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ORIGINAL PAPER

Spectrofluorimetric Assessment of Hydrochlorothiazide Using Optical Sensor Nano-Composite Terbium Ion Doped in Sol-Gel Matrix

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Abstract A new, simple, sensitive and selective spectrofluorimetric method for the determination of Hydrochlorothiazide was developed in acetonitrile at pH 6.2. The Hydrochlorothiazide can remarkably enhance the luminescence intensity of the Tb^{3+} ion doped in sol–gel matrix at λ_{ex}=370 nm. The intensity of the emission band of Tb^{3+} ion doped in sol–gel matrix was increased due to the energy transfer from the triplet excited state of Hydrochlorothiazide to ($^5D_4$) excited energy state of Tb^3 ion. The enhancement of the emission band of Tb^{3+} ion doped in sol–gel matrix at ($^5D_4 \rightarrow {}^7F_5$) 545 nm was directly proportion to the concentration of Hydrochlorothiazide with a dynamic ranges of 5.0×10^{-10}—5.0×10^{-6} mol L^{-1} and detection limit of 2.2×10^{-11} mol L^{-1}.

Keywords Hydrochlorothiazide · Terbium · Optical sensor · Energy transfer · Luminescence intensity · Sol–gel

Introduction

The thiazidic diuretics, such as hydrochlorothiazide (HCT) Fig. 1, increase the rate of urinary excretion of sodium and water by sodium reabsorption inhibition in the renal tubules. Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4 benzothiadiazine -7-sulphonamide-1,1- dioxide) is the prototype of the thiazide drugs. These drugs comprise an important class of diuretics. Hydrochlorothiazide is indicated for the treatment of edemas associated with the heart (congestive heart failure), liver (hepatic cirrhosis) and kidneys (nephrotic syndrome, chronic renal failure, acute glomerolonephritis). It has also been used for all degrees of hypertension, being efficient as antihypertensive agents of the other classes.

Various analytical methods have been described for the determination of hydrochlorothiazide (HCT) in pharmaceutical preparations, blood and plasma. These include electrochemical methods [1], Chemiluminescence methods [2], Capillary Zone Electrophoresis methods [3], Vierordt's method [4], spectrophotometric [4–15], HPLC [16–21], derivative spectroscopy methods [22–24]. High-Performance Thin-Layer Chromatography [25], Liquid Chromatographic [25–28] and Spectral analysis methods [29–33].

However, these published methods suffered from either requiring time-consuming derivatization technique or had low detection limits (i.e. in microgram level), Hence, there have been increasing demands for new, fast, simple, convenient and sensitive methods for the determination of HCT. Due to the inherent low temperature process, sol –gel technology has acquired great popularity in the field of optical sensors [34–38]. The driving force for these attempts is that, the sol—gel chemistry provides a relatively simple way to incorporate recognition species in a stable host environment. The sol–gel technology provides a unique means to prepare inorganic and organic–inorganic hybrid material for use in sensing devices. The simple doping of the sol–gel solution with the desired compound is the most popular technique for immobilization because of its generality, simplicity and retention of the properties of the compound in the immobilized state. A recent literature on the analytical applications of the terbium (III) ion has revealed no study on the use of this species in sol- gel for measuring the concentration of HCT in the pharmaceutical and serum samples. In this work, the HCT concentration was determined by the optical sensor

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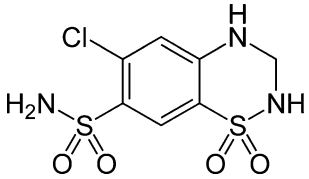

Fig. 1 Chemical structure of HCT

terbium doped in the sol- gel matrix. The absorption and emission spectra of HCT and Terbium were measured in sol –gel matrix. In comparison with other spectrofluorimetric techniques, this method is simple, relatively interference free from coexisting substances and can successfully be applied to the determination of HCT in pharmaceutical preparations and in serum samples with remarkably satisfactory results.

Experimental

Chemicals and Reagents

All chemicals used are analytical-reagent of higher grade. Pure standard of HCT is either purchased from Sigma or supplied by the National Organization for Drug Control and Research (Cairo, Egypt) Fig. 1. Pharmaceutical preparations, Capozide, 25 mg (Bristol-Myres Squib Company), and Ezapril 12.5 mg (Multypharm Company) were purchased from local market.

