

## Kinetic Study of the Hydrolysis of synthesized Ibuprofen Ester and its Biological Activity

*Noha Mohammad Yahya*\*

*Sabah G. AL-Dabbagh*\*\*

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### Abstract:

It is known that the oral administration of ibuprofen caused an irritation of stomach as a side effect due to its carboxylic moiety. Ibuprofen ester was synthesized by linking the carboxylic moiety of ibuprofen and the hydroxylic group of paracetamol to reduce its side effect. Study the kinetic hydrolysis of prepared ester was examined at different values of physiological pH (1.0, 5.8, 6.4 and 7.4) at  $37 \pm 0.1$  of 1 hour period. Measurements of absorbance were carried out by UV-Visible spectrophotometer to follow the stability of ester, it showed Pseudo first order hydrolysis. The pH- apparent rate profiles of ester was exhibited a good stability at pH 1.0 and pH 5.8. Pharmacological activity in vivo of prepared ester was evaluated in relation to analgesic and anti-inflammatory activity using the acetic acid method and the hind paw oedema inhibition, respectively. Acetyl salicylic acid (aspirin) was used as a reference drug for the above tests. The synthesized ester showed higher analgesic and anti-inflammatory action than aspirin.

**Key words:** ibuprofen, prodrug, ester hydrolysis, ester synthesis

### Introduction:

Ibuprofen is a 2-(4-isobutyl phenyl) propionic acid, it was introduced into clinical practice as anti-inflammatory drug in the treatment of rheumatoid arthritis with a lower incidence of side effects [1]. It may be used with caution in patients with gastro-intestinal disease due to its acidic moiety and is often tolerated by patients with peptic ulcer or intolerance to major anti-inflammatory drugs such as aspirin [2]. Prodrug approach is a promising way of overcoming gastrotoxicity associated with long term oral use of nonsteroidal anti-inflammatory drugs like ibuprofen [3]. The candidature of benzyl ester prodrug of ibuprofen was examined to assess its ability to reduce gastrotoxicity without affecting pharmacological response. It gave highly promising activity in established animal model like

carrageenan induced rat paw oedema and acetic acid induced writhing reflex assay. The aim of this study is to synthesize ibuprofen ester by estrification reaction of ibuprofen with paracetamol ( N-acetyl-p- amino phenol) between the acidic moiety of ibuprofen and OH group in phenol part of paracetamol. Hydrolysis of ibuprofen ester prodrug was examined kinetically at different pH values at  $37 \pm 0.1$  °C . The biological activity of synthesized ester was measured by testing anti-inflammatory and analgesia.

### Materials and Methods:

Ibuprofen, 2-(4- isobutyl phenyl) propionic acid, and paracetamol ( N-acetyl-p- amino phenol) drugs were supplied by the state entrtrprise for drug

\*Chemistry Dept. , College of Education For Girls, Mosul University

\*\*Pharmacology Dept., College of Pharmacy, Mosul University

industries and medical appliances Samarra-Iraq.

Thionyl chloride, dichloromethane, benzene and acetonitrile were purchased by Fluka company.

Albino young male of mouse ( $28 \pm 4.7$  g) and rats ( $168.8 \pm 17.5$  g) were obtained from drug control centre, Mosul University.

The infra red spectra were recorded as potassium bromide discs using SP-2000 Infra Red spectrophotometer, that manufactured by Beckman Acculab T.M. spectrometer.

Shimadzu Ultra Violet- Visible recording spectrophotometer which was used for kinetic study.

### Synthesis of Ester :

A solution of 8.25 g (1mM) of ibuprofen in 40 ml. of benzene and 6.5 g ( 54.5mM ) of thionyl chloride were refluxed by using steam water for 5

