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Research Article

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Stability Study of Tetracycline Drug in Acidic and Alkaline Solutions by Colorimetric Method

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ABSTRACT

The stability of tetracycline drug taken from capsule was studied in acidic solution with four conditions light, dark, in the present of metal ($Cu(NO_3)_2.3H_2O$) and in the present of surfactant (tween80) using colorimetric method at 440 nm. The stability of drug in light acidic solution was compared with the stability in light alkaline solution by measuring the absorbance at 380 nm for the last. The results showed that the stability was marked effected by changing the conditions, the light decreases the stability whereas the presence of metal and surfactant increase the stability.

Keywords: Tetracycline, stability, colorimetric method.

INTRODUCTION

Tetracycline is broad spectrum antibiotic. It inhibits cell growth by inhibiting translation. It binds to 16 S part of the 30 S ribosomal subunit & prevents the amino-acyl t-RNA from binding to the site of the ribosome[1]. Due to their broad antibacterial spectrum , loaded chitosan microspheres [2], drug delivery system[3] and economic advantages, tetracycline have been commonly used in human pathologies as well as in veterinary medicine, animal nutrition and feed additives for cattle growth. It is used for many different infections, such as respiratory tract infections, urethritis and severe acne. It also has a role in the treatment of multidrug resistant malaria.[4, 5]

Several methods have been reported for the determination of tetracycline in dosage forms including: Microbiological assay, non- Aqueous volumetric titration, TLC densitometry with fluoresence[6]. Also High Performance Liquid Chromatography (HPLC) methods have been used with different detections. Such as UV-detection or fluorescence detections using post column derivatization, tandem mass spectrometry, chemiluminescence detection, and electrochemical detection[7, 8]. Numerous flow injection method with amperometric[9, 10] and chemiluminometric[11] detections are also used for tetracycline determination in pharmaceutical preparation and honey.

Tetracycline undergoes reversible epimerisation in solution to the less active 4-epitetracycline; the degree of epimerisation is dependent on pH, and is greatest at a pH of about 3, with conversion of some 55% to the epimer at equilibrium. The rate at which epimerisation occurs is affected by a variety of factors including temperature and the presence of phosphate or citrate ions. Intravenous solutions of tetracycline hydrochloride with a pH between 3 and 5 have been reported to be stable for 6 hours, but to lose approximately 8 to 12% of their potency in 24 hours at room temperature. Although epimerisation has been observed to be the dominant degradation reaction at pH 2.5 to 5, outside this pH range other reactions become important, with the pH-dependent formation of anhydrotetracycline at pH 2 or less, and oxidation to isotetracycline at alkaline solution.[12] In contrast to the case in solution, suspensions of tetracycline hydrochloride with a pH between 4 and 7 are stable for at least 3 months. This is because

epimerisation, which continues until an equilibrium is achieved between tetracycline and its epimer, depends only on the portion in solution, and the solubility of tetracycline at this pH range is low.[13]

There are four chemical instability reactions that can potentially take place when it comes to tetracyclines. The first is conversion to anhydrotetracycline via dehydration, when stored under acidic conditions. This occurs when tetracyclines age and through improper storage, which leads to nephrotoxicity. In basic mediums, tetracycline will open its ring and form isotetracycline. In acidic solutions with a pH around 4, an inactive form will result. This occurs through epimerization of tetracycline at the 4-position from the α to the β position. This was accounted for in the old tetracycline capsule with an overfill of 15%. The last reaction that can take place is phototoxicity. This is common with compounds containing a chloro-substitution at the 7-position. This leads to sunburn from free radical formation with sun exposure.[14]

EXPERIMENTAL SECTION

Materials and Instruments

Tetracycline capsule (ZMC pharmaceutical Co., Ltd., China), Hydrochloric acid (Fluka Co.), Sodium hydroxide (Fluka Co.), Tween80 (Thomas Baker Co., Ltd., India) and Cupric nitrate trihydrate (Aldrech Co.). The absorbance of the solutions were measured using Pheonix Range Spectrophotometer in the technique laboratory of pharmaceutical chemistry department, college of pharmacy, Basra university, Iraq.