Distilled water and pure grade solvents from (Aldrich) are used for the preparation of all solutions and during the all determinations. A stock solution of HCT (1×10^{-2} mol L^{-1}) is freshly prepared and dissolved in ethanol and stored at 4° C when not in use.

A Tb^{3+} ion stock solution (1×10^{-2} mol L^{-1}) is prepared by dissolving $Tb(NO_3)_3$ (delivered from Aldrich, 99.99%) with a small amount of ethanol in 100 mL measuring flask, then diluting to the mark with ethanol.

Borate buffers (pH 6.2) were prepared by mixing appropriate volumes of 0.2 mol L^{-1} boric acid with 0.2 mol L^{-1} sodium hydroxide

Apparatus

All luminescence measurements are carried out on Shimadzu RF5301 Spectrofluorophotometer in the range (290–750 nm). The absorption spectra are recorded with a Unicam UV-Visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a Deuterium lamp, which has a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder.

TEM Measurements The JEOL JEM-1230 available at NRC, Dokki, Cairo was used. JEOL JEM-1230 is a high performance, high contrast, 40–120 kV transmission electron microscope with excellent imaging capabilities. Imaging modes include bright and dark field and electron diffraction. The electron gun is a standard tungsten filament. The instrument is capable of magnifications from 50× to 600,000× and resolution at 120 kV is 0.2 nm.

General Procedure

Preparation of Lanthanide Complex Doped in Sol–Gel Matrix

Mixture consisting of TEOS (Tetraethoxysilane), C_2H_5OH and H_2O in a molar ratio of 1 :5 :1 was refluxed for 1 h to give precursor sol solutions, using a few drops of diluted HCl solution as a catalyst. Subsequently, appropriate amount of the $Tb(NO_3)_3.6H_2O$ (0.02 gm) dissloved in 10 mL ethanol and the precursor solution were mixed and stirred together for 15 min until the mixture become homogeneous. The obtained terbium-dispersed sol solution was casted into polystyrene cup and kept at 25° C in air for 2 weeks then heating at 100–300° C for 24 h to give solidified and transparent composite sample [34–38] of 36 nm in size Fig. 2.

Preparation of HCT Solutions

To 10 mL clean and sterilized measuring flasks, the standard solutions of Hydrochlorothiazide are prepared by different additions of (2×10^{-4} mol L^{-1}) HCT solution to give different concentrations of Hydrochlorothiazide. The solutions are diluted to the mark with acetonitrile at room temperature. The above method was used for the subsequent measurements of absorption, emission spectra and effect of solvents. The luminescence intensity is measured at $\lambda_{ex}/\lambda_{em}$=370/545 nm.

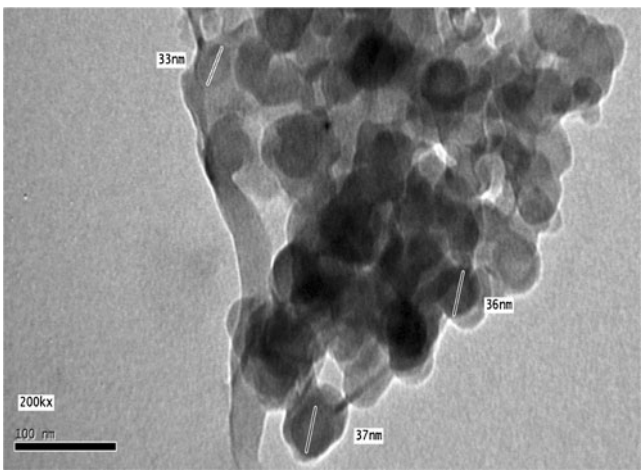

Fig. 2 TEM image of nano-composite optical sensor Tb^{3+} doped in sol–gel matrix

Measurement Procedures

After the preparation of the different standard solutions of **HCT** in acetonitrile according to the above procedures, the optical sensor nano-composite Tb^{3+} doped in sol–gel matrix will immerse in each standard solution of **HCT** in the cell of the Spectrofluorimetric device then the luminescence spectrum will be measured at the excitation wavelength. The optical sensor must be rinsed after each measurement by acetonitrile. Then draw the peak intensity at λ=545 nm on y axis against concentration of **HCT** on x axis (5×10^4, 1×10^4, 5×10^3, 1×10^3, 500, 100, 50, 10, 5, 1, 0.5, 0.1 nmol L^{-1}) on x axis.