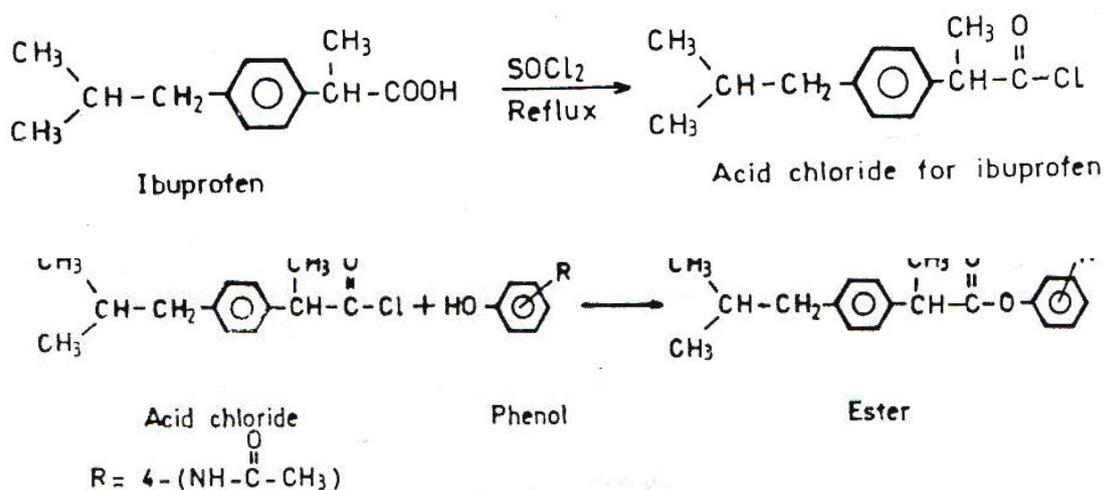
hours [4]. Then, benzene and excess of thionyl chloride were removed under vacuum. The resulting compound of ibuprofen was checked by IR spectra.

Ester compound was synthesized [5 ] from acid chloride of ibuprofen (1mole) that dissolved in dry dichloroethane, then added dropwise to one equivalent amount of dry pyridine. The reaction was taking place in cooling condition with stirring overnight to complete the reaction. To the extracted ester 30ml of 1N HCl added and the mixture transferred to a separating funnel. The water phase which contains the pyridinium salts was removed and the organic phase was extracted with 5% of sodium carbonate to remove any unesterified acid. Then it was dried over magnesium sulphate. The filtered solution was evaporated under vacuum to remove dichloroethane and the

### Kinetic study

Kinetic study [6] of ester compound was carried out at different pH values of 1.0, 5.8, 6.4 and 7.4 which are similar to the pH of stomach, small intestine, large intestine and plasma, [7] :

respectively. This study was performed by using UV-Visible spectra. pH 1.0 was prepared from 0.1M of HCl and the other pH solution were prepared using buffer phosphate solution as follows



Fig(1): synthesis of ibuprofen ester

resulting ester was purified by petroleum ether and dried under vacuum, see Figure 1. IR- spectra were measured for ibuprofen, ibuprofen chloride and synthesized ester.

pH	X	50-X
5.8	4.0	46
6.4	13.25	36.75
7.4	40.5	9.5

X indicates 0.2 M of  $\text{Na}_2\text{HPO}_4$

50-X indicates 0.2 M of  $\text{NaH}_2\text{PO}_4$

The above volumes of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  were completed using distilled water to 100 ml. The ionic strength of buffer solutions were adjusted by the addition of 0.5 M of KCl. Hydrolysis of ester [28] was carried out thermostatically maintained in water bath at  $37 \pm 0.1$  °C . The initial concentration of ester stock solution as  $10^{-3}\text{M}$  was freshly prepared in acetonitrile and the absorbance was measured at interval time for 10 min ( $\lambda_{\text{max}} = 241$  nm).

The absorbance of ester was measured against the corresponding buffer blank, that contains 0.6 ml. of acetonitrile and 2.4 ml. of the appropriate buffer solutions. Then, scanning for ester was performed to determine the wave length of maximum absorption [8] .

The pseudo first- order rate constant for ester disappearance was determined from the plot of  $\ln A_0/A_t$  versus time(t) by follows:

$$\ln A_0/A_t = k.t$$

where  $A_0$  is the absorbance of the initial ester concentration at different buffer solutions and  $A_t$  represents the absorbance of the remaining concentrations of ester in buffer solution at time (t), k is the rate constant of the reaction.

## Biological activity measurements

Liquid paraffin was used as a solvent for ester. To test analgesia, the acetic acid test ( Modified Koster test ) was performed [9] in male albino mice 28.9 g. Aspirin was considered as the drug reference. A drug solution, 100 mg/Kg body weight of mouse was given orally to mouse using a stomach tube. Each group of control, aspirin, ibuprofen and ibuprofen ester were included four mice. After 60 min. of drug administration 0.6% of acetic acid solution was injected intraperitoneal as 60 mg/Kg. Then, each control group of mice were given orally the same amount of paraffin solvent. Five minutes after the administration of acetic acid, mean stretching numbers were recorded during a period of 10 min. The analgesic activity was calculated according to the following equation:

$$\% \text{ Analgesic activity} = \frac{N - N'}{N} \times 100 \dots (1)$$

Where N is the mean stretching number of the control group and N' is the mean stretching number of the ibuprofen ester.

