH range of 6.5–8.5 while keeping the concentrations of Tb^{3+}, Dano and Hep at $1.0 \cdot 10^{-5}$ mol/l, $2.0 \cdot 10^{-5}$ mol/l, and 0.25 μg/ml, respectively, were measured at $\lambda_{ex}/\lambda_{em}$ = 347 nm/545 nm (Fig. 3). As shown in Fig. 3, the pH of the medium had a great effect on the fluorescence intensity of the system. The enhancement in the fluorescence intensity (ΔF%) of the Tb^{3+}–Dano complex with Hep reaches the maximum value at pH 7.2. Different buffers adjusted to pH 7.2 were also tested, and the ΔF% values were 24.9 (Tris-HCl), 17.2 (hexamethylene tetraamine), 6.7 (acetate), and 12.4 (ammoniacal). It can be seen that Tris-HCl is the most suitable buffer. Thus, Tris-HCl buffer with pH 7.2 was selected for the following experiments.

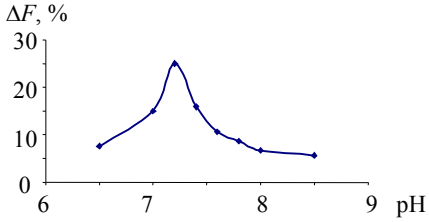

Fig. 3. Effect of pH; $[Tb^{3+}] = 1 \cdot 10^{-5}$ mol/l; [Dano] = $2 \cdot 10^{-5}$ mol/l; [Hep] = 0.25 μg/ml.

Effect of concentration of Tris buffer. The Tris buffer is known to have chelating properties with lanthanide ions. Hence, its concentration should be optimized so as to achieve the maximum sensitization of the Hep-Tb^{3+}–Dano system. The influence of the Tris buffer concentration on the luminescence intensity of Tb^{3+} was studied by varying the volume of the 0.1 mol/l buffer in the range of 0.25–2.00 ml while keeping the concentrations of Tb^{3+}, Dano, and Hep at $1.0 \cdot 10^{-5}$ mol/l, $2.0 \cdot 10^{-5}$ mol/l, and 0.25 μg/ml, respectively. The results indicated that the fluorescence intensity of the probe in the presence of the analyte reaches its maximum at the buffer volume of 0.5 ml. Therefore 0.5 ml of the 0.1 mol/l Tris–HCl buffer solution in the 10 ml mixture was the optimum buffer volume.

Effect of Tb^{3+} concentration. The effect of the Tb^{3+} concentration on the luminescence intensity enhancement (ΔF%) of Hep–Tb^{3+}-Dano system was studied at the constant concentrations of $2.0 \cdot 10^{-5}$ mol/l Dano and 0.25 ng/ml Hep. The ΔF% was highest when the concentration of Tb^{3+} in the mixture was in the range of $3.0 \cdot 10^{-5}$ to $5.0 \cdot 10^{-5}$ mol/l. Therefore, an intermediate value was chosen and the concentration of Tb^{3+} in the mixture was selected at $4.0 \cdot 10^{-5}$ mol/l.

Effect of the amount of Dano. The influence of the amount of Dano on the fluorescence intensity was studied and it was found that the enhancement in the fluorescence intensity of the Hep-Tb^{3+}-Dano system reached a maximum when the concentration of Dano was $2.5 \cdot 10^{-5}$ mol/l. Thus $2.5 \cdot 10^{-5}$ mol/l Dano was selected for further study.

Effect of reaction time. Under the optimum conditions, the effect of time on the fluorescence intensity was investigated. The results indicated that the chelation reaction of the Hep-Tb^{3+}-Dano system was immediately completed at room temperature; furthermore the fluorescence intensity remained stable for 90 min. This is due to the rapid complex formation reaction between Tb^{3+}, Dano and Hep. 5 min was set as the standard for all the fluorescence intensity measurements. As shown in Table 1, the primary advantage of this method compared to other sensitized fluorescence techniques for the determination of Hep is its temporary independence.

Effect of temperature. Under the optimum conditions, the effect of temperature on the $\Delta F\%$ in the range of 0–35 °C was investigated. Temperature had no significant influence on the $\Delta F\%$ of the system; therefore, we selected room temperature (25 °C) for further study. Another advantage of the proposed method is that $\Delta F\%$ is temperature independent.