Determination of HCT in Pharmaceutical Preparations

Ten tablets each of Capozide, Ezapril, and Tritacec, are carefully weighed and ground to finely divided powders. Accurate weights equivalent to 25 mg Capozide, Ezapril, and Tritacec are accurately transferred to 50 mL beaker and dissolved in acetonitrile and solutions are stand for about 10–15 min and filtered up using 12 mm filter papers then transferred to 100 mL volumetric flask and completed to the mark with acetonitrile to give the test solution. The concentration of the drug was determined by using 9 concentrations for each sample from the corresponding calibration graph.

Determination of HCT in Serum Solution

3 mL of citrate solution was added to 4.0 mL plasma of a real health volunteer and the solution was centrifuged for 15 min at 4,000 r/min to remove proteins, then the serum sample was placed in 10 mL volumetric flasks. 0.26 mL of borate buffer was added to sample then completed to the mark with acetonitrile to give the test solution then the optical sensor Tb^{3+} was immersed in the test solution. The luminescence intensity of the test solution was measured before and after addition of Tb^{3+} optical sensor. The change in the luminescence intensity was used for determination of **HCT** in serum sample.

Results & Discussions

Spectral Characteristics

The absorption spectrum of 2×10^{-4} mol L^{-1} of HCT in sol gel matrix shows two band at 274 and 322 nm were attributed to π→π* transitions Fig. 3. Upon addition of 1×10^{-4} mol L^{-1} of Tb^{3+} ion into **HCT** in sol–gel matrix, a red shift was observed in the two bands by 3 and 8 nm, respectively. The fluorescence excitation spectrum (1) Tb + **HCT** and emission spectra of (2) **HCT**, (3) Tb^{3+} and (4, 5 and 6) are emission spectra of Tb^{3+} in different concentrations of **HCT** in sol–gel matrix are shown in Fig. 4. From curve (3) in Fig. 4, it can be seen that single Tb^{3+} ion in sol–gel matrix has nearly no luminescence peak. Comparing curve (2) with curve (4) in Fig. 4, after the addition of Tb^{3+} ion into the **HCT** in sol–gel matrix, **HCT** can form a binary complex with Tb^{3+} ion. So it appears the characteristic luminescence peaks of Tb^{3+} ion ($^5D_4\rightarrow{}^7F_6$ at 490 nm, $^5D_4\rightarrow{}^7F_5$ at 545 nm, $^5D_4\rightarrow{}^7F_4$ at 590 nm, and $^5D_4\rightarrow{}^7F_3$ at 620 nm), respectively.

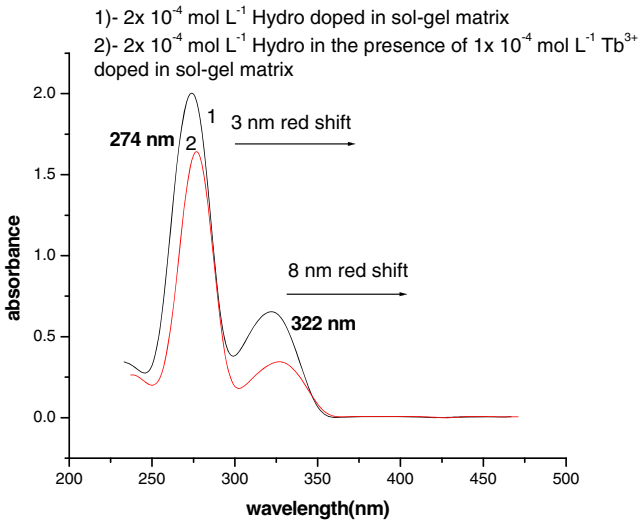

Fig. 3 Absorption spectra of (1) 2×10^{-4} mol L^{-1} of HCT and (2) 2×10^{-4} mol L^{-1} of HCT in the presence of 1×10^{-4} Tb^{3+} in sol–gel matrix

Comparing curve (3) with curves (4, 5 and 6) in Fig. 4. It can be seen that the addition of different concentrations of HCT enhance the characteristic peak of Tb^{3+} at 545 nm.