To test anti-inflammatory, the hind paw oedema was performed [10] in male albino rats weighing  $168 \pm 17.5$  g . Four groups of animals ( control, aspirin, ibuprofen and ibuprofen ester. Each groups consisting of four rats were used. Paw oedema was produced by the injection of 0.1 ml. of 2% formaldehyde solution to each rat, regardless of weight on the first and third days of the experiment. Then, paraffin, aspirin and ibuprofen ester were given daily for 10 days to all experimental animals. Drug solution 100 mg/kg body weight of rats was given orally to rats using stomach tube, the thickness of the hind paw was measured by vernia before treatment and then at tenth day after treatment.

The percent oedema and percent oedema inhibition were *calculated as follows*:

$$\% \text{oedema} = \frac{N'}{N} \times 100$$

$$\% \text{oedema inhibition} = \frac{N - N'}{N} \times 100 \dots (2)$$

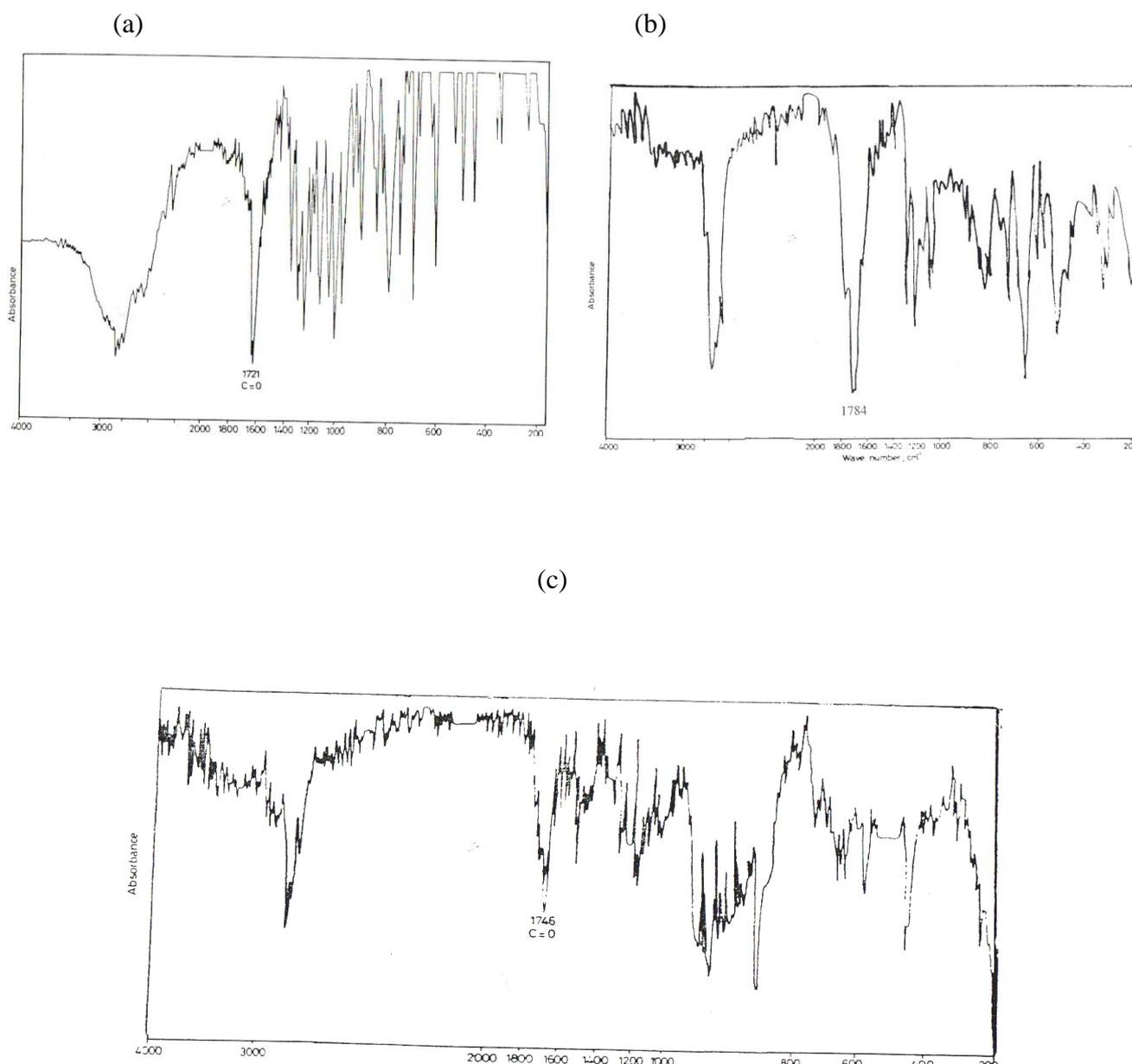
Where N is the oedema value, paw diameter on the tenth day – initial paw diameter before treatment ) of the

