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فراس طاهر ماهر
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بسم الله الرحمن الرحيم

اقرا بسم ربك الذي خلق ؟
خلق الإنسان من علق ؟
اقرا وربك الأكرم الذي علم
بالقلم ؟ علم الإنسان ما لم
يعلم ؟

صدق الله العظيم
(سورة العلق ١-٥)

REPUBLIC OF IRAQ
AL-Nahrain University
College of Science

Biochemical Study of some derivatives of *L*-Ascorbic acid as a pro-drug

A thesis submitted to the College of Science, University of Al-Nahrain As a partial fulfillment of the requirements for the PhD in Biochemistry

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B.Sc, Chemistry 1993
M.Sc, Chemistry 1999

2005

3.9: Biochemical study :

3.9.1 : Determination of Acetyl Choline Esterase (AChE) activity :

(AChE) activity was measured in the serum (*In vitro*) according to the method described in (2.8.4).The value of (AChE) in serum was between (4.41±0.61–8±1.14 µmol/3min/ml) this value is in the normal value of AChE activity in Iraqi population (men and women) .[152],[153]

3.9.2: effect of new compounds (19),(20),(21) on the (AChE) activity in serum :

Any compound that reduces the velocity of an enzyme-catalyzed reaction can be considered to be an "inhibitor". The inhibition of enzyme activity is one of the major regulatory devices of living cells, and the most important diagnostic procedure of the enzymatic inhibition studies often tells us something about the specificity of an enzyme , the physical and chemical architecture of active site , and the kinetic mechanism of the reaction . Enzyme inhibitor can found masquerading as drugs, antibiotics, poisons , and toxins .[154]

The effect of the new compounds (19),(20),(21) were evaluated on serum (*In vitro*). A stock solution (0.5 g/25 ml) of each compound was prepared in DMSO. Dilution was made to obtain the required concentration [($5.2x10^{-8}$ M) for (19) ,($3.7x10^{-8}$ M) for (20) and ($2.42x10^{-8}$ M) for (21)]. The substrate concentration was (0.1 M)

The evaluated compounds (19),(20),(21) were found to be inhibitor of (AChE) . The inhibitory effect was found to increase as the concentration of the compounds increase .

The result indicated that the (AChE) inhibition ranges were (88.75-10.75 %), (92.2-8.67 %) and (94.97-35.88 %) due to the synthesis compounds 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) , 2,3-di(acetyl salicyloyl)-

5,6 –O-isopropylidene-*L*-ascorbic acid (20) and 2,3,5,6 –tetra (acetyl salicyloyl)-*L*-ascorbic acid (21) and the inhibitory percentage of the compounds are summarized in the table (21,22,23) :

Table (21) inhibition effect of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) on Acetyl choline esterase activity.

Inhibitor conc. M	Enzyme Activity µmol/3min/ml	%Inhibition	% Recovery
Nil	4.975±١,٤٥	0	100
$5.2x10^{-8}$	4.44±0.40	١٠,٧٥	٨٩,٢٤
$5.2x10^{-7}$	4.04±0.35	١٨,٨٠	٨١,٢٠
$5.2x10^{-6}$	3.92±0.25	٢١,٢٠	٧٨,٧٩
$5.2x10^{-5}$	3.21±0.20	٣٥,٤٧	٦٤,٥٢
$5.2x10^{-4}$	2.38±0.125	٥٢,١٦	٤٧,٨٣
$5.2x10^{-3}$	1.41±0.1	71.65	28.34
$5.2x10^{-2}$	0.56±٠,٠٧٥	88.74	11.25

Table (22) inhibition effect of 2,3-di(acetyl salicyloyl)-5,6 – O-isopropylidene-*L*-ascorbic acid (20) on Acetyl choline esterase activity.

Inhibitor conc. M	Enzyme Activity µmol/3min/ml	%Inhibition	% Recovery
Nil	4.885±١,٣٢٥	0	100
$3.7x10^{-8}$	4.461±0.45	٨,٦٧	٩١,٣٢
$3.7x10^{-7}$	3.92±0.35	٣٦,٣٣	٥٧,٤٨
$3.7x10^{-6}$	3.11±0.25	٥٧,١٨	٦٣,٦٦
$3.7x10^{-5}$	2.33±0.15	٥٢,٣١	٤٧,٦٩
$3.7x10^{-4}$	1.84±0.125	٦٢,٣٤	٣٧,٦٦
$3.7x10^{-3}$	1.01±0.075	٧٩,٣٢	٢٠,٦٧
$3.7x10^{-2}$	0.381±٠,٠٢٥	٩٢,٢	٧,٨

Table (23) inhibition effect of 2,3,5,6 -tetra(acetyl salicyloyl)-*L*-ascorbic acid (21) on Acetyl choline esterase activity.

Inhibitor conc. M	Enzyme Activity µmol/3min/ml	%Inhibition	% Recovery
Nil	4.975±١,٢٢٥	0	100
$2.42x10^{-8}$	3.19±0.35	٣٥,٨٨	٦٤,١٨
$2.42x10^{-7}$	2.86±0.275	٤٢,٥١	٥٧,٤٨
$2.42x10^{-6}$	2.13±0.225	٥٧,١٨	٤٢,٨١
$2.42x10^{-5}$	1.73±0.15	٦٥,٢٢	٣٤,٧٧
$2.42x10^{-4}$	1.38±0.125	٧٢,٢٧	٢٧,٧٣
$2.42x10^{-3}$	0.761±0.075	٨٤,٧	١٥,٢٩
$2.42x10^{-2}$	0.25±٠,٠٢٥	٩٤,٩٧	٥,٠٢

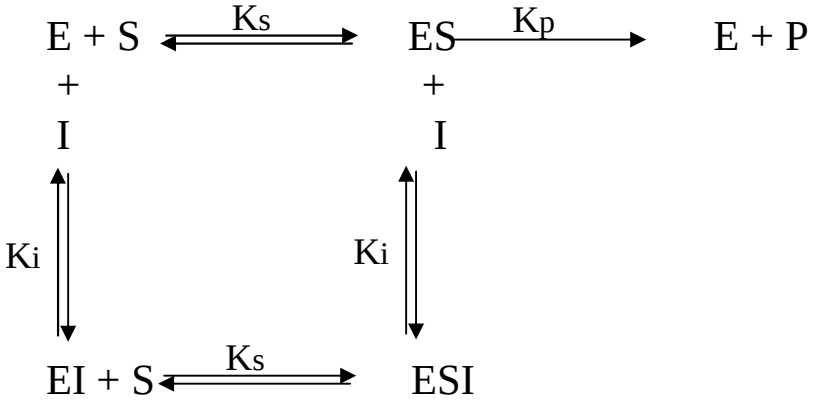
3.9.3 - Inhibition type study :

The inhibition type study was done by fixing the concentrations of compounds (Inhibitor) and changing the substrate concentration ,the applied concentration of each compound (inhibitor) were [($5.2x10^{-4}$) M,($3.7x10^{-4}$) M and ($2.42x10^{-4}$ M) for (19,20 and 21) respectively. While the substrate concentration was (0.1- 0.02 M).

The method described in section (2.8.4) was followed to evaluate the enzyme activity in the present and absence of an inhibitor in the same condition figure (30).

Linweaver – Burk relation was shown that the type of inhibition was non-competitive inhibition (Km value unchanged and the Vmaxi was less then Vmax observed in the absence of inhibitor) figures (31-33) . [155]

A classical non-competitive inhibitor has no effect on substrate binding, S and I were bound reversibly , randomly , and independently at different sites , so; I binds to E and to ES and S binds to E and to EI .The resulting ESI complex is catalytically inactive .



It can be stated from equilibria that at any inhibitor concentration , an infinitely high substrate concentration cannot drive all enzyme to the ES form . At any [I] a portion of the enzyme will remain as the nonproductive ESI complex .It can be predicted that Vmaxi would be less then Vmax observed in

the absence of inhibitor and Km value (measured as the [S] required for 0.5 Vmax) will be unchanged because at any inhibitor concentration, the enzyme forms can combine with S (E and EI) having equal affinities for S: [156]

$$\frac{1}{v} = \frac{Km}{V\ \max}(1 + \frac{[I]}{Ki})\frac{1}{[S]} + \frac{1}{V\ \max}(1 + \frac{[I]}{Ki})$$

Table (24) show value of Km,Vmax,Ki,Vmaxi :

Table (24) value of Km,Vmax,Ki,Vmaxi

Compound	Km [M]	Ki [M]	Vmax µmol/3min/ml	Vmaxi µmol/3min/ml
19	0.0434	$4.676x10^{-4}$	0.67	0.384
20	0.0416	$1.533x10^{-3}$	0.67	0.540
21	0.0455	$2.32x10^{-3}$	0.67	0.621

Table (25) value of (Velocity)(V) with and without the inhibitors (compounds)(19),(20),(21)

[S] M	(V)Normal µmol/3min/ml	(V) with (19) µmol/3min/ml	(V) with (20) µmol/3min/ml	(V) with (21) µmol/3min/ml
0.1	4.561±1.4	4.08±1.00	3.71±0.75	3.02±0.75
0.08	4.210±1.35	3.78±0.75	3.31±0.5	2.73±0.5
0.06	3.871±1.35	3.496±0.5	3.02±0.25	2.51±0.25
0.05	3.453±1.2	٣,٢٠٥±٠,٢٥	2.81±0.125	2.27±0.125
0.04	2.884±1.15	2.62±0.125	2.43±0.1	2.01±0.1
0.02	1.968±1.00	1.87±0.1	1.63±0.075	1.34±0.075

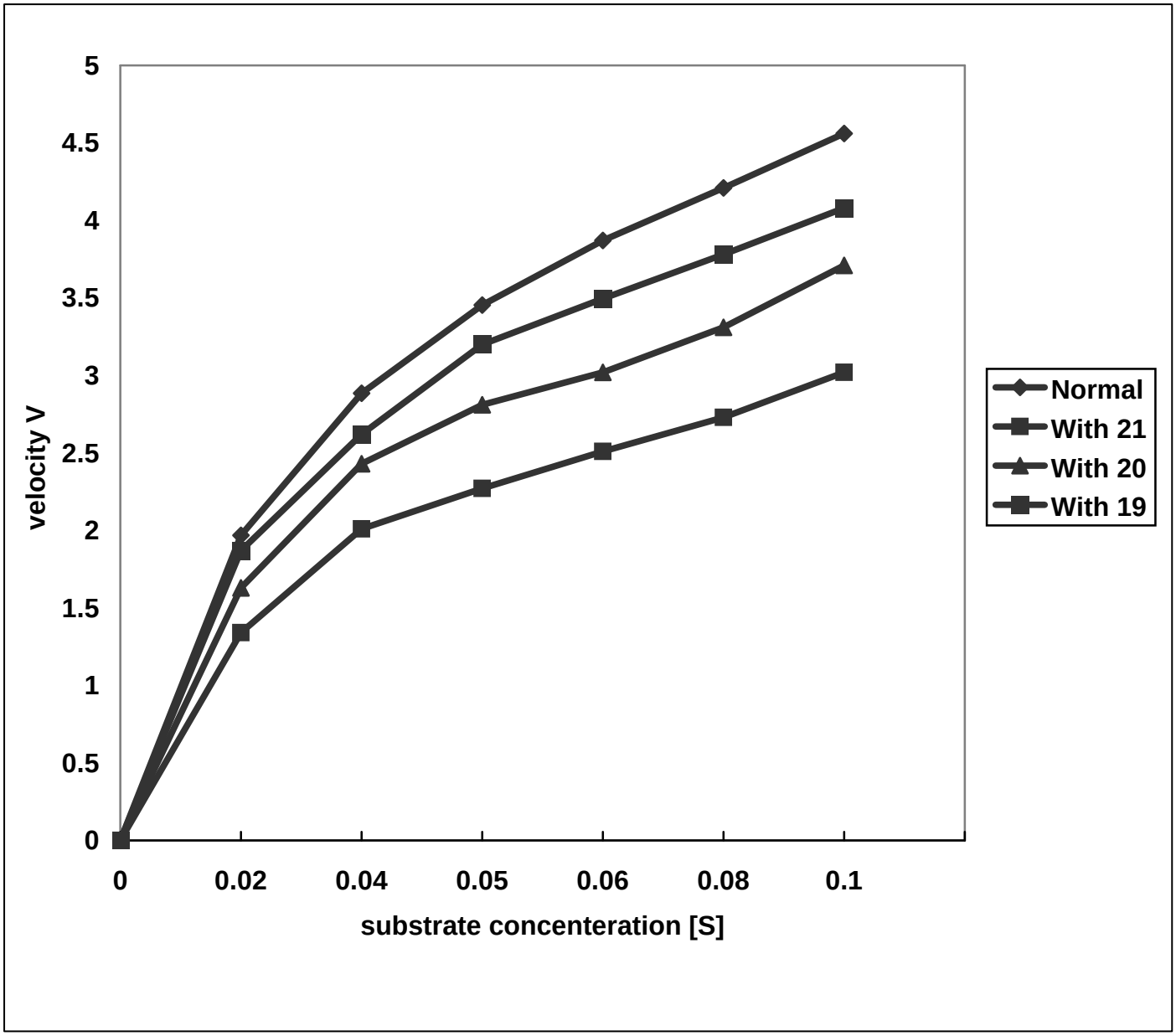


Figure (30) Velocity versus [S] with and without inhibitor

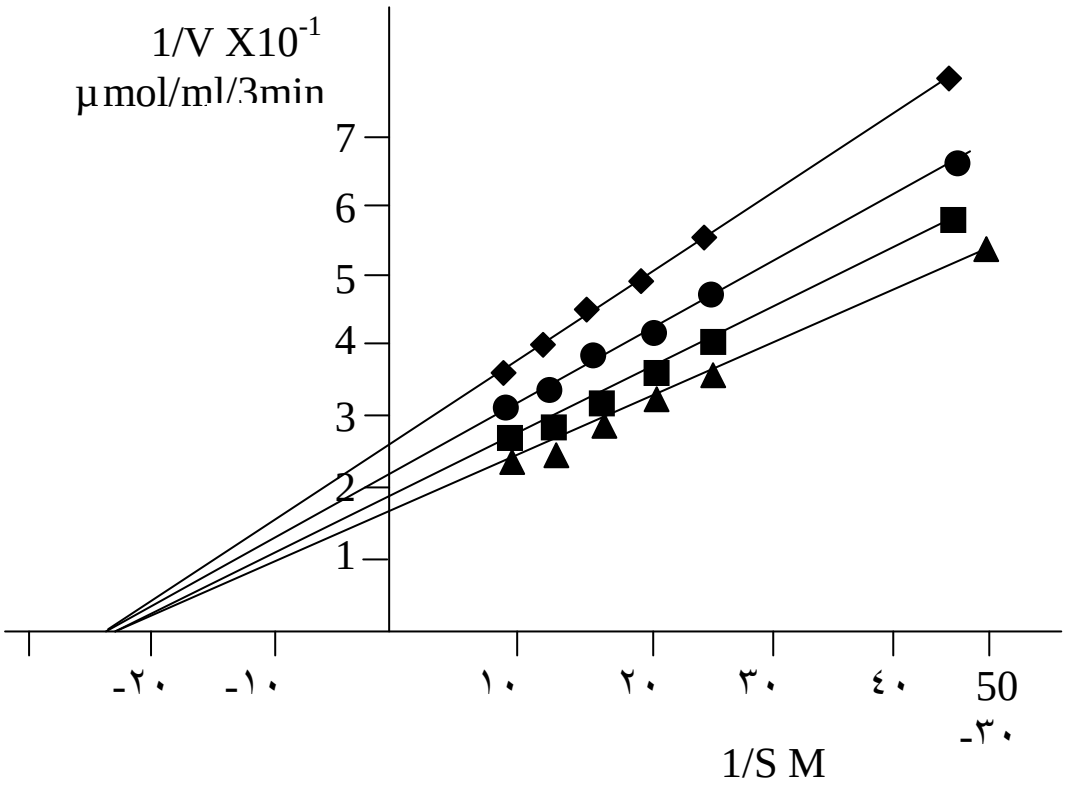


Figure (31) Linweaver – Burk plot for compounds (19,20,21)

▲ **: Control**

▬ **:3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19)**

● **: 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20)**

◆ **: 2,3,5,6-Tetra(acetyl salicyloyl)-*L*-ascorbic acid (21)**

3.9.4 Determination of Alkaline phosphatase (ALP) and acid phosphatase (ACP) activity:

(ALP) and (ACP) activities were measured in the serum (*In vitro*) according to the method described in (2.10.2),(2.12.2) respectively. The normal value of (ALP) activity is between (3-13 KAU/dl) . The activity ALP in serum assay (control) was (8±0.75 KAU/dl) (one Kind and King units is the amount of enzyme which , in the given condition , liberates 1 mg of phenol in 15 min. at 37^0C) . While the normal value of serum (ACP) activity (Total acid phosphate was up to 11 u/L , Prostatic acid Phosphate was up to 4 u/L). The activity ACP in serum assay (control) was (Total acid phosphate was 9.92 ±1.3 u/L , Prostatic acid Phosphate was3.12±0.7 u/L)

The effect of the new compounds (19),(20),(21) were evaluated on serum (*In vitro*).

The compounds (19),(20),(21) were found to increase the activity of (ALP) and (ACP) . The activity effect was found to increase as the concentration of the compounds increase:

1- compound 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) was found to have activation effect on activity of (ALP) ,(ACP) . The activation percent was between (365.25- 145.62 %) for (ALP), (164.11 - 101.91%) for Total acid phosphate and (190 – 110.02%) for Prostatic acid Phosphate.

2- compound 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20) was found to have activation effect on activity of (ALP) and (ACP) . The activation percent was between (412.5 - 175%) for (ALP) , (195.86 - 107.96%) for total acid phosphate and (248.71–123.07%) for Prostatic acid Phosphate.

3- compound 2,3,5,6 –tetra (acetyl salicyloyl)- *L*-ascorbic acid (21) was found to have activation effect on activity of (ALP) and (ACP) . The activation percent was between (500 –

228.75%) for (ALP) , (228.22 – 117.13%) for total acid phosphate and (305.76 – 132.37%) for Prostatic acid Phosphate.

The activation effect of the 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19), 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid(20),2,3,5,6-tetra(acetyl salicyloyl)-*L*-ascorbic acid (21) on (ALP) and (ACP) is shown in table (26-29) :

Table (26) effect of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) , 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20) , 2,3,5,6-terta(acetyl salicyloyl)-*L*-ascorbic acid (21) on Alkaline phosphatase activity.

(19)M	**Enzyme Activity** KAU/dl	**(20)M**	**Enzyme Activity** KAU/dl	**(21)M**	**Enzyme Activity** KAU/dl
0	8±0.75	0	8±0.75	0	8±0.75
$5.2x10^{-8}$	11.65±1.75	$3.7x10^{-8}$	14±1	$2.42x10^{-8}$	18.3±2
$5.2x10^{-7}$	14.2±1	$3.7x10^{-7}$	17.6±1	$2.42x10^{-7}$	20.1±1.5
$5.2x10^{-6}$	17±2	$3.7x10^{-6}$	21.1±1.4	$2.42x10^{-6}$	24.61±1
$5.2x10^{-5}$	21±1	$3.7x10^{-5}$	24.22±2	$2.42x10^{-5}$	28±1.87
$5.2x10^{-4}$	23.5±1.8	$3.7x10^{-4}$	26±1	$2.42x10^{-4}$	32±2.41
$5.2x10^{-3}$	25±1	$3.7x10^{-3}$	29.61±2.3	$2.42x10^{-3}$	37±2
$5.2x10^{-2}$	29.22±2	$3.7x10^{-2}$	33±2	$2.42x10^{-2}$	40±3

Table (27) effect of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) on Acid phosphatase activity.

(19) M	Total acid pho.	Prostatic acid pho.
0	9.92±1.3	3.12±0.7
$5.2x10^{-8}$	10.11±0.5	3.44±0.125
$5.2x10^{-7}$	11.21±0.5	3.97±0.125
$5.2x10^{-6}$	11.83±0.5	4.54±0.125
$5.2x10^{-5}$	12.44±0.5	4.96±0.125
$5.2x10^{-4}$	13.35±0.75	5.22±0.25
$5.2x10^{-3}$	14.74±0.75	5.67±0.25
$5.2x10^{-2}$	16.28±1	5.93±0.25

Table (28) effect of 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20) on Acid phosphatase activity.

(20)M	Total acid pho.	Prostatic acid pho.
0	9.92±1.3	3.12±0.7
$3.7x10^{-8}$	10.71±0.5	3.84±0.125
$3.7x10^{-7}$	11.55±0.5	4.11±0.125
$3.7x10^{-6}$	12.61±0.5	4.87±0.125
$3.7x10^{-5}$	13.28±0.75	5.21±0.25
$3.7x10^{-4}$	14.56±0.75	5.92±0.0.25
$3.7x10^{-3}$	16.78±1	6.19±0.25
$3.7x10^{-2}$	19.43±1.5	7.76±0.5

Table (29) effect of 2,3,5,6-tetra(acetyl salicyloyl)-*L*-ascorbic acid (21) on Acid phosphatase activity.

(21)M	Total acid pho.	Prostatic acid pho.
0	9.92 ± 1.3	3.12±0.7
$2.42x10^{-8}$	17.73±1.75	8.713±0.5
$2.42x10^{-7}$	15.67±1.5	7.68±0.5
$2.42x10^{-6}$	14.59±1.25	6.43±0.25
$2.42x10^{-5}$	14.59±1.25	6.43±0.25
$2.42x10^{-4}$	12.43±0.75	4.51±0.125
$2.42x10^{-3}$	11.62±0.5	4.13±0.125
$2.42x10^{-2}$	22.64±2	9.54±0.75

3.10: Biological study:

In this study several type of bacteria were tested (***Pseudomonas aeruginasa, Salmonella typhimuriua , Staphylococcus aureus, Candida (yeast) , Bacillus subtitles and E.Coli***) blood agar was used as culture media . The effect of the new compounds (19),(20),(21) were calculated at different concentrations.

The compounds (19),(20),(21) were found to have inhibition effect on growth of bacteria . The inhibitory effect was found to increase as the concentration of compounds increase:

1- compound 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) was found to have inhibition zone on growth of bacteria .The inhibition zone between (0.52-0 cm for Pseudomonas , 1.3 cm for Salmonella , 0.171 cm for Staphylococcus , 0.5 cm for Candida ,no inhibition for Bacillus subtitles and 0.18 cm for E.Coli)

2- compound 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20) was found to have inhibition zone on growth of bacteria .The inhibition zone between (1.1 cm for Pseudomonas , 2 cm for Salmonella , 0.261cm for Staphylococcus , 1 cm for Candida , no inhibition for Bacillus subtitles and 0.3 cm for E.Coli)

3- compound 2,3,5,6 –tetra (acetyl salicyloyl)-*L*-ascorbic acid (21) was found to have inhibition zone on growth of bacteria .The inhibition zone between (2 cm for Pseudomonas , 3.5 cm for Salmonella , 0.5 cm for Staphylococcus , 1.5 cm for Candida , no inhibition for Bacillus subtitles and 0.5 cm for E.Coli)

The inhibitory effect of the 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19), 2,3-di(acetyl salicyloyl)-

5,6 – O-isopropylidene-*L*-ascorbic acid (20),2,3,5,6-tetra (acetyl salicyloyl)-*L*-ascorbic acid (21) is shown in table (33-35) :

Table (30) effect (inhibition zone in cm) of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) on several type of bacteria

Conc. M	Pseudomonas cm	Salmonella *typhimuriua* cm	Staphylococcus cm	Candida cm	Bacillus subtitles cm	E.Coli cm
$5.2x10^{-2}$	0.52	1.3	1.71	0.5	0	0.18
$5.2x10^{-3}$	٠,٣٤	1.00	0.057	0.034	0	0.08
$5.2x10^{-4}$	0.21	0.71	0	0.021	0	0.03
$5.2x10^{-5}$	٠,١٦	0.58	0	0.05	0	0
$5.2x10^{-6}$	٠,٠٥٧	0.49	0	0	0	0
$5.2x10^{-7}$	0	0.16	0	0	0	0
$5.2x10^{-8}$	0	0	0	0	0	0

Table (31) effect (inhibition zone in cm) of 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20) on several type of bacteria

Conc. M	Pseudomonas cm	Salmonella *typhimuriua* cm	Staphylococcus cm	Candida cm	Bacillus subtitles cm	E.Coli cm
$3.7x10^{-2}$	1.1	2.0	0.261	1.0	0	0.3
$3.7x10^{-3}$	٠,٦	1.86	0.16	0.7	0	0.16
$3.7x10^{-4}$	0.41	1.1	0.054	0.42	0	0.1
$3.7x10^{-5}$	٠,٢٠	1.04	0	0.166	0	0
$3.7x10^{-6}$	٠,١١	0.87	0	0.048	0	0
$3.7x10^{-7}$	0.05	0.22	0	0	0	0

$3.7x10^{-8}$	0	0	0	0	0	0

Table (32) effect of (inhibition zone in cm)2,3,5,6-tetra (acetyl salicyloyl)-*L*-ascorbic acid (21) on several type of bacteria

Conc. M	**Pseudomonas cm**	**Salmonella *typhimuriua* cm**	**Staphylococcus cm**	**Candida cm**	**Bacillus subtitles cm**	**E.Coli cm**
$2.42x10^{-2}$	2.0	3.5	0.5	1.5	0	0.5
$2.42x10^{-3}$	١,٠	3.13	0.28	1.1	0	0.31
$2.42x10^{-4}$	0.86	2.77	0.13	0.72	0	0.2
$2.42x10^{-5}$	٠,٥٥	2.10	0	0.35	0	0
$2.42x10^{-6}$	٠,٣٢	1.5	0	0.11	0	0
$2.42x10^{-7}$	0.19	0.5	0	0	0	0
$2.42x10^{-8}$	0	0	0	0	0	0

3.11*: In vivo* study :

In this study 1.5 gm the compounds (19),(20),(21) were used (oral administration to a group of three rabbits).

Blood sample was taken from the rabbits then the serum was separated by centrifuge,150 µL from serum was diluted with methanol (25 ml) .The aspirin concentration was measured in serum at 277 nm .

-The aspirin concentration after (2 hr.) was ($3.71X10^{-5}$ M) , (3 hr.) was ($3.2X10^{-3}$ M) , (4 hr.) was ($4.08X10^{-3}$ M) , (6 hr.) was ($3.41X10^{-3}$) ,(8 hr.) was ($4.61X10^{-3}$ M) , (10 hr.) was ($3.601 X10^{-5}$ M) for the compound (19) .

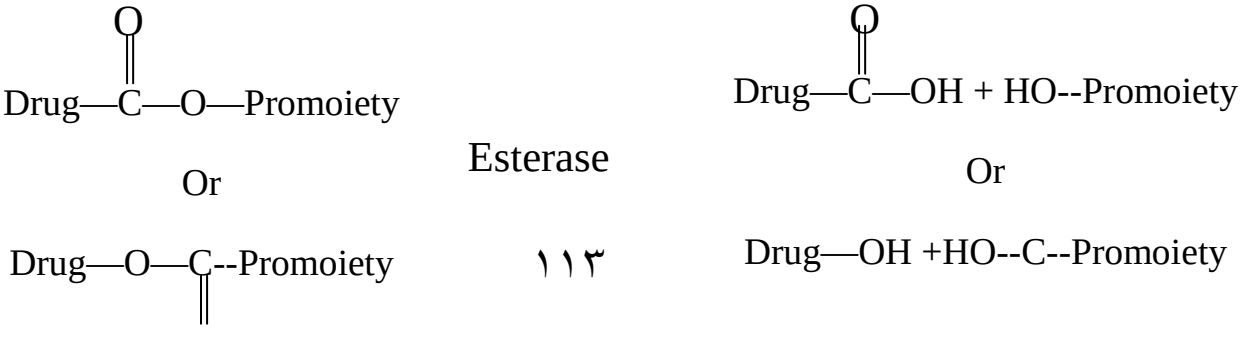
-The aspirin concentration after (2 hr.) was ($7.52X10^{-5}$ M) , (3 hr.) was ($6.40X10^{-3}$ M) , (4 hr.) was ($8.38X10^{-3}$ M) , (6 hr.) was ($6.68X10^{-3}$ M) ,(8 hr.) was ($9.18X10^{-4}$ M) , (10 hr.) was ($7.25X10^{-5}$ M) for the compound (20) .

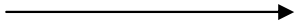
-The aspirin concentration after (2 hr.) was ($1.47X10^{-4}$ M) , (3 hr.) was ($1.27X10^{-2}$ M) , (4 hr.) was ($1.68X10^{-2}$ M) , (6 hr.) was ($1.34X10^{-2}$ M) ,(8 hr.) was ($1.84X10^{-3}$ M) , (10 hr.) was ($1.46X10^{-4}$ M) for the compound (21) .Table (36-38) ,Figure (34-36) .

The results show that the concentration of aspirin was low in the first hour and then increased with time increased until 4 hours (the maximum concentration of aspirin) .

After 4 hour (6,8,10 hr.) the concentration of aspirin was decreased .After 10 hours the concentration of aspirin was equal to the aspirin concentration in (2 hours).

Hydrolysis normally accomplished by esterase enzyme present in serum and other tissues capable or hydrolyzing a wide variety of ester linkages like (Ester hydrolase, Lipase ,Cholesterol esterase , Acetyl cholinesterase ,Carboxypeptidase) [161] .





T O er moiety is subsequently hydrolyze O the gastrointestinal tract and the agent is absorbed as aspirin and vitamin C [163].

Schnabelruch and coworker[162] (1990) refer to the hydrolytic release of the bioactive agent (carboxymethyl cellulose-2,2-dichloropropionates) (CMC ester) is mainly dependent on the hydrophilicity of the CMC ester . In the case of containing enzymatic cleavable the release can be accelerated by addition of esterase .The release of 2,2-dichloropropanic acid from CMC ester at 30^0C and pH 7 without addition of esterase was 8 % after 100 hours while The release of 2,2-dichloropropanic acid from CMC ester at 30^0C and pH 7 with addition of esterase was 50 % after 100 hours.

Yi-Nuo Pang et al (2002) [163] refers to that the recovery of dexamethasone (%) in colon after (1,3,4,5,6,7,9) hours (oral administration) was (0,0,20.5,18.0,10.2,15.2,18.2)% respectively. These results showed that the ester type prodrugs of dexamethasone/dextran (DSD) release dexamethasone preferentially on colonic contents after the hydrolysis of dextran to small oligosaccharides.The dextran conjugate survives the passage through upper GI tract although the high level of esterase in small intestine, indicating that dextran protects ester bond from hydrolysis by esterase. This result, together with the observation mentioned above, suggests that bacterial enzymes in the colon are responsible for hydrolysis of dextran conjugates. When DSD reached the colon, dextran was completely hydrolyzed into smaller oligosaccharides and exposed the ester bonds to esterase, which led to the rapid release of dexamethasone.

The bacterial count in the colon is much higher than that in upper GI tract[164]. The colonic micro flora produces a variety of

enzymes, including azoreductase, various glycosidases and amidases, which are not present in the stomach or the small intestine. Therefore, enzyme dependent drug release, which relies on the existence of enzyme-producing microorganisms in the colon, could be used to deliver drug to the colon after enzymatic cleavage of degradable carrier bonds.

Table (33) Concentration of aspirin in rabbit blood serum [compound (19)]

Time (hr)	Concentration M
0	0
2	$3.71X10^{-5}$
3	$3.2X10^{-3}$
4	$4.08X10^{-3}$
6	$3.41X10^{-3}$
8	$4.61X10^{-4}$
10	$3.601X10^{-5}$

Table (34) Concentration of aspirin in rabbit blood serum [compound (20)]

Time (hr)	Concentration M
0	0
2	$7.52X10^{-5}$
3	$6.40X10^{-3}$
4	$8.34X10^{-3}$
6	$6.68X10^{-3}$
8	$9.18X10^{-4}$
10	$7.25X10^{-5}$

Table (35) Concentration of aspirin in rabbit blood serum [compound (21)]

Time (hr)	Concentration M
0	0
2	$1.47X10^{-4}$
3	$1.27X10^{-2}$
4	$1.68X10^{-2}$
6	$1.34X10^{-2}$
8	$1.84X10^{-3}$
10	$1.46X10^{-4}$

Conclusion :

1. In this work new derivatives of *L*-ascorbic acid (Vitamin C) has been stynthezized: 3- (acetyl salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid , 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid and 2,3,5,6 -Tetra (acetyl salicyloyl)-5,6-*L*-ascorbic acid . This derivatives were obtained by simple and efficiency methods .
2. Drug released study : hydrolysis of derivatives increase when pH increase and the gastric irritation will be decrease by the condensation of aspirin with vitamin C because the ester linkage will expected to be cleaved in the colon librating aspirin and vitamin C.
3. The new derivatives were found to inhibition of acetyl choline esterase activity. The type of inhibition was non competitive inhibition (Vmax changed , Km unchanged) .
4. The new derivatives were found to increased alkaline phosphatase and acid phosphatase activity.
5. The new derivatives were found to inhibition of growth of some type of bacteria .
6. *In vivo* study showed us that the highest aspirin concentration was found after 4 hrs. of administration .

Future Studies Recommendation :

1. More kinetic studies should be conducted in the relation to the rate of hydrolysis of the compounds in blood and with specific colon's enzyme .
2. Toxicity studies should carried out intensively , as acute and chronic exposure .
3. Testing the effect of these compounds on cancer cell.
4. Testing the effect of these compounds on cyclooxygenase activity.

