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UTILITY OF SCANNING ELECTRON MICROSCOPE IN DETERMINATION OF CERTAIN
ANTIBIOTICS COMPLEXED WITH URANIUM

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Abstract

New sensitive and accurate procedure was developed for the determination of pure standard eight antibiotics with high accuracy (0.5-2.5 mg) of pure standard erythromycin, azithromycin, clarithromycin, amikacin, neomycin and streptomycin, (0.3-1.5mg) of doxycycline and oxytetracycline. The procedure based on the Scanning Electron Microscope (SEM) technique that used in this way for the first time in the medicinal chemistry field to analyze the studied pure drugs and their pharmaceutical formulations through the microscopic quantitative examination of uranium, pH of the solutions and other optimal reaction conditions were carefully studied and adjusted.

Keywords: Antibiotics; Uranium; Scanning Electron Microscope.

Introduction

A complex was obtained due to the interaction of uranyl ion with the studied drugs and their studied formulations, the four functional groups C = O, -OH, -NH and -COOH are responsible for chelation with metal ions.

SEM technique has been applied in this way for the first time in the medicinal chemistry field to analyze the studied pure drugs through the microscopic examination of the uranium.

Uranium was determined spectrophotometrically using piroxicam [1], meloxicam [2], 2-(2-Thiazolylazo)-p-Cresol (TAC) [3], arsenazo III [4], 2-ethanolimino-2-pentylidino-4-one [5], a mixture of

xylene and benzene [6], azide ions [7], acetylacetonate [8], di-2-pyridyl ketone benzoyl hydrazone [9], thiocyanate [10] and 7-iodo-8-hydroxyquinoline-5-sulfonic acid (ferron) [11] .

Uranium was used in the spectrophotometric determination of sulfur containing compounds [12], piroxicam and tenoxicam [13], phosphorylated proteins [14], propranolol hydrochloride [15], acylthiosemicarbazide [16], free fatty acids [17], phenylephrine [18], diiodoquin, clioquinol [19], phosphate [20] and serum cholesterol [21]. SEM is a type of electron microscope [22] that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern [23-25]. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography [26-27], composition and other properties such as electrical conductivity [28].

SEM technique used to measure the rates and amounts of minerals in the samples [29], phase identification [30] porous silicon were studied and analyzed by SEM [31].

Experimental

Apparatus

Scanning Electron Microscope (SEM) model Philips XL30

Working solutions

Working solutions

1-Erythromycin, azithromycin, clarithromycin, amikacin, neomycin, streptomycin 0.5 mgml⁻¹ solution in methanol.

2-Doxycycline and Oxytetracyclin: 0.3 mgml⁻¹ solution in methanol.

3-Uranyl acetate : prepared as 0.15% w/v solution in methanol.

4-Activated charcoal: 0.3g.

General procedures: 0.5-2.5mg of pure standard erythromycin, azithromycin, clarithromycin, amikacin, neomycin and streptomycin, 0.3-1.5mg of doxycycline and oxytetracyclin were transferred to a 25 ml volumetric flasks. 3ml of 0.15% uranyl acetate solution were added. The mixtures of amikacin, neomycin and

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streptomycin were diluted with 20 ml methanol and transferred into a heating tubes and heated in a water bath at 50°C for 5 minutes then cooled to room temperature and retransferred to 25 ml volumetric flask. All the mixtures volume were completed with methanol then extracted with 4×10 ml portions chloroform.

Chloroformic layer was evaporated to dryness. The residue was dissolved in 1 ml conc. HCl and the volume was completed with distilled water to 25 ml in a volumetric flask. 0.3g Activated charcoal were added to the solution, pH was adjusted to 4.8 by adding diluted nitric acid to give uranium maximum adsorption and stirred for 15 minutes. After filtering on quantitative paper, it was dried in an oven at 100°C for 30 minute to decrease the action of water which observed as well developed grooves on the surfaces of the grains. The separated powders was studied by using SEM. A blank (omitting addition of the drugs), the weight percent of uranium in adsorbed complexes of the pure drugs and their pharmaceutical formulations were compared with the weight percent of uranium in standard prepared (50ppm uranyl acetate) so we can calculate the concentration of drugs complexes .

Results obtained were recorded and compared with the official methods [32], tables 1-3.

The optimal reaction conditions were carefully studied as mentioned in the following paragraphs

Effect of pH

The uranium adsorption increased with the increasing of pH up to 4.8 for equilibrium solution and then decreased slightly because of colloid particles forming Fig.1.

Effect of solvent

Many solvents were tried, methanol represents the optimum diluting solvent with maximum uranium weight percent for all studied drugs .

