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ORIGINAL PAPER

Supramolecular Study on the Interaction Between Ofloxacin and Methyl β-Cyclodextrin by Fluorescence Spectroscopy and its Analytical Application

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Abstract The supramolecular interaction of ofloxacin (Oflo) and methyl β-cyclodextrin (Mβ-CD) has been examined by UV–vis, IR and fluorescence spectroscopy. The formation of inclusion complex has been confirmed based on the changes of the spectral properties. The results showed that Mβ-CD reacted with Oflo to form an inclusion complex. The Oflo and Mβ-CD complex formed a host-guest complex in 1:1 stoichiometry and inclusion constant ($K=7.8\times10^{-3}$ L mol^{-1}) was ascertained by the typical double reciprocal plots. Furthermore, the thermodynamic parameters (ΔH°, ΔS° and ΔG°) associated with the inclusion process were also determined. In addition, solid inclusion complex was synthesized. Based on the significant enhancement of the fluorescence intensity of Oflo produced through complex formation, a simple, accurate, rapid and highly sensitive spectrofluorometric method for the determination of Oflo in pharmaceutical formulation was developed. The measurement of relative fluorescence intensity was carried out at 497 nm with excitation at 296 nm. The factors affecting the inclusion complex formation were studied and optimized. Under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9995) were in the concentration range of 50–350 ng/mL for spectrofluorimetry. The limit of detection (LOD) was 11.5 ng/mL. The proposed method was successfully applied to the analysis of Oflo in pharmaceutical preparation.

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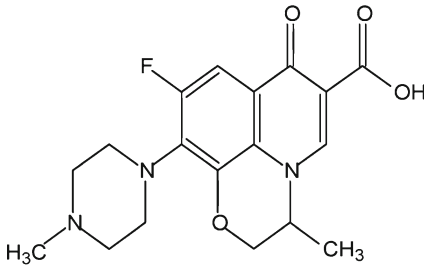
Introduction

Cyclodextrins (CDs) are cyclic, truncated cone shaped, chiral oligosaccharides composed of α-(1→4) glucopyranose unit [1]. The three industrially important forms, alpha (−α), beta (−β) and gamma (−γ) CDs, are composed of 6, 7 and 8 glucopyranose units respectively. The outer periphery of the macrocyclic ring is hydrophilic due to the presence of numerous hydroxyl groups. The internal cavity of CDs is relatively hydrophobic and can encapsulate a variety of compounds especially pharmaceuticals [2]. The formation of an inclusion complex greatly affects the physical chemical properties of the guest molecules, such as solubility, chemical reactivity and the spectroscopic and electrochemical properties, and most of these effects can be utilized in many fields including pharmaceutical industry [3–6] to improve the solubility, stability and bioavailability of pharmaceuticals, as a carriers of active substances in biological systems and to retard the release of active substances from the pharmaceutical matrix and various branches of analytical chemistry [7, 8]. From an analytical point of view the formation of inclusion complexes allows, to improve fluorescence intensity [9–13] and induce chiral separation in capillary electrophoresis (CE) [14–16]. Analysts have used this property of CDs, and many methods based on the fluorescence of inclusion complexes with CDs have been proposed for the determination of several pharmaceutical drugs, pesticides, and metal ion. Elbashir et al. [17], have recently made in-depth review on this topic.

Ofloxacin, Fig. 1, having the chemical name (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo7H-pyrido[1,2,3-de]-1,4-benzo oxazine-6-carboxylic acid, belongs to the quinolone class of antibiotics. The



Ofloxacin

Fig. 1 Chemical structure of Ofloxacin (Oflo)

fluoroquinolones are recently gaining much interest because they provide activity against both Gram positive (due to the fluorine atom) and Gram negative (due to the piperazine group) organisms, bearing little side effects.

Several analytical methods for the determination of Oflo in pharmaceutical formulations and biological fluids were reported in the scientific literature. These include high performance liquid chromatography (HPLC) [18–20], spectrophotometry [21], spectrofluorometry [22, 23] adsorptive stripping voltammetry [24], potentiometry [25], polarography,[26] differential pulse voltammetry [27] CE [28] and micellar electrokinetic capillary chromatography (MEKC) [29] among others.

