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# Structure elucidation and degradation kinetic study of Ofloxacin using surface enhanced Raman spectroscopy

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ABSTRACT

A simple, fast and sensitive surface enhanced Raman spectroscopy (SERS) method for quantitative determination of fluoroquinolone antibiotic Ofloxacin (OFX) is presented. Also the stability behavior of OFX was investigated by monitoring the SERS spectra of OFX after various degradation processes. Acidic, basic and oxidative force degradation processes were applied at different time intervals. The forced degradation conditions were conducted and followed using SERS method utilizing silver nanoparticles (Ag NPs) as a SERS substrate. The Ag NPs colloids were prepared by reduction of silver nitrate using polyethyelene glycol (PEG) as a reducing and stabilizing agent. Validation tests were done in accordance with International Conference on Harmonization (ICH) guidelines. The calibration curve with a correlation coefficient ($R = 0.9992$) was constructed as a relationship between the concentration range of OFX (100–500 ng/ml) and SERS intensity at 1394 $cm^{-1}$ band. LOD and LOQ values were calculated and found to be 23.5 ng/ml and 72.6 ng/ml, respectively. The developed method was applied successfully for quantitation of OFX in different pharmaceutical dosage forms. Kinetic parameters were calculated including rate constant of the degradation of the studied antibiotic.



## 1. Introduction

OFX, (±) 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-ppierazinyl)-7-oxo-7H-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid (Fig. 1). OFX is a fluorinated quinolone antibacterial agent used in the treatment of a wide range of infections. It is a member of the third generation of quinolone synthetic antibiotics with a broad spectrum of activity against Gram-positive and Gram-negative bacteria through inhibition of their DNA gyrase [1,2]. The current analytical procedures used for quantitative determination of OFX are UV/VIS spectrophotometry [3–5] in pharmaceuticals, chemiluminescence methods [6,7], spectrofluorimetric methods [8–11], HPLC with UV and fluorescence detection in more complicated samples [12–15] although electrochemical analysis methods are used for this purpose [16–19].

Flow injection methods are described in the quantitative determination of OFL using chemiluminescence detection [20,21]. Owing to their pharmacologically importance, the attention to the stability of fluoroquinolones has been concerned by many scientists. According to ICH, the stability testing has been established.

After careful survey in the literature review of the analysis of OFX, there is limited number of research articles discussing the stability indicating study of OFX using HPLC methods.

A few chromatographic methods were reported the simultaneous determination of OFX with its degradation products [22,23]. OFX was analyzed simultaneously with various pharmaceutical drugs, such as ketorlac [24], metronidazole [25] and piroxicam [26]. One method was developed for stability indicating assay of OFX and ceftriaxone using spectrophotometric method [5].

Literature survey reveals that the present research is the first investigation for stability indicating assay of OFX using SERS developed so far. In this research, force degradation studies of OFX were done under acidic, basic and oxidative degradation conditions. The degradation process was followed by SERS method at different time intervals. The SERS substrate was applied using Ag NPs prepared by the reduction of silver nitrate with polyethylene glycol (PEG 4000) in alkaline medium.

## 2. Experimental

### 2.1. Materials

OFX was purchased from Sigma Aldrich GmbH, China as working standard. Floxal eye drop (Bausch&Lomb GmbH, Berlin) and Ofloxacin STADA tablets (STADA Arzneimittel GmbH, Wien) were subjected to the analysis by the proposed method.

Silver nitrate ($AgNO_3$, 99.99%), polyethylene glycol (PEG4000), sodium hydroxide (NaOH) were used for the preparation of Ag NPs of analytical grade. Hydrochloric acid, sodium hydroxide and hydrogen

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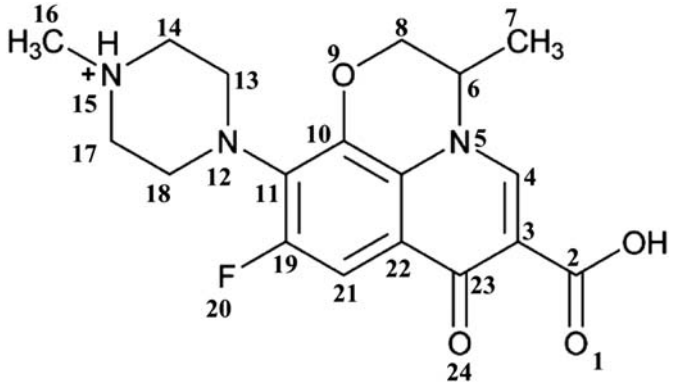

**Fig. 1.** Structure of Ofloxacin (OFX).

peroxide (Sigma Aldrich, USA) were used for the force degradation of the studied OFX. All chemicals and reagents were used without any purification step.

### 2.2. Instruments

A computerized, UV–visible spectrophotometer (UV-1601 PC, Shimadzu, Japan) with 1.0 cm quartz cells was used to record the extinction spectrum of Ag colloids in the UV–visible range of 200–800 nm.