control and N' is the oedema value of the experimental group.

## Results and Discussion:

### Identification of Ester:

The IR spectra of ibuprofen, acid chloride and ibuprofen ester are shown in Figure 2, which gave a comparison of these compounds according to the carbonyl band in



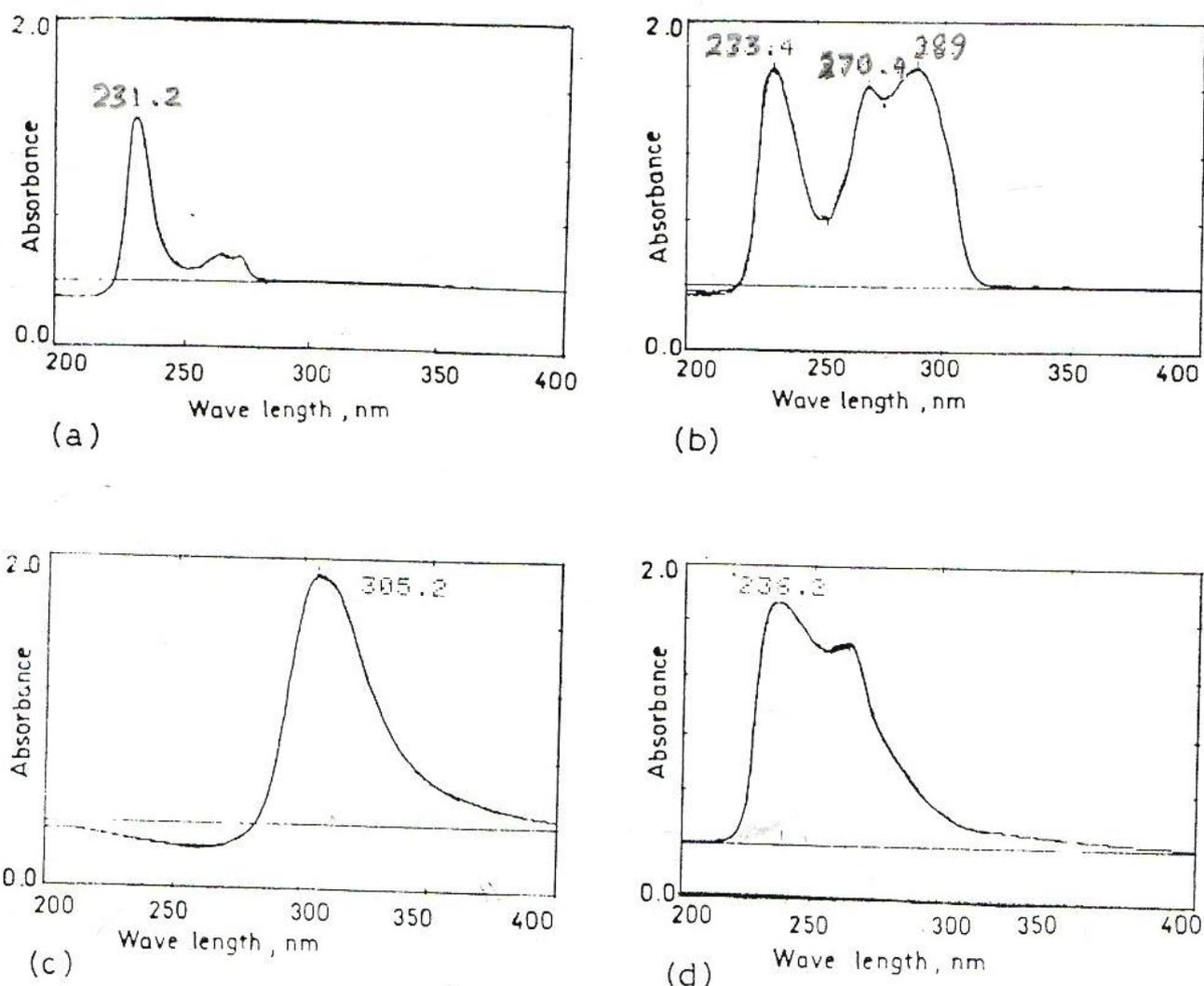
**Fig (2): IR- spectra of a) ibuprofen. b) ibuprofen acid chloride. c) ibuprofen ester .**