In this article, the host–guest inclusion interaction between Mβ-CD and Oflo was investigated using fluorescence, UV–vis, and IR spectroscopy. A series of conditions during the formation of the inclusion complex was studied. Based on the great enhancement of the fluorescence intensity of Oflo, a novel method spectrofluorometric was developed to determine Oflo in pharmaceutical formulation. Moreover solid complex of ofloxacin/MC β-CD was prepared by co-precipitation method.

Experimental

Chemicals and Reagents

Oflo reference standard and Mβ-CD were obtained from Sigma-Aldrich (St. Louis, USA). Optiflox (Sterile ophthalmic solution containing 3 mg/mL of Oflo was purchased from local market and manufactured by supplied Jamjoom pharmaceuticals (Jeddah, Saudi Arabia)). Deionized water was used to prepare all solutions in this study.

Instruments and Apparatus

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-1501 spectrofluorimeter (Shimadzu, Japan) equipped with a 150 W xenon lamp. Slit widths for both monochromators were set at 10 nm. All of the spectrophotometric measurements were made with a double beam UV1800 ultraviolet–visible spectrophotometer provided with matched 1-cm quartz cell (Shimadzu, Japan). IR was recorded using (FTIR) Perkin-Elmer spectrometer-8400S (Shimadzu, Japan). Samples were pressed into KBr pellets and recorded at frequencies from 4,000 to 200 cm^{-1}. pH meter model HI 255 (Hanna Instruments, Mumbai, India) was used for pH measurements and thermostat LAUDA Ecoline model RE220 (Gaithersburg, MD) was used.

Stock and Standard Solutions

A 100 μg/mL stock standard solution of Oflo was prepared by dissolving 10 mg of Oflo in1.0 mL of NaOH (0.1 M) and diluted to 100 mL with water. 0.1312 g of Mβ-CD was dissolved in deionized water, transferred into a 100 mL standard flask and diluted to the mark with deionized water and mixed well to prepare 1×10^{-3} M. The formulation was prepared by the same method.

Buffer Solutions

A buffer solution of pH 3.0 was prepared from 0.2 M H_3PO_4 and 0.2 M Na_2HPO_4 and adjusted by a pH meter. Buffer solutions of different pH values were also prepared according to the method reported in the literature [30].

Procedure

Preparation of Solid Complex of Oflo-Mβ-CD

Accurately weighting 0.363 g Mβ-CD was placed into a 50 mL conical flask and 30 mL distilled water was added, stirred, then 0.1 g Oflo was put into a 50 mL beaker and 20 mL of distilled water was added and put over electromagnetic stirrer to stir until it was dissolved. Then slowly the M-β-CD solution was poured into stirred Oflo solution, continually stirred for 20 h at room temperature [11, 12]. The reaction mixture was freeze dried. At this time, the formation of white crystal was observed. This is inclusion complex of Oflo with M-βCD was characterized by IR.

Spectrophotometric Method

A volume of 0.5 mL of Oflo solution was added into 10 mL volumetric flask, then, 3.0 mL of Mβ-CD solution was added into flask followed by 1.5 mL of 0.2 M phosphate buffer solution was used to control the pH value of the media and diluted to 10 mL with deionized water. The final mixture