Table (6) concentration of aspirin released by compound (19) in pH (8)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.69X10^{-5}$	0.1
0.167	$4.22X10^{-5}$	0.26
0.25	$5.07X10^{-5}$	0.32
0.34	$7.123X10^{-5}$	0.45
0.50	$7.346X10^{-5}$	0.46
1.00	$8.47X10^{-5}$	0.53
1.25	$3.178X10^{-4}$	2
1.5	$3.94X10^{-4}$	2.4
2.00	$4.40X10^{-4}$	2.7
2.75	$4.8X10^{-4}$	3
3.00	$5.45X10^{-4}$	3.4
4.00	$7.16X10^{-4}$	4.51
6.00	$1.015X10^{-3}$	6.4
8.00	$1.98X10^{-3}$	12.4
24.00	$6.60X10^{-3}$	41.6
26.00	$6.63X10^{-3}$	41.8
28.00	$6.74X10^{-3}$	42.5
29.00	$7.15X10^{-3}$	45.1
30.00	$7.24X10^{-3}$	45.6

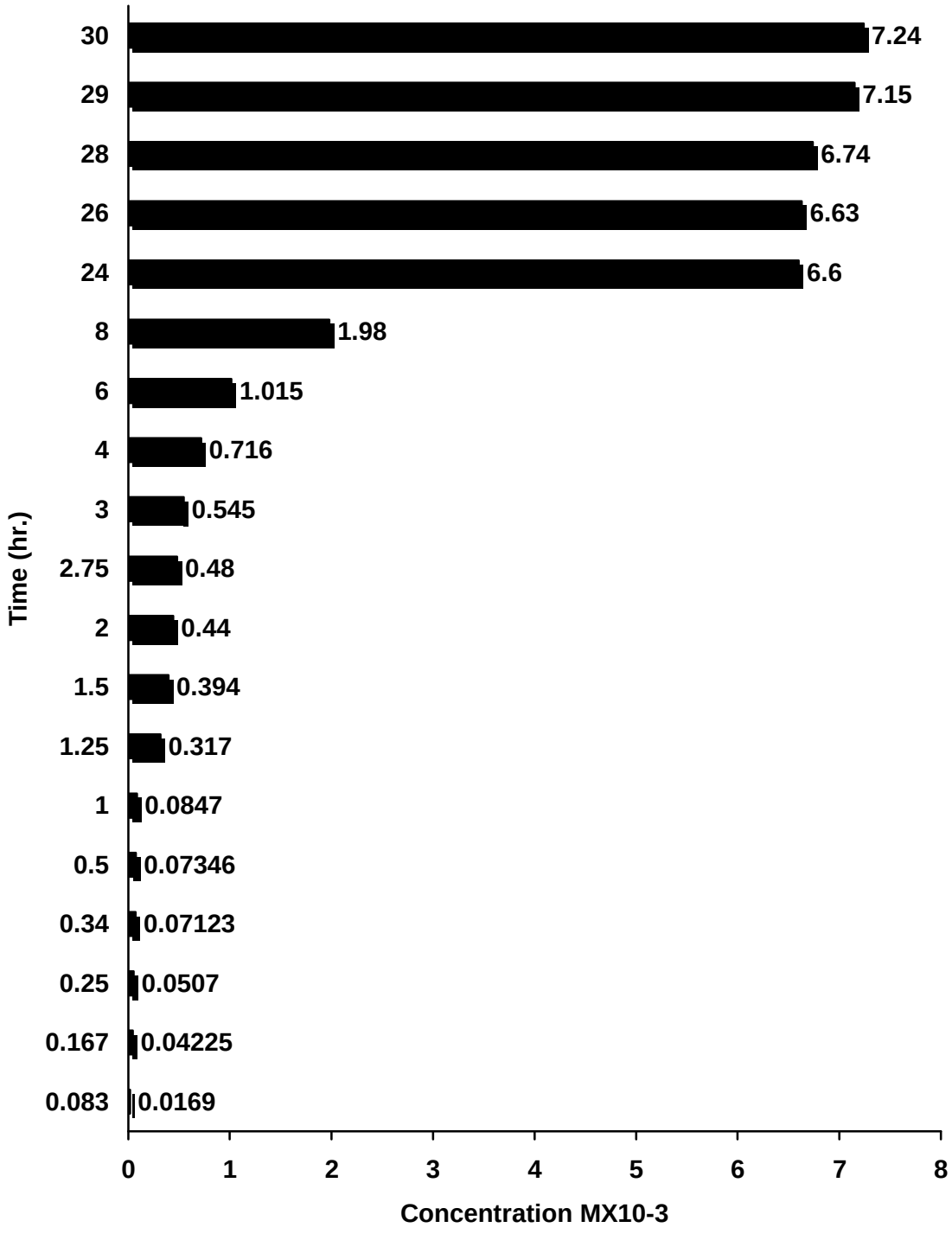


Figure (11) concentration of aspirin released by compound (19) in pH (8)

Table (7) concentration of aspirin released by compound (19) in pH (10)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$3.38X10^{-5}$	0.213
0.167	$7.623X10^{-5}$	0.48
0.25	$8.47X10^{-5}$	0.53
0.34	$9.32X10^{-5}$	0.588
0.50	$1.272X10^{-4}$	0.8
1.00	$1.684X10^{-4}$	1.06
1.25	$4.11X10^{-4}$	1.92
1.5	$4.22X10^{-4}$	2.66
2.00	$6.51X10^{-4}$	4.11
2.75	$6.81X10^{-4}$	4.3
3.00	$1.01X10^{-3}$	6.37
4.00	$1.57X10^{-3}$	9.9
6.00	$1.95X10^{-3}$	12.34
8.00	$2.94X10^{-3}$	18
24.00	$6.71X10^{-3}$	42.33
26.00	$6.942X10^{-3}$	43.7
28.00	$7.157X10^{-3}$	45.2
29.00	$7.303X10^{-3}$	46.1
30.00	$7.455X10^{-3}$	47

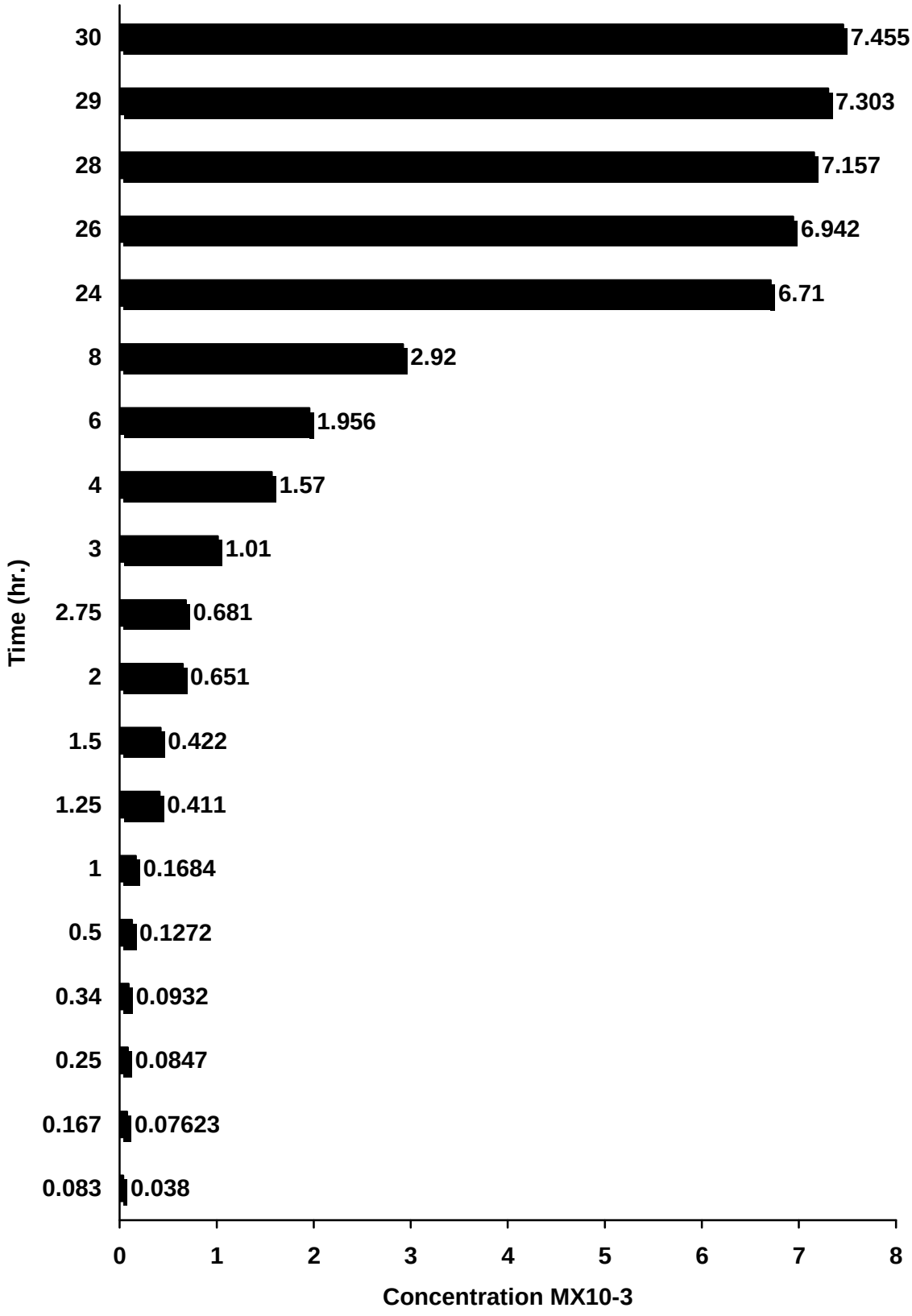


Figure (12) concentration of aspirin released by compound (19) in pH (10)

Table (8) concentration of aspirin released by compound (19) in pH (12)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$7.123X10^{-5}$	0.45
0.167	$1.272X10^{-4}$	0.8
0.25	$1.59X10^{-4}$	1
0.34	$1.99X10^{-4}$	1.26
0.50	$2.22X10^{-4}$	1.4
1.00	$3.178X10^{-4}$	2
1.25	$4.54X10^{-4}$	2.86
1.5	$7.58X10^{-4}$	4.71
2.00	$1.51X10^{-3}$	9.5
2.75	$1.956X10^{-3}$	12.34
3.00	$2.416X10^{-3}$	15
4.00	$2.88X10^{-3}$	18.19
6.00	$3.508X10^{-3}$	22.1
8.00	$4.21X10^{-3}$	26.5
24.00	$7.261X10^{-3}$	45.8
26.00	$7.41X10^{-3}$	46.68
28.00	$7.55X10^{-3}$	47.6
29.00	$7.583X10^{-3}$	47.8
30.00	$7.608X10^{-3}$	48

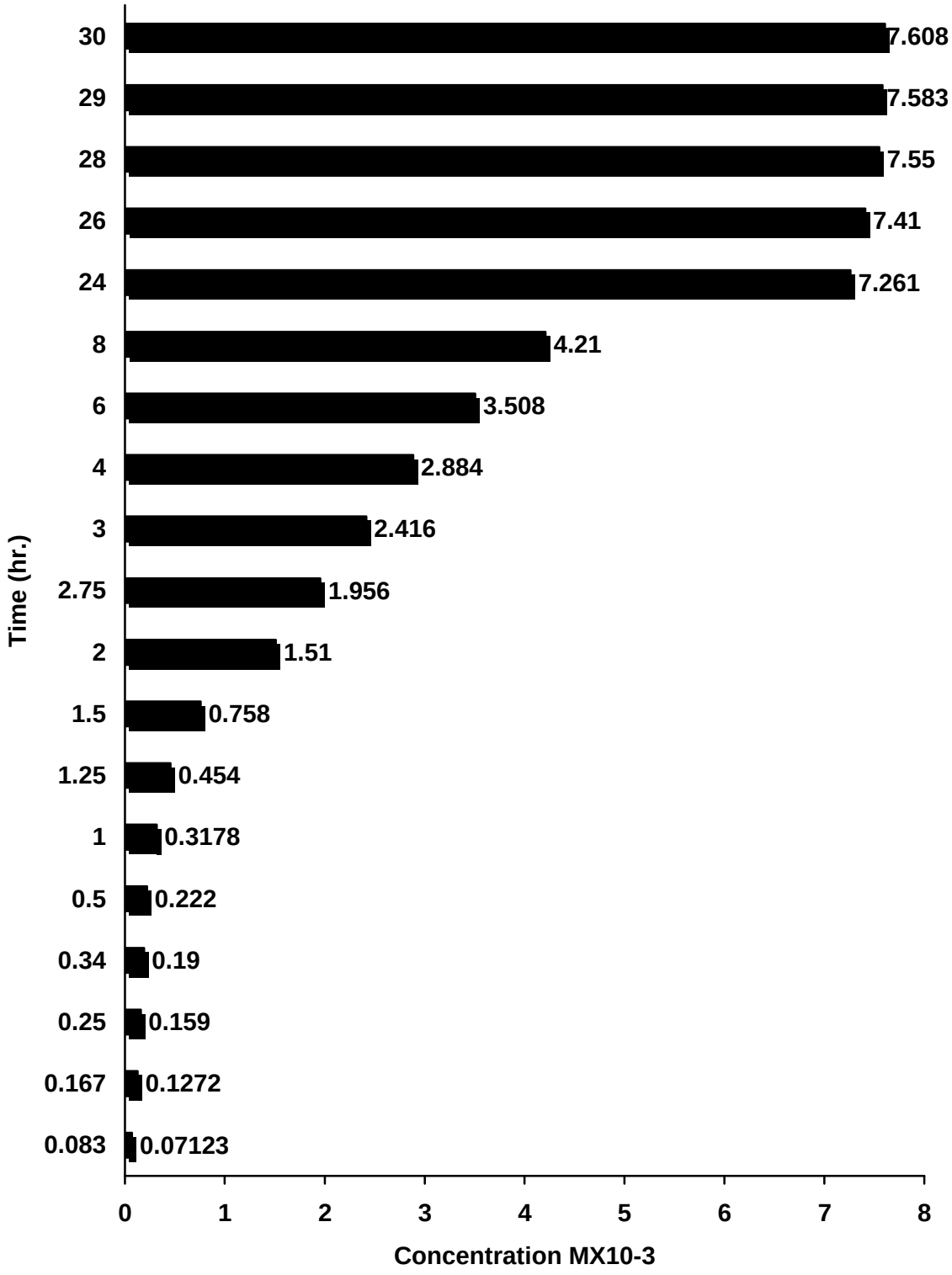


Figure (13) concentration of aspirin released by compound (19) in pH (12)

Table (9) concentration of aspirin released by compound (20) in pH (2)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	0	0
0.25	$8.45X10^{-6}$	0.076
0.34	$1.69X10^{-5}$	0.152
0.50	$2.535X10^{-5}$	0.221
1.00	$4.225X10^{-5}$	0.38
1.25	$7.346X10^{-5}$	0.66
1.5	$1.272X10^{-4}$	1.14
2.00	$1.99X10^{-4}$	1.79
2.75	$3.27X10^{-4}$	2.94
3.00	$3.325X10^{-4}$	3
4.00	$3.80X10^{-4}$	3.4
6.00	$4.70X10^{-4}$	4.2
8.00	$5.78X10^{-4}$	5.2
24.00	$2.97X10^{-3}$	26.7
26.00	$3.47X10^{-3}$	31.1
28.00	$3.8X10^{-3}$	34.2
29.00	$3.88X10^{-3}$	35.0
30.00	$3.996X10^{-3}$	36.0

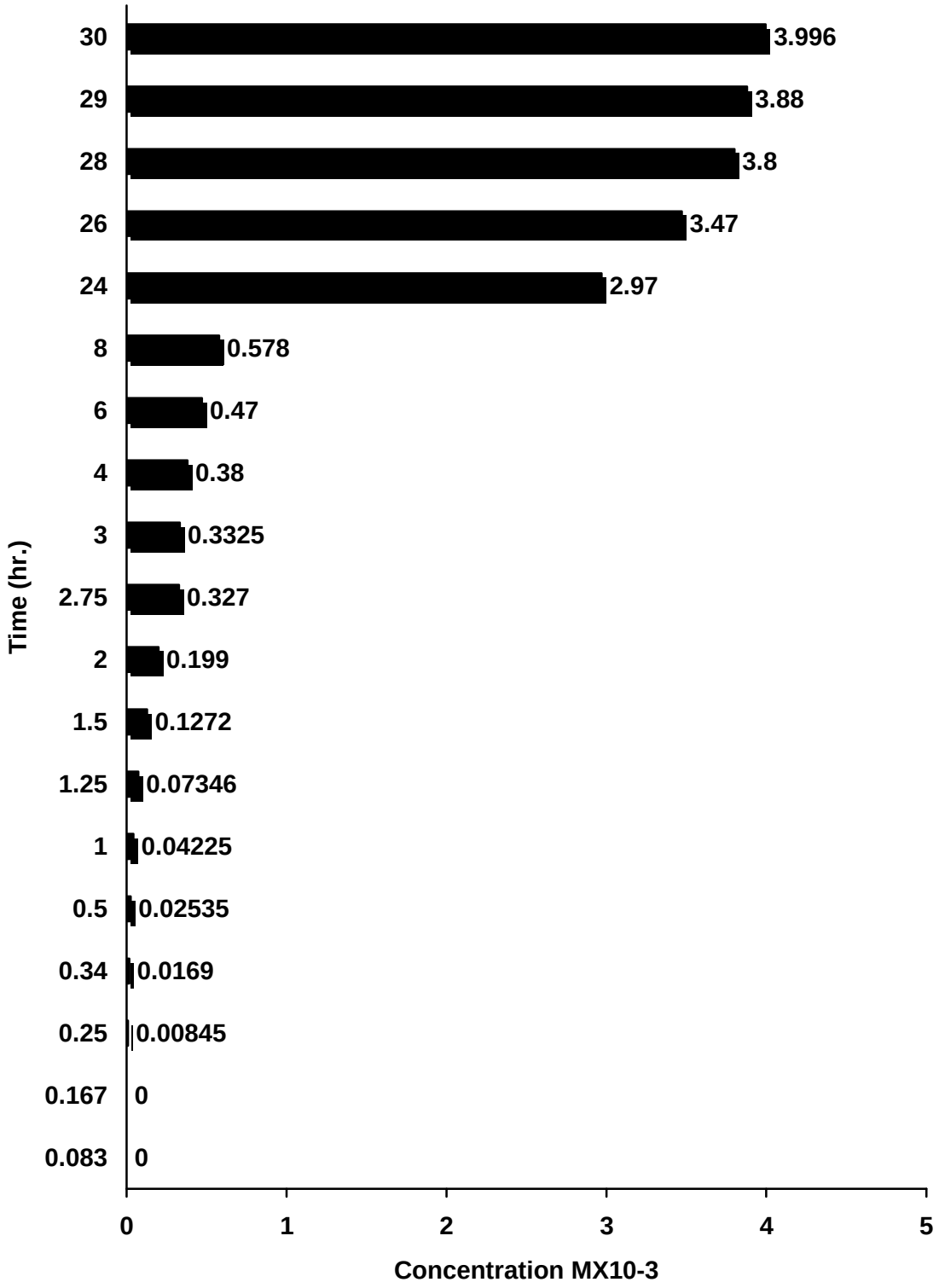


Figure (14) concentration of aspirin released by compound (20) in pH (2)

Table (10) concentration of aspirin released by compound (20) in pH (4)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	0	0
0.25	$1.69X10^{-5}$	0.152
0.34	$2.535X10^{-5}$	0.22
0.50	$3.380X10^{-5}$	0.304
1.00	$4.225X10^{-5}$	0.38
1.25	$7.623X10^{-5}$	0.68
1.5	$1.59X10^{-4}$	1.43
2.00	$2.22X10^{-4}$	2
2.75	$3.56X10^{-4}$	3.2
3.00	$3.64X10^{-4}$	3.27
4.00	$4.43X10^{-4}$	3.99
6.00	$4.63X10^{-4}$	4.17
8.00	$6.56X10^{-4}$	5.91
24.00	$3.42X10^{-3}$	2.08
26.00	$3.67X10^{-3}$	33.09
28.00	$3.98X10^{-3}$	35.91
29.00	$4.09X10^{-3}$	36.88
30.00	$4.16X10^{-3}$	37.5

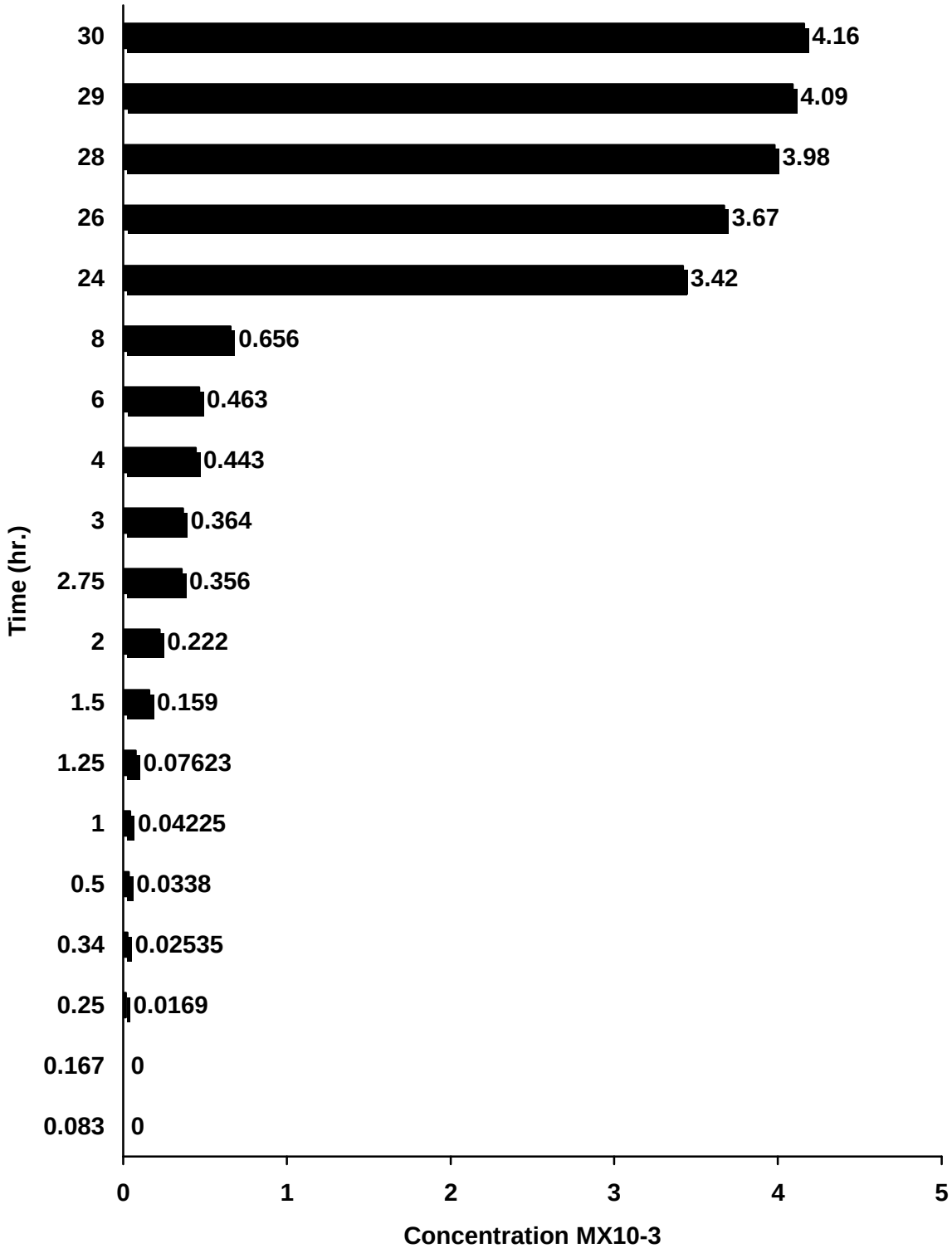


Figure (15) concentration of aspirin released by compound (20) in pH (4)

Table (11) concentration of aspirin released by compound (20) in pH (6)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$8.45X10^{-6}$	0.076
0.167	$1.69X10^{-5}$	0.152
0.25	$2.53X10^{-5}$	0.22
0.34	$3.38X10^{-5}$	0.305
0.50	$4.225X10^{-5}$	0.38
1.00	$5.07X10^{-5}$	0.45
1.25	$9.32X10^{-5}$	0.84
1.5	$1.77X10^{-4}$	1.6
2.00	$3.46X10^{-4}$	3.1
2.75	$3.95X10^{-4}$	3.5
3.00	$4.21X10^{-4}$	3.8
4.00	$4.56X10^{-4}$	4.1
6.00	$6.92X10^{-4}$	6.2
8.00	$2.18X10^{-3}$	19.6
24.00	$3.71X10^{-3}$	33.5
26.00	$4.17X10^{-3}$	37.5
28.00	$4.52X10^{-3}$	40.7
29.00	$4.73X10^{-3}$	42.5
30.00	$4.75X10^{-3}$	42.8

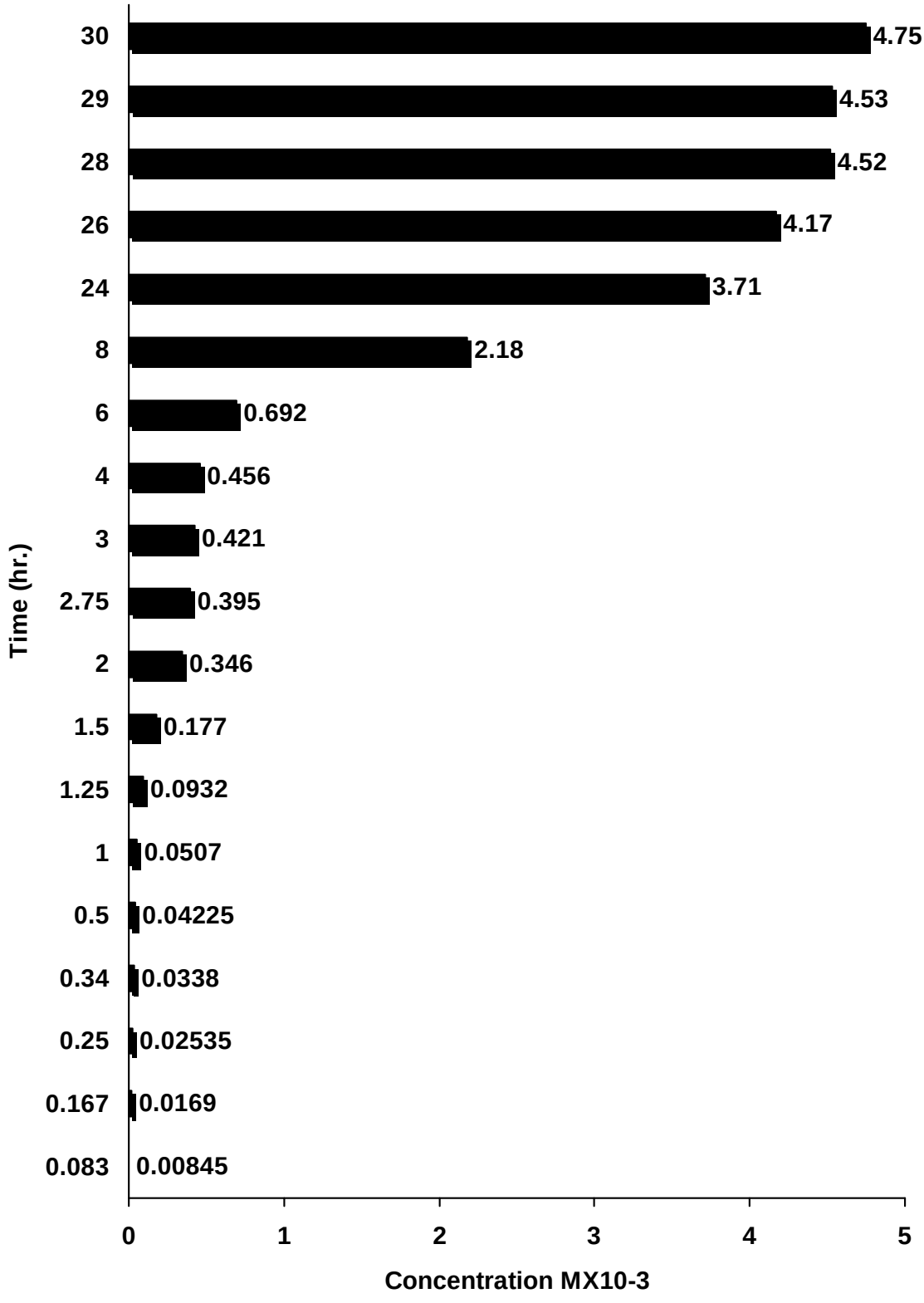


Figure (16) concentration of aspirin released by compound (20) in pH (6)

Table (12) concentration of aspirin released by compound (20) in pH (8)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$3.38X10^{-5}$	0.304
0.167	$8.47X10^{-5}$	0.76
0.25	$9.32X10^{-5}$	0.84
0.34	$1.01X10^{-4}$	0.91
0.50	$1.1X10^{-4}$	0.99
1.00	$1.99X10^{-4}$	1.79
1.25	$4.38X10^{-4}$	3.94
1.5	$4.63X10^{-4}$	4.17
2.00	$2.15X10^{-3}$	19.43
2.75	$2.36X10^{-3}$	21.3
3.00	$2.66X10^{-3}$	24
4.00	$3.29X10^{-3}$	29.6
6.00	$3.42X10^{-3}$	30.8
8.00	$3.93X10^{-3}$	35.4
24.00	$5.19X10^{-3}$	46.8
26.00	$5.25X10^{-3}$	47.3
28.00	$5.328X10^{-3}$	48
29.00	$5.48X10^{-3}$	49.4
30.00	$5.56X10^{-3}$	50.1

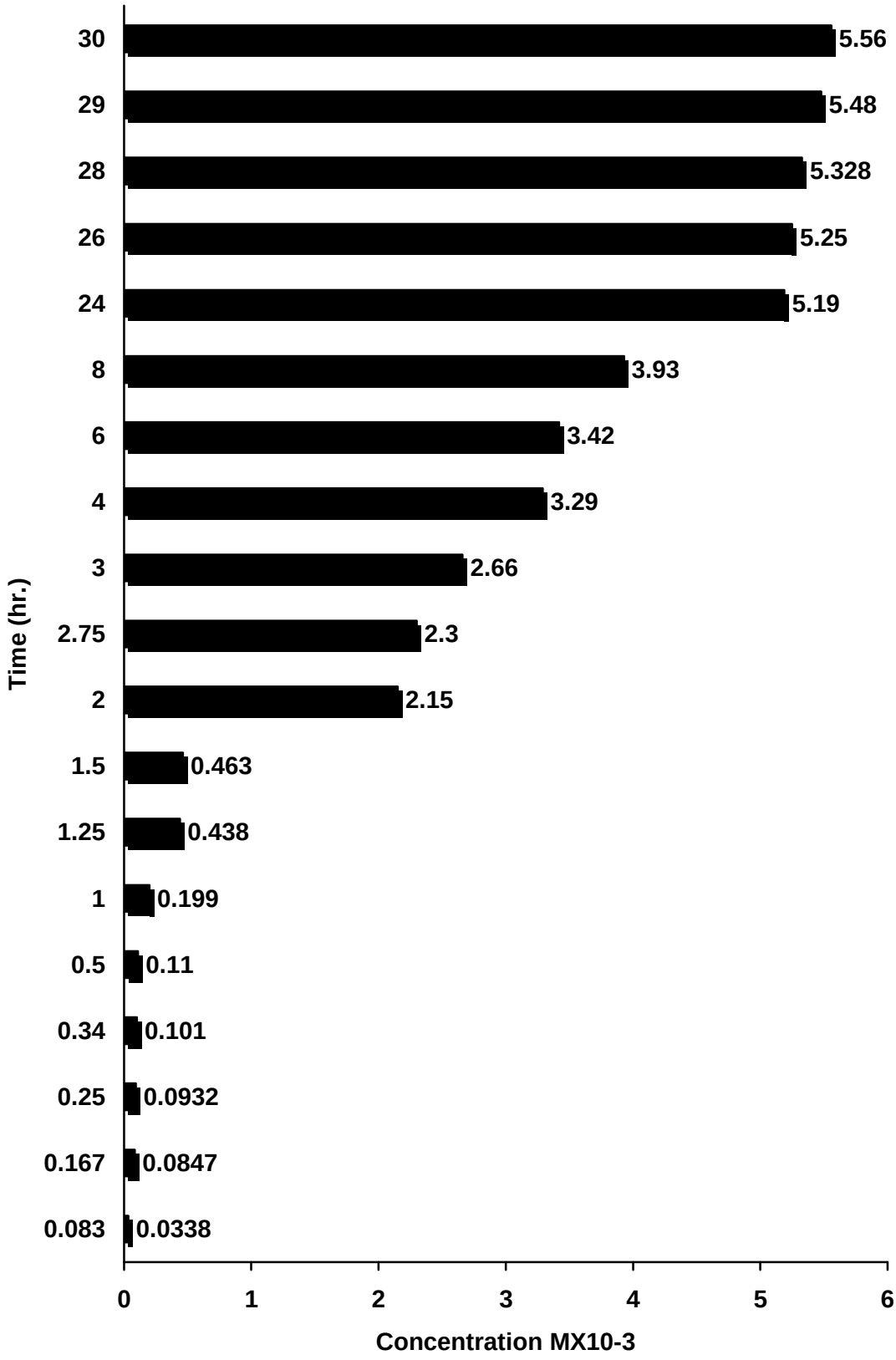


Figure (17) concentration of aspirin released by compound (20) in pH (8)

Table (13) concentration of aspirin released by compound (20) in pH (10)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$7.123X10^{-5}$	0.641
0.167	$1.59X10^{-4}$	1.4
0.25	$1.77X10^{-4}$	1.6
0.34	$2.22X10^{-4}$	2
0.50	$2.32X10^{-4}$	2.09
1.00	$3.46X10^{-4}$	3.1
1.25	$6.21X10^{-4}$	5.6
1.5	$2.04X10^{-3}$	18.4
2.00	$3.13X10^{-3}$	28.2
2.75	$3.22X10^{-3}$	29
3.00	$3.39X10^{-3}$	30.5
4.00	$3.48X10^{-3}$	31.3
6.00	$3.81X10^{-3}$	34.3
8.00	$4.45X10^{-3}$	40.1
24.00	$5.61X10^{-3}$	50.5
26.00	$5.81X10^{-3}$	52.3
28.00	$5.90X10^{-3}$	53.1
29.00	$5.97X10^{-3}$	53.7
30.00	$6.027X10^{-3}$	54.3