ibuprofen was  $1721\text{cm}^{-1}$ , for acid chloride of ibuprofen was  $1784\text{cm}^{-1}$  and for synthesized ester of ibuprofen was near to  $1750\text{cm}^{-1}$ . Hence the ester was distinguished [ 11] by shifting of strong stretching band of carbonyl group from  $1721\text{cm}^{-1}$  in ibuprofen to  $1750\text{cm}^{-1}$  region in ester of ibuprofen. In IR- spectra, additional band due to substituted benzene rings aromatic C-H stretching, aliphatic C-H

stretching and C-O stretching were also observed at the appropriate frequencies as shown in Figure 2.

### Kinetic Study of ester hydrolysis

Figure 3 shows the UV- spectra of ibuprofen, acid chloride of ibuprofen, paracetamol and ibuprofen ester in acetonitrile.



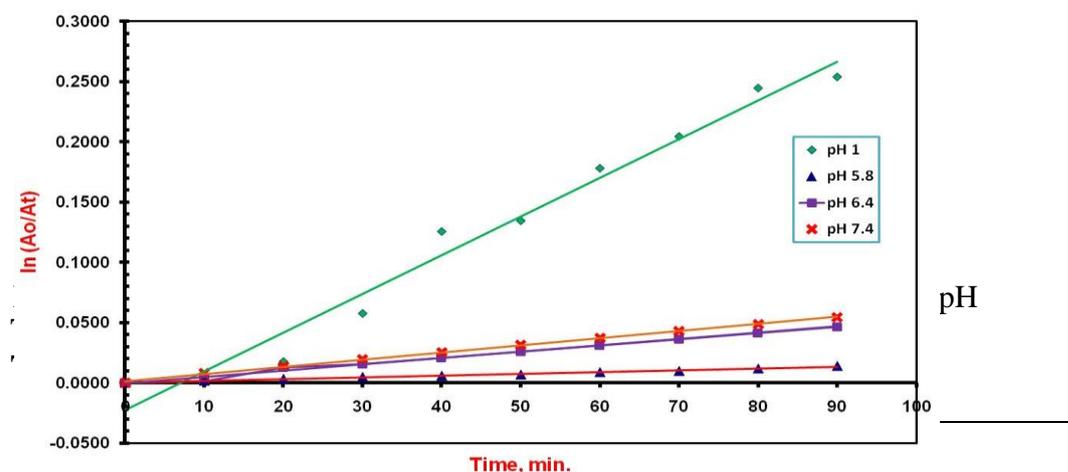
**Fig( 3): UV-spectra of a) ibuprofen. b) paracetamol c) ibuprofen acid chloride. d) ibuprofen ester, in acetonitrile .**

The absorbance values which are obtained from the hydrolysis of ester is shown in Table 1.

**Table( 1):The absorbance of ester hydrolysis at maximum wave length ( $\lambda_{max.}=241$  nm) at different pH and 37°C for 1.5 hour.**

Time, min.	pH 1.0 $A_t$	pH 5.8 $A_t$	pH 6.4 $A_t$	pH 7.4 $A_t$
0	1.929	1.997	1.817	1.990
10	1.926	1.993	1.807	1.973
20	1.895	1.990	1.794	1.962
30	1.821	1.987	1.788	1.959
40	1.701	1.985	1.779	1.939
50	1.686	1.982	1.770	1.928
60	1.614	1.979	1.761	1.917
70	1.572	1.970	1.752	1.906
80	1.510	1.965	1.743	1.894
90	1.496	1.950	1.734	1.884

**Fig (4) indicates the plot of  $\ln A_0/A_t$  versus time of ester hydrolysis at different pH values, giving a straight line at time period of 1.5 hour which confirmed Pseudo first order kinetic. The correlation coefficients ( R ) are shown in Table2**