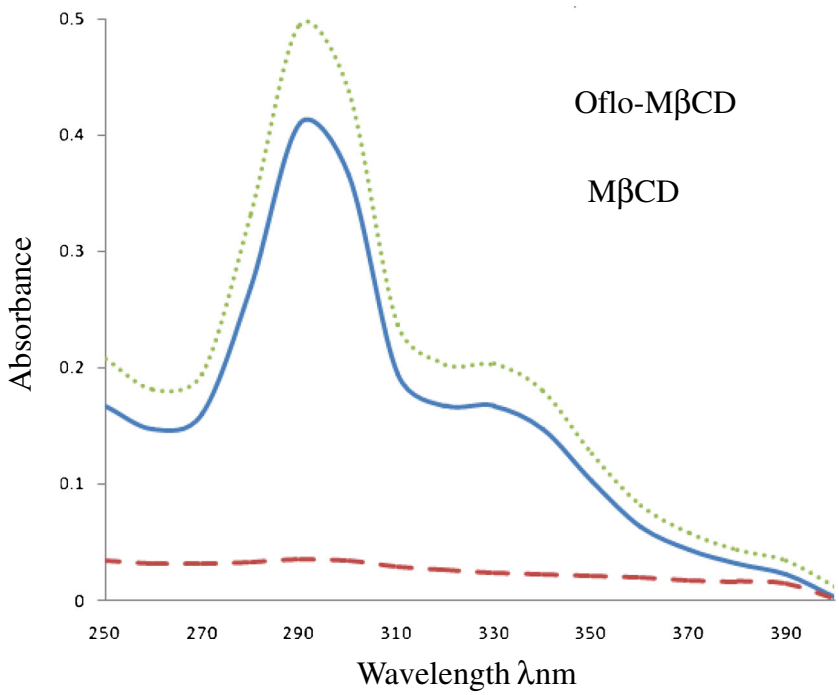


Fig. 2 Absorbance spectra Oflo and Oflo-Mβ-CD Inclusion complex,[Oflo] = 5 μg/mL, [Mβ-CD] = 3×10-4 M, at room temperature, time 25 min, pH 3.0

solution was oscillated at room temperature. The absorbance spectra were measured at 296 nm.

Spectrofluorimetric Method

Into a 10 mL volumetric flask, solutions were added in the following order: 100 ng/mL of Oflo, 1.5 mL of buffer solution pH 3.0 and appropriate amount of (0.001 M) Mβ-CD. The mixture was diluted to the mark with deionized water and oscillated at room temperature for 25 min. The fluorescence intensity of Oflo-Mβ-CD inclusion complex was measured at $\lambda_{ex}/\lambda_{em}$=296 nm/497 nm.

Result and Discussion

Absorbance Spectra

The Absorption spectra of Oflo in the absence and in the presence of 3×10^{-4} M, Mβ-CD was first recorded Fig. 2. The result showed that the wavelengths of maximum absorbance of Oflo at pH 3.0 was 296 nm, when Mβ-CD was added into the Oflo solution, the wavelength of maximum of absorbance did not change but the absorbance is slightly increased and the shape of the absorption spectrum of Oflo (see Fig. 2) remains constant in the broad spectral range 250–

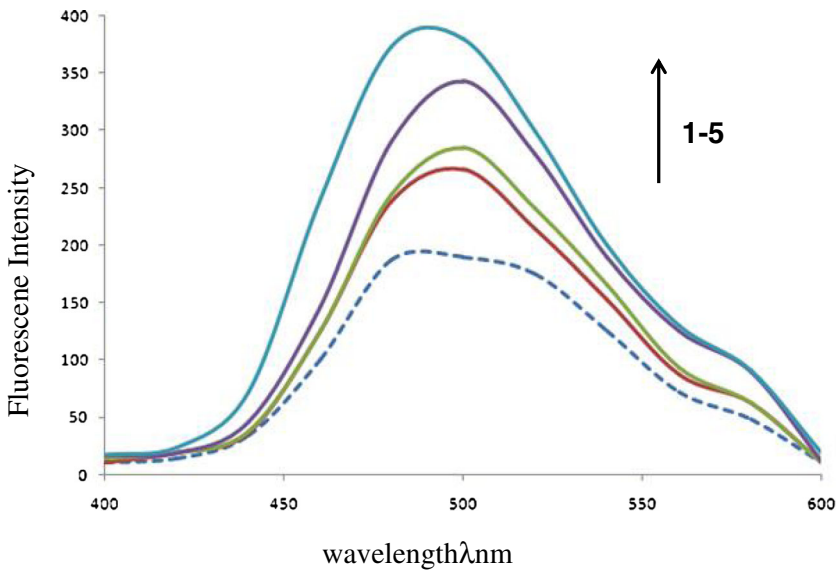


Fig. 3 Emission spectra of (1) Oflo100 ng/mL and Oflo-Mβ-CD inclusion complex (2) Oflo100 ng/mL + Mβ-CD 0.5×10^{-4} M, (3) Oflo100 ng/mL + Mβ-CD 1.0×10^{-4} M, (4) Oflo100 ng/mL + Mβ-CD 2.0×10^{-4} M, (5) Oflo100 ng/mL + Mβ-CD 3.0×10^{-4} M, at room temperature, time 25 min, pH 3.0