Methods

1-Preparation of stock solutions

Acidic solutions: four types of tetracycline solutions were prepared. The first two types were prepared by dissolving 10mg of tetracycline powder in 50ml of 0.01N of hydrochloric acid solution (TL and TD), whereas other two types prepared by dissolving 10mg of tetracycline powder and 0.1g of cupric nitrate trihydrate or 0.2ml tween80 in 50ml of 0.01N of hydrochloric acid solution (TM and TS), respectively.

Alkaline solution: this solution was prepared by dissolving 4mg of tetracycline powder in 50ml of 0.01N of sodium hydroxide (TLNa).

Five types of solutions were kept at room temperature and used in the future in the colorimetric measurements, as show in Table 1.

Table 1 Types of solutions and their symbols and conditions

Solution	Symbol	Condition of storage at room temperature
Acidic tetracycline	TL	Light
Acidic tetracycline	TD	Dark
Acidic tetracycline + Cu^{2+}	TM	Light
Acidic tetracycline + Tween	TS	Light
Alkaline tetracycline	TLNa	Light

2-Preparation of blanks

Acidic solution: three types of solutions were prepared. The first one was 0.01 N hydrochloric acid solution was used as blank in the colorimetric measurements of samples TL and TD. Other two types were 0.01N hydrochloric acid solution containing 0.1g of cupric nitrate trihydrate or 0.2ml of tween80 were used as blank in the colorimetric measurements of samples TM and TS, respectively.

Alkaline solution: solution of 0.01N sodium hydroxide was used as blank in the colorimetric measurements of sample TLNa.

Table 2 Absorbance of samples with time

Sample	Absorbance				
	1st day	2nd day	3rd day	4th day	5th day
TL	0.366±0.002	0.553	0.805	0.988	1.211
TD	0.307±0.005	0.492	0.543	0.725	0.793
TM	0.425±0.004	0.501	0.578	0.663	0.697
TS	0.297	0.477	0.629	0.801	0.929
TLNa	0.876	1.161	1.516	1.731	1.882

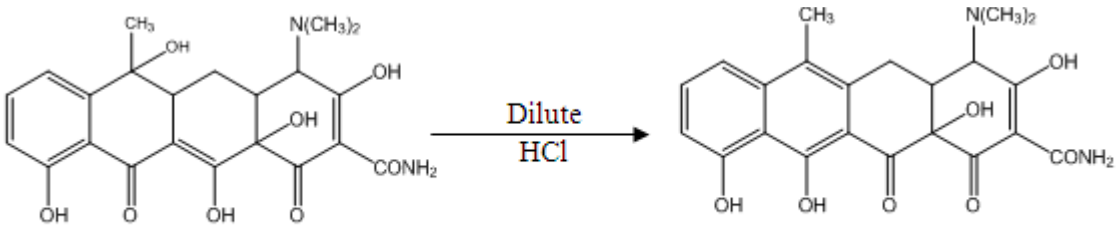
3- Colorimetric measurements

Two milliliters of samples (TL, TD, TM, TS and TLNa) were transfer to 2ml absorption cell and measured the absorbance at 440 nm (for the first four samples) and at 380nm (for the last) with time in days. Table 2 shows the data of the absorbance of samples per day, any absorbance value has been measured twice.

RESULTS AND DISCUSSION

We used colorimetric measurements to follow the stability of tetracycline by measuring the increasing in the intensity of absorbance in acidic solution of anhydrotetracycline which absorbed at 440nm.

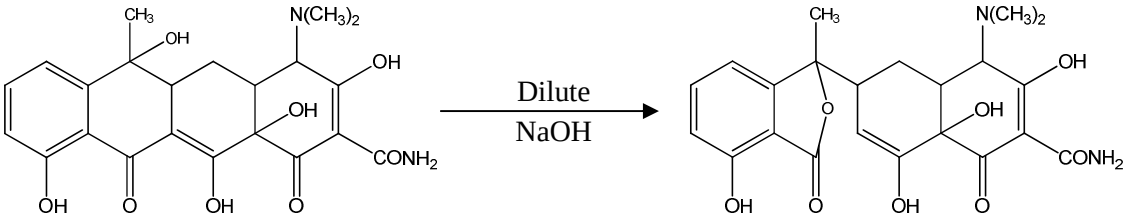
Tetracycline when exposed to dilute acid conditions undergoes dehydration to yield anhydrotetracycline[15], as shown in equation below:



Tetracycline Anhydrotetracycline

The increasing of absorbance results from increasing in color of solution because of the yellow color of anhydrotetracycline compound.