Effect of heating temperatures

Different temperatures varying from ambient to 70°C temperature were studied. It was found that heating did not enhance the complexation reaction for Erythromycin, Azithromycin, Clarithromycin, Doxycycline and Oxytetracyclin. All further experiments carried out at room temperature, but heating Amikacin, Neomycin, Streptomycin at 50°C on water bath reach the highest uranium weight percent.

Effect of heating time

Heating at 50°C for 5 minutes were sufficient for maximum uranium weight percent for Amikacin, Neomycin and Streptomycin. The reaction was carried out at $25 \pm 1^\circ\text{C}$ for Erythromycin, Azithromycin, Clarithromycin, Doxycycline and Oxytetracyclin so don't need any heating time, the complex formed of all studied drugs was stable more than 12 hours.

Effect of reagent volumes

It was found that 3 ml of 0.1% uranyl acetate achieves a suitable volume for maximum uranium weight percent.

Linearity and quantification

A linear relationship was obtained for the weight percent of uranium and the cited drugs concentration ranges of (20-100 $\mu\text{g ml}^{-1}$) for Erythromycin, Azithromycin, Clarithromycin, Amikacin, Neomycin and Streptomycin Figs.2,3. , (12-60 $\mu\text{g ml}^{-1}$) for Doxycycline or Oxytetracyclin Fig. 4.

Results

The previous procedures were applied to Different concentrations of pure Erythromycin, Azithromycin, Clarithromycin, Amikacin, Neomycin, Streptomycin, Doxycycline and Oxytetracyclin, the results obtained compared with the official methods [32] are shown in tables 1-3.

Conclusions

It was reasonable to expect that uranium would be adsorbed in the form of uranyl ion hydrolysis products that lead to increasing adsorption at optimum pH, the pH of the solutions was adjusted by adding 5% v/v of HNO₃ or 5% of v/v NH₄OH solutions.

Activated charcoal was chosen as the solid phase as a very popular and low cost substance, The final procedure is very simple, fast and showed appreciable uranium concentration rate when compared with direct water analysis.

SEM has many advantages more than traditional microscopes. SEM has a large depth of field, which allows more of the specimen to be in focus at one time. The SEM also has higher resolution, so closely spaced specimens can be magnified at much higher levels. Because of the SEM uses electromagnets rather than lenses, the researcher has much more control in the degree of magnification. All of these advantages, as well as the actual striking clear images, make the SEM one of the most useful instruments in research today.

Table -1: Determination of Erythromycin, Azithromycin and Clarithromycin through complex formation with uranyl acetate by (SEM) .

Erythromycin			Azithromycin			Clarithromycin		
Taken µg ml ⁻¹	Uranium ppm** *	Recovery** %	Taken µg ml ⁻¹	Uranium ppm***	Recovery** %	Taken µg ml ⁻¹	Uranium ppm***	Recover** %
20	23.85	99.4	20	23.16	98.4	20	24.12	100.5
40	47.47	98.9	40	47.66	99.3	40	47.56	99.1
60	71.42	99.2	60	71.71	99.6	60	72	99.6
80	95.71	99.7	80	95.04	99	80	94.75	98.7
100	120.96	100.8	100	117.64	98.3	100	118.92	99.1
Mean recovery*	99.26 ± 0.559		99.7±0.935			98.96±0.321		
N	5		5			5		
Variance	0.313		0.875			0.103		
S.E	0.25		0.425			0.143		
t-test	0.125		0.93			0.74		
Ftest	2.15		1.68			3.39		

(*) Mean ± S.D.

(**) Average of three experiments.

(***) Calculating from comparing the weight percent of the sample and the standard(50ppm).

Table-2:Determination of Amikacin, Neomycin and Streptomycin through complex formation with uranyl acetate by (SEM)

Amikacin			Neomycin			Streptomycin		
Taken µg ml ⁻¹	Uranium ppm***	Recovery** %	Taken µg ml ⁻¹	Uranium ppm***	Recovery** %	Taken µg ml ⁻¹	Uranium ppm***	Recovery** %
20	23.76	99	20	23.52	98	20	23.78	99.1
40	47.56	99.1	40	48.67	101.4	40	47.28	98.5
60	71.5	99.3	60	71.28	99	60	71.56	99.4
80	96.96	101	80	95.23	99.2	80	96.48	100.5
100	119.04	99.2	100	120.84	100.7	100	118.92	99.1
Mean recovery*	99.52 ± 0.834		99.66±1.37			99.32±0.736		
N	5		5			5		
Variance	0.697		1.878			0.542		
S.E	0.376		0.612			0.329		
t-test	0.97		1.09			1.19		
Ftest	1.35		6.01			1.33		

(*) Mean ± S.D.