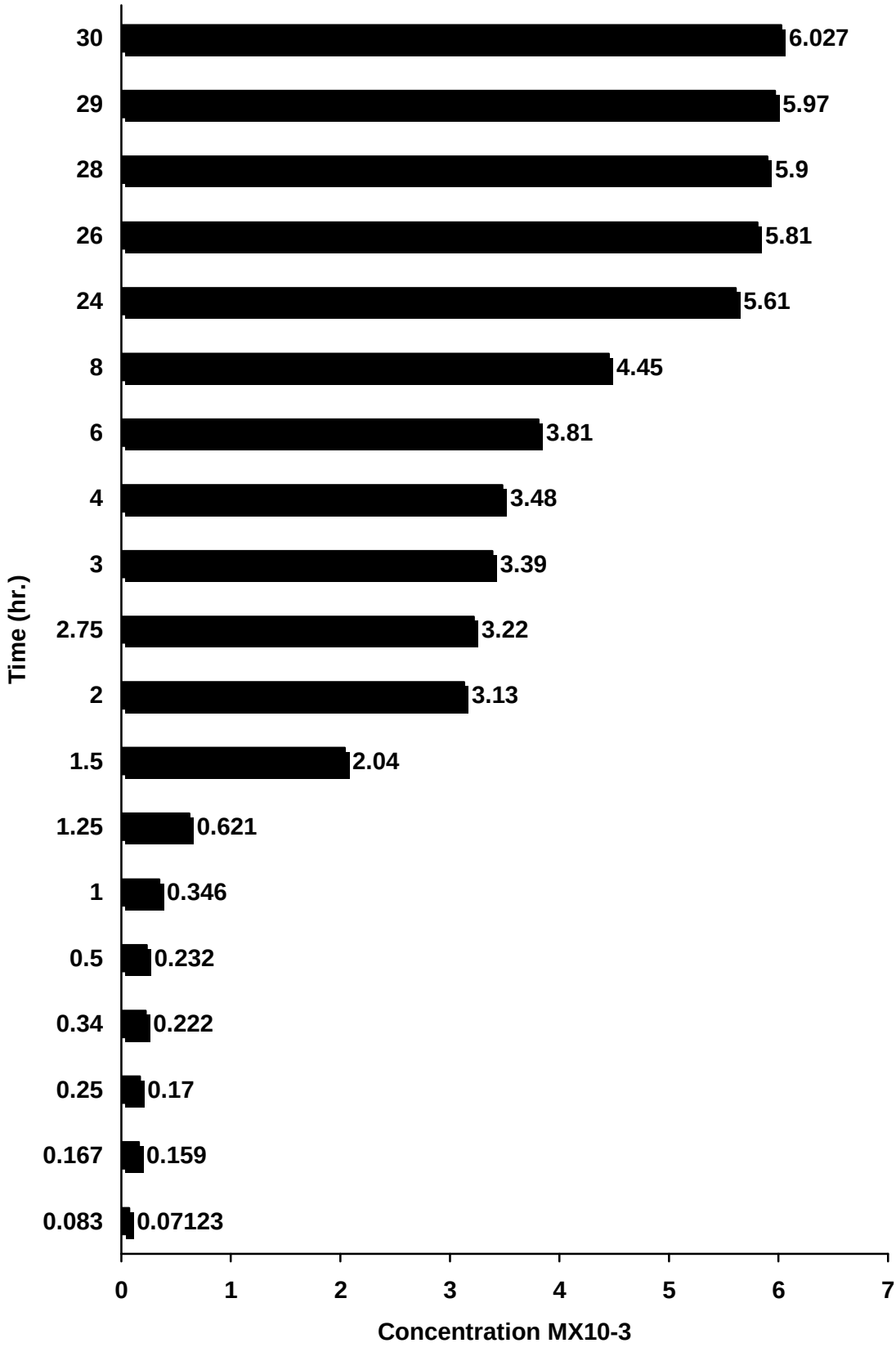


Figure (18) concentration of aspirin released by compound (20) in pH (10)

Table (14) concentration of aspirin released by compound (20) in pH (12)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.378X10^{-4}$	1.24
0.167	$3.11X10^{-4}$	2.88
0.25	$5.43X10^{-4}$	4.9
0.34	$5.88X10^{-4}$	5.3
0.50	$8.54X10^{-4}$	7.7
1.00	$1.80X10^{-3}$	16.4
1.25	$2.63X10^{-3}$	23.6
1.5	$3.29X10^{-3}$	29.7
2.00	$3.91X10^{-3}$	35.3
2.75	$4.11X10^{-3}$	37.1
3.00	$4.18X10^{-3}$	37.7
4.00	$4.45X10^{-3}$	40.1
6.00	$4.59X10^{-3}$	41.4
8.00	$4.95X10^{-3}$	44.6
24.00	$5.79X10^{-3}$	52.2
26.00	$5.97X10^{-3}$	53.8
28.00	$6.17X10^{-3}$	55.6
29.00	$6.38X10^{-3}$	57.5
30.00	$6.467X10^{-3}$	58.2

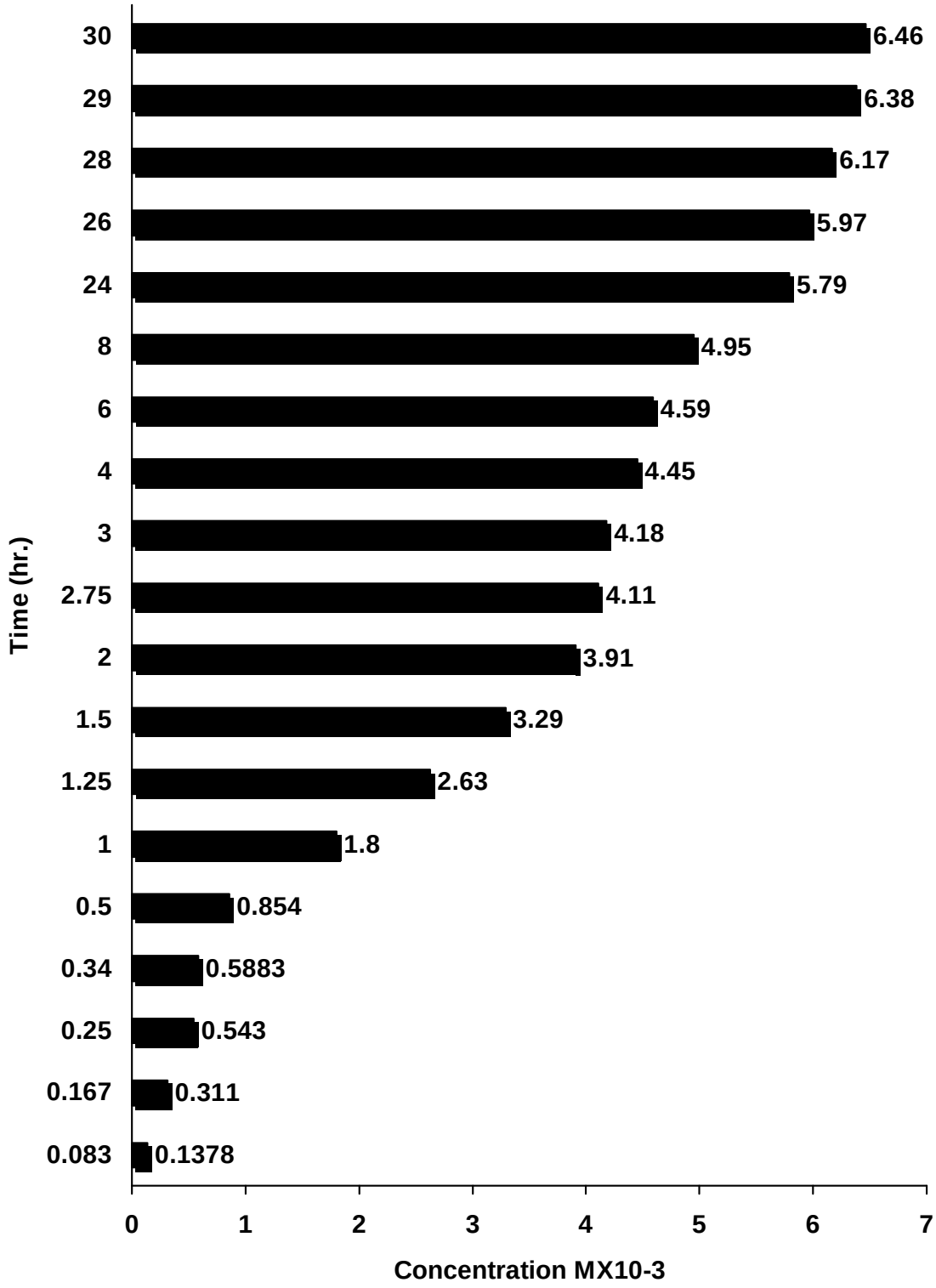


Figure (19) concentration of aspirin released by compound (20) in pH (12)

Table (15) concentration of aspirin released by compound (21) in pH (2)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	$1.69X10^{-5}$	0.23
0.25	$2.535X10^{-5}$	0.34
0.34	$4.225X10^{-5}$	0.58
0.50	$7.123X10^{-5}$	0.97
1.00	$7.623X10^{-5}$	1
1.25	$1.496X10^{-4}$	2
1.5	$2.92X10^{-4}$	4
2.00	$3.65X10^{-4}$	5
2.75	$4.70X10^{-4}$	6.4
3.00	$4.85X10^{-4}$	6.7
4.00	$6.35X10^{-4}$	8.73
6.00	$9.09X10^{-4}$	12.5
8.00	$1.214X10^{-3}$	16.7
24.00	$2.15X10^{-3}$	29.6
26.00	$2.37X10^{-3}$	32.7
28.00	$2.57X10^{-3}$	35.4
29.00	$2.82X10^{-3}$	38.8
30.00	$2.85X10^{-3}$	39.3

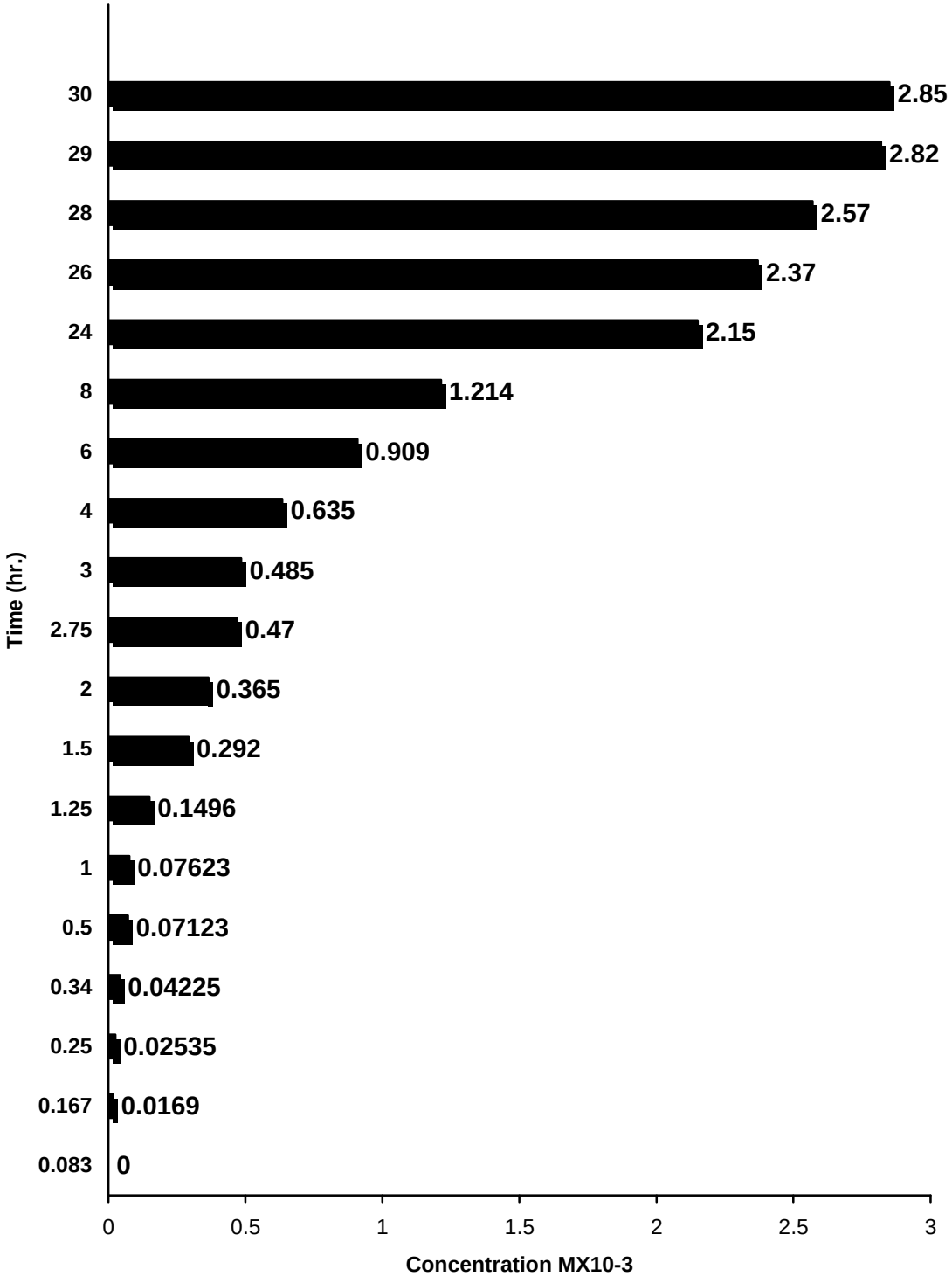


Figure (20) concentration of aspirin released by compound (21) in pH (2)

Table (16) concentration of aspirin released by compound (21) in pH (4)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.69X10^{-5}$	0.23
0.167	$2.535X10^{-5}$	0.34
0.25	$3.38X10^{-5}$	0.46
0.34	$5.07X10^{-5}$	0.69
0.50	$6.32X10^{-5}$	0.87
1.00	$7.90X10^{-5}$	1.08
1.25	$2.14X10^{-4}$	2.95
1.5	$2.44X10^{-4}$	3.36
2.00	$3.39X10^{-4}$	4.66
2.75	$5.83X10^{-4}$	8.02
3.00	$7.17X10^{-4}$	9.86
4.00	$1.19X10^{-3}$	16.22
6.00	$1.96X10^{-3}$	27.02
8.00	$2.48X10^{-3}$	34.18
24.00	$3.31X10^{-3}$	45.5
26.00	$3.42X10^{-3}$	47.1
28.00	$3.66X10^{-3}$	50.4
29.00	$3.84X10^{-3}$	52.8
30.00	$3.87X10^{-3}$	53.3

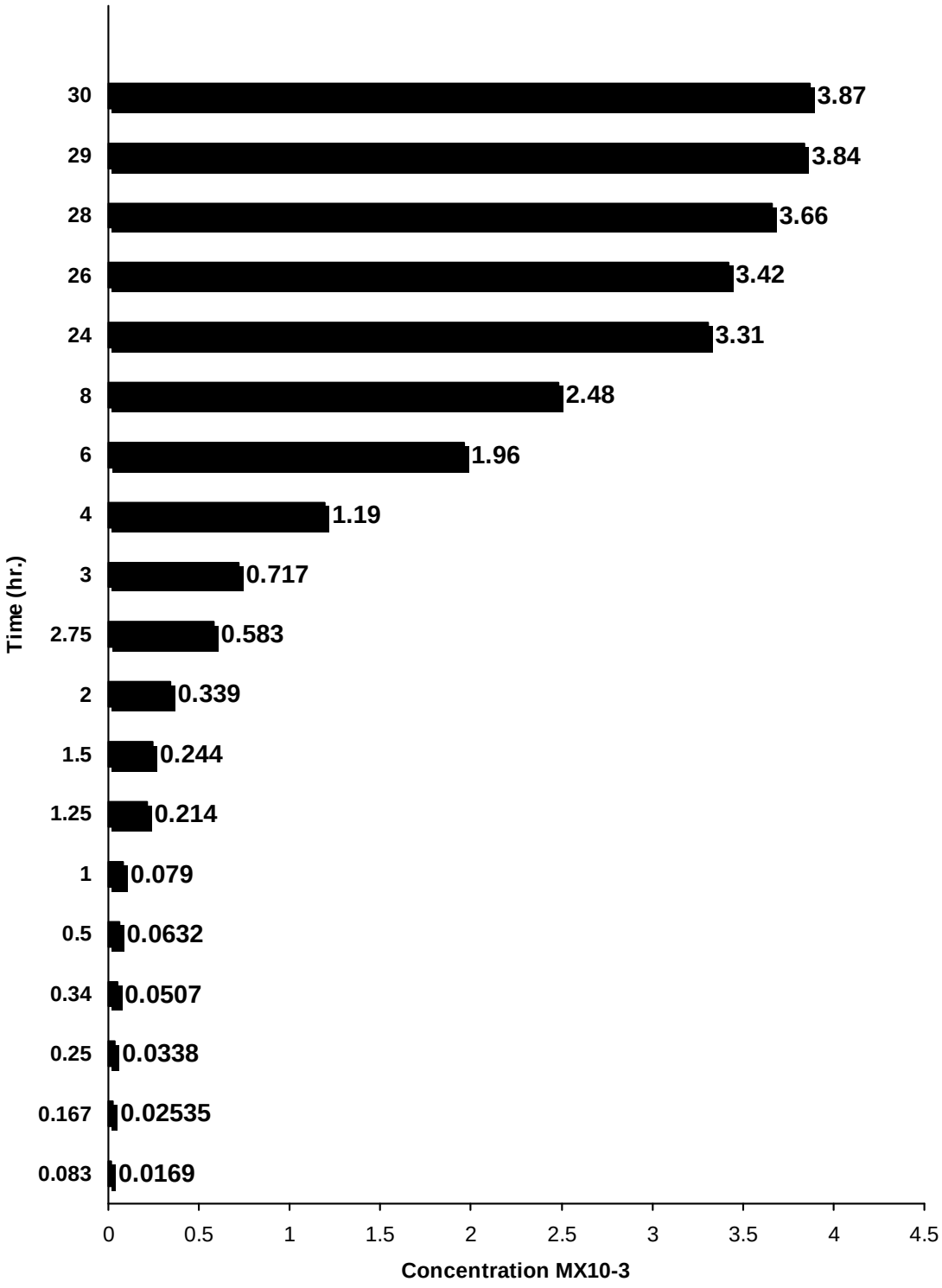


Figure (21) concentration of aspirin released by compound (21) in pH (4)

Table (17) concentration of aspirin released by compound (21) in pH (6)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.69X10^{-5}$	0.23
0.167	$2.535X10^{-5}$	0.34
0.25	$5.07X10^{-5}$	0.69
0.34	$6.32X10^{-5}$	0.87
0.50	$7.623X10^{-5}$	1.04
1.00	$9.32X10^{-5}$	1.28
1.25	$2.22X10^{-4}$	3.05
1.5	$4.662X10^{-4}$	9.1
2.00	$1.14X10^{-3}$	15.7
2.75	$1.39X10^{-3}$	19.2
3.00	$1.43X10^{-3}$	19.7
4.00	$1.86X10^{-3}$	28.6
6.00	$2.08X10^{-3}$	36.7
8.00	$3.47X10^{-3}$	47.8
24.00	$4.37X10^{-3}$	60.1
26.00	$4.61X10^{-3}$	63.4
28.00	$4.7X10^{-3}$	64.7
29.00	$4.74X10^{-3}$	65.2
30.00	$4.76X10^{-3}$	65.5

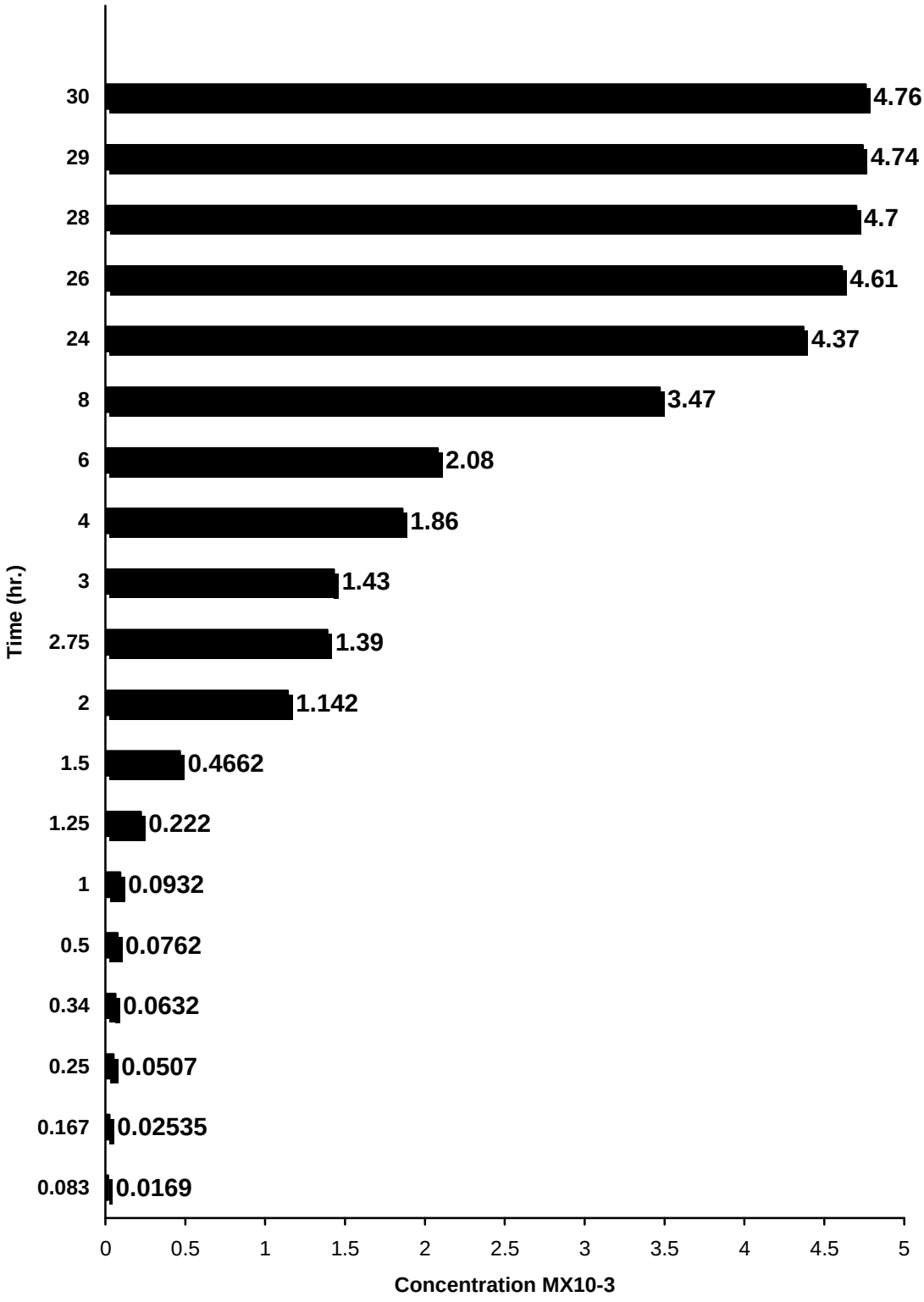


Figure (22) concentration of aspirin released by compound (21) in pH (6)

Table (18) concentration of aspirin released by compound (21) in pH (8)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$6.32X10^{-5}$	0.87
0.167	$1.09X10^{-4}$	1.5
0.25	$1.45X10^{-4}$	2
0.34	$2.03X10^{-4}$	2.8
0.50	$3.05X10^{-4}$	4.2
1.00	$7.57X10^{-4}$	10.4
1.25	$1.28X10^{-3}$	17.7
1.5	$1.65X10^{-3}$	22.8
2.00	$2.23X10^{-3}$	30.8
2.75	$2.64X10^{-3}$	36.3
3.00	$2.88X10^{-3}$	39.7
4.00	$3.02X10^{-3}$	41.6
6.00	$3.59X10^{-3}$	49.4
8.00	$4.01X10^{-3}$	55.2
24.00	$5.12X10^{-3}$	70.3
26.00	$5.40X10^{-3}$	74.2
28.00	$5.62X10^{-3}$	77.3
29.00	$5.65X10^{-3}$	77.7
30.00	$5.73X10^{-3}$	78.8

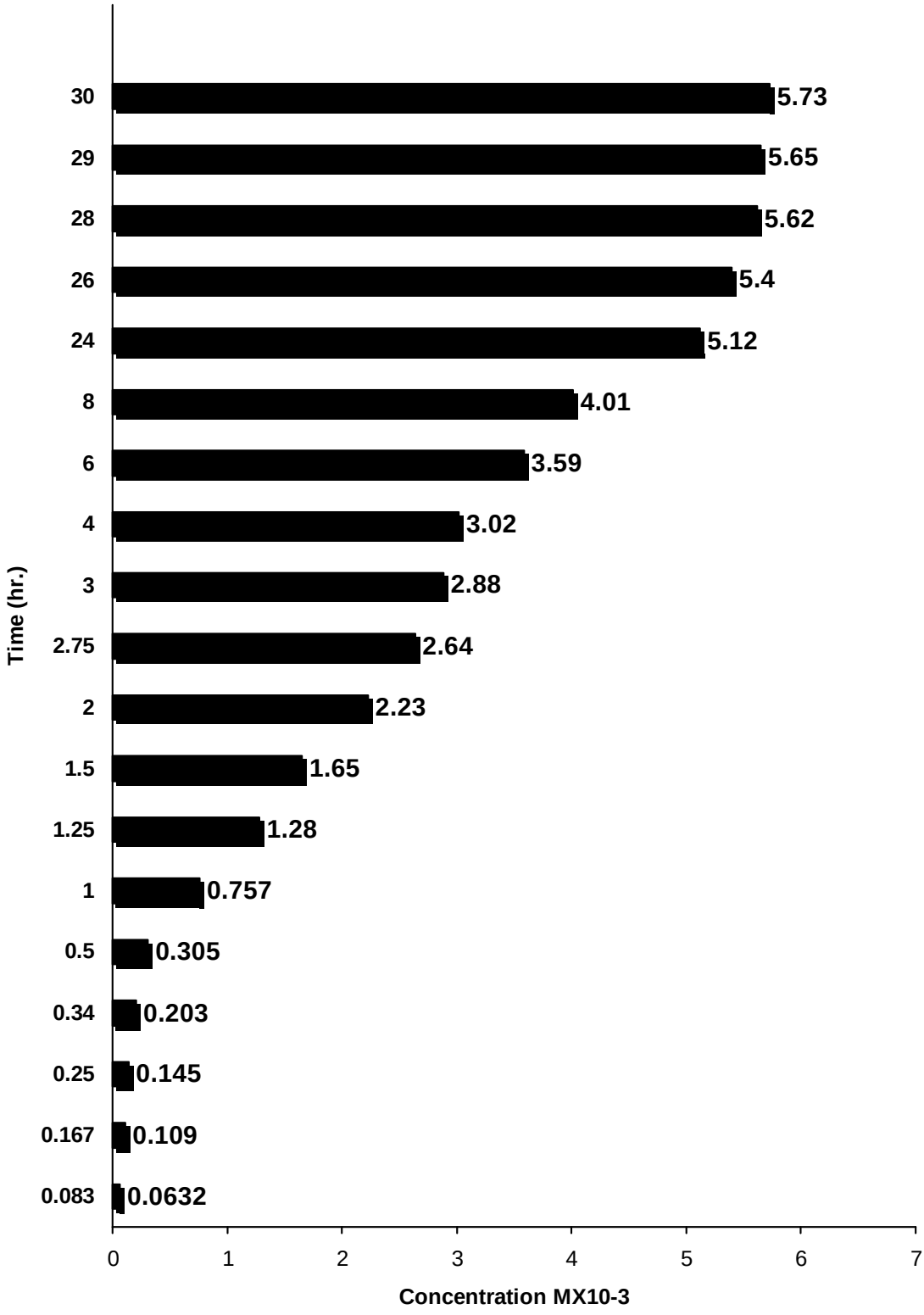


Figure (23) concentration of aspirin released by compound (21) in pH (8)

Table (19) concentration of aspirin released by compound (21) in pH (10)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.38X10^{-4}$	1.9
0.167	$1.74X10^{-4}$	2.4
0.25	$2.25X10^{-4}$	3.1
0.34	$3.20X10^{-4}$	4.4
0.50	$4.94X10^{-4}$	6.8
1.00	$1.05X10^{-3}$	14.5
1.25	$1.94X10^{-3}$	26.7
1.5	$2.49X10^{-3}$	34.3
2.00	$2.72X10^{-3}$	37.4
2.75	$3.04X10^{-3}$	41.8
3.00	$3.13X10^{-3}$	43.1
4.00	$3.55X10^{-3}$	48.8
6.00	$3.95X10^{-3}$	54.4
8.00	$4.38X10^{-3}$	60.2
24.00	$5.42X10^{-3}$	74.5
26.00	$5.64X10^{-3}$	77.6
28.00	$5.83X10^{-3}$	80.2
29.00	$5.91X10^{-3}$	81.3
30.00	$5.97X10^{-3}$	82

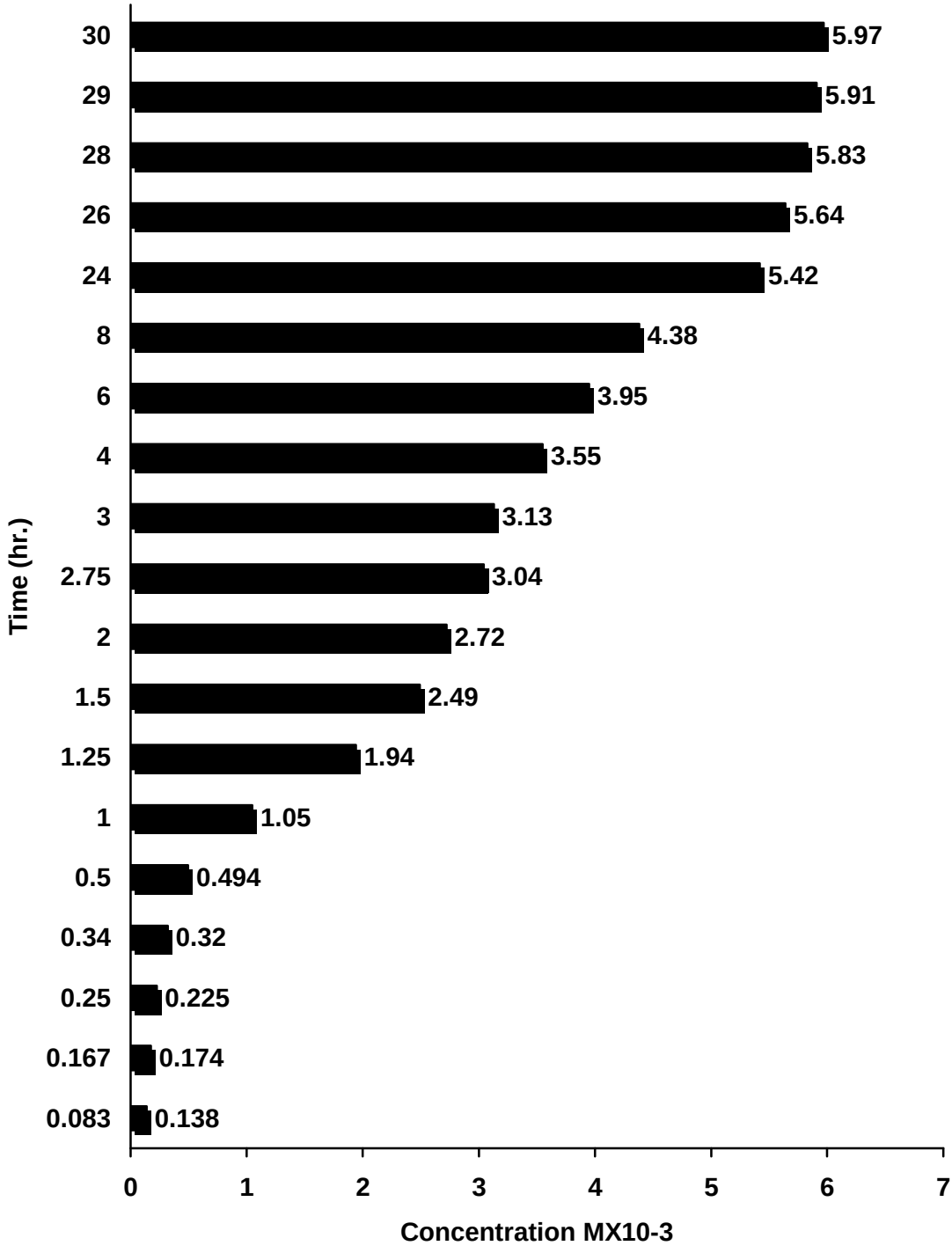


Figure (24) concentration of aspirin released by compound (21) in pH (10)

Table (20) concentration of aspirin released by compound (21) in pH (12)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$2.62X10^{-4}$	3.6
0.167	$4.24X10^{-4}$	5.9
0.25	$7.12X10^{-4}$	9.8
0.34	$8.07X10^{-4}$	11.1
0.50	$1.40X10^{-3}$	19.3
1.00	$1.69X10^{-3}$	23.3
1.25	$2.33X10^{-3}$	32.6
1.5	$2.89X10^{-3}$	39.7
2.00	$3.15X10^{-3}$	43.3
2.75	$3.53X10^{-3}$	48.6
3.00	$3.61X10^{-3}$	49.7
4.00	$4.18X10^{-3}$	57.5
6.00	$4.71X10^{-3}$	64.8
8.00	$5.33X10^{-3}$	73.3
24.00	$5.83X10^{-3}$	80.14
26.00	$5.95X10^{-3}$	81.8
28.00	$6.08X10^{-3}$	83.5
29.00	$6.17X10^{-3}$	84.8
30.00	$6.21X10^{-3}$	85.3

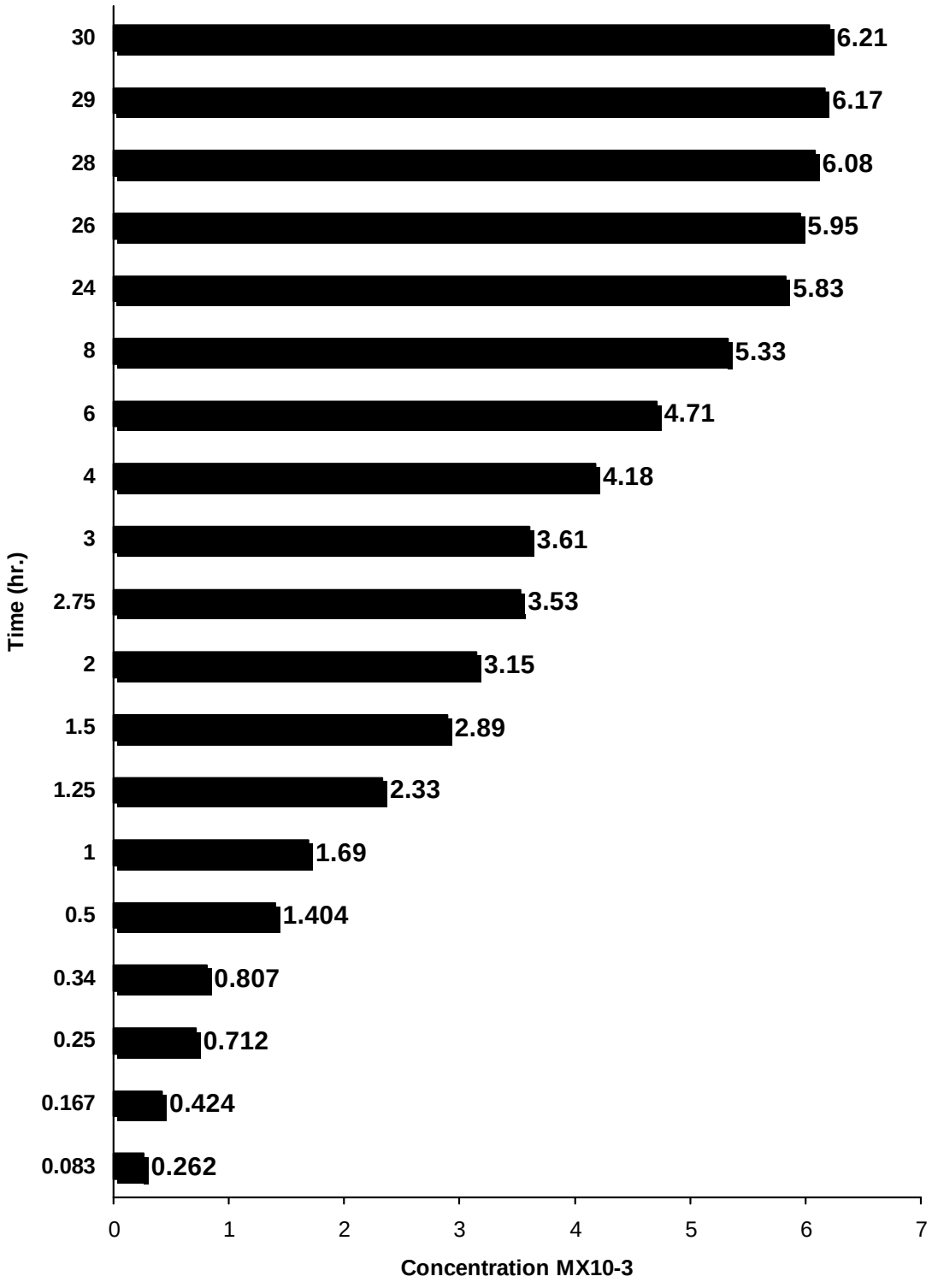


Figure (25) concentration of aspirin released by compound (21) in pH (12)

3.8 :Drug release study :

The aim of this study is to clarify the release and degradation of aspirin in acidic and alkaline media since the pro drug will persist to about (2 hrs.) in the stomach .