Effect of the addition order of reagents. Different addition orders of the reagents with the optimum concentrations were studied. The fluorescence intensities (F) of the prepared solutions and their corresponding blank solutions (F_0) were measured at $\lambda_{ex}/\lambda_{em}$ = 347 nm/545 nm. The results shown in Table 2 indicate that the addition of various reagents in different orders has a great influence both on the fluorescence intensities (F and F_0) and on $\Delta F\%$. Therefore considering the maximum intensity and stability of the fluorescence probe, the following addition order: Tb^{3+}>Dano>Hep>buffer – was chosen for further study.

TABLE 1. Comparison of the Reaction and Stability Times of the Sensitized Fluorescence Methods for the Determination of Hep

Subject	Minimum time required to complete the chelation reaction, min	Stability time after completion of the reaction, hr	References
Spectrofluorimetric determination of trace heparin using lomefloxacin-terbium probe	15	0.4	[14]
Fluorimetric study of the interaction between heparin and norfloxacin-terbium complex and its application	30	4	[15]
Spectrofluorimetric determination of heparin using a tetracycline–europium probe	30	4	[16]
Determination of heparin using ciprofloxacin-Tb^{3+} as a fluorescence probe	20	2	[17]
Determination of heparin using norfloxacin-cerium complex as a fluorescence probe by spectrofluorimetry	30	2	[18]
This method	Immediately	1.5	

TABLE 2. Effect of the Addition Order of the Reagents

Reagent	Reagent	Reagent	Reagent	$\Delta F\%$	Reagent	Reagent	Reagent	Reagent	$\Delta F\%$
Tb^{3+}	Dano	Hep	Buffer	21.0	Buffer	Tb^{3+}	Dano	Hep	8.2
Tb^{3+}	Dano	Buffer	Hep	12.0	Buffer	Tb^{3+}	Hep	Dano	2.5
Tb^{3+}	Buffer	Dano	Hep	6.6	Buffer	Hep	Dano	Tb^{3+}	12.9
Tb^{3+}	Buffer	Hep	Dano	7.4	Buffer	Hep	Tb^{3+}	Dano	2.5
Tb^{3+}	Hep	Dano	Buffer	5.7	Buffer	Dano	Hep	Tb^{3+}	5.5
Tb^{3+}	Hep	Buffer	Dano	2.8	Buffer	Dano	Tb^{3+}	Hep	4.6
Dano	Tb^{3+}	Hep	Buffer	16.3	Hep	Tb^{3+}	Dano	Buffer	9.5
Dano	Tb^{3+}	Buffer	Hep	5.7	Hep	Tb^{3+}	Buffer	Dano	3.5
Dano	Buffer	Tb^{3+}	Hep	6.4	Hep	Dano	Buffer	Tb^{3+}	3.9
Dano	Buffer	Hep	Tb^{3+}	11.6	Hep	Dano	Tb^{3+}	Buffer	12.9
Dano	Hep	Buffer	Tb^{3+}	7.3	Hep	Buffer	Tb^{3+}	Dano	3.6
Dano	Hep	Tb^{3+}	Buffer	19.8	Hep	Buffer	Dano	Tb^{3+}	4.7

Experimental conditions: [Tb^{3+}]: $4 \cdot 10^{-5}$ mol/l; [Dano]: $2.5 \cdot 10^{-5}$ mol/l; [Hep]: 0.25 μg/ml; pH 7.2.

Solvent effect. The effect of different solvents was investigated at optimum conditions. Although some solvents (e.g. acetonitrile) improved the $\Delta F\%$, the effect was negligible for the rest of the solvents, and the fluorescence intensities of the probe decreased both with and without Hep. The increased deviation and lower repeatability resulted from the reduced probe fluorescence intensity. So water as a non-toxic and environment-friendly solvent was chosen for further studies (Fig. 4).

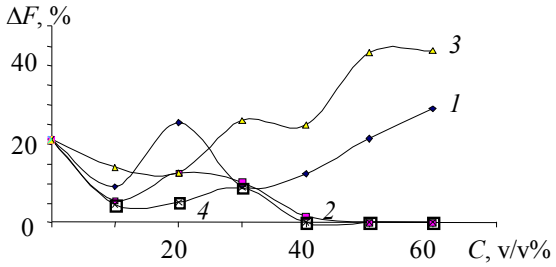

Fig. 4. Solvent effect for methanol (1), ethanol (2), acetonitrile (3), and propanol (4); $[Tb^{3+}] = 4 \cdot 10^{-5}$ mol/l; [Dano] = $2.5 \cdot 10^{-5}$ mol/l; [Hep] = 0.25 μg/ml; pH 7.2.