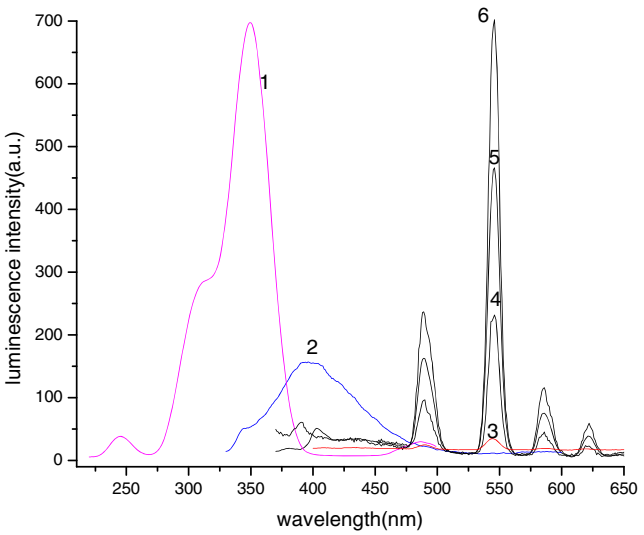

Fig. 4 The fluorescence excitation spectrum (1)- Tb + (HCT) and emission spectra of (2)- (HCT), (3)- Tb^{3+}, (4)- 1×10^{-7} mol L^{-1}, (5)- 1×10^{-6} mol L^{-1} and (6)- 1×10^{-5} mol L^{-1} of HCT+1×10^{-4} mol L^{-1} of Tb^{3+} in sol- gel matrix at $\lambda_{ex}/\lambda_{em}$=370/545 nm

Effect of Different Experimental Conditions

Effect of the Amount of (Hydrochlorothiazide)

The influence of the amount of (**HCT**) on the luminescence intensities of the Tb^{3+} ion doped in the sol–gel matrix was studied. The luminescence intensity of Tb- **HCT** complex was increased upon increasing the concentration of **HCT** till 2×10^{-4} mol L^{-1} then becomes constant Fig. 5.

Effect of the Amount of Tb^{3+}

The influence of the amount of Tb^{3+} ion on the luminescence intensities of Tb-**HCT** in sol–gel matrix was studied under the conditions established above. The luminescence intensity of Tb- **HCT** complex at 545 nm was increased upon increasing the concentration of Tb up to 1×10^{-4} mol L^{-1} then becomes constant. When the concentration of Tb^{3+} ion is 1.0×10^{-4} mol L^{-1}, the composition ratio between the Tb^{3+} and (**HCT**) in the Tb^{3+}-**HCT** system is 1:2. Thus, 1.0×10^{-4} mol L^{-1} Tb^{3+} ion concentration was used for further study in the sol–gel matrix.

Effect of pH

The pH of the medium has a great effect on the luminescence intensity of the Tb- **HCT**. The appropriate structure of **HCT** for the perfect energy transfer from triplet state of **HCT** to the excited energy state of Tb^{3+} $^5D_4$ in sol–gel matrix was found at pH 6.2 (borate buffer).