In the alkaline solution, tetracycline transformed to isotetracycline which could determined by colorimetric method by measuring the absorbance at 380nm. The absorbance of solution with time was increased because of increasing the concentration of yellow color of isotetracycline compound.[16]



Tetracycline Isotetracycline

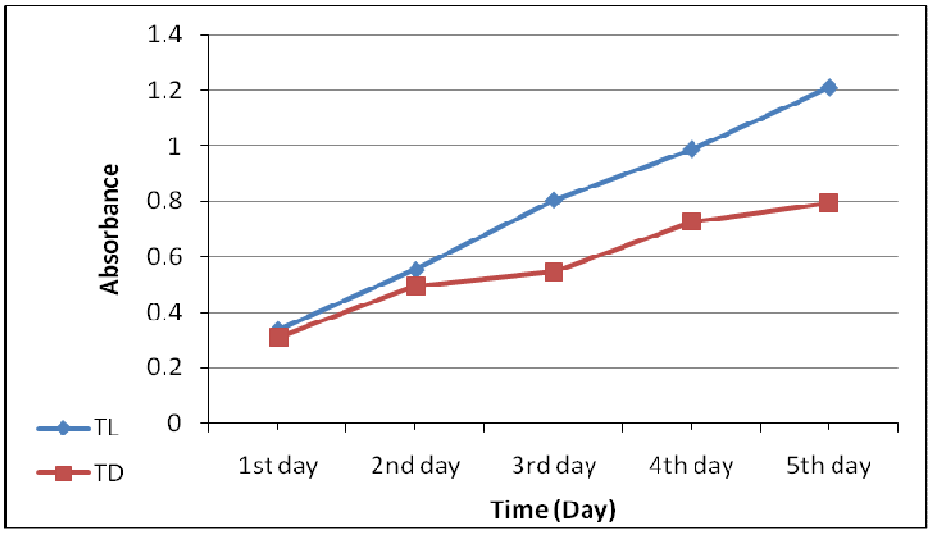


Figure 1 Absorbance of samples TL and TD

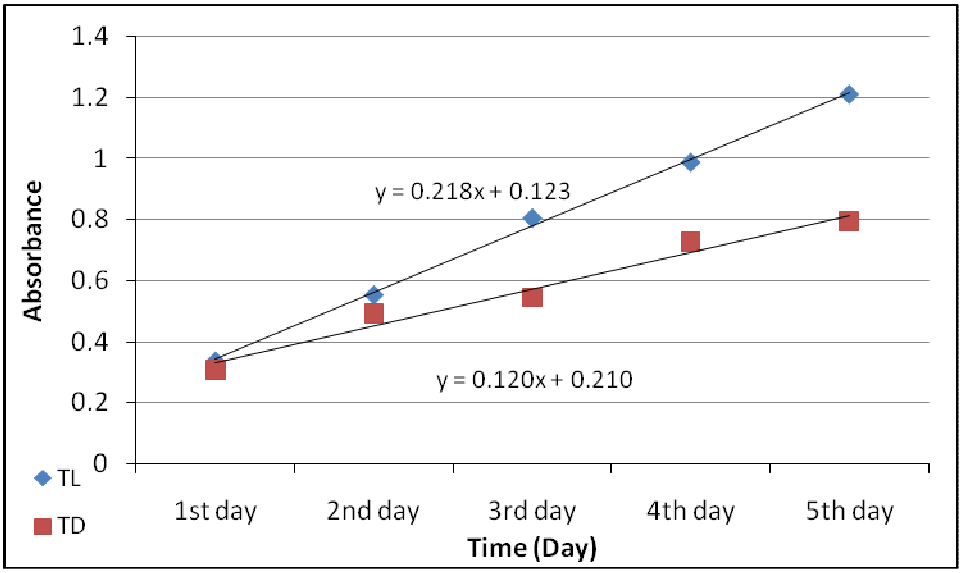


Figure 2 Slopes of curves of samples TL and TD

Stability of tetracycline

Five types of tetracycline solutions have been prepared and followed through five days by colorimetric measurements, four of them in acidic solutions with different conditions, and last one in alkaline solution.