(**) Average of three experiments.

(***) Calculating from comparing the weight percent of the sample and the standard(50ppm).

Table 3: Determination of Doxycycline and Oxytetracycline through complex formation with uranyl acetate by (SEM).

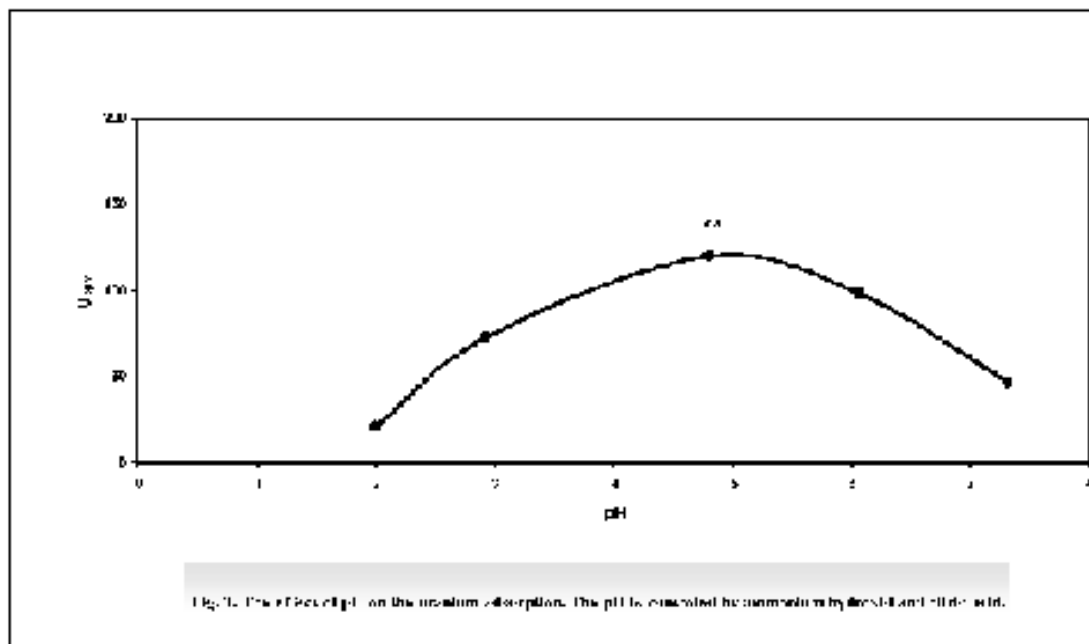
Doxycycline			Oxytetracycline		
Taken $\mu\text{g ml}^{-1}$	Uranium ppm***	Recovery** %	Taken $\mu\text{g ml}^{-1}$	Uranium Ppm***	Recover** %
12	28.78	99.9	12	28.64	99.5
24	57.26	99.4	24	57.14	99.2
36	85.16	99.2	36	85.44	98.9
48	115.76	100.5	48	115.2	100
60	142.56	99	60	143	99.3
Mean recovery*	99.6 \pm 0.604		Mean recovery*	99.38 \pm 0.408	
N	5		N	5	
Variance	0.365		Variance	0.167	
S.E	0.27		S.E	0.182	
t-test	0.45		t-test	0.14	
F-test	1.63		F-test	3.25	