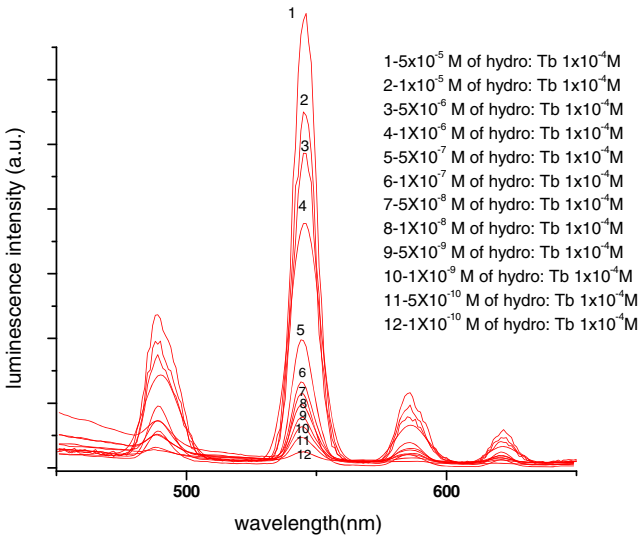

Fig. 5 Luminescence spectra of 1×10^{-4} mol L^{-1} of Tb^{3+} in the presence of different molar concentration of (HCT) in acetonitrile at λ_{ex}=370 nm

Effect of Solvent

The influence of the solvent on the luminescence intensity of the Tb^{3+} in the complex of 2.0×10^{-4} mol L^{-1} of (**HCT**) with 1.0×10^{-4} mol L^{-1} of $Tb(NO_3)_3$. 6 H_2O in sol–gel matrix was studied under the conditions established above. The results show that there is no quenching in the energy of Tb^{3+}-(**HCT**) in sol-gel matrix in the presence of acetonitrile [30–33, 39–42].

Analytical Parameters

Linear Range and Limit of Detection

Under the chosen experimental conditions, there is an established linear relationship between luminescence intensity of Tb^{3+}-(**HCT**) complex and concentration of **HCT** within the range of 5×10^{-10} to 5.0×10^{-6} mol L^{-1} with a correlation coefficient of 0.9998. The regression equation was luminescence intensity=2.2 $X10^{11}\times$Concentration (mol L^{-1})+37.7 LOD=3.3 S/b and LOQ=10 S/b, [43] (where S is the standard deviation of blank luminescence intensity values, and b is the slope of the calibration plot) are also presented in Table 1. The low values of LOD and LOQ 2.2×10^{-11} and 6.6×10^{-11} mol L^{-1}, respectively, indicate the high sensitivity of the proposed method. The nano-composite optical sensor Tb^{3+}- **HCT** displayed constant luminescence intensity from day to day in the presence of **HCT** solutions and the calibration slope did not change over a period of 1 year, this may be due to the high thermal stability of the sol–gel host up to 500° C.

Accuracy and Precision of the Method

To compute the accuracy and precision, the assays described under "general procedures" were repeated three times within

Table 1 Sensitivity and regression parameters for optical sensor

Parameter	Method
λ_{em}, nm	545
Linear range, mol L^{-1}	5×10^{-10} -5×10^{-6}
Limit of detection (LOD), mol L^{-1}	2.2×10^{-11}
Limit of quantification (LOQ), mol L^{-1}	6.6×10^{-11}
Regression equation, Y*	
Intercept (a)	37.76
Slope (b)	2.2×10^{11}
Standard deviation	1.54
Variance (Sa^2)	2.37
Regression coefficient (r)	0.9551

* Y = a+bX, Where Y is luminescence intensity, X is concentration in n mol L^{-1}, a is intercept, b is slope

Table 2 Evaluation of intra-day and inter-day accuracy and precision

Method	HCT taken*	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
		HCT Average Found* ± CL	% RE	% RSD	HCT average found* ± CL	% RE	% RSD
Corpril, (5 mg), Aventis Co. Egypt	2.0	2.03±0.23	1.50	0.09	2.06±0.32	3.00	0.13
	3.0	2.95±0.20	1.66	0.08	3.05±0.31	1.66	0.12
	4.0	4.09±0.21	2.25	0.09	4.12±0.28	3.00	0.11
Hydrochlorothiazide, (5 mg), Aventis Co. Egypt	0.50	0.51±0.16	2.00	0.06	0.49±0.30	2.00	0.11
	1.00	1.02±0.18	2.00	0.07	1.04±0.41	4.00	0.16
	1.50	1.49±0.13	0.60	0.05	1.53±0.31	1.50	0.12
Serum sample	0.60	0.61±0.40	1.60	0.16	0.62±0.42	3.33	0.17
	0.80	0.82±0.39	2.50	0.15	0.83±0.39	3.75	0.15
	1.00	1.02±0.36	2.00	0.14	1.03±0.46	3.00	0.18