Prodrugs agent that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester. This is the most commonly seen type of prodrug, due to the ease with which the ester can be hydrolyzed to give the active drug .

Decreasing the water solubility of a drug by the formation of a prodrug may have additional benefits beyond simply increasing absorption .A number of agent have unpleasant taste when given orally .This results when the drug begins to dissolve in the mouth and then is capable of interacting with taste receptors .This can a significant problem especially in children , and may lead to low compliance . A prodrug with reduced water solubility does not dissolved to an appreciable extent in the mouth and therefore dose not interact with taste receptors [142].

This approach has been utilized in the case of the compounds (19),(20),(21) . The hydrophobic salicylate ester dose not dissolve to appreciable extent in the mouth , so there is a little chance for interaction with taste receptor .

In this study the concentration of aspirin at different times and pH was followed at fixed wave length at 277 nm , we know from the results that aspirin concentration increase when the pH increase .

The synthesized compounds are insoluble in water and are more stable in stomach . Concentration of aspirin released from (3-(acetyl salicyloyl)-5,6-O- isopropylidene-*L*-ascorbic acid (19)) at (pH = 2,4,6,8,10,12) after (2 hr) was ($8.47X10^{-5}$M (0.53%), $9.32X10^{-5}$M (0.588%) , $1.77X10^{-4}$M (1.1%), $4.4X10^{-4}$M(2.7%),$6.51X10^{-4}$M (4.11%) , $1.58X10^{-4}$ M (9.5%) respectively ,While concentration of aspirin released from (2,3-di(acetyl salicyloyl)-5,6-O- isopropylidene-*L*-ascorbic acid (20)) at (pH = 2,4,6,8,10,12) after (2 hr) was ($1.99X10^{-4}$M (1.79%) , $2.22X10^{-4}$M (2%) ,$3.46X10^{-4}$M (3.1%) ,$2.15X10^{-3}$M (19.43%) ,$3.13X10^{-3}$M(28.2%) ,$3.91X10^{-3}$M(35.3%)) ,While

concentration of aspirin released form (2,3,5,6 -tetra(acetyl salicyloyl)-*L*-ascorbic acid (21)) at (pH = 2,4,6,8,10,12) after (2 hr) was ($3.65X10^{-3}$M (5%),$3.39X10^{-3}$M (4.66%), $1.142X10^{-3}$M (16.7%), $2.23X10^{-3}$M(30.7%), $2.72X10^{-3}$M(37.4%), $3.15X10^{-3}$M(43.3%)) . Moreover that aspirin concentration at the first two hours in the acidic media was very low while aspirin concentration was increase when increased the pH.

Drug released study was conducted (on compounds 19, 20, 21) to prove that the compounds was insoluble in water and slightly soluble in gastric juices. So that will give these compounds a chance to pass throw the stomach with less acid harmless. it would be absorbed in the large intestine where the hydrolysis would also start to begin from the ester linkage

F.Arranz and M.Sanchez[161] (1998) refers to that the hydrolysis of amylase –α-naphthylacetic acid showed that the released of bioactive compound (α-naphthylacetic acid) is dependent on the hydrophilic character and on the pH value of the medium. When the pH value increased the hydrolysis was increased.

The gastric irritation will be decrease by the condensation of aspirin with vitamin C because the ester linkage will expected to be cleaved in the colon librating aspirin and vitamin C.[151]

Table (3-20) and figure (8-25) show the data of hydrolysis of compounds (19),(20),(21) in different pH and different time :

Table (3) concentration of aspirin released by compound (19) in pH (2)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	0	0
0.25	0	0
0.34	$8.45X10^{-6}$	0.053
0.50	$1.69X10^{-5}$	0.1
1.00	$2.535X10^{-5}$	0.16
1.25	$3.38X10^{-5}$	0.213
1.5	$7.123X10^{-5}$	0.45
2.00	$8.47X10^{-5}$	0.53
2.75	$1.59X10^{-4}$	1
3.00	$2.11X10^{-4}$	1.3
4.00	$2.42X10^{-4}$	1.52
6.00	$3.27X10^{-4}$	2.06
8.00	$3.80X10^{-4}$	2.4
24.00	$2.20X10^{-3}$	13.9
26.00	$2.61X10^{-3}$	16.4
28.00	$2.81X10^{-3}$	17.7
29.00	$2.88X10^{-3}$	18.2
30.00	$3.03X10^{-3}$	19.1

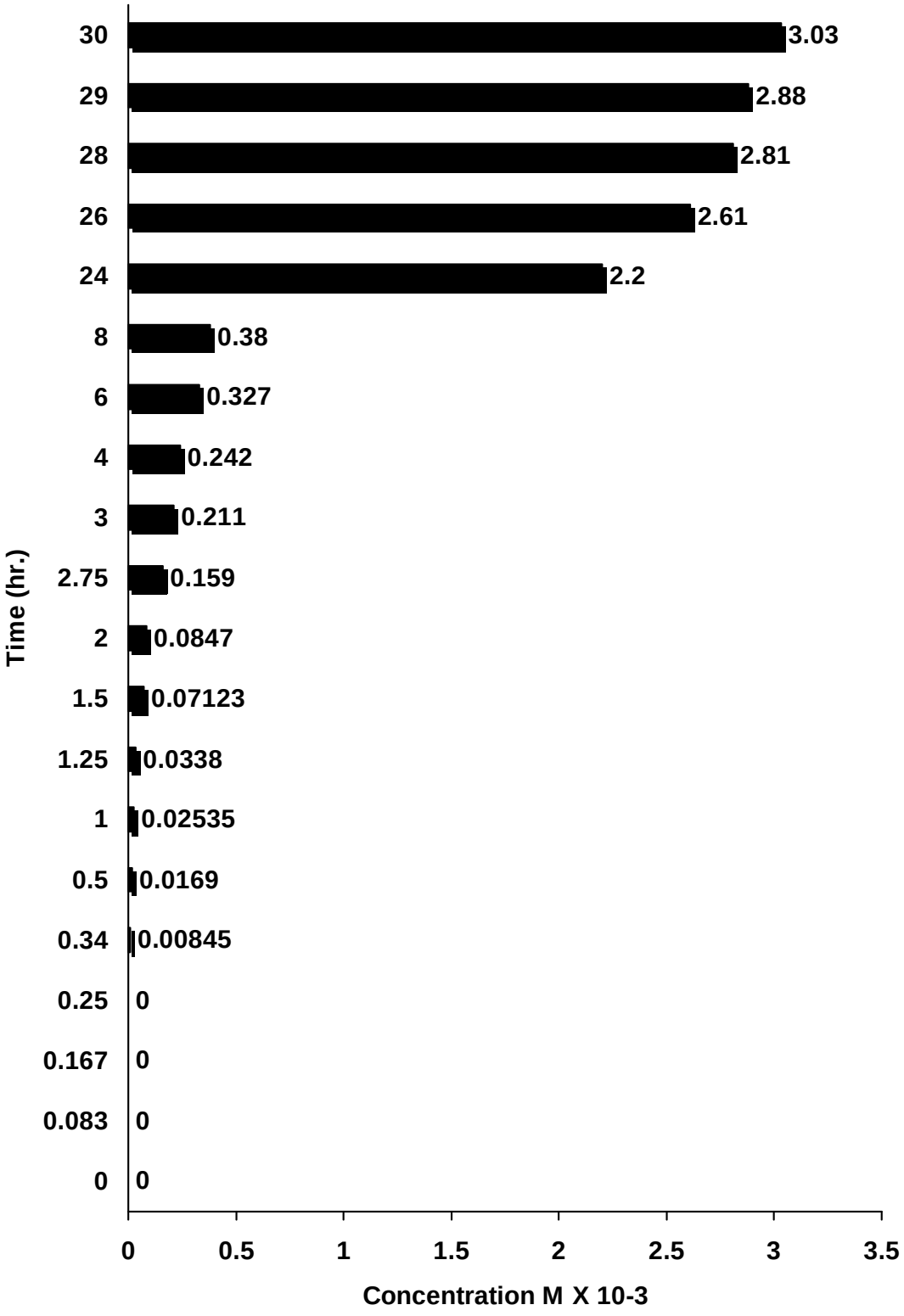


Figure (8) Concentration of aspirin released by compound (19) in pH (2)

Table (4) concentration of aspirin released by compound (19) in pH (4)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	0	0
0.25	$8.45X10^{-6}$	0.053
0.34	$1.69X10^{-5}$	0.1
0.50	$2.535X10^{-5}$	0.16
1.00	$3.38X10^{-5}$	0.213
1.25	$4.22X10^{-5}$	0.26
1.5	$7.346X10^{-5}$	0.46
2.00	$9.32X10^{-5}$	0.588
2.75	$1.77X10^{-4}$	1.1
3.00	$2.32X10^{-4}$	1.46
4.00	$2.82X10^{-4}$	1.78
6.00	$3.12X10^{-4}$	2
8.00	$4.43X10^{-4}$	2.8
24.00	$2.52X10^{-3}$	15.9
26.00	$2.7X10^{-3}$	17
28.00	$2.97X10^{-3}$	18.7
29.00	$3.01X10^{-3}$	19
30.00	$3.06X10^{-3}$	19.3

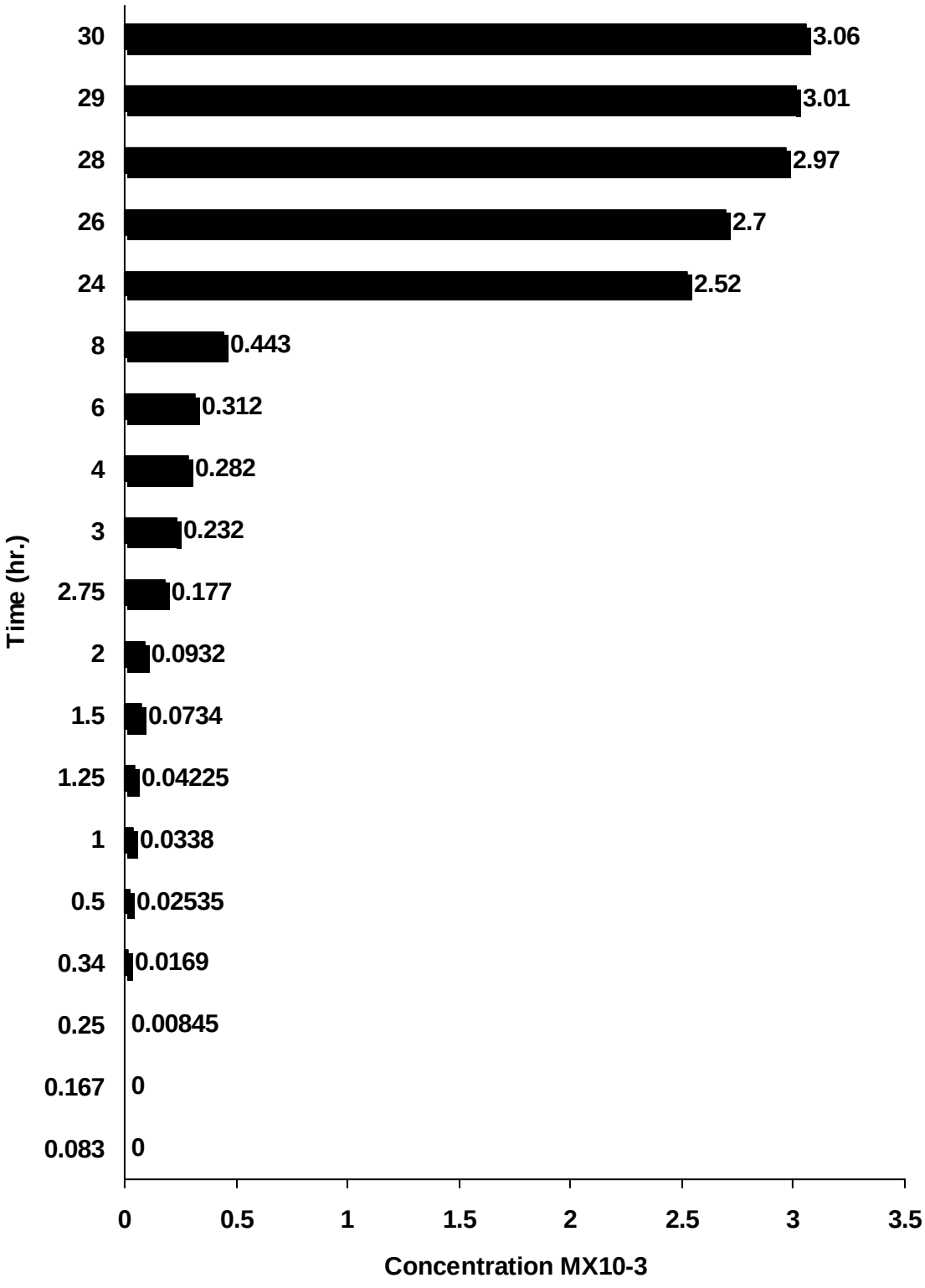


Figure (9) concentration of aspirin released by compound (19) in pH (4)

Table (5) concentration of aspirin released by compound (19) in pH (6)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	$8.45X10^{-6}$	0.053
0.25	$1.69X10^{-5}$	0.1
0.34	$2.535X10^{-5}$	0.16
0.50	$3.38X10^{-5}$	0.213
1.00	$4.22X10^{-5}$	0.26
1.25	$7.123X10^{-5}$	0.45
1.5	$7.623X10^{-5}$	0.48
2.00	$1.77X10^{-5}$	1.1
2.75	$2.42X10^{-4}$	1.52
3.00	$3.02X10^{-4}$	1.9
4.00	$3.17X10^{-4}$	2
6.00	$4.2X10^{-4}$	2.6
8.00	$4.51X10^{-4}$	2.8
24.00	$3.41X10^{-3}$	21.5
26.00	$3.84X10^{-3}$	24.2
28.00	$4.3X10^{-3}$	27.1
29.00	$4.35X10^{-3}$	27.6
30.00	$4.44X10^{-3}$	28.0

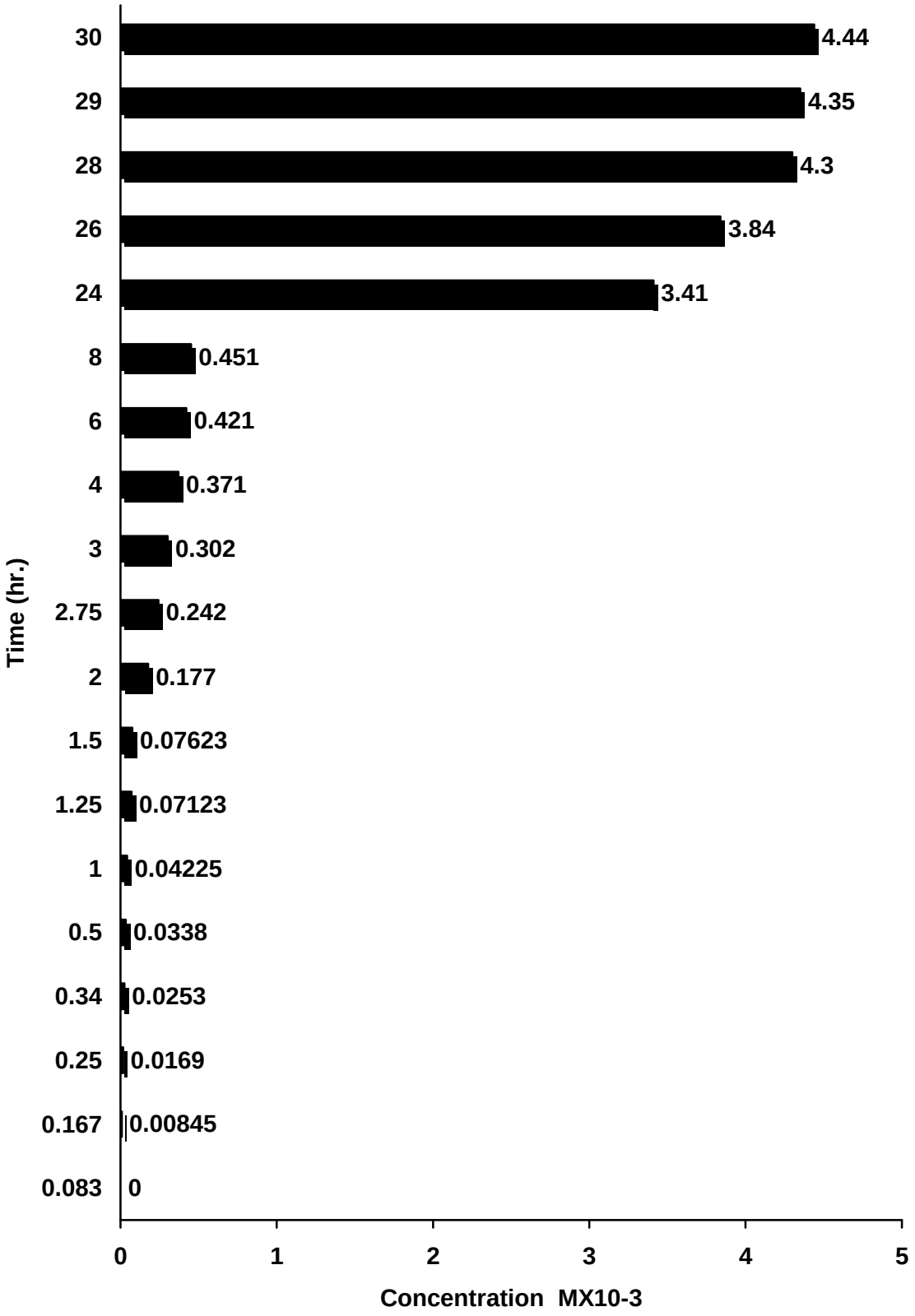


Figure (10) concentration of aspirin released by compound (19) in pH (6)

Table (6) concentration of aspirin released by compound (19) in pH (8)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.69X10^{-5}$	0.1
0.167	$4.22X10^{-5}$	0.26
0.25	$5.07X10^{-5}$	0.32
0.34	$7.123X10^{-5}$	0.45
0.50	$7.346X10^{-5}$	0.46
1.00	$8.47X10^{-5}$	0.53
1.25	$3.178X10^{-4}$	2
1.5	$3.94X10^{-4}$	2.4
2.00	$4.40X10^{-4}$	2.7
2.75	$4.8X10^{-4}$	3
3.00	$5.45X10^{-4}$	3.4
4.00	$7.16X10^{-4}$	4.51
6.00	$1.015X10^{-3}$	6.4
8.00	$1.98X10^{-3}$	12.4
24.00	$6.60X10^{-3}$	41.6
26.00	$6.63X10^{-3}$	41.8
28.00	$6.74X10^{-3}$	42.5
29.00	$7.15X10^{-3}$	45.1
30.00	$7.24X10^{-3}$	45.6

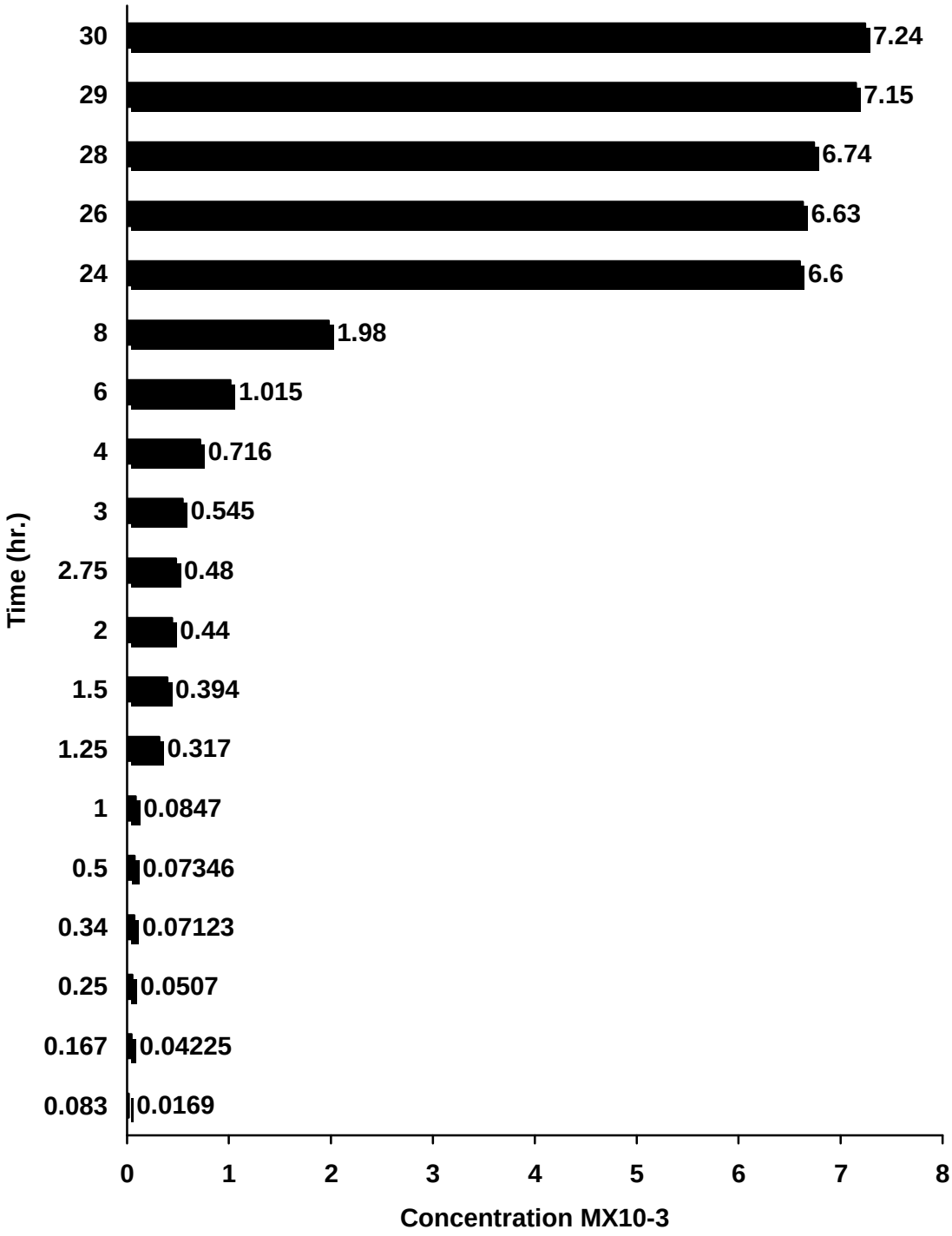


Figure (11) concentration of aspirin released by compound (19) in pH (8)

Table (7) concentration of aspirin released by compound (19) in pH (10)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$3.38X10^{-5}$	0.213
0.167	$7.623X10^{-5}$	0.48
0.25	$8.47X10^{-5}$	0.53
0.34	$9.32X10^{-5}$	0.588
0.50	$1.272X10^{-4}$	0.8
1.00	$1.684X10^{-4}$	1.06
1.25	$4.11X10^{-4}$	1.92
1.5	$4.22X10^{-4}$	2.66
2.00	$6.51X10^{-4}$	4.11
2.75	$6.81X10^{-4}$	4.3
3.00	$1.01X10^{-3}$	6.37
4.00	$1.57X10^{-3}$	9.9
6.00	$1.95X10^{-3}$	12.34
8.00	$2.94X10^{-3}$	18
24.00	$6.71X10^{-3}$	42.33
26.00	$6.942X10^{-3}$	43.7
28.00	$7.157X10^{-3}$	45.2
29.00	$7.303X10^{-3}$	46.1
30.00	$7.455X10^{-3}$	47

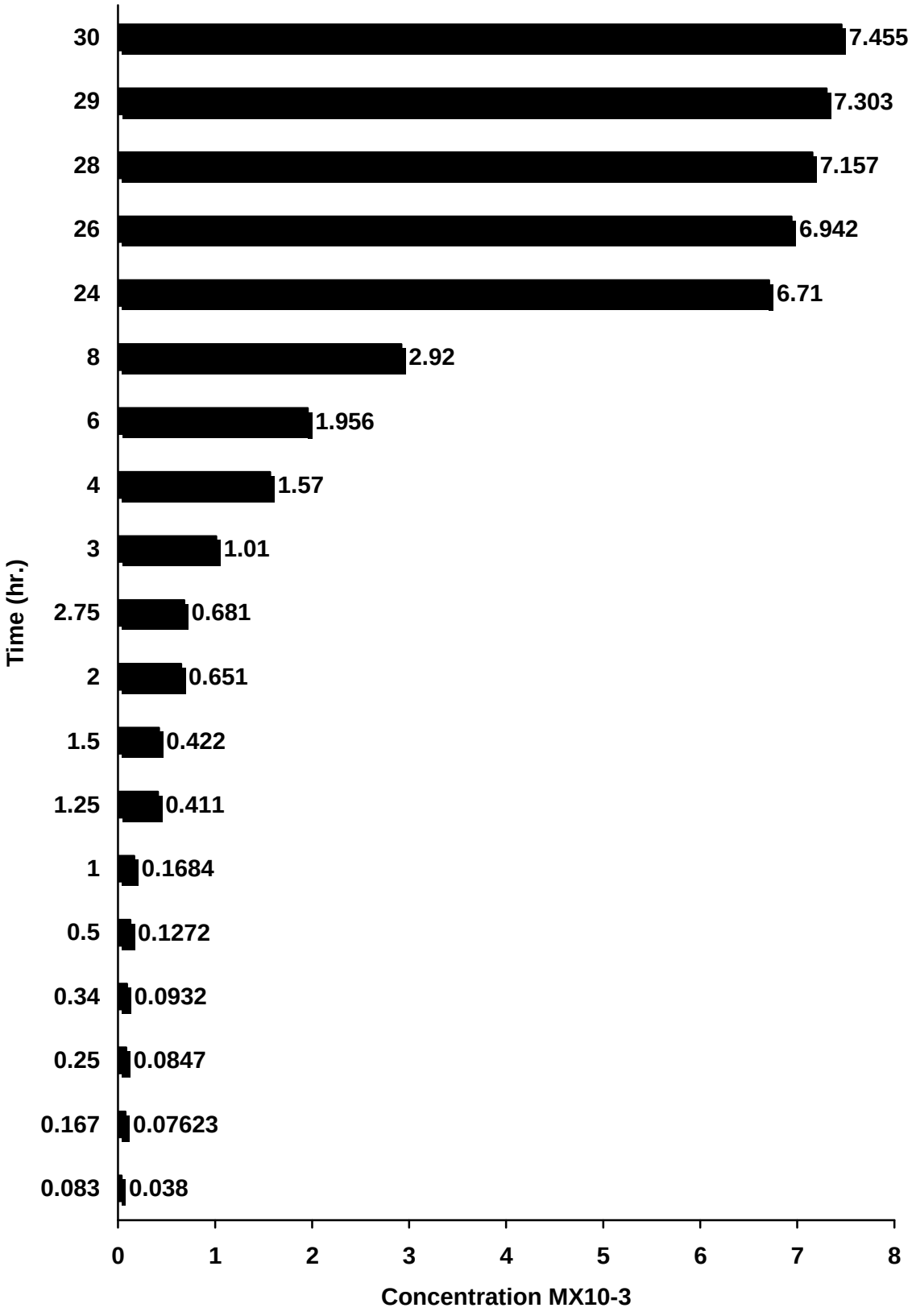


Figure (12) concentration of aspirin released by compound (19) in pH (10)

Table (8) concentration of aspirin released by compound (19) in pH (12)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$7.123X10^{-5}$	0.45
0.167	$1.272X10^{-4}$	0.8
0.25	$1.59X10^{-4}$	1
0.34	$1.99X10^{-4}$	1.26
0.50	$2.22X10^{-4}$	1.4
1.00	$3.178X10^{-4}$	2
1.25	$4.54X10^{-4}$	2.86
1.5	$7.58X10^{-4}$	4.71
2.00	$1.51X10^{-3}$	9.5
2.75	$1.956X10^{-3}$	12.34
3.00	$2.416X10^{-3}$	15
4.00	$2.88X10^{-3}$	18.19
6.00	$3.508X10^{-3}$	22.1
8.00	$4.21X10^{-3}$	26.5
24.00	$7.261X10^{-3}$	45.8
26.00	$7.41X10^{-3}$	46.68
28.00	$7.55X10^{-3}$	47.6
29.00	$7.583X10^{-3}$	47.8
30.00	$7.608X10^{-3}$	48

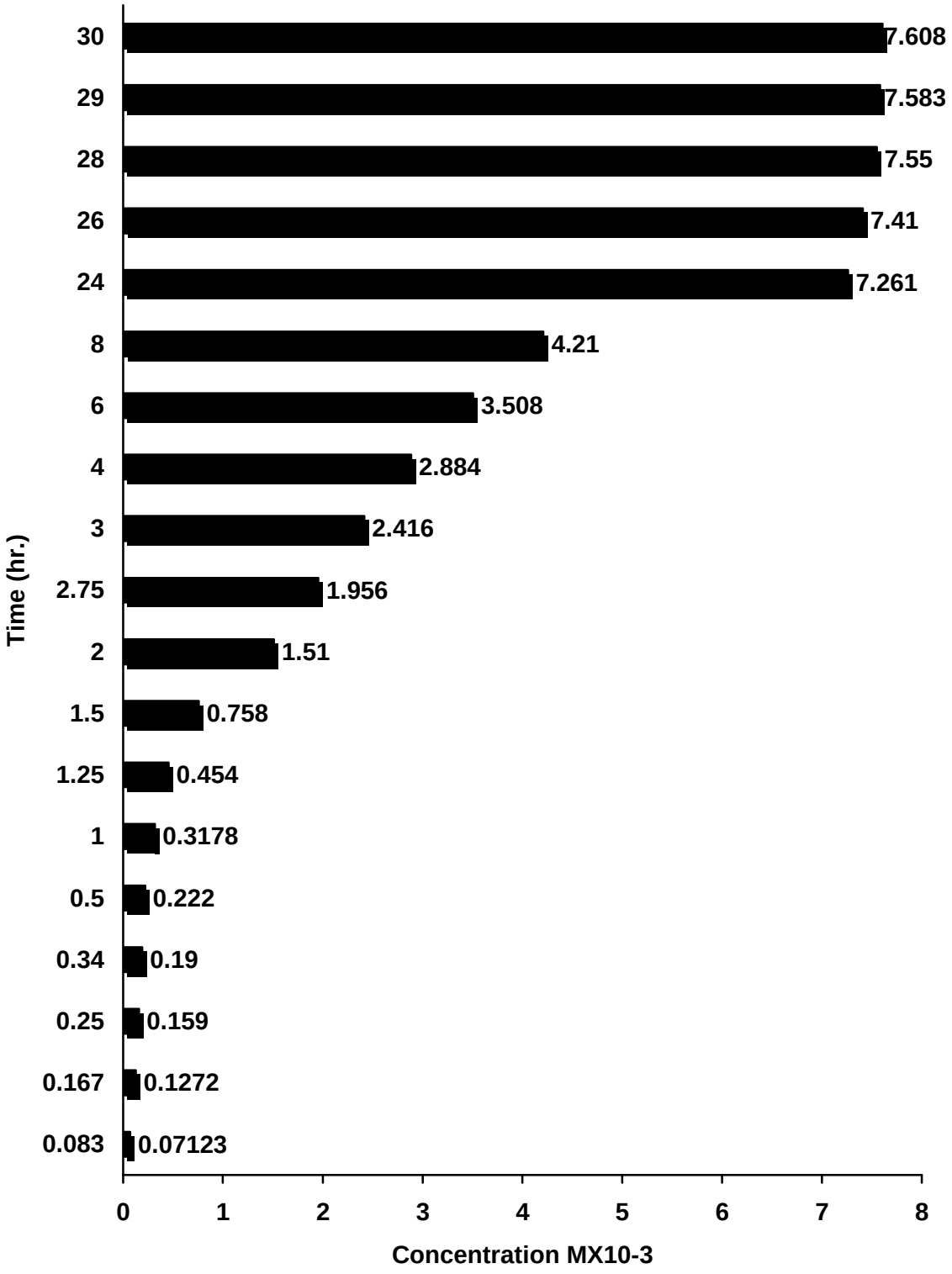


Figure (13) concentration of aspirin released by compound (19) in pH (12)