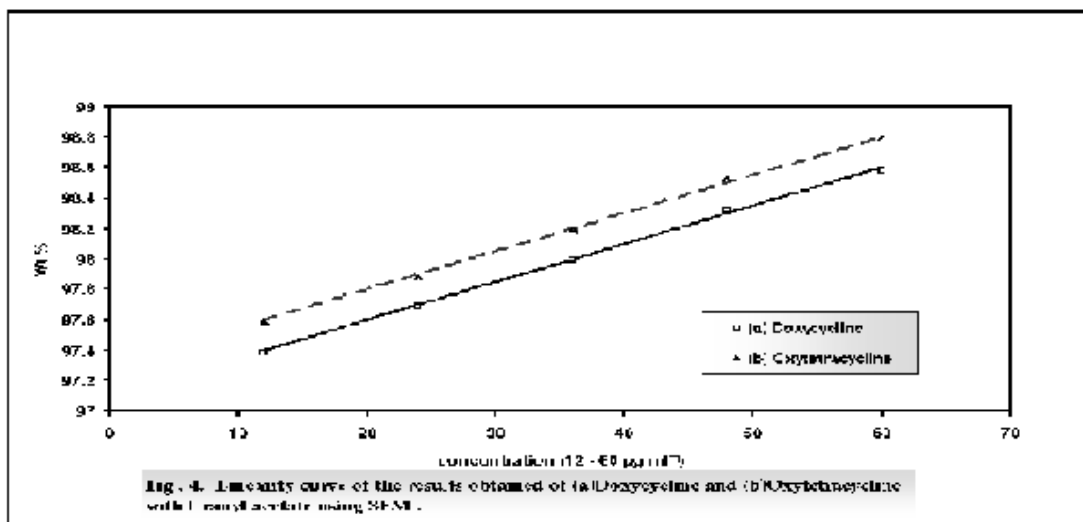
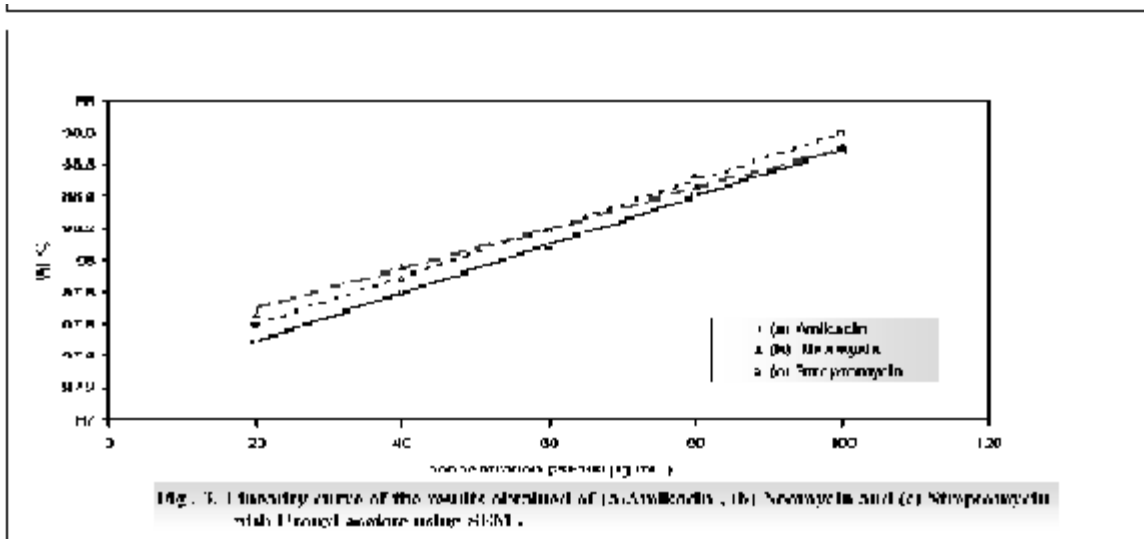
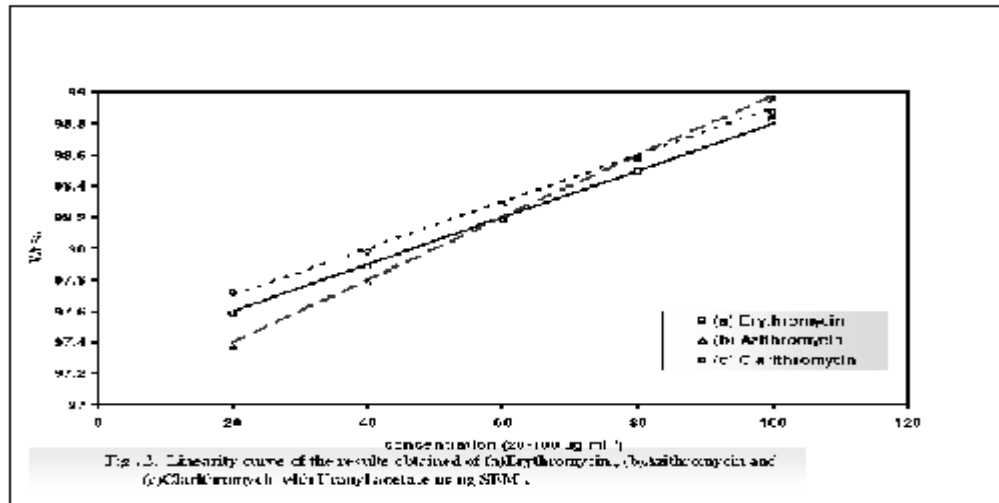
Mean \pm

(*) S.D.

(**) Average of three experiments.

(***) Calculating from comparing the weight percent of the sample and the standard(50ppm).





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