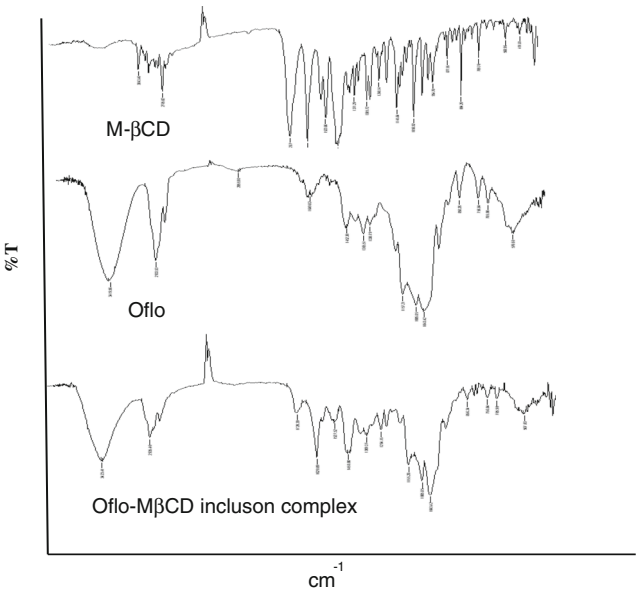


Fig. 4 IR spectra of M-βCD, Oflo, Oflo-MβCD incluson complex

390 nm, giving rise to molar absorptivity coefficients from 31850 to 38000 ε ($Lmol^{-1}$ cm^{-1}). This is due to the complexation of Oflo with Mβ-CD.

Emission Spectra

The spectral characteristics of Oflo were studied and the result showed that the wavelength of maximum emission of Oflo at pH 3.0 was 497 nm Fig. 3. When Mβ-CD was added into the Oflo solution, the wavelength of maximum of emission did not change but the fluorescence intensity dramatically increased. This can be rationalized by having Oflo enters the hydrophobic cavity of Mβ-CD and binding takes place through non-covalent bonding van der Waal forces and hydrogen bonding.

Infrared Spectra Studies

The solid complex formation was confirmed by FT-IR spectroscopy Fig. 4.

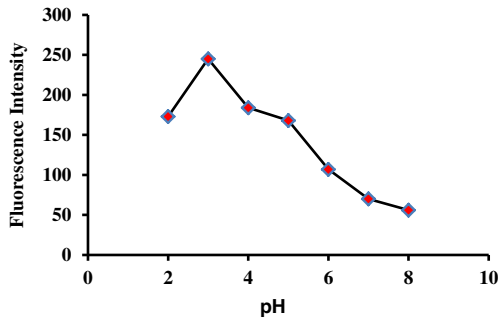


Fig. 5 Effect of pH on the fluorescence intensity of inclusion complex Oflo–Mβ-CD, [Oflo] = 100 ng/mL M-βCD = 2×10^{-4} M, at room temperature, time15 min

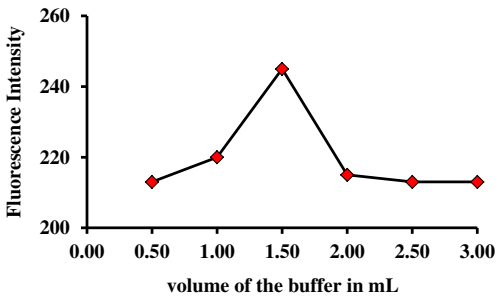


Fig. 6 Effect of the volume of the buffer on the fluorescence intensity of Oflo-Mβ-CD inclusion complex, [Oflo] = 100 ng/mL,[M-βCD] = 2×10^{-4} M, at room temperature, time 15 min, pH 3.0

However some Oflo peaks were either absent (C-N stretching) or shifted (C-O stretching) which suggested change in environment due to inclusion complex formation between Oflo and M-βCD.

Optimization of Experimental Variables

In order to optimize the reaction conditions between the Oflo and Mβ-CD the following parameters were investigated pH of the buffer, the volume of the buffer, reaction time, and temperature and Mβ-CD concentration.

Effect of pH on the Fluorescence Intensity

The effect of pH on the fluorescence intensity of the Oflo-Mβ-CD complex was studied in the pH range 2–11, the solutions were prepared as described in the general procedure and the obtained results are presented in Fig. 5. The pKa values for Oflo has been reported as 8.0, and 6.0 for the basic and acidic group, respectively [31]. Increase in fluorescence intensity was observed when the pH values were increased from 2.0 to 3.0 and then decreased from pH 3.5 to 8.0. This results suggested that inclusion complex of oflo-Mβ-CD was stable in acidic form. The formation constant (K) of the inclusion complex was determined by fluorescence measurement.