Table (9) concentration of aspirin released by compound (20) in pH (2)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	0	0
0.25	$8.45X10^{-6}$	0.076
0.34	$1.69X10^{-5}$	0.152
0.50	$2.535X10^{-5}$	0.221
1.00	$4.225X10^{-5}$	0.38
1.25	$7.346X10^{-5}$	0.66
1.5	$1.272X10^{-4}$	1.14
2.00	$1.99X10^{-4}$	1.79
2.75	$3.27X10^{-4}$	2.94
3.00	$3.325X10^{-4}$	3
4.00	$3.80X10^{-4}$	3.4
6.00	$4.70X10^{-4}$	4.2
8.00	$5.78X10^{-4}$	5.2
24.00	$2.97X10^{-3}$	26.7
26.00	$3.47X10^{-3}$	31.1
28.00	$3.8X10^{-3}$	34.2
29.00	$3.88X10^{-3}$	35.0
30.00	$3.996X10^{-3}$	36.0

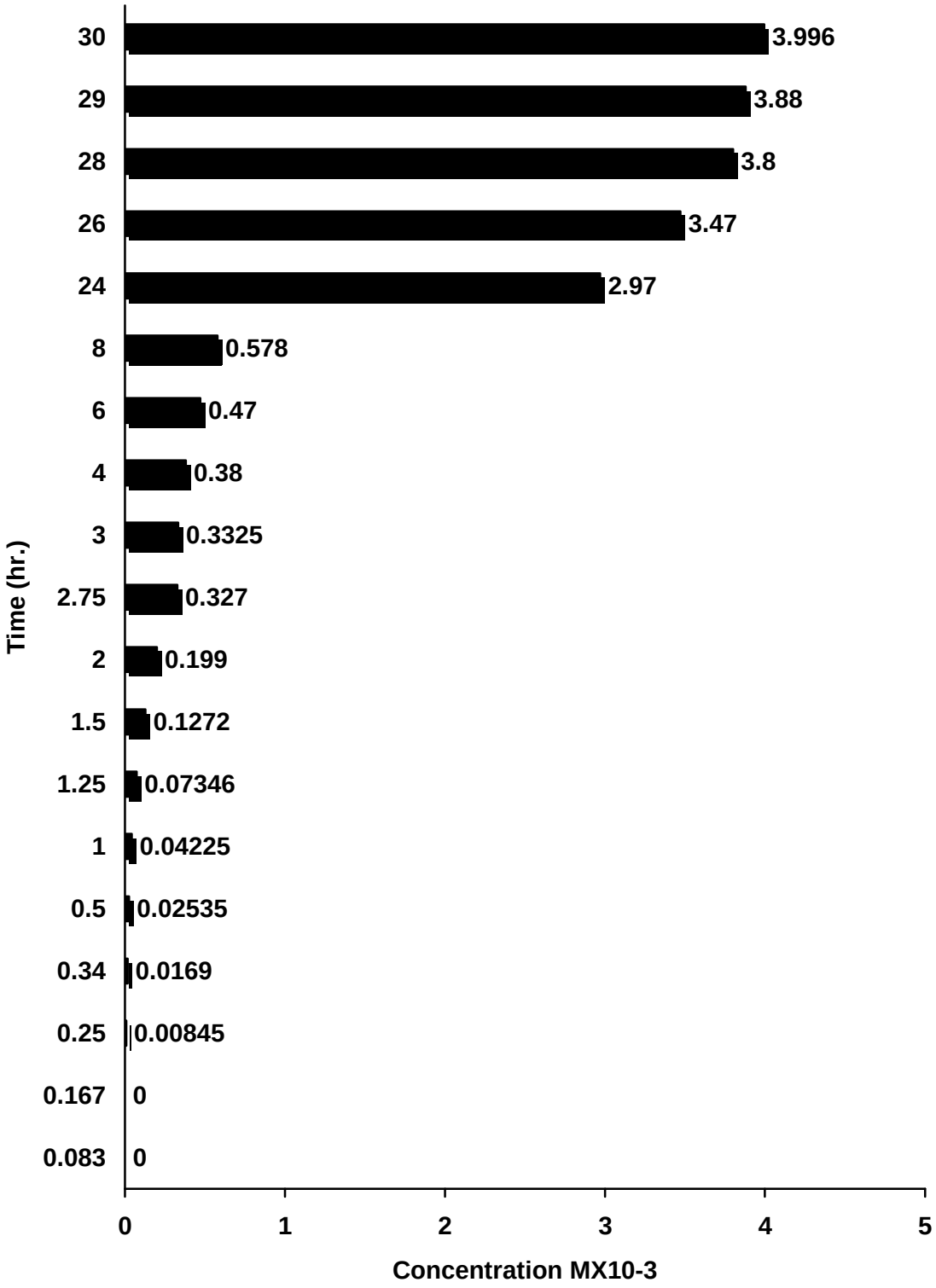


Figure (14) concentration of aspirin released by compound (20) in pH (2)

Table (10) concentration of aspirin released by compound (20) in pH (4)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	0	0
0.25	$1.69X10^{-5}$	0.152
0.34	$2.535X10^{-5}$	0.22
0.50	$3.380X10^{-5}$	0.304
1.00	$4.225X10^{-5}$	0.38
1.25	$7.623X10^{-5}$	0.68
1.5	$1.59X10^{-4}$	1.43
2.00	$2.22X10^{-4}$	2
2.75	$3.56X10^{-4}$	3.2
3.00	$3.64X10^{-4}$	3.27
4.00	$4.43X10^{-4}$	3.99
6.00	$4.63X10^{-4}$	4.17
8.00	$6.56X10^{-4}$	5.91
24.00	$3.42X10^{-3}$	2.08
26.00	$3.67X10^{-3}$	33.09
28.00	$3.98X10^{-3}$	35.91
29.00	$4.09X10^{-3}$	36.88
30.00	$4.16X10^{-3}$	37.5

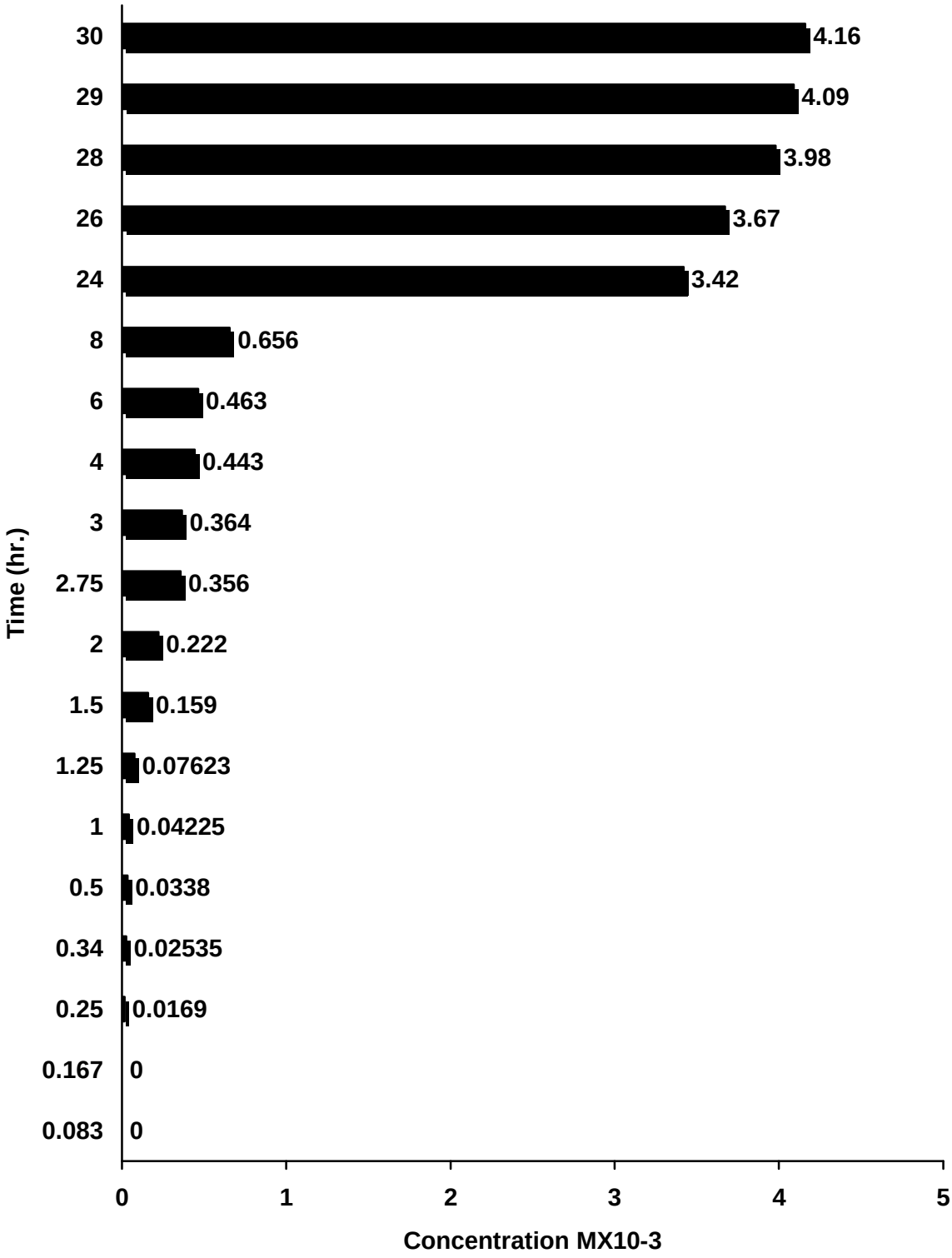


Figure (15) concentration of aspirin released by compound (20) in pH (4)

Table (11) concentration of aspirin released by compound (20) in pH (6)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$8.45X10^{-6}$	0.076
0.167	$1.69X10^{-5}$	0.152
0.25	$2.53X10^{-5}$	0.22
0.34	$3.38X10^{-5}$	0.305
0.50	$4.225X10^{-5}$	0.38
1.00	$5.07X10^{-5}$	0.45
1.25	$9.32X10^{-5}$	0.84
1.5	$1.77X10^{-4}$	1.6
2.00	$3.46X10^{-4}$	3.1
2.75	$3.95X10^{-4}$	3.5
3.00	$4.21X10^{-4}$	3.8
4.00	$4.56X10^{-4}$	4.1
6.00	$6.92X10^{-4}$	6.2
8.00	$2.18X10^{-3}$	19.6
24.00	$3.71X10^{-3}$	33.5
26.00	$4.17X10^{-3}$	37.5
28.00	$4.52X10^{-3}$	40.7
29.00	$4.73X10^{-3}$	42.5
30.00	$4.75X10^{-3}$	42.8

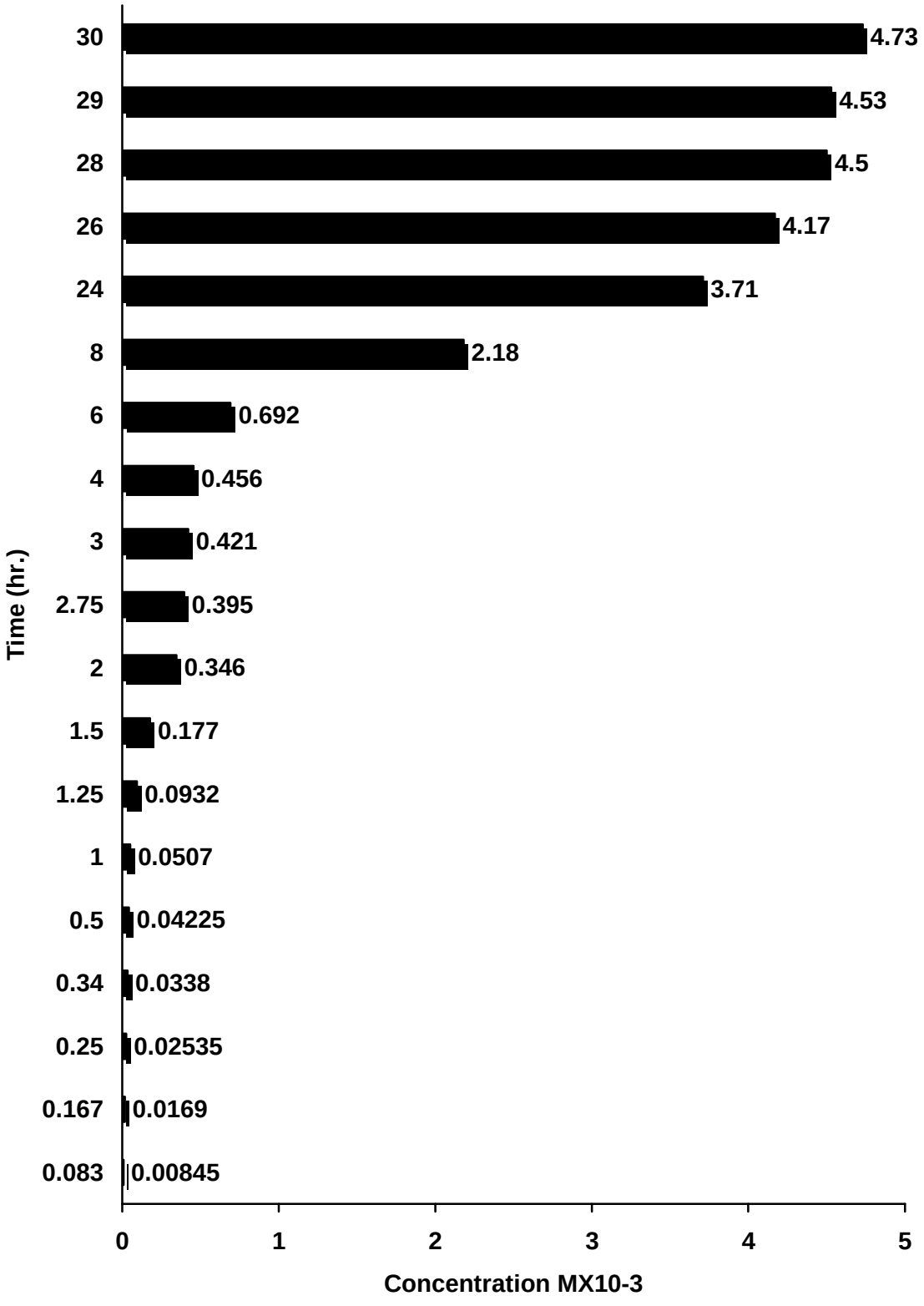


Figure (16) concentration of aspirin released by compound (20) in pH (6)

Table (12) concentration of aspirin released by compound (20) in pH (8)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$3.38X10^{-5}$	0.304
0.167	$8.47X10^{-5}$	0.76
0.25	$9.32X10^{-5}$	0.84
0.34	$1.01X10^{-4}$	0.91
0.50	$1.1X10^{-4}$	0.99
1.00	$1.99X10^{-4}$	1.79
1.25	$4.38X10^{-4}$	3.94
1.5	$4.63X10^{-4}$	4.17
2.00	$2.15X10^{-3}$	19.43
2.75	$2.36X10^{-3}$	21.3
3.00	$2.66X10^{-3}$	24
4.00	$3.29X10^{-3}$	29.6
6.00	$3.42X10^{-3}$	30.8
8.00	$3.93X10^{-3}$	35.4
24.00	$5.19X10^{-3}$	46.8
26.00	$5.25X10^{-3}$	47.3
28.00	$5.328X10^{-3}$	48
29.00	$5.48X10^{-3}$	49.4
30.00	$5.56X10^{-3}$	50.1

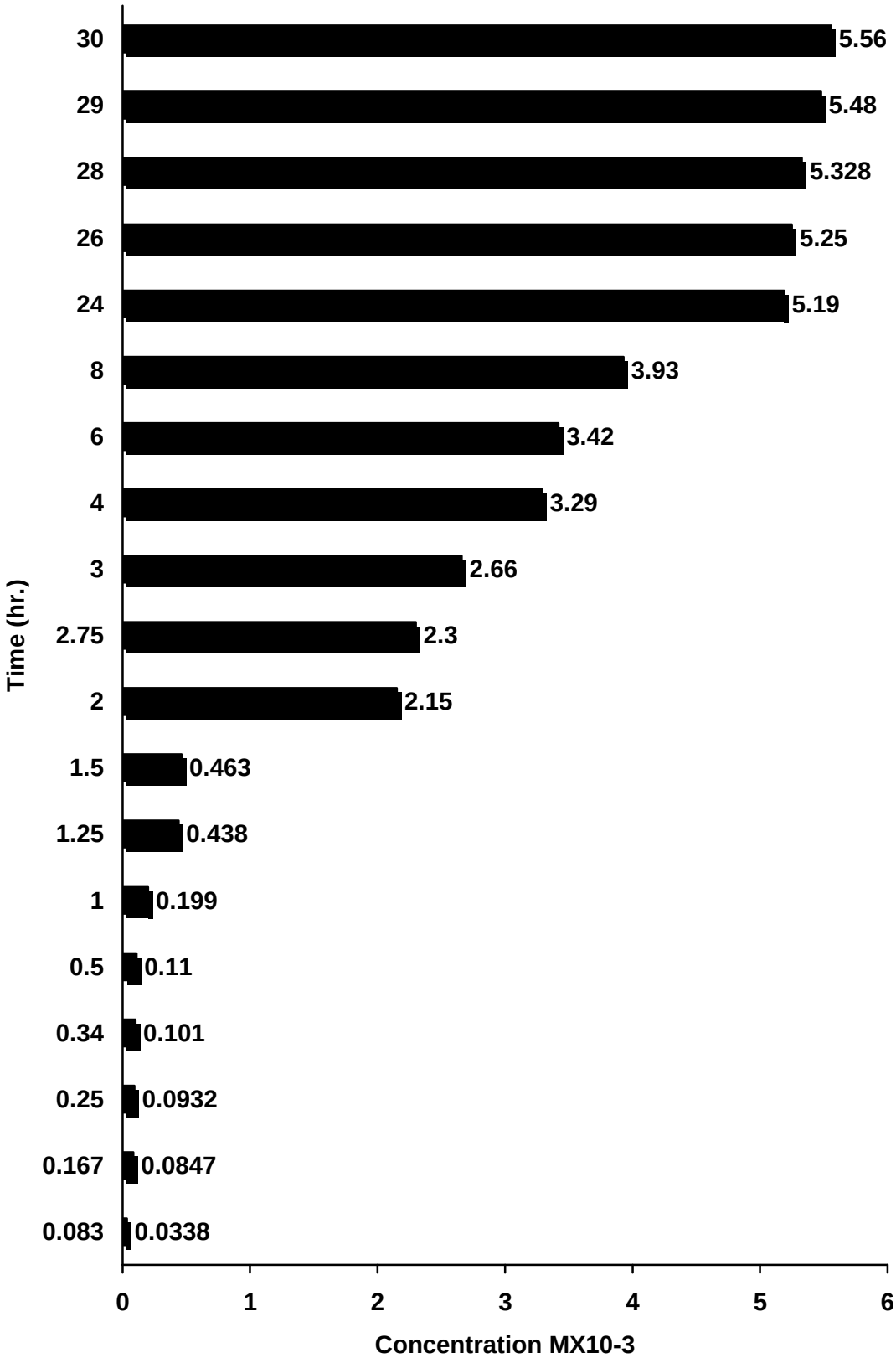


Figure (17) concentration of aspirin released by compound (20) in pH (8)

Table (13) concentration of aspirin released by compound (20) in pH (10)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$7.123X10^{-5}$	0.641
0.167	$1.59X10^{-4}$	1.4
0.25	$1.77X10^{-4}$	1.6
0.34	$2.22X10^{-4}$	2
0.50	$2.32X10^{-4}$	2.09
1.00	$3.46X10^{-4}$	3.1
1.25	$6.21X10^{-4}$	5.6
1.5	$2.04X10^{-3}$	18.4
2.00	$3.13X10^{-3}$	28.2
2.75	$3.22X10^{-3}$	29
3.00	$3.39X10^{-3}$	30.5
4.00	$3.48X10^{-3}$	31.3
6.00	$3.81X10^{-3}$	34.3
8.00	$4.45X10^{-3}$	40.1
24.00	$5.61X10^{-3}$	50.5
26.00	$5.81X10^{-3}$	52.3
28.00	$5.90X10^{-3}$	53.1
29.00	$5.97X10^{-3}$	53.7
30.00	$6.027X10^{-3}$	54.3

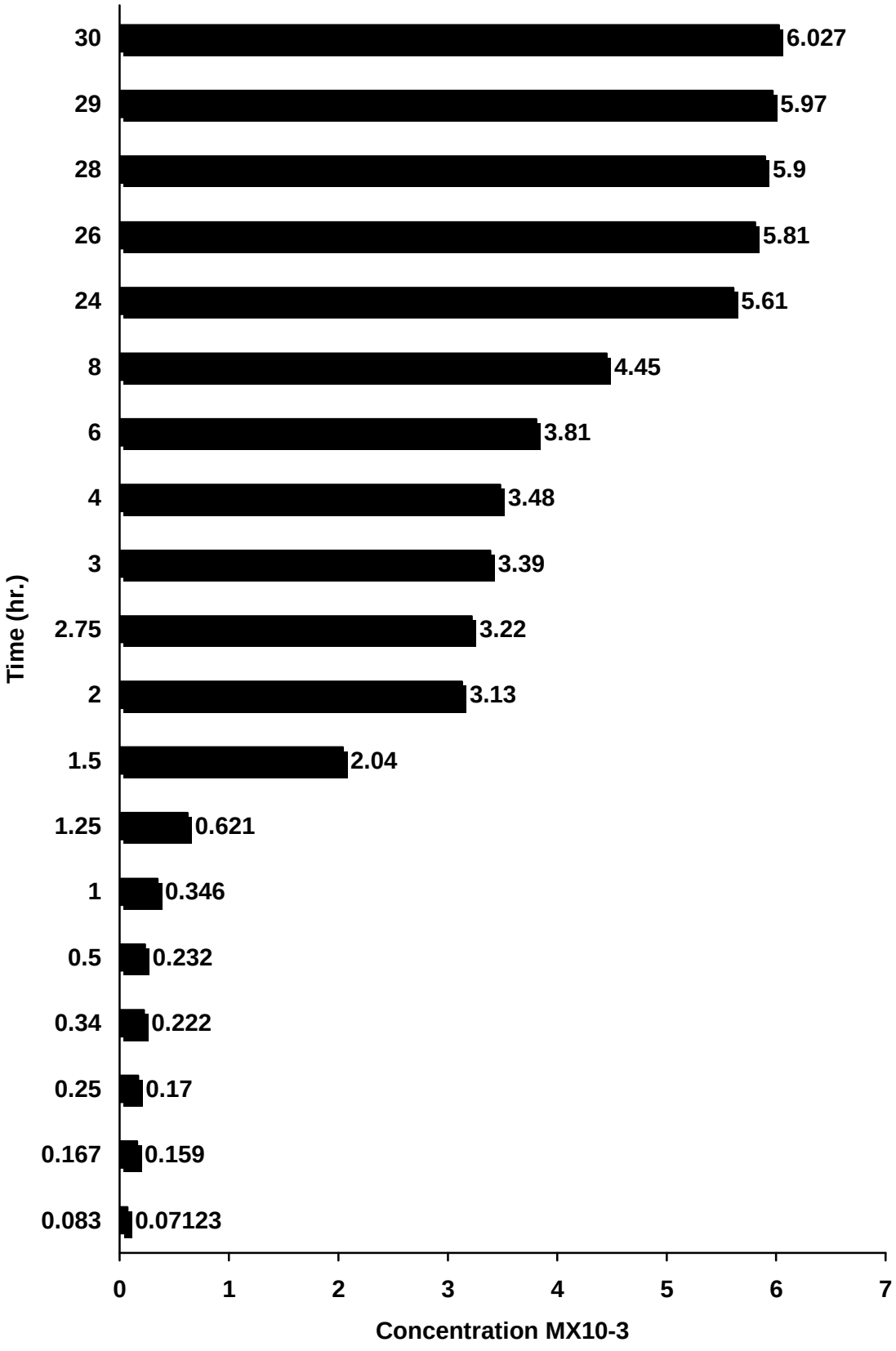


Figure (18) concentration of aspirin released by compound (20) in pH (10)

Table (14) concentration of aspirin released by compound (20) in pH (12)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.378X10^{-4}$	1.24
0.167	$3.11X10^{-4}$	2.88
0.25	$5.43X10^{-4}$	4.9
0.34	$5.88X10^{-4}$	5.3
0.50	$8.54X10^{-4}$	7.7
1.00	$1.80X10^{-3}$	16.4
1.25	$2.63X10^{-3}$	23.6
1.5	$3.29X10^{-3}$	29.7
2.00	$3.91X10^{-3}$	35.3
2.75	$4.11X10^{-3}$	37.1
3.00	$4.18X10^{-3}$	37.7
4.00	$4.45X10^{-3}$	40.1
6.00	$4.59X10^{-3}$	41.4
8.00	$4.95X10^{-3}$	44.6
24.00	$5.79X10^{-3}$	52.2
26.00	$5.97X10^{-3}$	53.8
28.00	$6.17X10^{-3}$	55.6
29.00	$6.38X10^{-3}$	57.5
30.00	$6.467X10^{-3}$	58.2

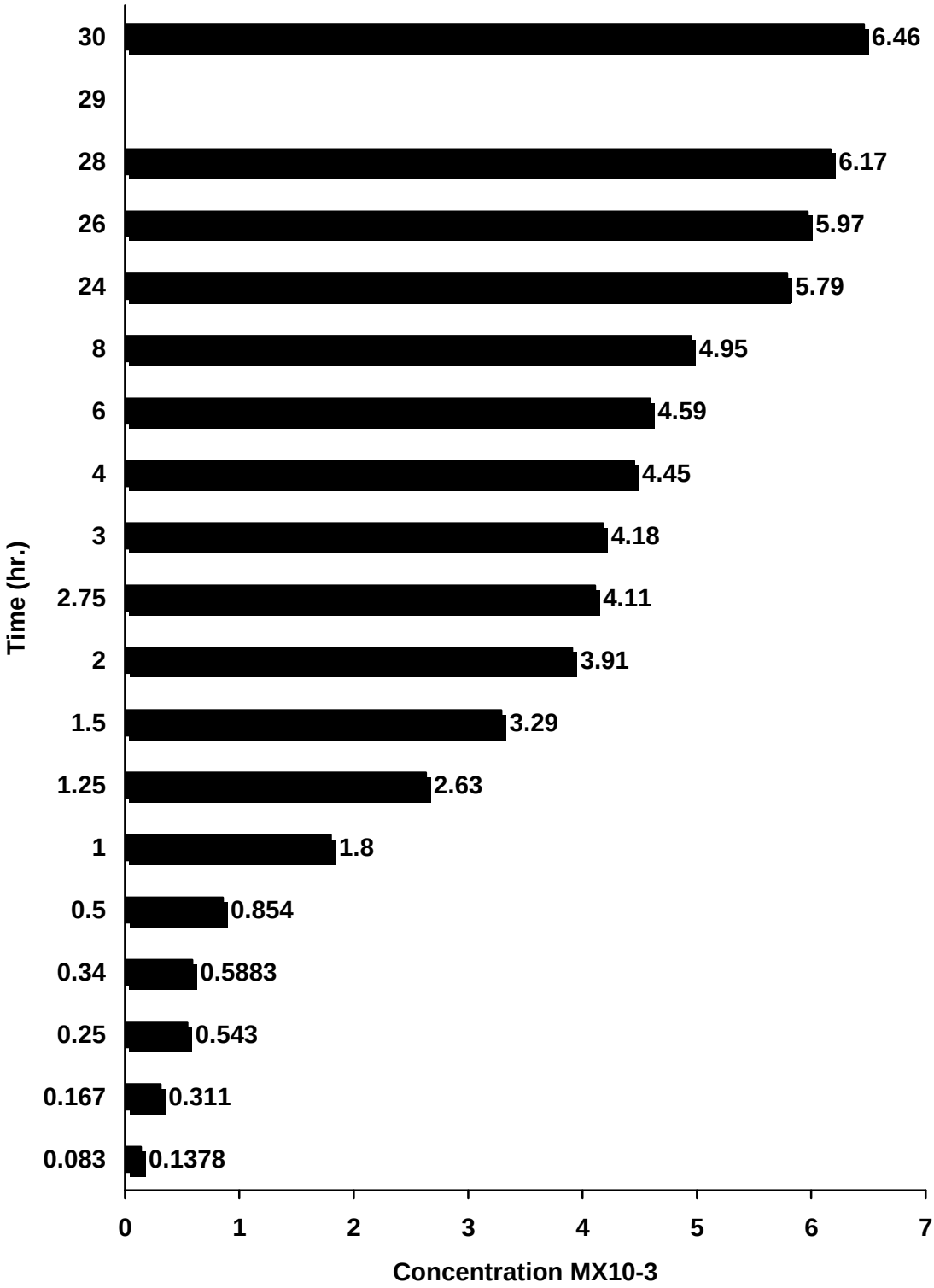


Figure (19) concentration of aspirin released by compound (20) in pH (12)

Table (15) concentration of aspirin released by compound (21) in pH (2)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	0	0
0.167	$1.69X10^{-5}$	0.23
0.25	$2.535X10^{-5}$	0.34
0.34	$4.225X10^{-5}$	0.58
0.50	$7.123X10^{-5}$	0.97
1.00	$7.623X10^{-5}$	1
1.25	$1.496X10^{-4}$	2
1.5	$2.92X10^{-4}$	4
2.00	$3.65X10^{-4}$	5
2.75	$4.70X10^{-4}$	6.4
3.00	$4.85X10^{-4}$	6.7
4.00	$6.35X10^{-4}$	8.73
6.00	$9.09X10^{-4}$	12.5
8.00	$1.214X10^{-3}$	16.7
24.00	$2.15X10^{-3}$	29.6
26.00	$2.37X10^{-3}$	32.7
28.00	$2.57X10^{-3}$	35.4
29.00	$2.82X10^{-3}$	38.8
30.00	$2.85X10^{-3}$	39.3

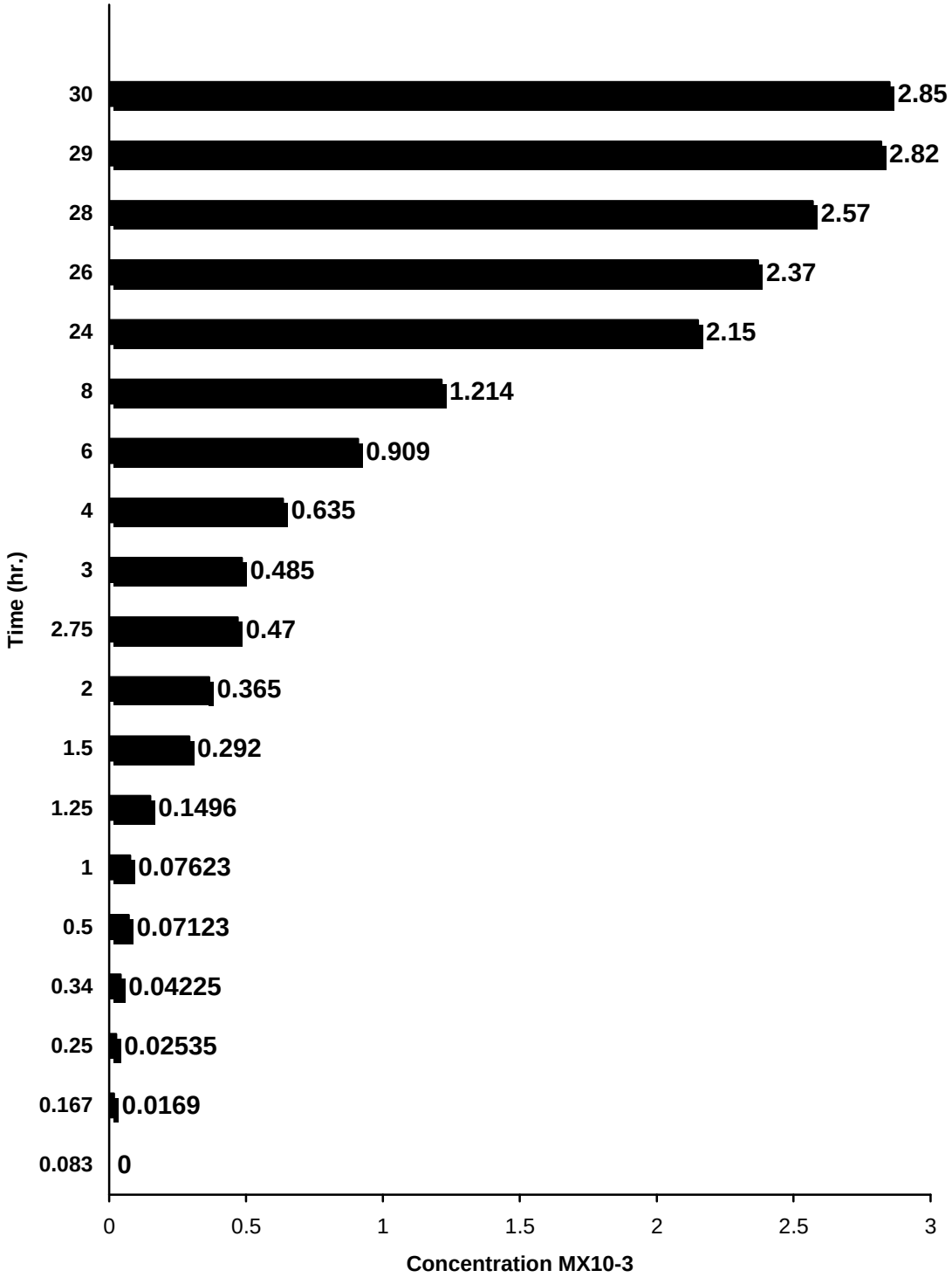


Figure (20) concentration of aspirin released by compound (21) in pH (2)