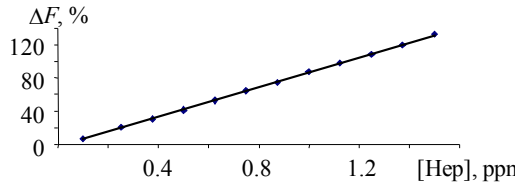

Fig 5. The calibration curve of heparin in optimum conditions.

Interference studies. Under the optimum conditions, the effects of various cations and anions on the fluorescence of the Hep–Tb^{3+}-Dano system were systematically studied. The criterion for the interference was fixed at a ±10% variation of the average fluorescence intensity calculated for the established level of Hep, and the experimental results are shown in Table 3. From the table, it can be seen that most coexisting substances show no interference.

Analytical applications and assay validation. *Linear range and limit of detection.* Under the optimum conditions defined, the calibration graph for Hep was obtained (Fig. 5) and the details are shown in Table 4. It can be seen that there is a linear relationship between the fluorescence intensity of the system and the concentration of Hep in the range of 0.1 to 1.5 μg/ml. The detection limit ($S/N = 3$) was 24.62 ng/ml. The comparison of the linear ranges and the detection limits of different methods for the determination of Hep is shown in Table 5.

TABLE 3. Results of Interference Studies

Substance	Concentration of Interfering Substance	Change in ΔF%	Substance	Concentration of Interfering Substance	Change in ΔF%
$Na^+(Cl^-)$	$1.5 \cdot 10^{-4}$	3.17	$Co^{2+}(Cl^-)$	$4 \cdot 10^{-6}$	1.89
$K^+(Cl^-)$	$1.1 \cdot 10^{-4}$	2.11	$Cd^{2+}(Cl^-)$	$2 \cdot 10^{-7}$	3.48
$Ag^+(NO_3^-)$	$1.65 \cdot 10^{-5}$	9.72	$Zn^{2+}(Cl^-)$	$2.2 \cdot 10^{-6}$	4.18
$Ca^{2+}(Cl^-)$	$6 \cdot 10^{-5}$	–2.76	Glucose	$1 \cdot 10^{-3}$	–1.27
$Al^{3+}(Cl^-)$	$1 \cdot 10^{-5}$	–7.43	Human serum albumin	$1 \cdot 10^{-7}$	8.51
$Mn^{2+}(Cl^-)$	$4 \cdot 10^{-5}$	–6.21	Calf thymus DNA	$1.2 \cdot 10^{-8}$	4.32
$Cu^{2+}(SO_4^{2-})$	$8 \cdot 10^{-6}$	4.70	Saccharose	$1 \cdot 10^{-3}$	3.23
$Mg^{2+}(SO_4^{2-})$	$6 \cdot 10^{-5}$	3.09	Glycine	$5 \cdot 10^{-3}$	–0.87
$Fe^{3+}(SO_4^{2-})$	$2.5 \cdot 10^{-6}$	–5.88			

Experimental conditions: $[Tb^{3+}] = 4 \cdot 10^{-5}$ mol/l; [Dano] = $2.5 \cdot 10^{-5}$ mol/l; [Hep] = 0.25 μg/ml; pH 7.2.

TABLE 4. Validation Data of the Proposed Method for Quantification of Hep

Parameter	Hep	Parameter	Hep
Linear range (μg/ml)	0.1–1.5	Correlation coefficient	0.999
Slope	88.95	Number of data points	13
Intercept	–2.486	LOD (ng/ml)	24.62

TABLE 5. Comparison of Linear Ranges and Detection Limits of Different Methods to Determine Hep

Method	Linear Ranges, μg/ml	Detection Limits, ng/ml	Reference
Lomefloxacin-terbium probe	0.6–2.0	45.22	[14]
Norfloxacin-terbium probe	0.03–1.50	51.04	[15]
Tetracycline–europium probe	0.02–1.6	4.45	[16]
Ciprofloxacin-terbium probe	0.1–1.2	6.89	[17]
Norfloxacin-cerium probe	0.06–1.5	60	[18]
Danofloxacin-terbium probe	0.1–1.5	24.62	This study

Precision and accuracy. The data on intra-assay precision and accuracy of calibration standards are shown in Table 6. All relative standard deviations (RSD%) were below 5% for the standard sample. Inter- and intra-assay precision along with accuracy for the quality control samples are listed in Table 7. The similar results obtained for these validation experiments showed that the developed method is both accurate and precise.