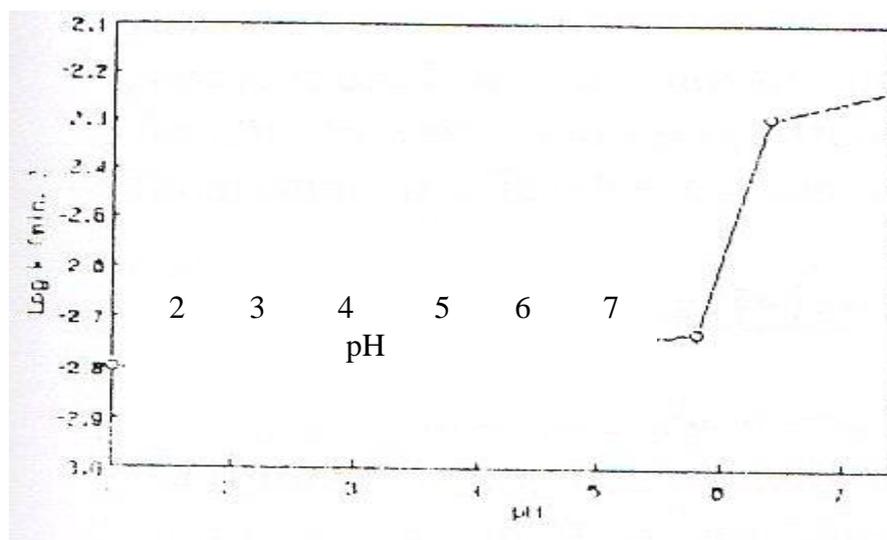


1.0	$1.597 \times 10^{-3}$	0.97	$1.41 \times 10^{-3}$
5.8	$1.874 \times 10^{-3}$	0.93	$2.43 \times 10^{-3}$
6.4	$5.089 \times 10^{-3}$	0.99	$6.87 \times 10^{-4}$
7.4	$5.894 \times 10^{-3}$	0.99	$6.43 \times 10^{-4}$

**a** slope is the rate constant (k) in  $\text{min.}^{-1}$

**b** represents the mean of standard error

Plot of pH versus log k was shown in Figure 5, to investigate ester hydrolysis.



Fig(5):The apparent pH–rate profiles of ester hydrolysis in different pH

Hydrolysis of ester at pH range of 1.0 to 5.8 shows a steady straight line, while ester undergoes to increase in the rate of hydrolysis at pH 6.4 and 7.4. Ester hydrolysis was treated as a unidirectional process in buffer solutions. This is because the magnitude of the equilibrium constant of the carboxylic acid and phenol substituent formed after complete hydrolysis [11]. Therefore, complete dissociation of ester is favored and the contribution from the slow reverse reaction towards the observed rate constant is insignificant. This is further assured by the magnitude of  $A_{\infty}$  (over night) which indicates the absorbance

reading of ester at infinity time that equal to zero practically.

### Biological Activity

Analgesic activity percentage and anti-inflammatory activity data were obtained in Table 3 and in Table 4, respectively. According to analgesic test ibuprofen was insignificant compared to aspirin due to its action as anti-inflammatory, whereas ester of ibuprofen was exhibited highly significance analgesic activity as shown in Table 3. For anti-inflammatory test, in Table 4, ester of ibuprofen was found more potent than aspirin.

Table (3): Analgesic activity following 100 mg/kg doses

Treatment	Mean stretching Number $\pm$ SD	Analgesic activity %
Control	30.0 $\pm$ 2.7	-----
Aspirin	20.2 $\pm$ 1.3	33.3
Ibuprofen	29.2 $\pm$ 1.7	2.5
Ester	9.2 $\pm$ 2.2	69.2

**Table (4) Antiinflammatory activity following 100 mg/kg doses**

Treatment	% Oedema	% Oedema inhibition $\pm$ SD
Control	100	-----
Aspirin	66.7	33.32 $\pm$ 7.25
Ibuprofen	78.46	21.78 $\pm$ 4.91
Ester	64.0	37.17 $\pm$ 6.45

**Conclusion:**

It can be concluded from kinetic study, that the hydrolysis of ibuprofen ester followed Pseudo first order kinetic as a decreasing of ester concentration, at different pH . It was stable at the pH of stomach (1.0) and hydrolyzed gradually that will prolong its release in stomach and then prevent its irritation. At pH 6.4 and above the ester hydrolyzed faster into active ingredients of ibuprofen and paracetamol. Therefore, ibuprofen ester can be considered as a prodrug which can prevent the side effect of ibuprofen which causes peptic ulcer. It was found that ester exhibited an anti-inflammatory potent higher than aspirin. Also, ibuprofen ester shows a double effect of analgesia compared to aspirin.