Table (16) concentration of aspirin released by compound (21) in pH (4)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.69X10^{-5}$	0.23
0.167	$2.535X10^{-5}$	0.34
0.25	$3.38X10^{-5}$	0.46
0.34	$5.07X10^{-5}$	0.69
0.50	$6.32X10^{-5}$	0.87
1.00	$7.90X10^{-5}$	1.08
1.25	$2.14X10^{-4}$	2.95
1.5	$2.44X10^{-4}$	3.36
2.00	$3.39X10^{-4}$	4.66
2.75	$5.83X10^{-4}$	8.02
3.00	$7.17X10^{-4}$	9.86
4.00	$1.19X10^{-3}$	16.22
6.00	$1.96X10^{-3}$	27.02
8.00	$2.48X10^{-3}$	34.18
24.00	$3.31X10^{-3}$	45.5
26.00	$3.42X10^{-3}$	47.1
28.00	$3.66X10^{-3}$	50.4
29.00	$3.84X10^{-3}$	52.8
30.00	$3.87X10^{-3}$	53.3

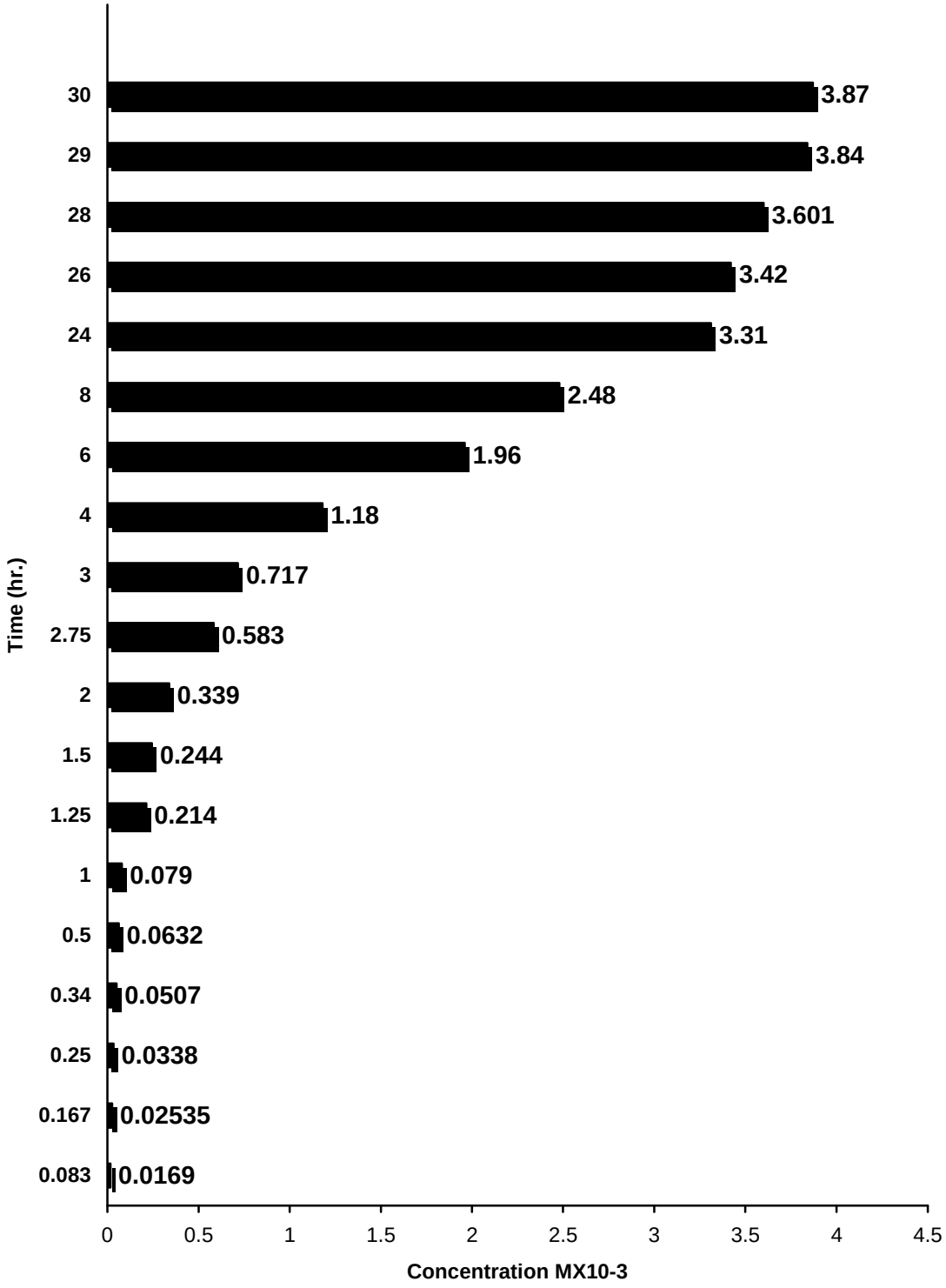


Figure (21) concentration of aspirin released by compound (21) in pH (4)

Table (17) concentration of aspirin released by compound (21) in pH (6)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.69X10^{-5}$	0.23
0.167	$2.535X10^{-5}$	0.34
0.25	$5.07X10^{-5}$	0.69
0.34	$6.32X10^{-5}$	0.87
0.50	$7.623X10^{-5}$	1.04
1.00	$9.32X10^{-5}$	1.28
1.25	$2.22X10^{-4}$	3.05
1.5	$6.62X10^{-4}$	9.1
2.00	$1.14X10^{-3}$	15.7
2.75	$1.39X10^{-3}$	19.2
3.00	$1.43X10^{-3}$	19.7
4.00	$1.86X10^{-3}$	28.6
6.00	$2.08X10^{-3}$	36.7
8.00	$3.47X10^{-3}$	47.8
24.00	$4.37X10^{-3}$	60.1
26.00	$4.61X10^{-3}$	63.4
28.00	$4.7X10^{-3}$	64.7
29.00	$4.74X10^{-3}$	65.2
30.00	$4.76X10^{-3}$	65.5

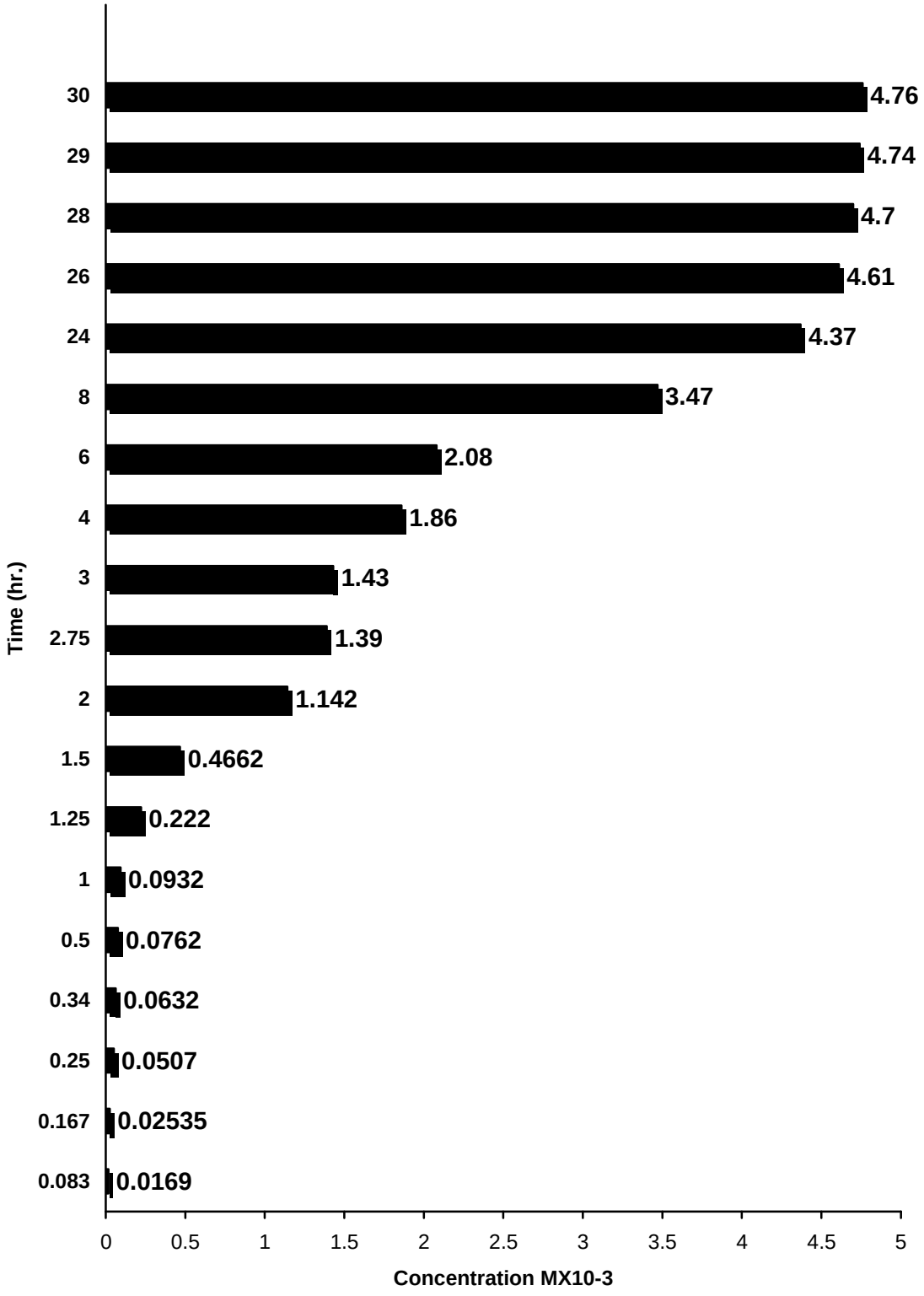


Figure (22) concentration of aspirin released by compound (21) in pH (6)

Table (18) concentration of aspirin released by compound (21) in pH (8)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$6.32X10^{-5}$	0.87
0.167	$1.09X10^{-4}$	1.5
0.25	$1.45X10^{-4}$	2
0.34	$2.03X10^{-4}$	2.8
0.50	$3.05X10^{-4}$	4.2
1.00	$7.57X10^{-4}$	10.4
1.25	$1.28X10^{-3}$	17.7
1.5	$1.65X10^{-3}$	22.8
2.00	$2.23X10^{-3}$	30.8
2.75	$2.64X10^{-3}$	36.3
3.00	$2.88X10^{-3}$	39.7
4.00	$3.02X10^{-3}$	41.6
6.00	$3.59X10^{-3}$	49.4
8.00	$4.01X10^{-3}$	55.2
24.00	$5.12X10^{-3}$	70.3
26.00	$5.40X10^{-3}$	74.2
28.00	$5.62X10^{-3}$	77.3
29.00	$5.65X10^{-3}$	77.7
30.00	$5.73X10^{-3}$	78.8

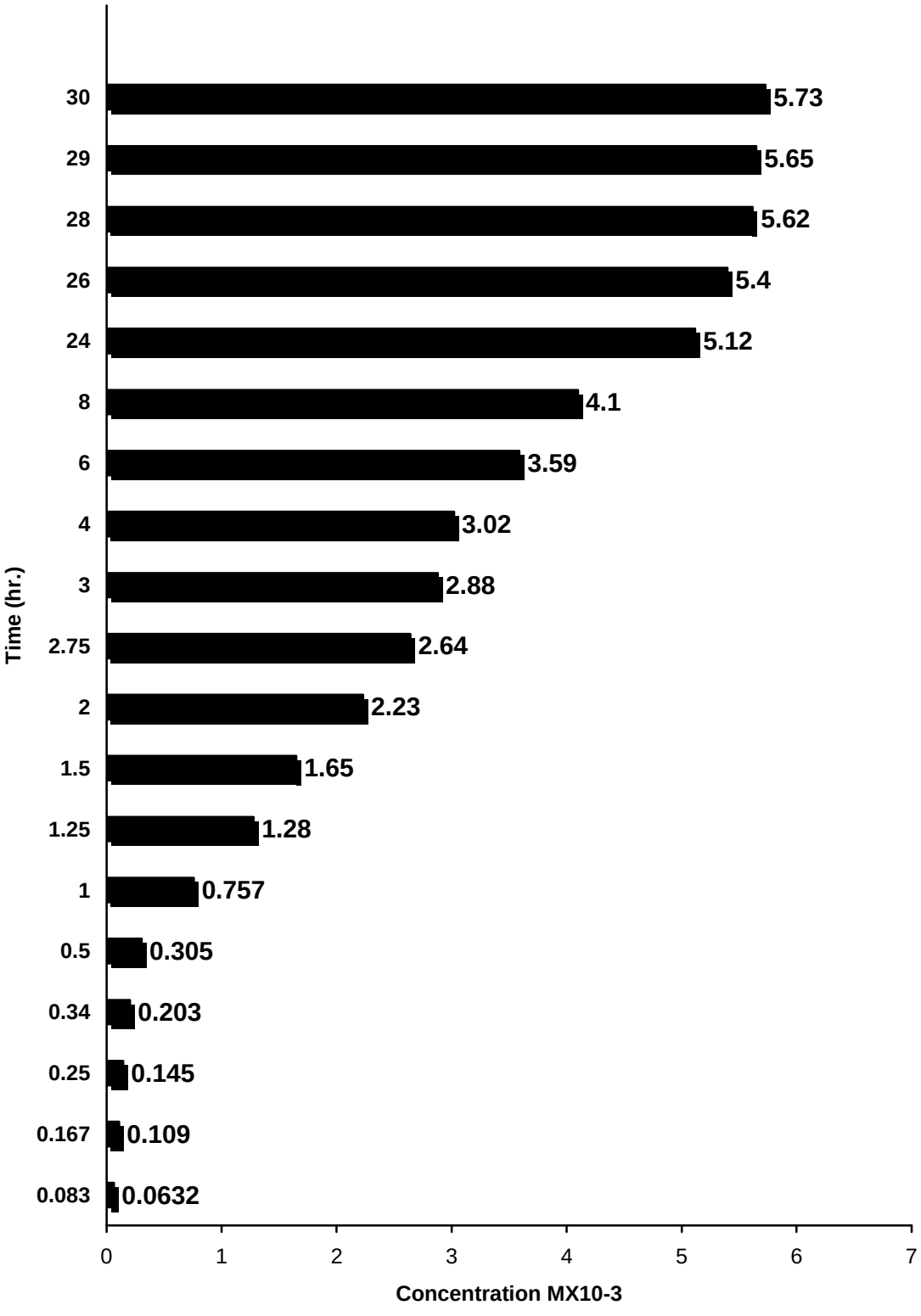


Figure (23) concentration of aspirin released by compound (21) in pH (8)

Table (19) concentration of aspirin released by compound (21) in pH (10)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$1.38X10^{-4}$	1.9
0.167	$1.74X10^{-4}$	2.4
0.25	$2.25X10^{-4}$	3.1
0.34	$3.20X10^{-4}$	4.4
0.50	$4.94X10^{-4}$	6.8
1.00	$1.05X10^{-3}$	14.5
1.25	$1.94X10^{-3}$	26.7
1.5	$2.49X10^{-3}$	34.3
2.00	$2.72X10^{-3}$	37.4
2.75	$3.04X10^{-3}$	41.8
3.00	$3.13X10^{-3}$	43.1
4.00	$3.55X10^{-3}$	48.8
6.00	$3.95X10^{-3}$	54.4
8.00	$4.38X10^{-3}$	60.2
24.00	$5.42X10^{-3}$	74.5
26.00	$5.64X10^{-3}$	77.6
28.00	$5.83X10^{-3}$	80.2
29.00	$5.91X10^{-3}$	81.3
30.00	$5.97X10^{-3}$	82

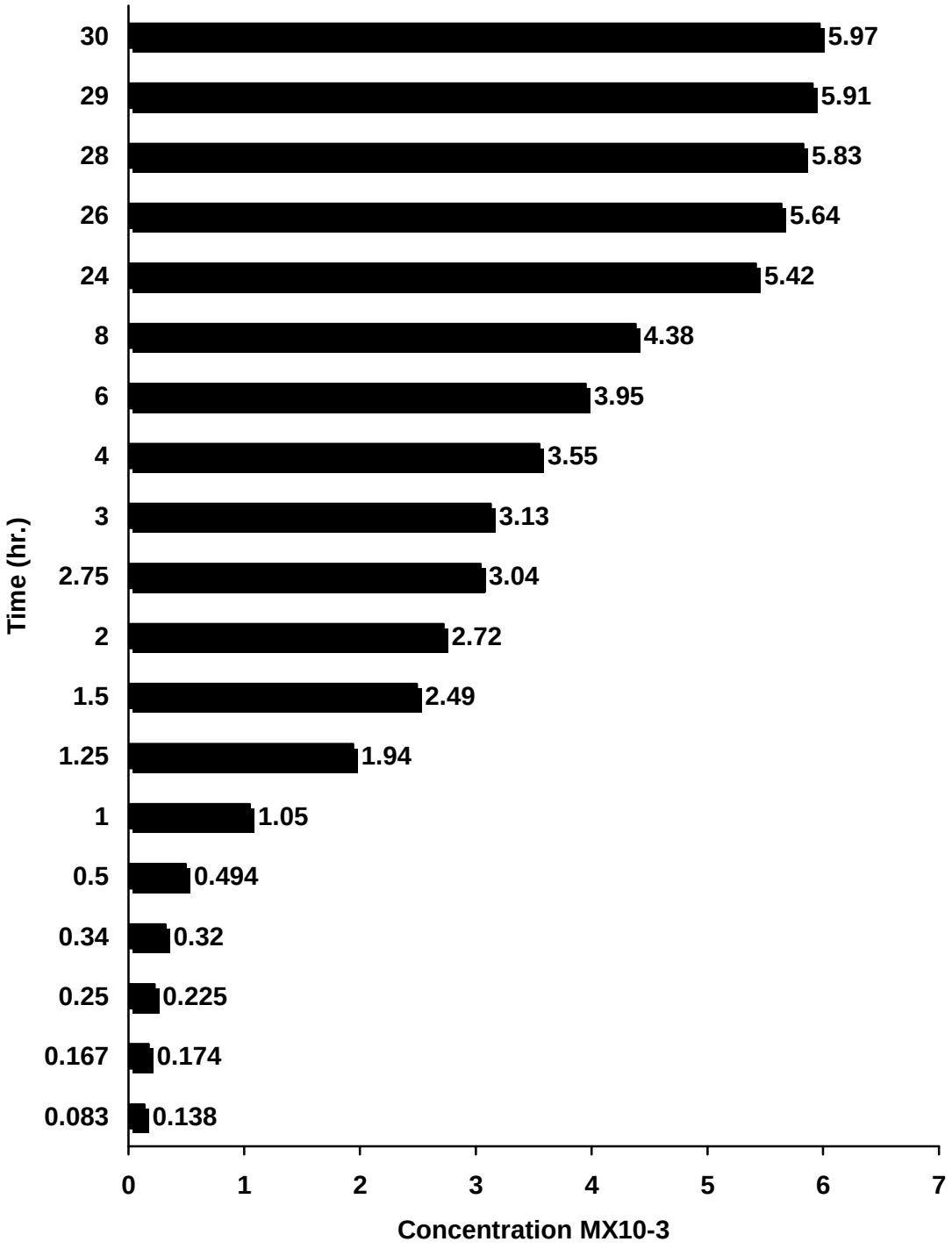


Figure (24) concentration of aspirin released by compound (21) in pH (10)

Table (20) concentration of aspirin released by compound (21) in pH (12)

Time (hr.)	Concentration of aspirin released M	% released
0	0	0
0.083	$2.62X10^{-4}$	3.6
0.167	$4.24X10^{-4}$	5.9
0.25	$7.12X10^{-4}$	9.8
0.34	$8.07X10^{-4}$	11.1
0.50	$1.40X10^{-3}$	19.3
1.00	$1.69X10^{-3}$	23.3
1.25	$2.33X10^{-3}$	32.6
1.5	$2.89X10^{-3}$	39.7
2.00	$3.15X10^{-3}$	43.3
2.75	$3.53X10^{-3}$	48.6
3.00	$3.61X10^{-3}$	49.7
4.00	$4.18X10^{-3}$	57.5
6.00	$4.71X10^{-3}$	64.8
8.00	$5.33X10^{-3}$	73.3
24.00	$5.83X10^{-3}$	80.14
26.00	$5.95X10^{-3}$	81.8
28.00	$6.08X10^{-3}$	83.5
29.00	$6.17X10^{-3}$	84.8
30.00	$6.21X10^{-3}$	85.3

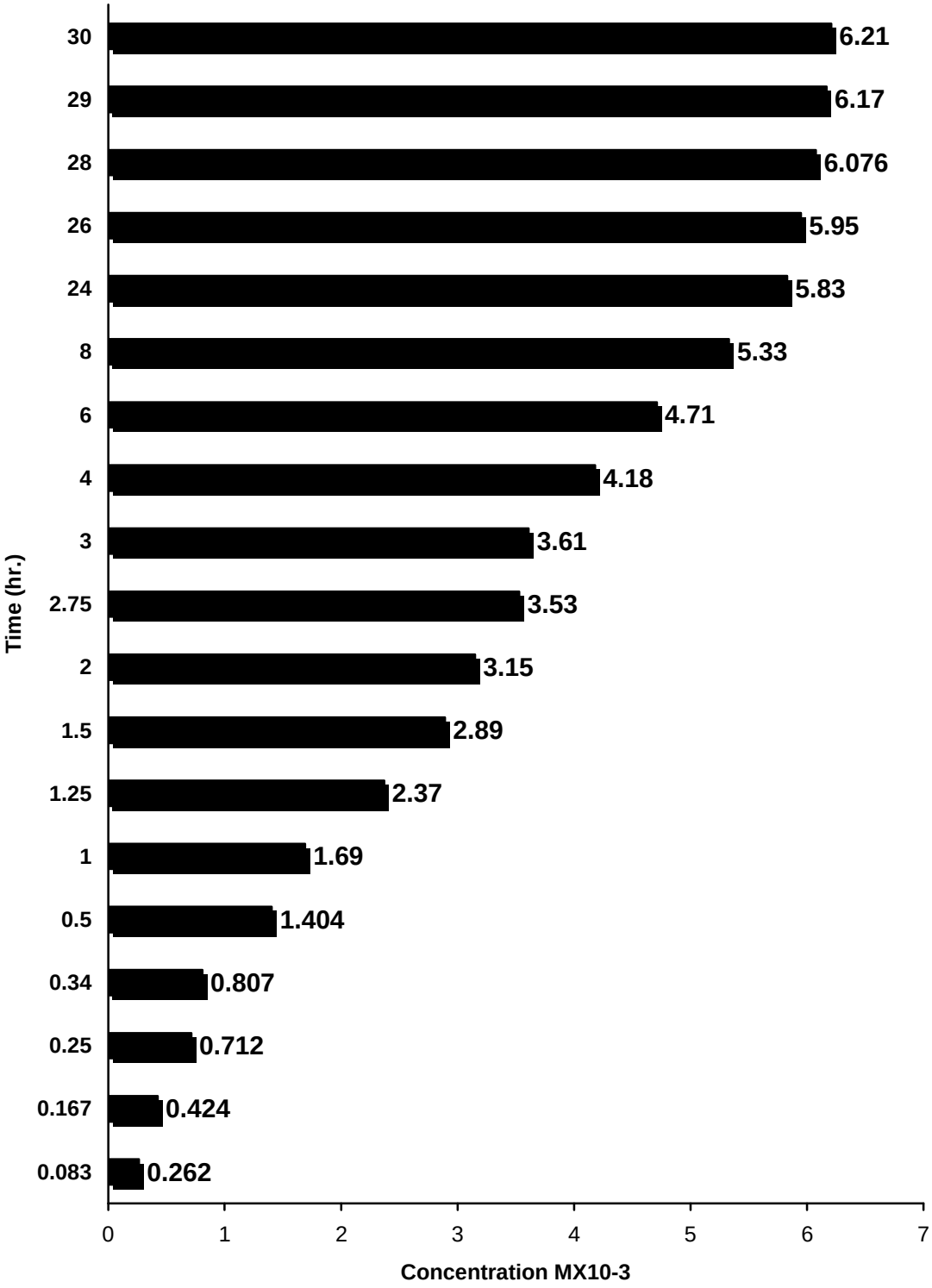


Figure (25) concentration of aspirin released by compound (21) in pH (12)

2.6 : Identification of the new derivatives:

2.6.1 : Identification of the new derivatives by Thin Layer Chromatography (TLC) :

The identity compounds (8),(10),(17),(18),(19),(20),(21) were determined by TLC using aluminum plates coated with (0.25 mm) layer of silica gel F_{254} as stationary phase, while the mobile phase is (Benzene : ether)(8:2) compounds were detected by iodine vapor . All synthesized compounds were purified using silica – gel column .

2.7 : Drug released study:

The Drug released study of aspirin released from the following compounds 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid , 2,3-di (acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid and 2,3,5,6 -Tetra (acetyl salicyloyl)-*L*-ascorbic acid was carried out in six buffer solutions; three under acidic hydrolysis and three under basic hydrolysis .150 mg of each compound was comprised at high pressure to form discs which is placed with stirring in 25 ml of buffer solution and the concentration of aspirin released from the hydrolysis was followed in the supernatant (3 ml) by recording the UV absorbance at 277 nm for the period 0-30 hrs. with previously prepared standard curve .Results were plotted in figures (8-25) .

2.7.1 : Solution :

buffer solution :

A - The buffer solution (Phosphate buffer) , (pH = 2,4,6) was prepared by dissolving (0.79 g , 1.58 g , 2.37 g) respectively from (Na_2HPO_4) in (100 ml) distilled water (few drop of H_3PO_4 were added to fixed the pH) .

B - The buffer solution, (pH = 8,10,12) by was prepared by dissolving (5.04 g , 6.3 g , 7.56 g) respectively from (Na_2CO_3) and (2.69 g , 3.36 g , 4.03 g) ($NaHCO_3$) in (1L) distilled water. (142)

2.8: Biochemical study :

2.8.1 : Blood sample :

The blood samples were collected from healthy men, the history of the men was investigated, the blood was left at room temperature for about half an hour then the serum was separated by centrifuging for 15 minutes at 3500 rpm.

2.8.2 : Determination of Acetyl Choline Esterase (AChE) activity :

2.8.3: Solutions:

1- Buffer solution : The buffer solution (Phosphate buffer), (pH= 7.2- 7.4) was prepared by dissolving (2.89 g) from (Na_2HPO_4) in (100 ml) distilled water to give 0.2 M solution (some drops of H_3PO_4 were added to fix the pH) .

2- Indicator DTNB (Ellman's reagent) : The indicator DTNB [5,5'-Dithiobis(2-nitrobenzoic acid) , 0.001 M] was prepared by dissolving (0.01g) DTNB (MWt = 396.36g/mol) in 25 ml distilled water , stirring , heating and a small amount of ($NaHCO_3$) was added to complete solubility . The solution was stored in amber flask to protect from light.

3- Substrate solution (S-Acetyl thiocholine Iodide) : (0.01735 g) was dissolved in (1 ml) distilled water .

2.8.4 : Procedure :

1- (2.25 ml) from the buffer solution in a test tube, and (50 µL) of DNTB indicator and (10µL) from the serum were added and mixed and the absorbance was recorded as blank.

2- (2 ml) from solution (1), in a test tube and (34µL) from substrate solution were mixed and the difference in

absorbance at 412 nm after and before adding of substrate solution was recorded in each (3 min.). The enzyme activity was determined using (µmol/ml/3min) unit (This experimental was repeated 3 times and the statistical average was taken). [143]

2.9 : Effect the new compound (19) , (20) , (21) on the (AChE) activity in serum :

The effect of the new compounds (19),(20),(21) were calculated at different concentrations. A stock solution (0.5 g/25 ml) of each compound was prepared in DMSO. Dilution was made to obtain the required concentration [($5.2x10^{-8}$ M) for (19) ,($3.7x10^{-8}$ M) for (20) and ($2.42x10^{-8}$ M) for (21)].The substrate concentration was (0.1 M)

Measurement of enzyme activity was carried out using the method described in section (2.8.4), by adding (1 ml) from the compound to the substrate buffer .

The inhibition percentage was calculated by comparing the activity with and without using the tested compound under the same conditions .[144]

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{The activity with inhibitor}}{\text{The activity without inhibitor}} \right] X100$$

2.9.1 – Type of inhibition:

Inhibition type study was done as described in section (2.8.4) at different concentration of the substrate (0.1- 0.02 M) with fixed concentrations of the tested compounds (Inhibitor) [(0.005 g/25 ml) [($5.2x10^{-4}$) M for (19) ,($3.7x10^{-4}$) M for (20) and ($2.42x10^{-4}$ M) for (21)]. [145]

The enzyme activity was measured in present and absence of inhibitor at the same conditions. Linweaver – Burk plot was applied to obtain: 1- Km 2-Ki, 3- Vmax 4- Vmaxi, 5-Type of inhibition for the three type of inhibitor.

2.10 : Determination of Alkaline phosphatase (ALP) activity

Colorimetric determination of alkaline phosphatase activity was carried out according to the following reaction

$$\text{Phenyl phosphate} \xrightarrow[\text{pH 10}]{\text{Alkaline phosphatase}} \text{phenol + phosphate}$$

The phenol liberated is measured in the presence of amino 4-antipyrine and potassium ferricyanide.The presence of sodium arsenate in the reagent stops the enzymatic reaction

The ALP activity was measured in serum according to the method of Kind and Belfleld .[146,147]

2.10.1:Procedure :

2.10.1.1 : Reagent :

R1	Reagent No. 1 substrate Buffer	Disodium phenyl phosphate Carbonate-bicarbonate buffer pH 10	5 mmol/L 50mmol/L
R2	Reagent No. 2 standard	Phenol	Equal 20 kind and king U
R3	Reagent No. 3 Blocking reagent	Amino-4-antipyrine Sodium arsenate	60 mmol/L 75 g/L
R4	Reagent No. 4 Color reagent	Potassium ferricynide	150mmol/L

2.10.2 : Assay :

Set up the following tubes

Table (2) measurement of total ALP activity in serum

	Serum Sample	Serum blank	Standard	Reagent blank
R1	2ml	2ml	2ml	2ml
Incubate for 5 min at 37^0C				
Serum	50µl	-	-	-
R2	-	-	50µl	-
Mix , incubate for exactly 15 min at 37^0C				
R3	0.5ml	0.5ml	0.5ml	0.5ml
Mix well				
R4	0.5ml	0.5ml	0.5ml	0.5ml
Serum	-	50µl	-	-
Distilled water	-	-	-	50µl

The reagent were mixed and left for 10 min. at dark the absorbance at 510 nm was measure against reagent blank ,the color intensity is stable for 45 min.

$$\textbf{Calculation(ALP activity)} = \frac{\textbf{A}\text{ serum sample} - \textbf{A}\text{serum blank}}{\textbf{A}\text{Std.}} \quad \textbf{20}$$

The calculate value was compared to the normal range.
Normal Range : Children : 10-20 KAU / dl
Adults : 3-13 KAU /dl

2.11 - Effect of the new compounds (19) , (20) , (21) on the Alkaline phosphates (ALP) activity in serum :

The effect of the new compounds (19),(20),(21) were calculate at different concentrations. A stock solution (0.5 g/25 ml) of each compound was prepared in DMSO. Dilution was

made to obtain the required concentration [($5.2x10^{-8}$ M) for (19) ,($3.7x10^{-8}$ M) for (20) and ($2.42x10^{-8}$ M) for (21)].

The measurement of enzyme activity was determined by the method described in section (2.10.2), (1 ml) from each compound was added to the substrate buffer .

2.12 : Determination of Acid phosphatase (ACP) activity

2.12.1 : Solutions :

1- Buffer solution : Citrate buffer (5.5 mmol /L) , pH = 4.8
2- Substrate : ρ – nitro phenyl phosphate (5.5 mmol/L)
3- Sodium tartrate (200 mmol/L)
4- NaOH (200mmol/L)
5- The contents of bottle 2 (substrate) were reconstitute with 10 ml buffer (1) .They were stable for (5) days at +2 to +8 ^{0}C
6- NaOH was diluted (10 ml NaOH + 90 ml distilled water)

2.12.2 : procedure of assay :

The following tubes were set up as follow :

Table (3) measurement of total ACP activity in serum

	Reagent blank	Sample 1	Sample 2
Substrate (2)	1.0 ml	1.0ml	1.0ml
Tartrate (3)	-	-	0.1 ml
Incubate for 5 min at 37^{0}C			
Serum	-	0.2 ml	0.2 ml
Incubate exactly for 30 min. at 37^{0}C			
Dilute NaOH	10 ml	10 ml	10 ml
Serum	20 ml	-	-

Mix , read the absorbance of the sample against the reagent blank at 405 nm .