Figure 1 shows the absorbance of samples TL and TD with time. We showed that the increasing of absorbance for sample TL in the light condition is more than for dark condition sample TD, which referred the effect of light on the stability of tetracycline solution[13], as shown in Figure 2.

Figure 3 shows the effect of addition of copper ion on the stability of tetracycline solution TM compared with TL. Figure 4 shows the slope of these curves which indicate that, the greater the slope, the higher the decomposition.

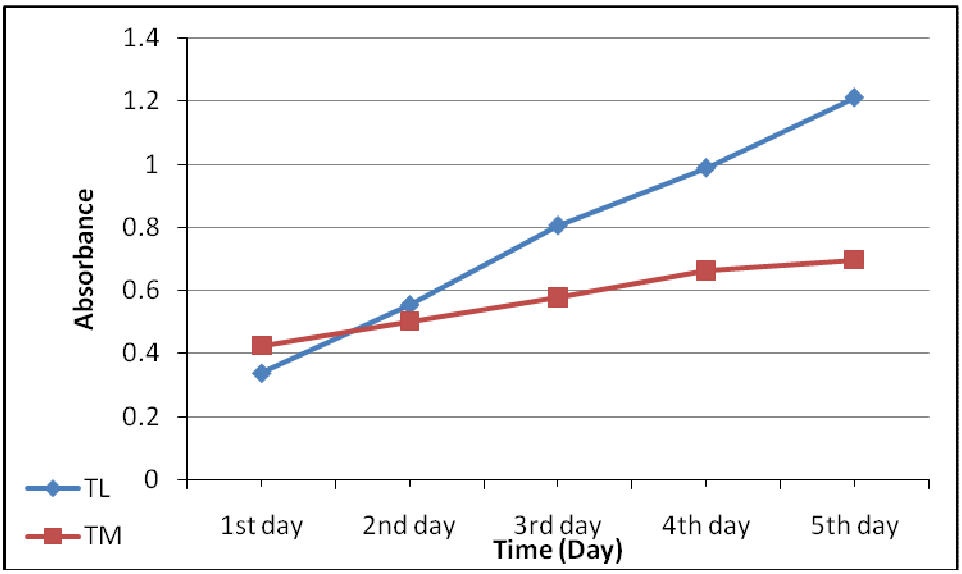


Figure 3 Absorbance of samples TL and TM

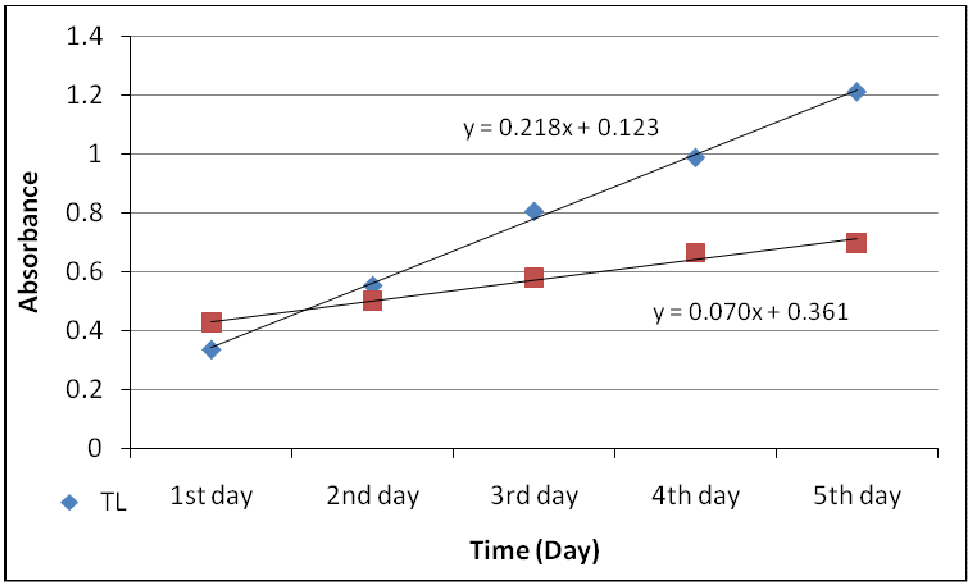
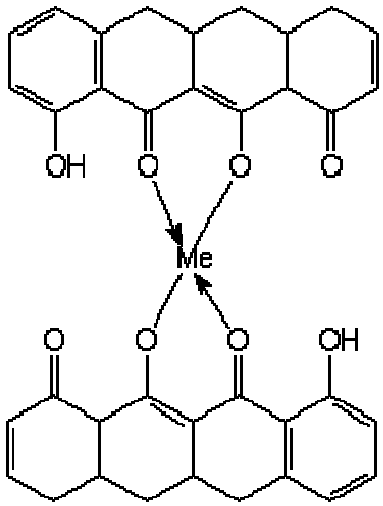