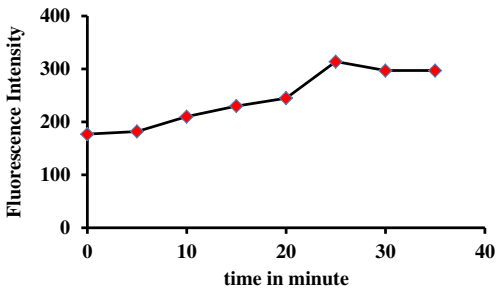


Fig. 7 Effect of time on the fluorescence intensity of Oflo-Mβ-CD inclusion complex, [Oflo] = 100 ng/mL, [M-βCD] = 2×10^{-4} M, at room temperature, time15 min, buffer volume 1.5 mL, pH 3.0

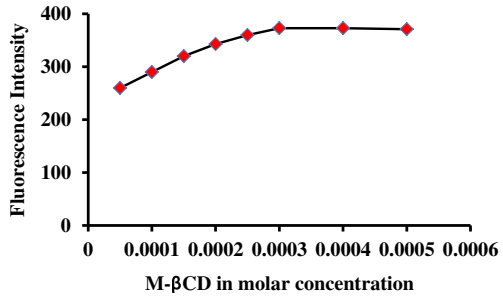


Fig. 8 Effect of M-βCD concentration on the fluorescence intensity of Oflo-Mβ-CD inclusion complex, [Oflo] = 100 ng/mL, at room temperature, time15 min, buffer volume 1.5 mL, pH 3.0

Influence the Volume of Buffer Solution

The effect of the volume of the phosphate buffer pH 3.0 on oflo-Mβ-CD was also studied. It was found that increasing the buffer volume resulting in subsequent increase fluorescence intensity up to 1.5 mL, after which effect of fluorescence intensity remained constant. A volume of 1.5 phosphate buffer pH 3.0 was recorded as optimum in this study Fig. 6.

Influence of Temperature and Time

Another factor that affects the fluorescence intensity is the temperature . The effect of temperature on the fluorescence intensity of oflo-Mβ-CD was studied by varying the temperature from 20 to 60 °C. The fluorescence intensity decreases when temperature above 60 °C (data not shown). Effect of time in room temperature was studied; the highest readings are obtained after 25 min (Fig. 7) therefor the fluorescence measured at room temperature after 25 min in this study.

Influence of Mβ-CD

The effect of Mβ-CD concentration on the fluorescence intensity of Oflo was examined. The concentration of Oflo was fixed at 100 ng/mL (2.7×10^{-7} M) and the concentration of Mβ-CD varied from (0.5, to 5×10^{-4} mol L^{-1}). Figure 8, illustrates that with the increase of Mβ-CD concentration the fluorescence intensity enhanced until the stable inclusion complex formed at about 3×10^{-4} M, above which it remained constant. The data collected in this experiment were used to determine the formation constant.

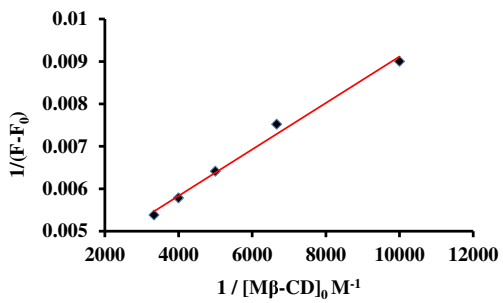


Fig. 9 Plot of $1/(F - F_0)$ vs. 1/[Mβ-CD] of Oflo-Mβ-CD complex; [Oflo] = 100 ng/mL, pH 3.0

Table 1 Thermodynamic parameters

Thermodynamic parameters	Value Kj/mol
ΔH°	−500
ΔS°	−1.2
ΔG°	−124.5

Stoichiometry of Inclusion Complex

The stoichiometry of the inclusion complex were studied under the established experimental condition: assuming that the composition of the complex was 1:1, using the typical double reciprocal (or Benesi–Hildebrand) plots:$1/F-F_0=1/(F_\infty-F_0)K[M\beta\text{-CD}] + 1/F_\infty-F_0$ where $[M\beta\text{-CD}]_0$ denotes the Mβ-CD concentration; F_0 the fluorescence intensity of Oflo in the absence of β-CD; F_∞ the fluorescence intensity when all of the Oflo molecules are essentially complexed with Mβ-CD; and F the observed fluorescence intensity at each M β-CD concentration tested. When a plot of $1/F - F_0$ vs. 1/[Mβ-CD] is constructed (Fig. 9) straight line is obtained which indicative of a 1:1 stoichiometry for Mβ-CD-Oflo complex. The inclusion constant (K) was 7.8×10^3 L mol^{-1}.