Calculation :

Total acid phosphate : 101X A Sample 1

Prostatic acid phosphates : 101X (A Sample 1 – A Sample 2)

Normal value : Total acid phosphate : up to 11 u/L

Prostatic acid phosphates: up to 4 u/L

2.13 - Effect of the new compounds (19) , (20) , (21) on the Acid phosphates (ACP) activity in serum :

The effect of the new compounds (19),(20),(21) were calculate at different concentrations. A stock solution (0.5 g/25 ml) of each compound was prepared in DMSO. Dilution was made to obtain the required concentration [($5.2x10^{-8}$ M) for (19) ,($3.7x10^{-8}$ M) for (20) and ($2.42x10^{-8}$ M) for (21)].

The measurement of enzyme activity was done according to the method described in section (2.12.2), then (1 ml) from compound was added to the substrate buffer .

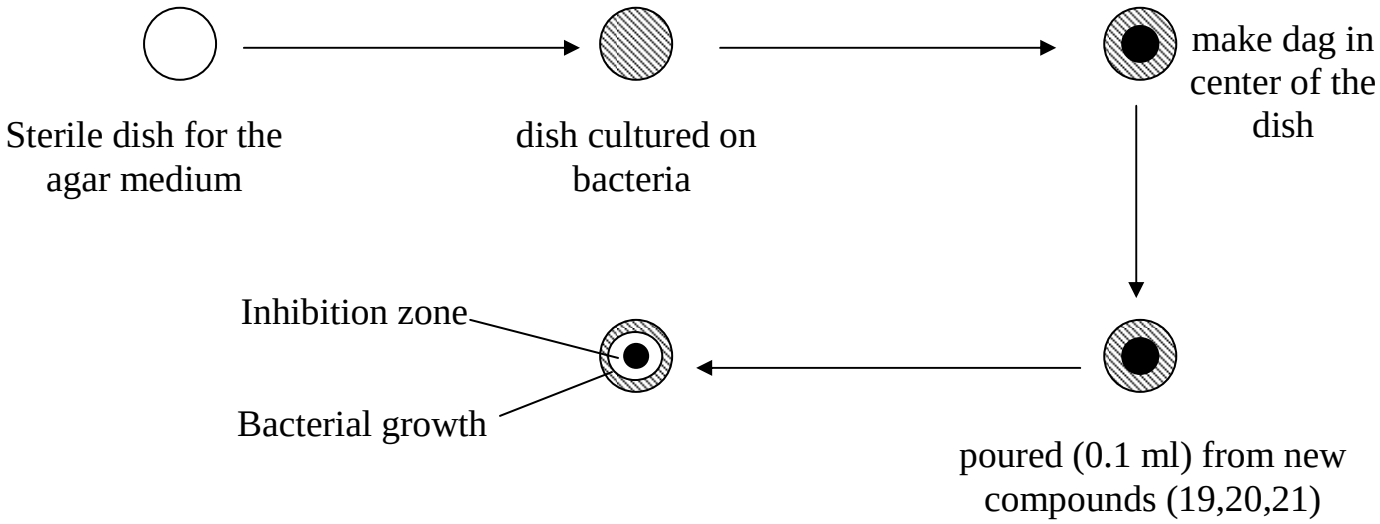
2.14 - Biological study :

In the current study five types of bacteria were tested : (*Staphylococcus aureus*), (*Pseudomonas aeruginasa*) , (*Salmonella typhimuriua*), (*Bacillus subtilus*), (*Escherichia coli*)., those bacteria were chosen because of their importance in the public healyh as pathogenic bacteria .The study also include (*Candida*) (yeast).

2.14.1 - Procedure:

Agar diffusion method was applied to the measurement the sensitivity of these bacteria toward the new compound:

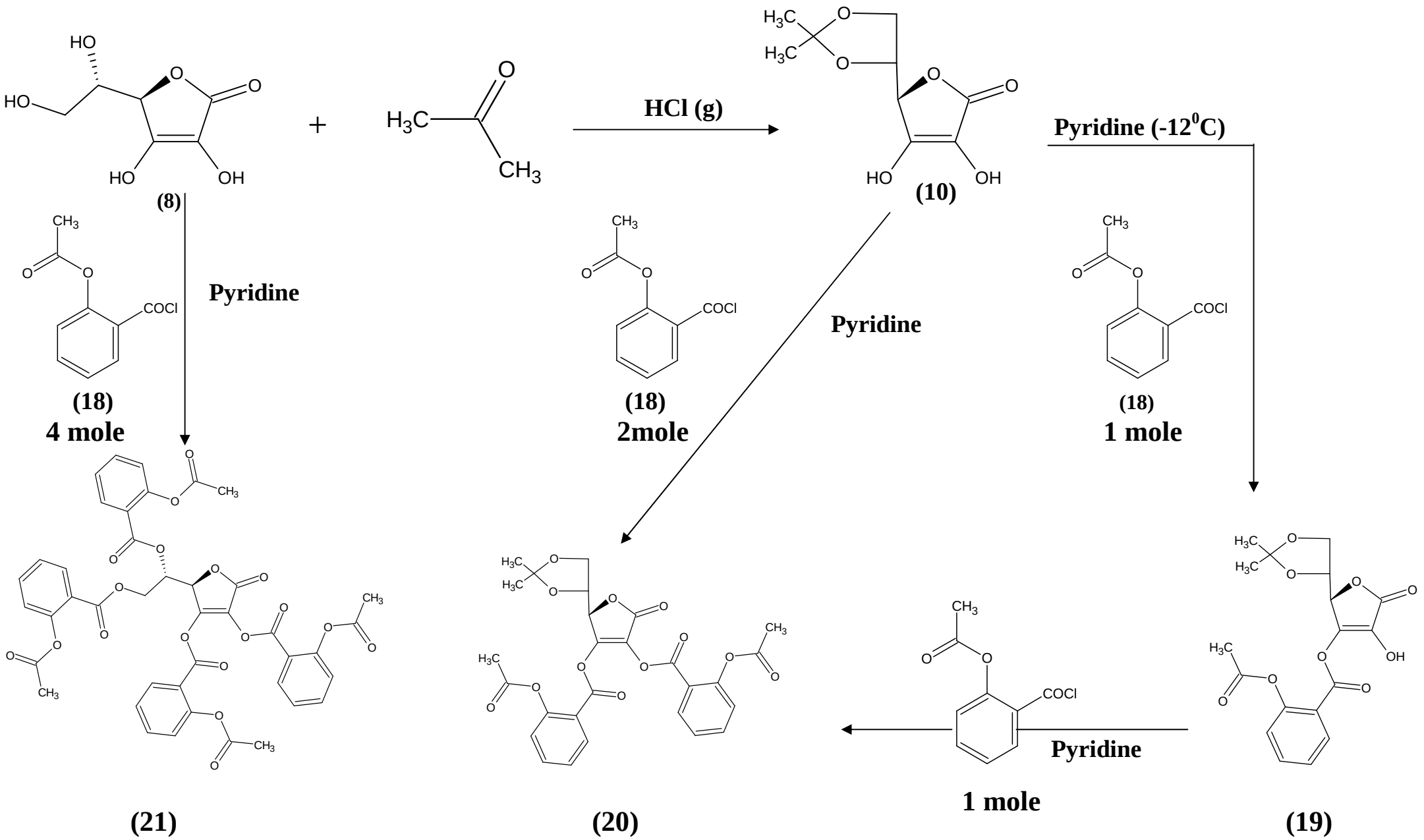
1- Dag was made in the center of bacterial agar dish
2- (0.1 ml) from the new compounds (19,20,21) was poured in the dag. The concentrations of the new compounds were calculated at different concentrations (0.5g/25ml) of each compound was prepared in DMSO. Dilution was made to obtain the required concentration [($5.2x10^{-8}$ M) for (19) , ($3.7x10^{-8}$ M) for (20) and ($2.42x10^{-8}$ M) for (21)] .
3- The dishes were incubated at 37^0C for 24 hr.
4- The inhibition zone of bacteria was measured.[148]



Scheme (2) procedure of biological study

2-15 : *In vivo* study :

Three individuals of English Angora rabbits weighing (1.80,1.92,2.1) Kg were fasted for 48 hrs. prior to drug administration with free access to drinking water. Each single rabbit forced fed 1.5g of tested drug (19,20,21); the drug introduced by a pice of cucumber contaminated with a tested dose. Blood samples were collected after and before the drug administration at different intervals of time (0,2,3,4,6,8,10 hr.) the blood was left at room temperature for about half an hour then the serum was separated by centrifuging for 15 minutes at 3500 rpm. and stored at -20^{o}C until analysis was performed . The concentration of aspirin was followed at fixed wave length at 277 nm[165] .


HCl (g)
Pyridine (-12^{0}C)
Pyridine
Pyridine
Pyridine
(8)
(10)
(18)
4 mole
(18)
2mole
(18)
1 mole
1 mole
(21)
(20)
(19)

We certify that this thesis was prepared under our supervision as a partial requirement for Ph.D.in biochemistry.

Signature :
Advisor: Pro.Dr.Abdul-Jabbar Mukhlis
Date :

Signature :
Advisor: Dr.Adnan Ibrahim Abuod
Date :

Signature :
Advisor: Dr.Nazar Ahmad Naji
Date :

In view of the available recommendations I Forward this thesis for debate by the Examining Committee.

Signature :
Name: Dr.Shahbaz Ahmed Maki
Head of Department of Chemistry/ College of Science University of Al-Nahrain.

Acknowledgements

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My special affection is extended to all my colleagues with whom I shared our common problems and ambitions.

Finally I would like to express my utmost respect to my family and wife who gave me all their love and support during my study.


To My Father, My Mother, My Wife & My Family & My children
With My Love And Respect
Firas 2005

SUMMARY

In this work new derivatives of *L*-ascorbic acid (Vitamin C) have been stynthezized . These derivatives have been obtained by the esterfication .

Selective esterfication of *C*-3 and *C*-2 hydroxyl group required protecting the two hydroxyl group at 5,6-position by conversion of vitamin C to 5,6-*O*-isopropylidene derivative (10)

3- (acetyl salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid (19) was synthesized by treatment of (10) with acetyl salicyloyl Chloride (18) .

2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid (20) was synthesized by treatment of (10) with two moles of acetyl salicyloyl Chloride (18) or treatment of (19) with one mole of acetyl salicyloyl Chloride (18).

2,3,5,6 -Tetra (acetyl salicyloyl)-5,6-*L*-ascorbic acid (21) was synthesized by treatment of vitamin C with four moles of acetyl salicyloyl Chloride (18)

The purity of the compounds were characterized by thin later chromatography (TLC) and infrared spectroscopy (IR).

The drug released study for hydrolysis of compounds (19,20,21) was carried out using different buffer at pH (2,4,6,8,10,12) over 30 hours at different intervals using UV spectroscopy which showed that these compounds were hydrophobic , but a period of 8-13h post starting to be hydrophilic . The released aspirin was increased as the pH is increased and extended time

The biochemical tests revealed that the evaluated compounds (19,20,21) showed non competitive inhibition behavior to the activity of acetyl choline esterase (AChE)

enzyme while these compounds showed an activation on alkaline phosphatase and acid phosphatase enzymes activity .

Bacterial inhibition zone revealed a positive inhibitory impact .

The *In vivo* study was carried out by (1.5 g) oral administration of the three compounds (19,20,21) and measurement of aspirin concentration in rabbits blood after (2,3,4,6,8,10) hrs. showing that the highest aspirin concentration was found after 4 hrs. of administration .

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General notes

1- Melting points were recorded on Elctrothermal melting point apparatus .

2- Infrared spectra were recorder on Shamadzu – FTIR 8000 series (KBr disk) at department of chemistry , college of science , Al-Nahrain university .

3- UV-spectra were recorder using Philips Pu-8720 uv/vis scanning spectrophotometer.

4- Thin layer chromatography (TLC) was performed on aluminum plates coated with (0.25 mm) layer of silica gel F_{254} (fluka) , compounds were detected by iodine vapor . all synthesized compounds were purified by silica – gel column .

5- All chemicals used supplied from Merk Chemicals , Fluka and BDH chemicals .

6- English Angora rabbits were used as laboratory animals .

2.1 :Synthesis of 5,6-O-isopropylidene-*L*-ascorbic acid (10) (61)

A saturated solution of *L*-ascorbic acid ($C_6H_8O_6$) M.Wt=176 g/mol (10.00 g ,57.00mmol) in 100 ml of freshly distilled acetone , (HCl) gas was bubbled at room temperature for 20 minutes , to this solution n-hexane (80 ml) was added , stirred , and decanted . The residue was washed with acetone – hexane (4:7) four times , then the solvent was removed under reduced pressure to give (10) (11.37 gm ,92% yield) as a white crystalline residue , **m.p.** 219-220^{0}C , **IR(KBr disk)** (υ cm^{-1}) 3240 (O-H) ,2995 (C-H) aliphatic 1755 (C=O) lactone .

2.2 : Synthesis of O- acetyl salicyloyl Chloride : (18) (141)

To a dry powder acetyl salicylic acid (17) (19.5mmol) in claisen flask was added redistilled thionyl chloride (1.74 ml) and the mixture was refluxed for 6 hours or until evolution of hydrogen chloride ceases .The reaction mixture was left to cool, the condenser was removed and the flask was heated at 60^{0}C for 3 minutes with occasional shaking .Excess thionyl chloride was removed under reduced pressure to give (18) . formula $C_9H_7O_3Cl$.(4.09 g , 95.94% yield) B.P. 135^{0}C . IR spectra (υ cm^{-1}) show stretching band at 1755 for (C=O) carboxylic acid chloride .

2.3 : Synthesis of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid : (19)

To an ice – cooled solution (-12^{0}C) of 5,6-O-isopropelidine-*L*-ascorbic acid (10) (4.21gm, 19.5 mmol) in pyridine , was added , drop wise , acetyl salicyloyl chloride (18) (4.37 gm ,19.5 mmol).The resulting reaction mixture was kept at room temperature for 24 hours , and then the cold distilled water (300 ml) and chloroform (400 ml) were added . The organic layer was separated , dried over anhydrous ($MgSO_4$) , filtered , and the solvent was removed under reduced pressure , (trace of

pyridine was removed under reduced pressure by co evaporation with toluene (3x50 ml)) . The remaining syrup was purified on a silica gel column to give (19) (6.80 gm , 86.38 % yield) , IR (υ cm^{-1}) ,3400-3082 (O-H), 2895 (C-H) aliphatic 1753 (C=O) ester , 1616,1485 (C=C) aromatic , 758 (C-H) out of plan .

2.4.A.:Synthesis of 2,3 - di (acetyl salicyloyl) - 5,6 – O – isopropylidene – *L* - ascorbic acid : (20)

To a solution of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene -*L*- ascorbic acid (19) (19.5mmol) in pyridine , was added , drop-wise, acetyl salicyloyl chloride (18) (4.37 gm ,19.5 mmol).The resulting mixture was kept at room temperature for 24 hours , and then the cold distilled water (300 ml) and chloroform (400 ml) were added . The organic layer was separated , dried over anhydrous ($MgSO_4$) , filtered , and the solvent was removed under reduced pressure , (trace of pyridine was removed under reduced pressure by co evaporation with toluene (3x50 ml)) . The remaining semi solid was purified on a silica gel column to give (20) (8.60 gm , 81.6 % yield) as semi solid , IR (υ cm^{-1}) , 2925 (C-H) aliphatic 1741 (C=O) ester , 1608,1485 (C=C) aromatic , 756 (C-H) out of plan.

2.4.B.: Synthesis of 2,3 – di (acetyl salicyloyl) - 5,6 – O – isopropylidene – *L* - ascorbic acid : (20)

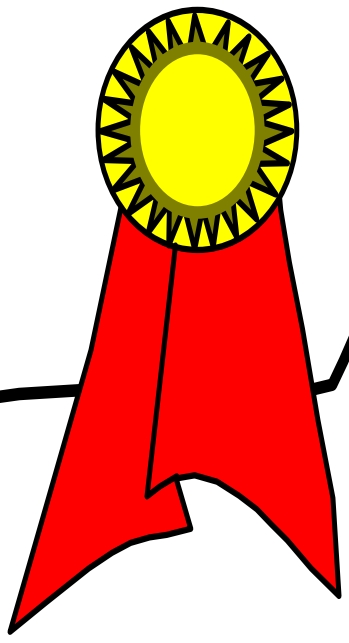
To a solution of 5,6 –O-isopropylidene -*L*- ascorbic acid (10) (4.21gm, 19.5 mmol) in pyridine , was added , drop wise , acetyl salicyloyl chloride (18) (8.74 g ,39.0 mmol).The resulting reaction mixture was kept at room temperature for 24 hours , and then the cold distilled water (300 ml) and chloroform (400 ml) were added . The organic layer was separated , dried over anhydrous ($MgSO_4$) , filtered , and the solvent was removed under reduced pressure , (trace of pyridine was removed under reduced pressure by co evaporation with toluene (3x50 ml)) . The remaining semi solid was purified on a silica gel column to

give (20) (8.20 gm , 81.1 % yield) as semi solid , IR (υ cm^{-1}) , 2925 (C-H) aliphatic 1741 (C=O) ester , 1608,1485 (C=C) aromatic , 756 (C-H) out of plan.

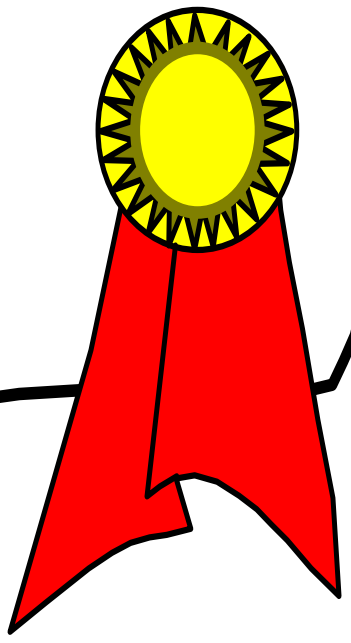
2.5: Synthesis of 2,3,5,6 -Tetra (acetyl salicyloyl)-*L*-ascorbic acid : (21)

To a solution of *L*- ascorbic acid (8) (4.21g, 24mmol) in pyridine , was added , drop-wise , acetyl salicyloyl chloride (18) (17.0 g ,96.0 mmol).The resulting reaction mixture was kept at room temperature for 24 hours , and then the cold distilled water (300 ml) and chloroform (400 ml) were added . The organic layer was separated , dried over anhydrous ($MgSO_4$) , filtered , and the solvent was removed under reduced pressure , (trace of pyridine was removed under reduced pressure by co evaporation with toluene (3x50 ml)) . The remaining syrup was purified on a silica gel column to give (21) (18.51 gm , 84.38 % yield) , IR (υ cm^{-1}) , 2858 (C-H) aliphatic 1730 (C=O) ester , 1568,1444 (C=C) aromatic , 758 (C-H) out of plan.

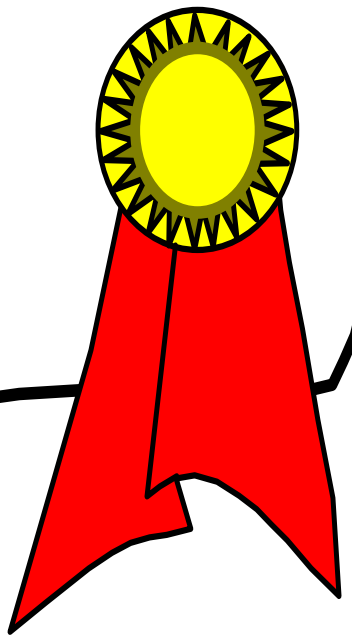
Chapter One
Introduction



Chapter Two Experimental



Chapter Three Result and discussion

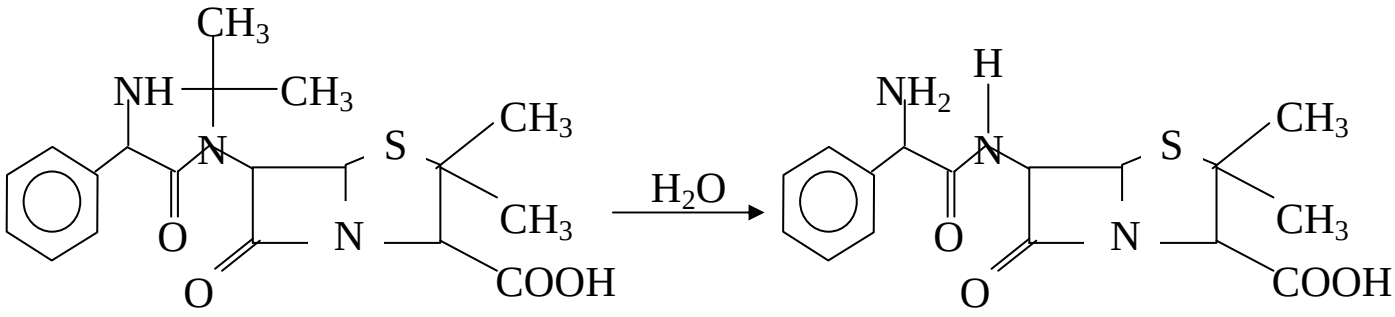


1.1 The Pro-drugs:

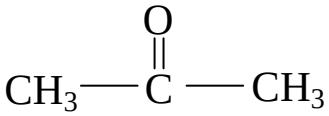
In 1958 Albert initially coined the term “pro-drug” and used it to refer to pharmacologically inactive compound that is transformed into an active substance by either chemical or metabolic means.[1,2]

Design and synthesis of pro-drug have been used to solve various problems associated with the use of many drugs . There are several advantages that may be gained by generating a pro-drug , such as increase absorption , alteration of pain at the site of injection if the agent is given parentally , elimination of an unpleasant taste associated with the drug ,decrease toxicity ,decrease metabolic inactivation , increase chemical stability , and a prolong or shorten action ,whichever is desired in particular agent[2].

Some pro-drugs relay upon chemical mechanisms for conversion of the pro-drug into its active form , for example , hetacillin is a pro-drug form of ampicillin in which the amide nitrogen and α- amino functionalities have been allowed to react with acetone to give an imidazolidinone ring system [2,3,4,5,6] . This has the effect of decreasing the basicity of the α- amino group and reducing protonation in the small intestine so that the agent would become more lipophilic. In this manner , the absorption of hetacillin from the small intestine will be increased after oral administration and chemical hydrolysis to regenerate ampicillin .In such approach , it is necessary to add a moiety or a promoiety to ampicillin which is in this case acetone for being non toxic and can easily be removed after performing its function

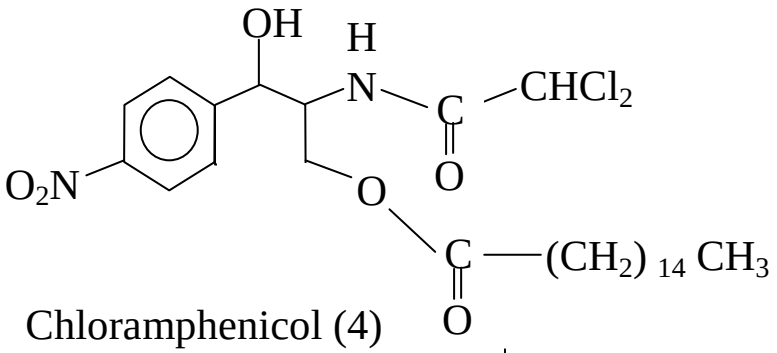


Hetacillin (1) Ampicillin (2)

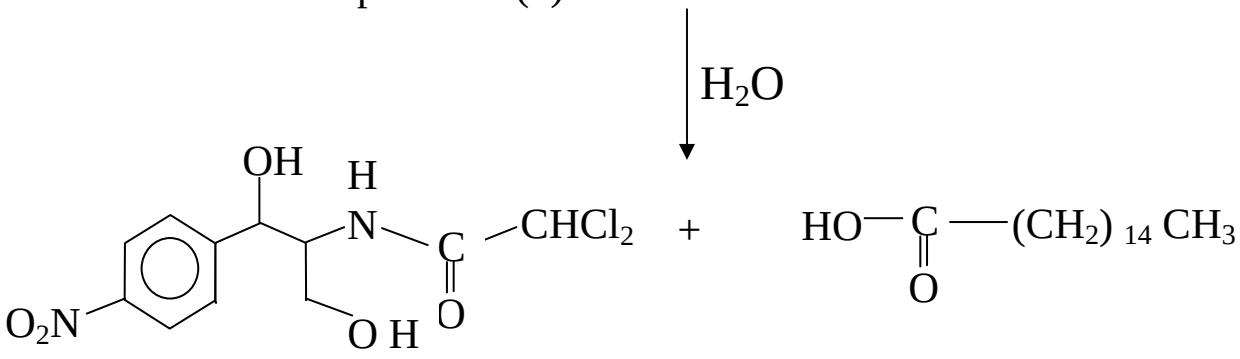
Acetone (3)  +

Pro-drug agents that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester . This is the most commonly seen type of pro-drug , due to the ease in which the ester can be hydrolyzed to give the active drug . Hydrolysis is normally accomplished by esterase enzyme which is present in plasma and other tissues which is capable of hydrolyzing a wide variety of ester linkages .[6,7]

A pro drug with low water solubility behavior has been utilized in the case of the antibacterial Chloramphenicol , which produces a bitter taste when given as the parent drug [7] . The ester moiety is subsequently hydrolyzed in the gastrointestinal tract , and the agent is absorbed as Chloraphenicol .



Chloramphenicol (4)



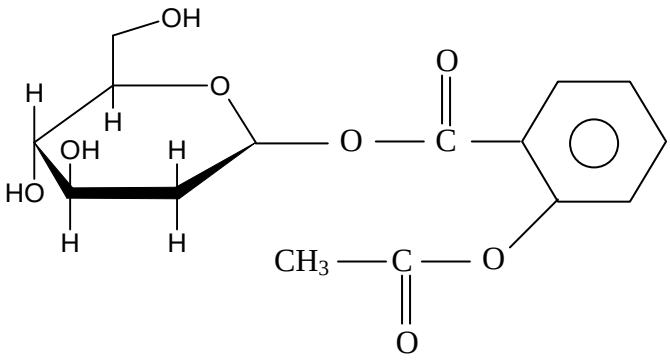
Chloraphenicol (5) Palmatic acid (6)

In another approach several hundreds analogues of aspirin have been made and tested in order to produce a compound that is more potent, longer acting with less gastric irritation .

Oral administration of aspirin induces gastric irritation and sometimes bleeding because of local irritation of the gastric mucosal membrance by the very acidic aspirin particles . In this approach and in order to minimize this side effect is to mask the acid carboxyl group of aspirin reversibly via a pro-drug which upon administration , dissolves first and then hydrolyzed to generate aspirin in the solution .

The transient blocking of the acidic carboxylic group of aspirin by acetal-linked derivative can result in a pro-drug that regenerates aspirin at an acceptable rate independent pH [8,9] .

The 1-*O* – (2-acetoxy) benzoyl – α- D-2 –deoxyglucopyranose (7) is an example of these pro-drugs [10]



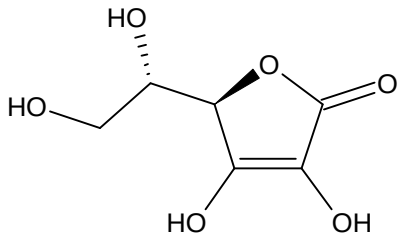
(7)

1.2:Vitamin C (L-Ascorbic Acid)

A vitamin may broadly defined as substance that is essential for the maintenance of normal metabolic function but not synthesized in the body and therefore must be furnished from an exogenous source .

Vitamin can be classified into water – soluble vitamin and fat – soluble vitamin , vitamin C is one of water soluble vitamin group[11]

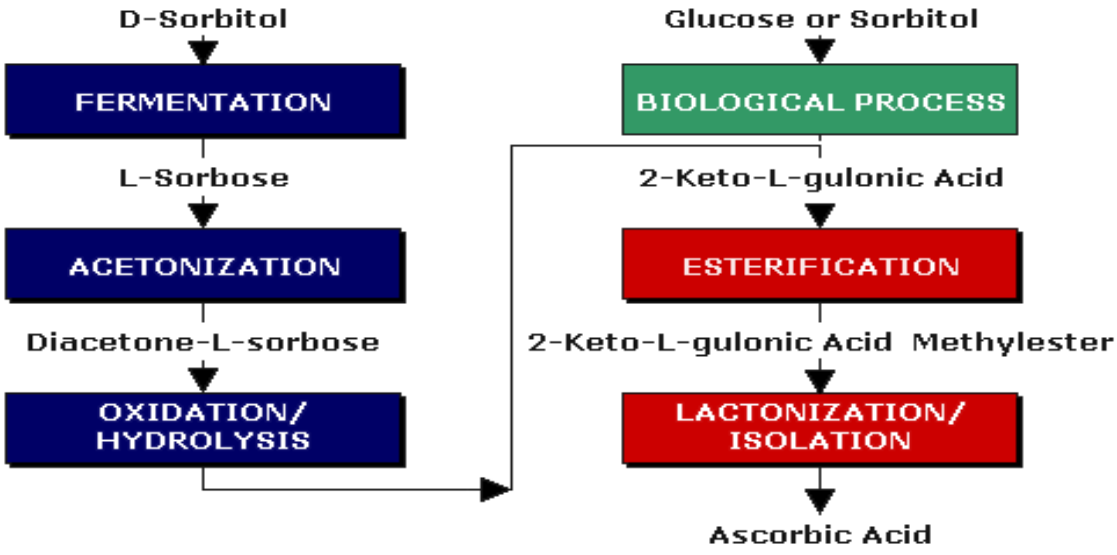
Vitamin C is well known as L-ascorbic acid because of its acidity character and its effectiveness in the treatment and prevention of scurvy [1] .Vitamin C was first isolated in pure form in 1925 by Bezsonoff [12,] , but did not recognize it as an anti scorbutic agent until 1928 [13,] , and the structure was not elucidated until 1933[14,15] and synthesized by Reichstein [16] having the structure shown :



Vitamin C (L-Ascorbic Acid)

(8)

Ascorbic acid is derived from glucose via the glucuronic acid pathway. The enzyme L-gluconolactone oxidase which is responsible for the conversion of gluconolactone to ascorbic acid which is absent in primates; making ascorbic acid required in the diet.[166]



The active form of vitamin C is ascorbic acid itself. The main function of ascorbate is as a reducing agent in a number of various reactions. Vitamin C has the potential to reduce cytochromes a and c of the respiratory chain as well as molecular oxygen. The most important reaction requiring ascorbate as a cofactor is the hydroxylation of proline residues in collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing since synthesis of connective tissue is the first event in wound tissue remodeling. Vitamin C is also necessary for bone remodeling due to the presence of collagen in the organic matrix of bones. Vitamin C is very important reducing agent for reduction Fe^{+++} to Fe^{++} as anti anemic factor,

Several other metabolic reactions require vitamin C as a cofactor. These include the catabolism of tyrosine and the synthesis of epinephrine from tyrosine and the synthesis of the bile acids. It is also believed that vitamin C is involved in the process of steroidogenesis since the adrenal cortex contains high levels of vitamin C which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland.

Deficiency in vitamin C leads to the disease named scurvy due to the role of the vitamin in the post-translational modification of collagens. Scurvy is characterized by easily bruised skin, muscle fatigue, soft swollen gums, decreased wound healing and hemorrhaging, osteoporosis, and anemia. Vitamin C is readily absorbed and so the primary cause of vitamin C deficiency is poor diet and/or an increased requirement. The primary physiological state leading to an increased requirement for vitamin C is severe stress (or trauma). This is due to a rapid depletion in the adrenal stores of the vitamin. The reason for the decrease in adrenal vitamin C levels is unclear but may be due either to redistribution of the vitamin to areas that need it or an overall increased utilization [127].

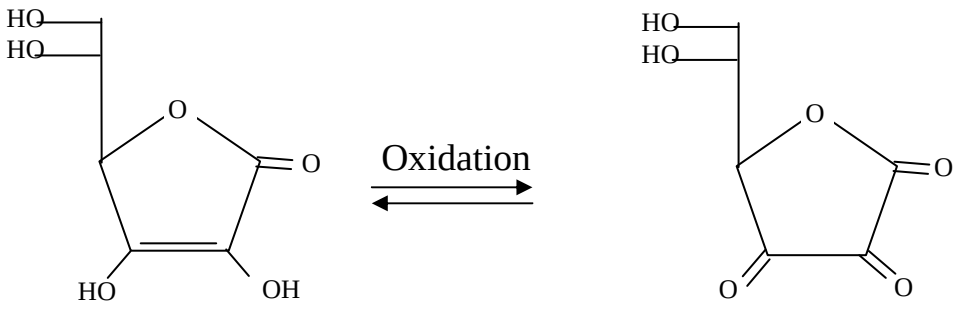
1.2.1:Properties and chemistry :

Vitamin C occurs as a white or slightly yellow , water – soluble crystals (usually plates , sometime needles, monoclinic system) ,it is odorless and gradually darkens upon exposure to light while stable to air when dry [16] .

Vitamin C is known as L- ascorbic acid because of its acidity character , which is due to the two enolic hydroxyls , this hydroxyl group on carbon -3 has the Pk_a value 4.1 and the hydroxyl group on carbon -2 has the Pk_a 11.6 [1] .

One gram of L-ascorbic acid dissolves in about 3ml water and in about 30 ml alcohol , 1% aqueous solution has pH 4.7 [1,17] .

Vitamin C is stable in solution only in the absence of oxygen; it is a strong reducing agent being very easily dehydrogenated to dehydroascorbic acid (9) in the body and this reaction is reversible. [17,18,19,20]



(8) (9)

Vitamin C is derived from 2-keto-gluconic acid [21], whereas the lactone ring of L-ascorbic acid is particularly stable, that of oxidized product is easily opened to give 2,3-diketo-L-gluconic acid which undergoes further rapid decomposition. [22]

The effect of pH on degradation of aqueous solution of L-ascorbic acid is widely investigated; Rogers and Yacomeni found that the maximum and minimum degradation occurred near pH 4 and 5.6 respectively .