*The values are mulitiplied by 10^{-7} mol L^{-1} for method

%RE. Percent relative error, %RSD. relative standard deviation and CL. Confidence limits were calculated from: CL = ± tS/√n. The tabulated value of t is 4.303, at the 95% confidence level; S = standard deviation and n = number of measurements

the day to determine the repeatability (intra-day precision) and three times on different days to determine the intermediate precision (inter-day precision) of the method. These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤0.16% (intra-day) and ≤0.18% (inter-day) indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of Hydrochlorothiazide. Bias {bias % = [(Concentration found—known concentration) x 100 / known concentration]} was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤4.0% demonstrates the high accuracy of the proposed method.

Robustness and Ruggedness

The robustness of the method was evaluated by making small incremental changes in the concentration of Tb^{3+}, **HCT** and contact time, and the effect of the changes was studied on luminescence intensity of the optical sensor. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (≤2.22%). Method ruggedness was expressed as the RSD of the same procedure applied by three different analysts. The inter-analysts RSD were within 1.81% for the same **HCT** concentrations ranged from 1.45 to 1.99% suggesting that the developed method was rugged. The results are shown in Table 3.

Selectivity

The proposed method was tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing talc (200 mg), starch (200 mg), lactose (20 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (50 mg) and magnesium stearate (80 mg) was extracted with water and the solution made as described under “analysis of

Table 3 Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	HCT taken#	Robustness			Ruggedness
		Parameter altered			Inter-analysts, (%RSD) (n=3)
		Concentration of Tb^{3+}* (%RSD)	Concentration of HCT * (%RSD)	Reaction time**	
Corpril, (5 mg), Aventis Co. Egypt	6.0	1.48	2.22	0.68	1.45
Hydrochlorothiazide, (5 mg), Aventis Co. Egypt	1.0	1.62	1.97	0.66	1.89
Serum sample	0.5	1.95	1.75	0.98	1.99

The values are muiltiplied by 10^{-7} mol L^{-1}. * Concentrations of Tb^{3+} were 2, 5 and 6×10^{-4} mol L^{-1}; and the concentrations of HCT were 5, 8 and 10×10^{-4} mol L^{-1}. ** The reaction times studied were 19, 20 and 21 min

Table 4 Determination of (HCT) in serum and pharmaceutical preparations using Tb^{3+} -(HCT) optical sensor

Drug	Added (x 10^{-8} M)	Found (x 10^{-8} M)	Average*	Average recovery ± R.S.D. (%)	B.P. (LC)
Corpril, (5 mg), Aventis Co. Egypt	10	10.12, 10.19, 10.11			
	5	5.07, 5.05, 5.03	1.008	100.8±0.75	99.5±1.0
	1	1.01, 1.08, 1.09			
Hydrochlorothi azide, (5 mg), Aventis Co. Egypt	10	10.02, 10.09, 10.11	1.004	100.4±0.42	99.5±0 .21
	5	5.02, 5.03, 5.05			
	1	1.03, 1.06, 1.01			
Serum sample	10	9.98, 10.03, 10.01			
	5	4.95, 4.96, 4.93	0.999	99.9±0.40	98.3±0.4
	1	1.01, 1.03, 1.06			

*Average of nine measurements

dosage forms". A convenient aliquot of solution was subjected to analysis according to the recommended procedures. In the method of analysis, there was no interference by the inactive ingredients.

A separate test was performed by applying the proposed method to the determination of **HCT** in a synthetic mixture. To the placebo blank of similar composition, different amounts of **HCT** of different products were added, homogenized and the solution of the synthetic mixture was prepared as done under "analysis of dosage forms". The filtrate was collected in a 100-mL flask. Five mL of the resulting solution was assayed (n=3) by proposed method which yielded a % recovery of 100.4–100.8±0.75 for tablets and 99.9±0.4 for serum samples Table 4.