**References:**

1. Robert F. Doerge, Wilson and Gisvolds textbook of organic medicinal and pharmaceutical chemistry 1982 8<sup>th</sup> Ed., London, J.B. Lippincott company, pp. 644-663.
2. Laurence D. R. and Bennett P. N.; Clinical pharmacology 1992 7th Ed., London, ELBS with Churchill Livingstone Medical Division of Longman group U.K. Ltd. pp. 273-285.
3. Bansal AK., Khar RK., Dubbey R. and Sharma AK. 2001 . Prodrug of ibuprofen. *Boll chim. Farm.*, 140(2): 79-82.
4. Delvin A. and Ober C. K 1988 Liquid crystalline polyesters by staged –addition polycondensation. *Polymer Bulletin*, 20(1): 45-50.
5. Todd D. 1979 . Experimental organic chemistry, Pretice-Hall, Inc., Englewood Cliff, New Jersey, p.272-278.
6. Naringrekar V. H. and Stella V. J. 1990 . Mechanism of hydrolysis and structure stability relationship of enamionones as potential prodrug of model primary amines. *J. Pharmaceutical Sci.*, 79(2):138-146.
7. Perrin D. D., Armarego and Dawn R. P 1981 Purification of Laboratory Chemicals, 2nd Ed., London, Headington Hill Hall, Pregamon press Ltd., pp. 309-316.
8. Valentina O., Cenzo C., Emmelie B. and Emma S. 2010 . Synthesis and evaluation of paracetamol ester as novel fatty acid amide hydrolyase inhibitors. *J. Med. Chem.*, 53 (5):2286-2298.
9. Domer F. R. 1971 Animal experimental in pharmacological

- analysis. Charles Thomas: Spring field Ltd., pp. 465-470.
10. Koster R., Anderson M. and Deber E. L. 1959 Acetic acid for analgesic screening. Fed Proc. 18, pp.412-413.
11. Zhong Y. , Dai Z. , Teny Y. and Wu B. 2012 . Synthesis, stability and pharmacological evaluation of a noval codrug consisting of amivndine and ursolic acid. Eur. J. Pharm. Sci., 23: 45:110-115.

## دراسة حركية التحلل المائي لاستر الايبوبروفين المحضر وفعاليتها البايولوجية

صباح غانم الدباغ\*\*

نهى محمد يحيى\*

\* قسم الكيمياء، كلية التربية للبنات جامعة الموصل  
\*\* فرع الادوية، كلية الصيدلة جامعة الموصل

### الخلاصة:

من المعروف ان تناول الايبوبروفين عن طريق الفم يعمل على تهيج غشاء المعدة بسبب احتوائه على جزء حامضي في تركيبه ( الجزء الكربوكسيلي ) و لتقليل مثل هذا التأثير ، تم تحضير استر الايبوبروفين وذلك بربط الجزء الحامضي للايبوبروفين مع مجموعة الهيدروكسيل للفينول المعوض في الباراسيتامول . لقد تم قياس الاستقرار من خلال اختبار حركية التحلل المائي للاستر المحضر ، في اوساط حامضية مختلفة (عند الدالات الحامضية 1.0,5.8,6.4,7.4) عند درجة حرارة  $0.1 \pm 37^\circ \text{C}$  و لمدة ساعة. تمت هذه الدراسة بقياس الامتصاصية بجهاز مطيافية الاشعة فوق البنفسجية .  
وقد تم قياس الفعالية الدوائية ( داخل الخلية ) لاستر الايبوبروفين ، كمسكنات باستخدام طريقة حامض الخليك و كمضادات للالتهابات العضوية باستخدام طريقة قياس الورم في الرجل الخلفي للحيوان المختبري . واستخدم الاسبرين كمصدر عند مقارنة الفعالية الدوائية في كلتا الحالتين. ولقد اظهر استر الايبوبروفين فعالية بايولوجية ذات تاثير فعال اقوى من الاسبرين في كلا الاختبارين.