Figure 4 Slopes of curves of samples TL and TM

We showed that addition of metal to the tetracycline solution leads to increase the stability of the drug by complexation of Cu^{2+} with tetracycline and prevent it to convert to anhydrotetracycline, as shown in Scheme 1.



Scheme 1 Complexation of tetracycline with metal

Figure 5 shows the absorbance of sample TL and TS with time, and Figure 6 represents the slopes of these curves.

Addition of surfactant to the tetracycline solution lead to increase the stability of drug compound by forming aggregations including the drug molecules and prevent them to decompose to anhydrotetracycline.[17]

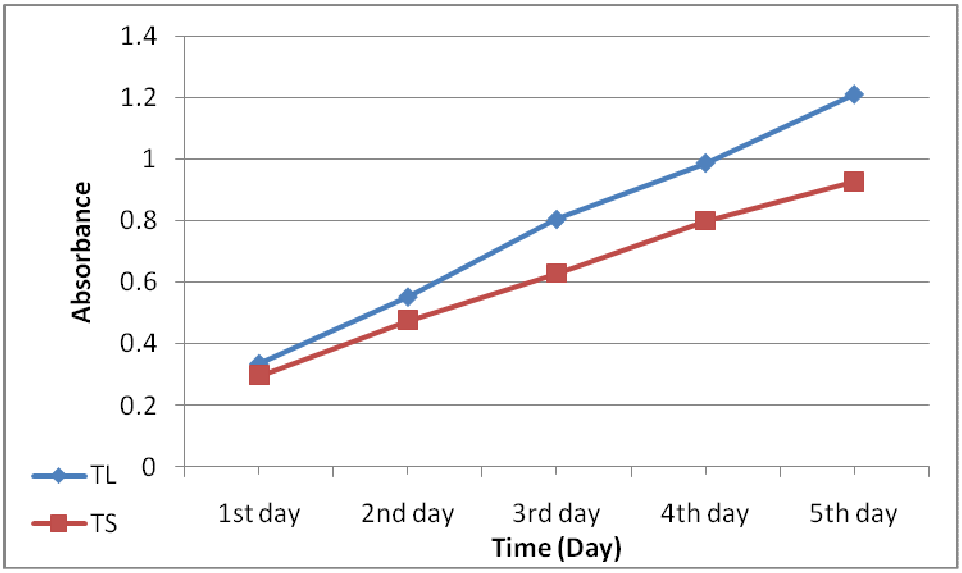


Figure 5 Absorbance of samples TL and TS

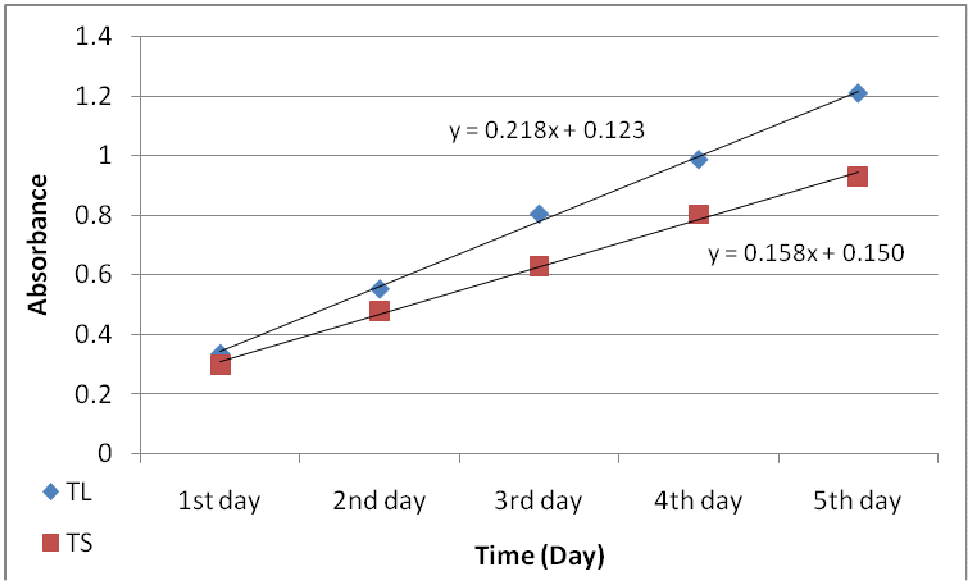


Figure 6 Slopes of curves of samples TL and TS

Finally, we studied the effect of acidic and alkaline solutions on the stability of tetracycline compound. Figure 7 shows the absorbance of sample TL and TLNa with time, and Figure 8 represents the slopes of these curves. We show that the stability of tetracycline in acidic solution is grater that its stability in alkaline solution.