Inclusion Complex Thermodynamics

The thermodynamic parameters (ΔH°, ΔS° and ΔG°) for the formation of inclusion complex were determined from temperature dependence of apparent association constants, by using classical van't Hoff equation ($\ln K = -\,°\Delta H/RT + \Delta S°/R$), and plotting ln K versus $1/T$ [32]. The corresponding enthalpy and entropy can be obtained from the slope and intercept, respectively, which indicate the marked tendency

Table 2 Summary of quantitative parameters and statistical data using the proposed procedure

Parameter	
Linear range(ng/ml)	50–350
LOD(ng/ml)	11.5
LOQ (ng/ml)	38.2
Slope	2.354
Intercept	−6.023
Correlation coefficient(r)	0.9995
Oflo Molar absorptivity, ε ($Lmol^{-1}$ cm^{-1})	31850
OfloM β- CD Molar absorptivity, ε ε ($Lmol^{-1}$ cm^{-1})	38000

Table 3 Precision

Taken	Found μg/mL	Recovery% ± SD*
100	99	99±0.577
300	290	97±1.52

*Values are mean of 3 determinations

of Oflo to complex with β-CD. G◦ was obtained according to the equation:

$\Delta G^o = \Delta H^o - T\Delta S^o$. The results were shown in Table 1.

Validation of the Method

Linearity and Limits of Detection

In the proposed method, linear plots (n =6) with good correlation coefficients were obtained in concentration range 50–350 ng/mL. The limits of detection (LOD) and quantification (LOQ) were determined using the formula: LOD or LOQ = K S.Da/b, where K=3.3 for LOD and 10 for LOQ, S.Da is the standard deviation of the intercept, and b is the slope. The values of LOD and LOQ were 11.5 and 38.2 ng/mL, respectively (Table 2).

Accuracy and Precision

The accuracy and precision of the proposed method were determined at three concentration levels of Oflo by analyzing three replicate samples of each concentration. The relative standard deviations for the results did not exceed 2 % (Table 3) indicating high reproducibility of the results and precision of the method. This good level of precision was suitable for quality control analysis of Oflo in its pharmaceutical formulations.

Table 4 Robustness of the proposed spectrofluroimetric method

Recommended condition		Recovery% ± SD*
Standard condition		99±0.577
pH	3.2	97±1.527
	2.8	99.2±0.577
Methylβ-cyclodextrin concentration × 10^{-4} M	3.2	96±1.1
	2.8	96.1±0.577
Temperature C°	20	102±1.00
	30	100±1.1
Reaction time(min)	27	104±0.577
	23	101±0.577

*Values are mean of 3 determinations

Table 5 Recovery of the proposed method

Sample content (ng/mL)	Standard added (ng/mL)	Found (ng/mL)	Recovery (% ± RSD)*
50	50	106.5	106.5±0 .235
50	150	192.4	96.2±0.2237
50	250	290.6	97±0.1475

Recovery was calculated as the amount found/amount taken × 100. Values are mean ± R.S.D. for 3 determinations

Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed, whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were shown in Table 4.

Applications of the Method

The proposed method was applied for the analysis of ofloxacin in its pharmaceutical formulations as shown in Table 5 which indicate high accuracy and recovery. The proposed method has the advantage of being virtually free from interferences by excipients.

Conclusions

In the present study we demonstrated that Mβ-CDs can be used as guest complexing agent, which acted as substrate reservoir in a dosage-controlled manner. The proposed method is simple, sensitive, accurate and cost-effective. The described method has advantages over previously reported spectrofluorimetric methods in terms of simplicity and sensitivity. The proposed method is fully validated and successfully applied for the analysis Oflo pharmaceutical formulations.

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