TABLE 6. Intra-Assay Precision and Accuracy of Calibration Standards (*N*=3)

Nominal, μg/ml	Found, μg/ml	Precision, RSD%	Accuracy, RE %	Nominal, μg/ml	Found, μg/ml	Precision, RSD%	Accuracy, RE %
0.375	0.372	3.19	–0.81	1.00	1.011	1.16	1.07
0.50	0.490	1. 83	–1.97	1.25	1.241	1.15	–0.75
0.75	0.753	1.45	0.42				

TABLE 7. Assay Precision and Accuracy of Quality Control Samples (*N*=5)

Nominal, μg/ml	Intra-assay precision, RSD%	Inter-assay precision, RSD%	Accuracy, RE%
0.375	0.54	2.80	–2.25
0.75	1.82	1.52	–1.59
1.25	1.07	0.84	–0.75

Determination of Hep in injection samples. The developed method was applied to determine Hep in pharmaceutical preparations (i.e. injection solutions). For the assay of Hep, the samples were diluted appropriately within the linear range of the determination of Hep. The samples were analyzed by the developed method. The results are shown in Table 8. The developed method can be performed easily with acceptable precision and accuracy.

TABLE 8. Determination of Hep in Injection Samples

Sample number	Hep added, μg/ml	Found concentration, μg/ml	Average, μg/ml	Recovery, %	RSD, %
Caspian Tamin Pharmaceutical Co. (Rasht, Iran)	1.00	0.991, 1.000, 0.996, 0.973, 0.977	0.988	98.8	1.2
Alborz Darou Pharmaceutical Co. (Qazvin, Iran)	1.00	1.080, 1.027, 1.049, 0.987, 1.006	1.030	103.0	3.5
Rotexmedica Pharmaceutical Co. (Trittau, Germany)	1.00	0.989, 0.976, 0987, 0.971, 0.987	0.982	98.2	0.8

The formation of Tb^{3+}-Dano binary complex. Dano contains an α-carbonyl carboxylic acid configuration (see Fig. 6 for its chemical structure). A literature survey shows that the α -carbonyl carboxylic acid configuration is needed for efficient energy transfer from a ligand to the Tb^{3+} ion, high fluorescence quantum yield, large Stokes shift, narrow emission bonds, and a large fluorescence lifetime. These characteristics help to avoid potential background fluorescent emission interferences from the biological matrix [19]. Therefore, Dano is an ideal ligand for the Tb^{3+} ion and it can possibly sensitize the fluorescence intensity of the Tb^{3+} ion via intramolecular energy transfer [20]. The coordination number of the Tb^{3+} ion is generally 8. According to the experimental data on the mole ratio for Dano to Tb^{3+} given above, we can see that the coordination of the Tb^{3+} ion is unsaturated. There are still a lot of positive charges and empty orbitals in the Tb^{3+}-Dano complex.

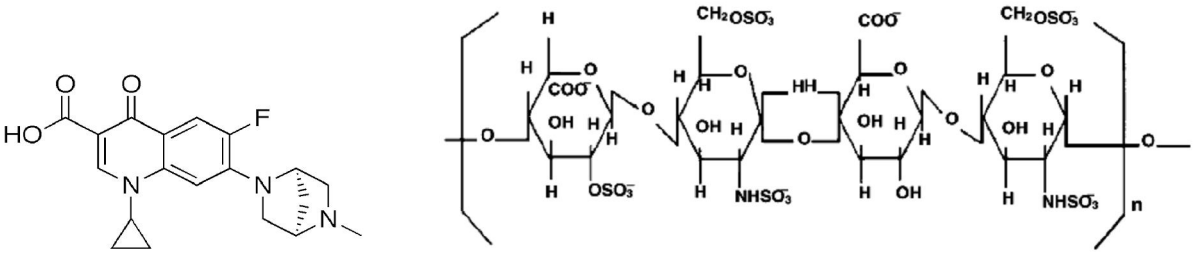

Fig. 6. Structures of Danofloxacin and of the tetrasaccharide unit of heparin.

Conclusions. A new fluorescence method for the determination of Hep based on the fluorescence enhancement has been reported. Under optimum conditions, the enhanced intensity of fluorescence is in proportion to the concentration of Hep. In comparison with the other luminescence techniques, the reported method has some advantages such as lower detection limit, simpler assay process, wider linear range, and very fast complex formation rate. Therefore, it is considered that the Tb^{3+}-Dano complex can be used as a fluorescence probe in the determination of Hep in pharmaceutical preparations.

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