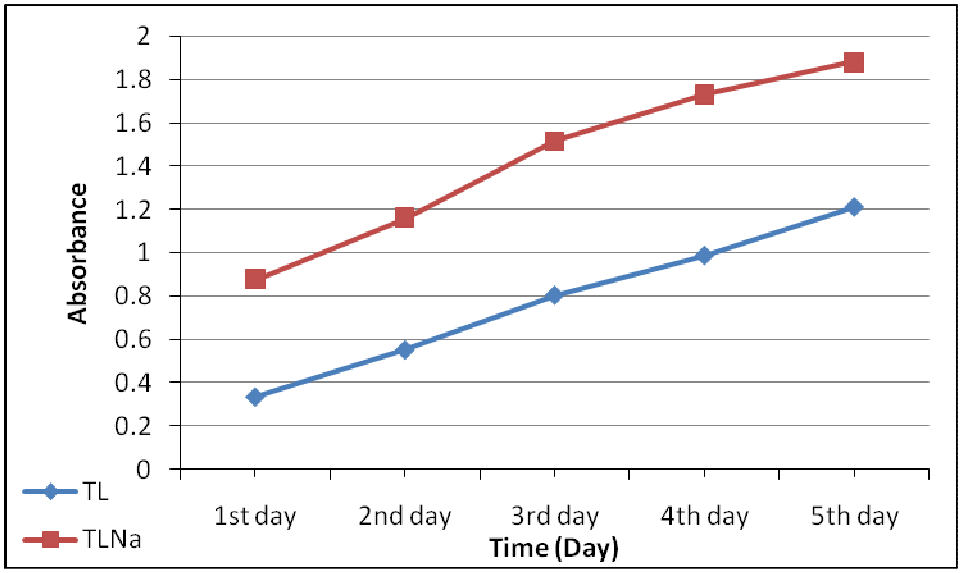


Figure 7 Absorbance of samples TL and TLNa

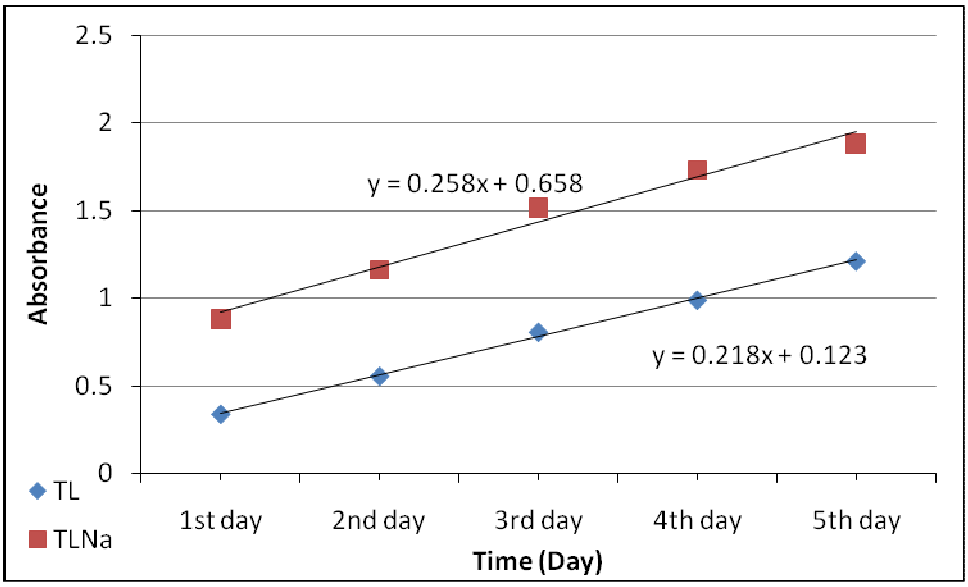


Figure 8 Slopes of curves of samples TL and TLNa

We found finally that these conditions differ in the effect on the stability of tetracycline, tetracycline in the alkaline solution is less stable than in acidic solution and other solutions. Whereas, the addition of metal yielded increase the stability of tetracycline as compared with other conditions, as shown in Figure 9.

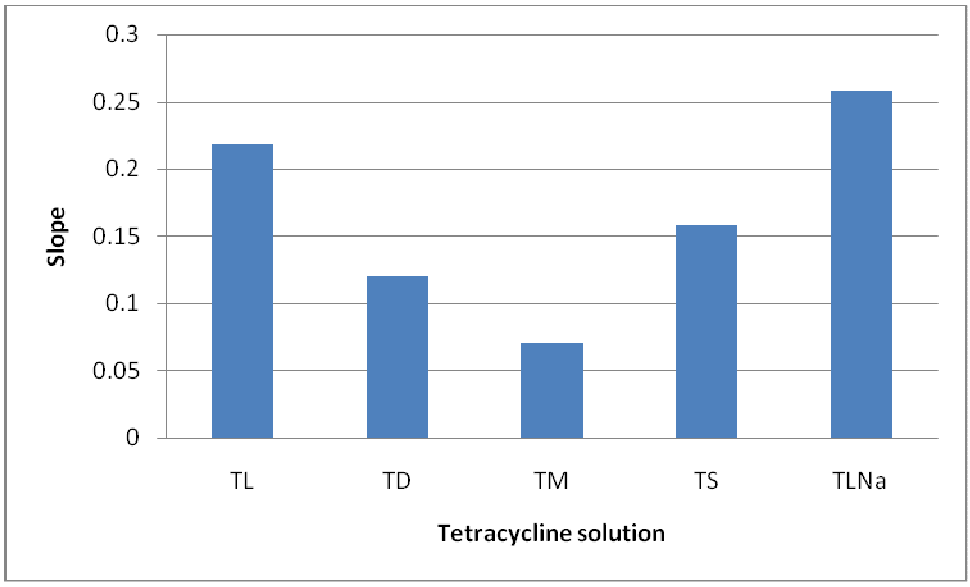


Figure 9 The slopes of the previous figures

CONCLUSION

This study indicated that the stability of tetracycline with time could be measured by colorimetric method, the previous methods used HPLC in this type of measurement. This study showed the effect of some factors like humidity, light and pH on the stability of tetracycline solution. Addition of some materials as metal and surfactant somewhat prevent the decomposition of drug. Effect of time on the changing of color from light yellow to brown and dark brown after exposure to the acidic and alkaline solution over 10-20 days. Results also indicated that light, which is the well known factor responsible for color transformation of tetracycline, is not the only factor to cause color change.

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