Recovery

The average recoveries of **HCT** were evaluated at three concentration levels of (1, 10, and 100 n mol L^{-1}) each one was repeated three times and from peak intensity of assayed samples comparison to the one of reference standards prepared in acetonitrile, then recoveries were calculated using the formula:

$$\%\text{Recovery} = \text{peak intensity serum} \,/\, \text{peak intensity acetonitrile} \times 100$$

The recommended procedure under "Calibration Curve" was performed. A blank experiment was carried out simultaneously. Determine the nominal content of **HCT** using the following equation:

$$\text{Recovery } \textit{in vivo} = \text{Delivery } \textit{in vivo} \times \text{Recovery } \textit{in vitro} \,/\, \text{Delivery } \textit{in vitro}$$

This means that % recovery for **HCT** in real human serum = Concentration of the drug in real serum X % recovery in spiked serum/Concentration of the drug in spiked serum .The results in Table 4 show that the method

Table 5 Freeze–thaw stability of HCT in pharmaceutical tablets and human serum (n=3)

Drug	Normal concentration nmoll^{-1}	Found Average recovery ± S.D. nmoll^{-1}			R.S.D. (%)
		0 day	15 days	30 days	
Corpril, (5 mg), Aventis Co. Egypt	1	1.2	1.4	1.5	
	10	10.0	10.1	10.2	0.25
	100	100.2	100.2	100.5	
Hydrochlorothi azide, (5 mg), Aventis Co. Egypt	1	1.1	1.3	1.4	0.32
	10	10.3	10.6	10.7	
	100	100.0	100.2	100.3	
Serum sample	1	1.0	1.1	1.3	
	10	10.2	10.4	10.6	0.36
	100	100.3	100.7	100.7	

is successful for the determination of **HCT** and that the excipients in the dosage forms did not interfere. The results obtained (Table 4) were statistically compared with the official British Pharmacopoeia [B.P] method [44]. The average recovery and R.S.D for the tablet in this method were found to be (100.8% and 0.75%) and (99.9% and 0.40%) for serum samples. Data obtained by B. P method showing average recovery 99.99% and R.S.D 0.4% and 99.8% and R.S.D 0.2% for serum samples were also presented for comparison and show a good correlation with those obtained by the proposed method.

Stability

No significant loss of **HCT** (0.75%, R.S.D.) was observed after storage of pharmaceutical tablet samples and serum samples at room temperature for at least 24 h Table 5. Pharmaceutical tablet samples and serum samples were stable over at least three freeze–thaw cycles Table 5 indicating that the pharmaceutical tablet samples and serum samples can be frozen and thawed at least three times prior to analysis (0.36%, R.S.D.).

Analytical Application

The developed method was applied to the determination of (**HCT**) in pharmaceutical preparations as shown in Table 4. For the assay of (**HCT**), the samples must be diluted appropriately within the linear range of determination of (**HCT**) and the sample solution is analyzed by the method developed above, using the standard calibration method. The average recovery and relative standard deviation (R.S.D) are (100.5% and 0.53%) respectively. Data obtained by Liquid Chromatography method of British Pharmacopoeia [44] (average recovery 99.0% and R.S.D 0.3%) are also presented for comparison and show a good correlation with those obtained by the proposed method. The developed method can be easily performed and offers good precision and accuracy when applied for the determination of (**HCT**) in pharmaceutical preparations.

The developed method was also, applied to the determination of (**HCT**) in human serum sample. Proteins in human serum interfere seriously for the system. So, 1.0 mL serum is centrifuged for 15 min at 4,000 r/min with 3 mL of citrate solution to remove proteins. Then 100 micron of the serum of real patients is added to 9.8 mL of acetonitrile then analyzed by the proposed method as mentioned above. The experimental results in Table 4 show that an average recovery of 99.99% with relative standard deviation of 0.30, which indicates that the developed method can be easily performed and offers good precision and accuracy when applied to human serum sample.

Conclusion

The Tb^{3+} ion doped in sol–gel matrix has high sensitivity and selectivity characteristic peaks. The intensities of these peaks are enhanced by increasing the concentration of **HCT** due to energy transfer from **HCT** to Tb^{3+} ion in the excited state by collision. Therefore, the enhancement of the emission band at 545 nm of Tb^{3+} can be used for determination of **HCT** in pharmaceutical preparations and in serum samples.

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