For recording SERS spectra, the confocal Raman spectrometer LabRam HR800 (Horiba Jobin Yvon, Bensheim, Germany) based on an Olympus BX41 optical microscope was used. Nd:YAG laser with output at 532 nm is focused on the sample located on the microscopic slide, a Nikon objective (×20, NA 0.35, WD 20.5) and a 600 lines/mm grating were applied along with a charge coupled device detector (CCD). Furthermore, neutral density filters were adjusted to attenuate the laser beam. The slit width was set to 800 μm and spectra in the relevant range from 200 to 3200 $cm^{-1}$ were recorded using a 10 s integration time.

Characterization of the prepared Ag NPs was done with transmission electron microscopy (TEM). For this purpose TEM images were taken with a JEM-100CX II (Japan). Images were performed with electron beam energy of 80.0 kV and detecting back scattered electrons.

### 2.3. Preparation of Ag NPs as a SERS substrate

The silver colloids were prepared using PEG 4000 as reducing and stabilizing agent by following the procedure of Stiufiuc [27]. The resulting colloids (PEG-Ag NPs) were mixed carefully until a homogenous mixture was obtained. The resulting Ag NPs showed a significant color of grayish yellow having a pH value 7.5. To characterize the morphology of the produced colloids, UV–visible spectroscopy and the transmission electron microscope (TEM) were used.

### 2.4. Preparation of standard and sample solutions

#### 2.4.1. Standard solution

Stock solution of OFX was prepared by dissolving 25 mg of the studied antibiotic in 50 ml of methanol to obtain a concentration of 0.5 mg/ml. A series of working standard solutions containing 100–500 ng/ml was prepared by suitable dilution of the stock solution with methanol.

#### 2.4.2. Sample solution

Ten tablets of Ofloxacin STADA were accurately weighed and then powdered. The average weight of one tablet was taken from the powdered tablets and transferred into a 100 ml volumetric flask. A 25 ml of methanol was added into the powder. The sample was stirred for 15 min for complete dissolution, and then completed with the mark with the same solvent. The resulting solution was filtered through a filter paper (Whatman no. 42). A suitable aliquot of the resulting filtrate solution was then diluted to obtain a concentration within the linearity range of the drug and to be suitable for quantitation.

Floxal ® eye drop contains 5 ml (0.3%) of OFX, was subjected to the proposed method in order to determine OFX quantitatively. One milliliter was taken and diluted with methanol to obtain a suitable concentration within the studied linearity range.

For recording SERS spectra of OFX, a 10 μl of the diluted working sample solution was transferred into a 1 ml Eppendorf tube followed by addition of 10 μl of PEG-AgNPs. After careful vortexing, an aliquot of 20 μl of the mixed solution was transferred onto a microscopic glass slide for SERS measurements.

### 2.5. Force degradation procedures of the studied OFX

Different force degradation procedures were carried out using ICH described stress conditions. In order to establish whether the proposed method is stability indicating, the degradation behavior of the studied OFX was investigated by recording the obtained products using SERS. Blank experiment of each of the three procedures was carried out under the same conditions without adding the standard solution of OFX.

### 2.6. Preparation of acid induced degradation products

A 5 ml of the standard stock solution was mixed with 5 ml of 1 N HCl in a 25 ml- volumetric flask. The mixed solution was refluxed for 1, 15, 30, 45 and 60 min at 70 °C. After completion of the stress procedure, all the solutions were neutralized by using 1 N NaOH and completed up to the mark with methanol.

Likewise, SERS spectra of the degradation products were recorded by taking 10 μl of the neutralized solution into an Eppendorf tube followed by the addition of 10 μl of PEG-Ag NPs, vortexed carefully. A 20 μl of the mixed solution was transferred onto a microscopic glass slide for the SERS spectra monitoring.

### 2.7. Preparation of base induced degradation products

The same procedure was followed as the acidic degradation, using 1 N NaOH instead of the acid. After completion of the stress tests, all the solutions were pH adjusted by using 1 N HCl and completed to the mark with methanol. SERS recordings of the degradation products were conducted as mentioned previously.

### 2.8. Preparation of oxidative induced degradation products

Two concentrations of hydrogen peroxide were used in the oxidative force degradation procedure of the studied OFX. A 5 ml of the standard stock solution was transferred into two different 25 ml volumetric flasks. The first one was treated with 5 ml of 1% $H_2O_2$ while the second flask with 5 ml of 3% $H_2O_2$. The two flasks were kept at room temperature at different time intervals (from 30 to 480 min.). After completing the degradation process, the flasks were completed with methanol.

For SERS measurements, the same procedure was followed and a 20 μl of the mixed solutions was transferred onto a microscopic glass slide for the SERS monitoring. Blank experiments were done by mixing 10 μl of 1 M HCl or NaOH or 3% $H_2O_2$ with 10 μl of PEG-Ag NPs and vortexed well before recording the SERS spectra.

### 2.9. SERS Determination of kinetic parameters of the degradation rate of OFX solution

At zero time ($I_o$), SERS intensity of OFX peak at 1394 $cm^{-1}$ is considered the initial concentration; the intensity of OFX peak ($I_t$) after degradation is taken as proportional to the remaining concentration as a function of time (t). A plot of log ($I_o$: $I_t$) versus time was constructed.