Vitamin C as an antiviral agent is in controversial , but some scientists still argue that L-ascorbic acid is an effective curative behavior or preventative of " common colds " [1,17,23] . In recent years there has been growing interest in the therapeutic application of L-ascorbic acid and it's derivatives as anticancer agents. [24] It has been demonstrated in cell-culture studies that L-ascorbic acid both alone and in combination with copper ions, is selectively toxic to melanoma cancer cells [1,25,26] .

The deficiency of vitamin C may result in several diseases such as scurvy, anemia and dental carriers. [20,27,28] .

1-2-2: Interaction effects of vitamin C with drugs

The administration of several drug can cause different impacts upon vitamin C behavior in the biological system as follows :

1-**Acetaminophen** [29,30,31]

Oral administration of 3gms. of ascorbic acid for 1.5 hour after an oral dose of 1 gm of acetaminophen caused pronounced decrease in the excretion rate of acetaminophen sulfate, so it might be able to use lower doses of acetaminophen to achieve equal or superior clinical result, and reduce side effects from the drug by combining with some form of vitamin C.

2-**Acetyl salicylic acid (aspirin)**[32,33]

Taking aspirin has been associated with increased loss of vitamin C in urine and has been linked to depletion of vitamin C . People who take regularly should consider supplementing at least a few hundred milligrams of vitamin C per day .Such an amount is often found in a multivitamin .

3-**Corticosteroids** [34]

Steroidal, anti-inflammatory drugs cause increased loss of vitamin C through the urine. However high doses of vitamin C (equal to or greater than 500 mg per day) may raise the blood levels of anti-inflammatory and other acidic medications.

4-**Cyclophosphamide** [35,36]

Individuals taking cyclophosphamide might experience greater efficiency of their medication with the simultaneous use of vitamin C.

5-**Deferoxamine** [37]

Vitamin C supplementation, even at low levels (200 mg per day), has consistently enhanced the ability of deferoxamine to let the body to get rid of excess iron even though vitamin C may slightly increase iron absorption.

6-**Doxorubicin** [38]

Vitamin C could potentially inhibit the therapeutic mechanism of doxorubicin, which relies upon the cytotoxic effect of free radical formation.

7-**Haloperidol** [39,40,41,42,43]

Some evidences indicate that ascorbic acid modulates dopamine transmission in the portion of the brain called the striatum and increases the activity of haloperidol.

8-**Isoniazid** [44]

In vitro, research indicates beneficial effects from vitamin C supplementation upon the formation of the free radical intermediates due to isoniazid and its metabolites.

9-**Furosemide** [45]

In a research involving rats; they found that significant increases in the diuretic and natriuretic effects of furosemide with vitamin C could be the result of increases in the re-absorption of furosemide at the renal tubular receptor sites.

10-**Nitroglycerin** [46,47,48]

The co-administration of vitamin C and nitroglycerin fully maintained the nitroglycerin-induced changes in the orthostatic blood pressure. These findings demonstrate that dietary supplementation with vitamin C eliminates vascular tolerance during long-term administration of nitroglycerin.

11-**Oral Contraceptive** [49,50,51]

The use of oral contraceptives has been consistently associated with decreased levels of vitamin C as a result of their interference with the metabolism of ascorbic acid.

12-**Tetracycline** [52,53,54,55]

Some research indicated that tetracycline can interfere with the activity of vitamin C, several studies have suggested that vitamin C can play a valuable role through its protective effect against tetracycline-induced kidney and liver damage.

13-**Warfarin** [56,57,58,59,60]

A variety of cases reports have suggested a possible interaction between warfarin and vitamin C in which the drug's activity was increased, while the large doses of vitamin C may diminish the anticoagulant activity of warfarin.

1-2-3: Derivative of L-ascorbic acid

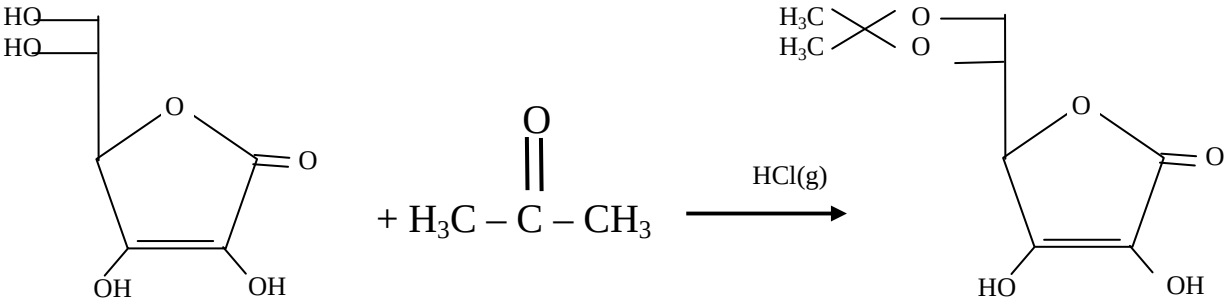
1: 5,6-O-cyclic acetal derivatives

All 5,6-O-cyclic acetal derivatives possess pharmaceutical activity similar to L-ascorbic acid superior in crystalline, stability and antioxidant effect to L-ascorbic acid and its known derivatives [61,62,63]

These derivatives have been shown to exert anticancer effect in patients without causing side effects [64,65,66,67], they are known as anti tumor substances [68,69,70,71], exhibit potent antioxidant effects, as free radical scavengers [72,73] and reduce the arterial blood pressure and heart rate [74].Below are some of these derivatives

- 5,6-O-isopropylidene- L-ascorbic acid (10) [61]

5,6-O-isopropylidene- L-ascorbic acid (10) was first synthesized in 1963 by bubbling HCl gas into a solution of powder L-ascorbic acid (8) in dry acetone, n-hexane added and the mixture stirred to yield 85-90 % of the Isopropylidene derivative (10).

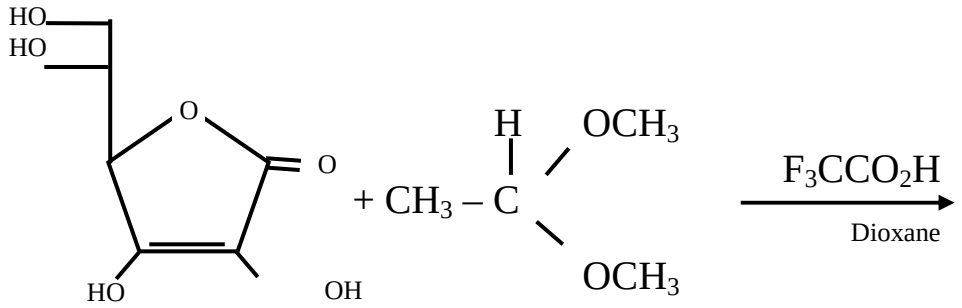


(8) (10)

5,6-O-isopropylidene- L-ascorbic acid

❖ 5,6-O-ethylidene- L-ascorbic acid (11) [62]

5,6-O-ethylidene- L-ascorbic acid (11) was first synthesized in 1963 by heating (8) on a steam bath with 1,1-di methoxy ethane in Dioxane and F_3CCO_2H, to yield (11) in 90 %.



L – Ascorbic Acid

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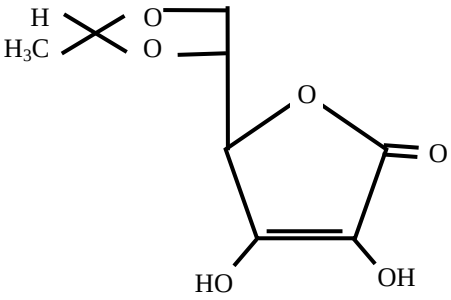
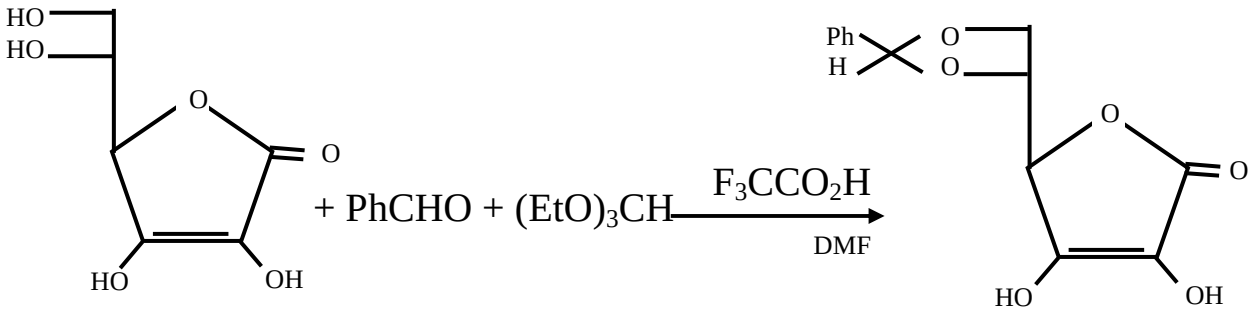


5,6 – O – Ethylidene – L – Ascorbic Acid

(11)

❖ 5,6-O-benzylidene- L-ascorbic acid (12) [63]

5,6-O-benzylidene- L-ascorbic acid (12) was prepared in 1971 by treatment of L-ascorbic acid (8) with benzaldehyde and triethyl orthoformate in dimethyl sulphoxide (DMSO) and trifluoroacetic acid (F_3CCO_2H).



L – Ascorbic Acid

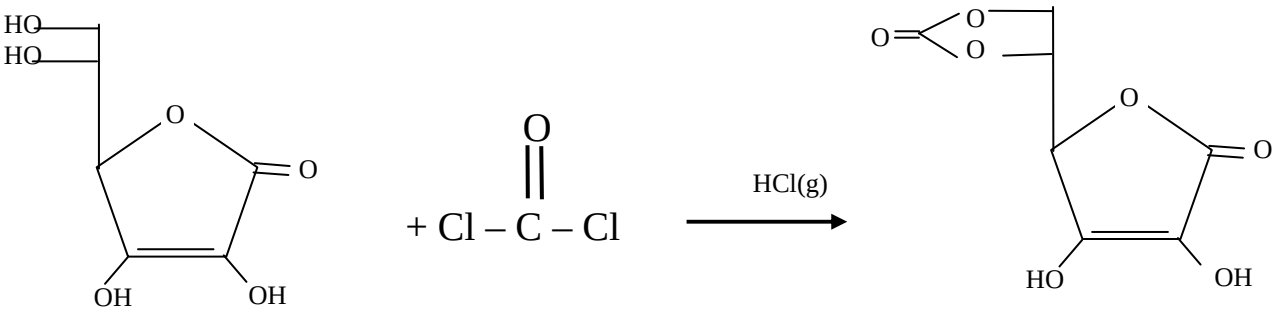
(8)

5,6 – O – Beznylidene – L – Ascorbic Acid

(12)

2: 5.6-O-carbonyl- L-ascorbic acid (13) [75,76]

This derivative was synthesized in 1966 by treating L-ascorbic acid (8) with carbonyl dichloride (phosgene) in anhydrous Tetrahydrofuran (THF) at room temperature.



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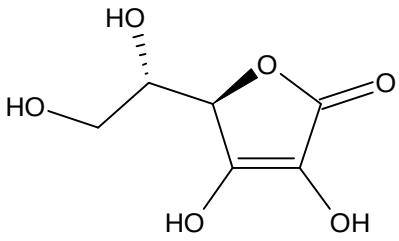
5.6-O-carbonyl- L-ascorbic acid

3: L-Ascorbic acid esters

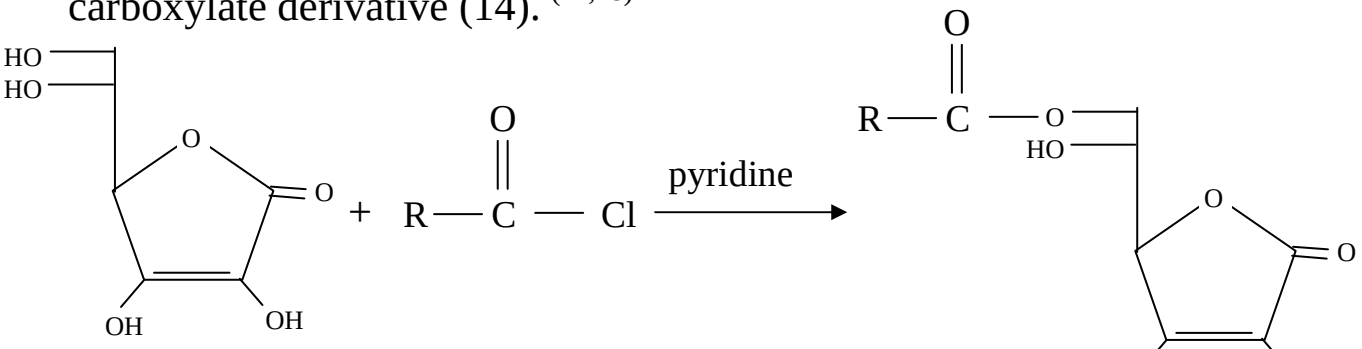
L-ascorbic acid (8) has four hydroxyl groups, and all these groups are active for classical esterfication. Carbon-6 hydroxyl group , (a primary hydroxyl group) is the most reactive group. [77,78]



Vitamin C (L-Ascorbic Acid)

Esterfication at 6-position

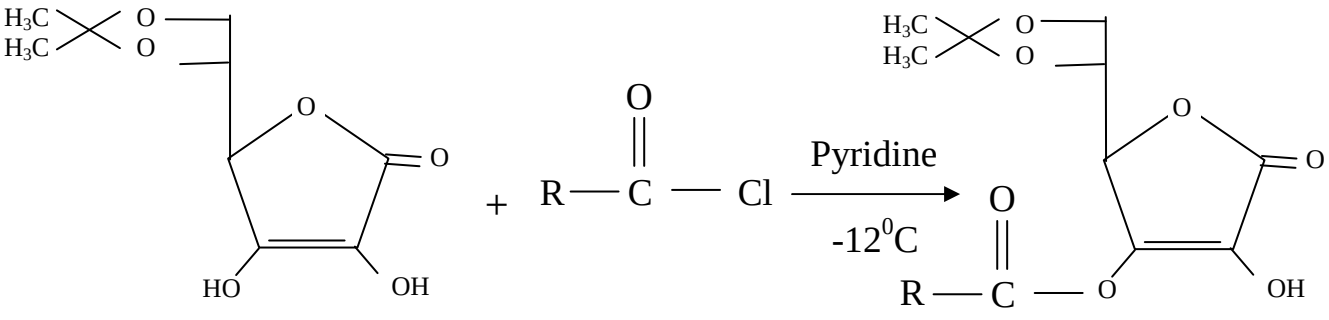
When one mole of L-ascorbic acid was added to a mole of acid chloride in pyridine at room temperature the reaction will yield a carboxylate derivative (14). [77,78]



(8) (14)

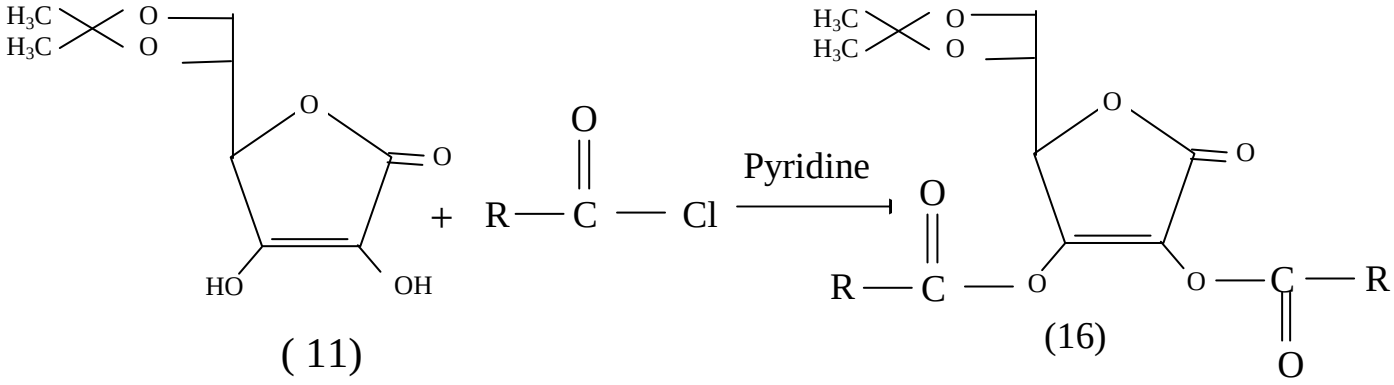
Esterfication at 3-postion

The esterfication of carbon-3 hydroxyl group requires blocking on the two-hydroxyl groups at 5-and 6-positions. The hydroxyl group at 3-position is more reactive toward esterfication than the hydroxyl group at 2- position . These esters may by prepared by treatment of (11) with acid chloride in pyridine at –12 ◌C and the reaction mixture is kept at room temperature overnight to produce (15), which upon hydrolysis in a mixture of dioxane and (1N) HCL (2:1) gives L-ascorbic acid –3-ester (15).[79,80]



(11) (15)

Treatment of (11) with excess of acid chloride in pyridine at room temperature overnight will afford the fully acylated product (16). [81]



(11) (16)

1.2.4:Physical properties

The pKa value of ascorbic acid is exceedingly decreased when esterification of the 2 and 3- positions is taken place [77].

The tri acylated L-ascorbic acid at 2,3,6-positions is more stable than L-ascorbic acid. Esterification with palmitoyl chloride at 6-position did not prevent the hydrolysis of molecule, either in solution or in emulsion, only the special preparation of products with high viscoelastic properties was able to reduce the typical behavior of these compound conversely, esterification at 2,3-position protected the molecule from break-up to the enediol system, these esters are very stable derivatives of L-ascorbic acid and may be easily used in various types of cosmetic products. [81,82,83]

Palmatic acid esters of L-ascorbic acid are slightly soluble in water and in vegetable oils. Long-chain fatty acid esters of L-ascorbic acid are more soluble and suitable for use with lipids than L-ascorbic acid.[1] These lipophilic antioxidants incorporated into cellular membranes are effective protectors agent against lipid peroxide-induced endothelial injury. [84]

1.2.5: Metabolism , Doses ,and Toxicity[121] :

Ascorbic acid is readily absorbed and metabolized . However , after oral administration of large quantities , only small amount are excreted in the urine while there is a steady rise in the level of ascorbic acid in blood serum . If the oral ingestion is continued for a sufficient period , the serum concentration rises to a maximum , after which a rapid urinary execretion of a large part of the ingested ascorbic acid occurs

a- Doses

The minimum daily requirement of ascorbic acid in adults is between 10-30 mg daily , the normal blood-level being 0.4 to 1.5 mg% . it is estimated that the daily dietary intake is between 30-100 mg .

b-Toxicity

LD50 (oral) **:** in mice and rats is 5 mg/Kg

1-1-4: Biological activity of L-ascorbic acid derivatives

Some of L-ascorbic acid derivatives with their biological activities are shown in table (1).

Table (1): shows some of L-ascorbic acid derivatives and their biological effects.

L-ascorbic acid derivatives	Biological effects	Ref.
2-O-poly phosphate L-ascorbic acid	1. As a vitamin C source.	85,86
2-O-Sulfate- L-ascorbic acid	1- As a vitamin C source. 2- Antiascorbutic agent.	87,88, 89 ,90,91
2-O-phosphate-L-ascorbic acid	1- as a vitamin C source . 2- Antiascorbutic agent . 3- Stamulation of collagen synthesis .	88,92,93,94 91 95,96,97,98

	4- Increase in activity of alkaline Phosphatase in human osteoblast cell line .	99
(2 or6)-*O*- stearoyl-L-ascorbic acid	1- Lipophilic antioxidant agent it has protective effect on lipid peroxide-induced endothelial injury. 2- Increased the antioxidant activity of alpha-tocopherol. 3- Antineoplastic agent. 4- Inhibition of human glioma cell proliferation and glutathione-s-transferase enzyme.	84 100 101 102,103,104
(2 or 6)-O-palmitoy- L-ascorbic acid	1-Antioxidant effect on stability of novel ditheranol ointment. 2-Antioxidant agent and free radical scavenger. 3-Lipophilic antioxidant agent it has protective effect on lipid peroxide-induced endothelial injury. 4-Antimutagen with membrane action. 5-Anti skin tumor. 6-Antineoplastic agent. 7-Inhibition of human glioma cell proliferation and glutathione-s-transferase. 8-Can be used as a retaining substance for cosmetic oils.	105 106 84 107 108 101 104,105 109
6-O-phosphate- L-ascorbic	1- Antioxidant agent.	110

acid		
2,6-O-di palmitoyl- L-ascorbic acid	1- Lipophilic antioxidant agent which has protective effect on lipid peroxide-induced endothelial injury.	84

1-3: Analgesic Agents

An analgesic agent may be defined as a drug bringing about insensibility to pain without loss of consciousness.[1] , Tainter has divided the history of analgesic drugs into 4 major, as following: [111]

1- The period of discovery and the use of naturally occurring plant drug.
2- Isolation of pure plant principles, e.g. alkaloids from the natural sources and their identification with analgesic action.
3- Development of organic chemistry and the first synthetic analgesics.
4- Development of modern pharmacologic techniques, making it possible to undertake a synthematic testing of new analgesic.

In the third eras, the first synthetic analgesics used in medicine were the salicylates, these originally were found in the nature (methyl salicylate , salicin) and then they were synthesized by chemists. [1]

1-3-1: Nonsteroidal anti-inflammatory antipyretic analgesics

anti-inflammatory , analgesics and antipyretic drugs are heterogenous group of compounds, often chemically unrelated (although most of them are organic acid) which nevertheless share certain therapeutic action and side effects. [112]

These drugs inhibit the enzyme cyclooxygenase, which catalyzes the synthesis of cyclic endoperoxides, which is important in the formation of prostaglandins. [113]

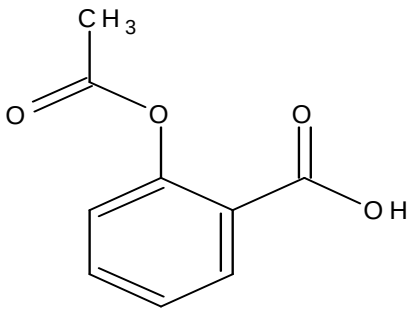
1-3-2 : Salicylic acid derivatives (the salicylates)

Salicylic acid (orthohydroxy benzoic acid) is highly irritating agent that can be used only externally, and therefore various derivatives of this acid have been synthesized for systemic use. These comprise two large classes, namely, esters of salicylic acid obtained by substitution in the carboxyl group, and salicylate esters of organic acids in which the carboxyl group of salicylic acid is retained and substitution is made in the OH group, for example aspirin is an ester of acetic acid.

Substitution on the carboxyl or hydroxyl groups serves only to change the potency or toxicity of the compound , the ortho position of the OH is an important feature for the action of the salicylate[(11)], the salicylates are readily absorbed from the stomach and the small intestine , being quite dependent on the pH of the media , the absorption is considerably slower as the pH rises (more alkaline) , due to the acidic nature of these compounds and the necessary for the presence of undissociated molecule for absorption through the lipodal membrane of the stomach and the intestine .

Aspirin (17) or O-acetyl salicylic acid was first prepared in 1853 by Gerhard but remained obscure until Felix Hoffmann discovered pharmacological activates in 1899 , it was tested and introduced to medicine by Dreser who named it aspirin by taking the **“a”** from acetyl and adding it to **“spirin”** an old name for salicylic or spiric acid derived form its natural source of spirea plant [(1)] .

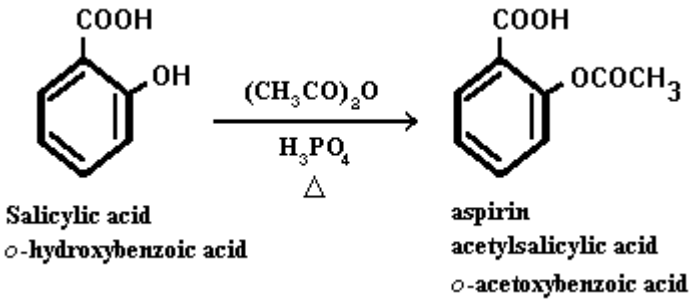
Aspirin occurs as white crystals or as a white crystalline powder , it is slightly soluble in water (1:300) , alcohol (1:5) , chloroform (1:17) and ether (1:15) , it is stable in dry air , but in the presence of moisture , it slowly hydrolyzes to acetic acid and salicylic acid [(1)] .



(17)

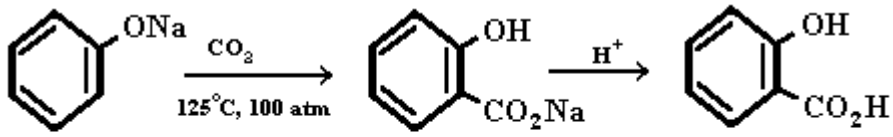
Aspirin , a weak acid with pK_a (3.5) , is rapidly and completely absorbed from the upper gastrointestinal tract .Factor affecting the absorption of aspirin including dosage , intragastric pH , and the rate of gastric emptying [114] .

One of the best known aromatic acetates is acetylsalicylic acid, or aspirin, which is prepared by the esterification of the phenolic hydroxyl group of salicylic acid.



Aspirin possesses a number of properties that make it the most often recommended drug. It is an analgesic, effective in pain relief, It is also an anti-inflammatory agent, providing some relief from the swelling associated with arthritis and minor injuries. Aspirin is also an antipyretic compound, which means it reduces fever. Each year, more than 40 million pounds of aspirin is produced in the united states alone, that translates to about 300 tablets per year for every man, woman and child. However, it is not so innocuous as drug as one might imagine from its widespread use and ready availability. Repeated use may cause gastrointestinal bleeding, and large doses can provoke a host of reactions including vomiting, diarrhea, vertigo and hallucinations. The average dose is 0.3-1 g; single doses of 10-30 g can be fatal.[115]

The key compound in the synthesis of aspirin, salicylic acid, is prepared from phenol by a process discovered over 100 years ago by the German chemist Hermann Kolbe. In the *Kolbe synthesis* (also known as the *Kolbe-Schmitt reaction*) sodium phenoxide is heated with CO_2 under pressure and the reaction mixture is subsequently acidified to yield salicylic acid.[116]



Sodium phenoxide Sodium salicylate Salicylic acid (79%)

The Kolbe Synthesis

1-3-3 : Drug Interactions and Dosage

1-Gastrointestinal reactions:

Doses of 1,000 mg per day of aspirin caused gastrointestinal symptoms and bleeding that, in some cases, were clinically significant. In the largest postinfarction study; the Aspirin Myocardial Infarction Study (AMIS) with 4,500 people, the percentage of incidences of gastrointestinal symptoms for the aspirin (1,000 mg of a standard, solid-tablet formulation) and placebo-treated subjects, respectively, were stomach pain (14.5%, 4.4%), heartburn (11.9%, 4.8%), nausea and/or vomiting (7.6%, 2.1%), hospitalization for GI disorder (4.9%, 3.5%). aspirin-treated patients had increased rates of gross gastrointestinal bleeding. Symptoms and signs of gastrointestinal irritation were not significantly increased in subjects treated for unstable angina with buffered aspirin in solution.

2-Cardiovascular and Biochemicals:

The dosage of 1,000 mg per day of aspirin was associated with small increases in systolic blood pressure (BP) (average 1.5 to 2.1 mm) and diastolic BP (0.5 to 0.6 mm), depending upon whether maximal or last available readings were used. Blood urea, nitrogen and uric acid levels were also increased but by less than 1.0 mg percent. Subjects with marked hypertension or renal insufficiency had been excluded from the trial so that the clinical importance of these observations for such subjects or for any subjects treated over more prolonged periods is not known. It is recommended that patients placed on long-term aspirin treatment, even at doses of 300

mg per day, be seen at regular intervals to assess changes in these measurements.

3-In Transient Ischemic Attacks:

At dosages of 1,000 milligrams or higher of aspirin per day, cause gastrointestinal side effects include stomach pain, heartburn, nausea and/or vomiting, as well as increased rates of gross gastrointestinal bleeding in some patients.

4-Uricosuric Agents:

Aspirin may decrease the effects of probenecid , sulfinpyrazone, and phenylbutazone.

5-Alcohol:

Has a synergistic effect with aspirin in causing gastrointestinal bleeding.

6-Corticosteroids:

Concomitant administration with aspirin may increase the risk of gastrointestinal ulceration and may reduce serum salicylate levels.

7-Pyrazolone Derivatives (phenylbutazone, oxyphenbutazone, and possibly dipyrone):

Concomitant administration with aspirin may increase the risk of gastrointestinal ulceration.

8-Nonsteroidal Antiinflammatory Agents:

Aspirin is contraindicated in patients who are hypersensitive to nonsteroidal antiinflammatory agents.

9-Urinary Alkalinizers:

Decrease aspirin effectiveness by increasing the rate of salicylate renal excretion.

10-Phenobarbital:

Decreases aspirin effectiveness by enzyme induction.

11-Phenytoin:

Serum phenytoin levels may be increased by aspirin.

12-Propranolol:

May decrease aspirin's anti-inflammatory action by competing for the same receptors. Enteric Coated Aspirin should not be given concurrently with antacids, since an increase in the pH of the stomach may effect the enteric coating of the tablets [140].

13-ACE inhibitors: The effects of ACE inhibitors may be blunted by aspirin administration, particularly at higher dosages.

- Buspirone increases aspirin's free % *in vitro*. [١١٧]

- Carbonic anhydrase inhibitors and corticosteroids have been associated with alteration in salicylate serum concentrations.

- Heparin and even at low molecular weight heparins in the Concurrent use may increase the risk of bleeding. [١١٨]

- Methotrexate serum levels may be increased; consider discontinuing aspirin 2-3 days before high-dose methotrexate treatment or avoid concurrent use.

- Probenecid effects may be antagonized by aspirin. [١١٩]

- Sulfonylureas: The effects of older sulfonylurea agents (tolazamide, tolbutamide) may be potentiated due to displacement from plasma proteins. This effect does not appear to be clinically significant for newer sulfonylurea agents (glyburide, glipizide, glimepiride). [١٢٥]

1-3-4 : Metabolism , Dosage and Toxicity[122]:

Metabolism

Aspirin appears to be absorbed intact from the gastrointestinal tract and persists in the blood for a short time in the unhydrolysed form; 10 minutes after ingestion 50% of the blood – salicylate is in the acetylated form ; after 20 minutes this dropped to 30% .

Normal therapeutic blood-level do not exceed 5% ; a dosage of 4 to 8 g daily will give a serum salicylate level of 18 to20 mg% but the high doses given in the treatment of rheumatoid arthritis may give rise to level 25 mg% . A single dose of 12 g may produce a level of 35 mg % together with symptom of acute poisoning within few hours .The rate of excretion of aspirin varies with pH of the urine and increased as the pH rises . One and half hour after ingestion 1.5 % of the dose may be found unchanged in the urine .The reminder is hydrolyzed to salicylic acid which is excreted partly unchanged , partly as glucuronide conjugates .

Doses

Usually up to 1 g ; in acute rheumatism, up to 8 g daily

Toxicity

The estimated lethal dose (LD50) in man is in the range of 5 to 15 g .A serum salicylate level above 30% is usually indicative of poisoning by aspirin . An important toxic effect which may occur even with small doses is irritation of the gastric mucosa leading to gastro-duodenal bleeding . The oral LD 50 of aspirin was 1.2 mg/Kg for mice and rats. In a case study it was pointed out that a 59 year old man estimated that within 15 days he had taken 227 g of aspirin; one examination on the 15th day ,the serum salicylate level was 61 % [121,122]

1-3-5 : Reference Range :

Timing of serum samples: Peak levels usually occur 2 hours after ingestion. Salicylate serum concentrations correlating with the pharmacological actions and adverse was effects observed. The serum salicylate concentration (µg/ml) and the corresponding clinical correlations are as follows [123]

Serum Salicylate: Clinical Correlations		
Serum Salicylate Concentration (µg/mL)	**Desired Effects**	**Adverse Effects/Intoxication**
~100	• Antiplatelet • Antipyresis • Analgesia	GI intolerance and bleeding, hypersensitivity, hemostatic defects
150-300	Anti-inflammatory	Mild salicylism
250-400	Treatment of rheumatic fever	Nausea/vomiting, hyperventilation, salicylism, flushing, sweating, thirst, headache, diarrhea, and tachycardia
>400-500		Respiratory alkalosis, hemorrhage, excitement, confusion, asterixis, pulmonary edema, convulsions, tetany, metabolic acidosis, fever, coma, cardiovascular collapse, renal and respiratory failure

1-4 : Enzymes as tools in clinical diagnosis :

Human serum contains a number of enzymes that modulate the majority of chemical changes in the body. The variation in their levels were suggested to be a great value in the clinical diagnosis .

Diagnosis from changes in serum level is possible only if any change specifically and sensitivity reflects damage in a particular organ . Because the presence of these enzymes in the serum indicates that tissue or cellular damage has occured resulting in the release of intracellular components into the blood [124] . Moreover , the method used in estimation of enzyme serum levels itself , must be specific and sensitive . Measurements , of serum enzyme have been shown to be a great value in diagnosis of deferent diseases,[125] for example the amylase enzyme has been increaseed in pancareatitis disease, while the peptidase has been increased in serum in shock state fever , tarmac injury and hemolytic anemia[126]

The typical "liver enzymes" measured are AST (aspartate aminotransferase), AST (also referred to as serum glutamate-oxaloacetate aminotransferase, s-GOT) and ALT (amino transferases: alanine transaminase, (sometimes still referred to as serum glutamate-pyruvate aminotransferase, s-GPT). ALT is particulary diagnostic of liver involvement as this enzyme is found predominantly in hepatocytes. When assaying for both ALT and AST the ratio of the level of these two enzymes can also be diagnostic. Normally in liver disease or damage that is not of viral origin the ratio of ALT/AST is less than 1. However, with viral hepatitis the ALT/AST ratio will be greater than 1. Measurement of AST is useful not only for liver involvement but also for heart disease or damage [127].

The measurement of LDH (lactate dehydrogenase) is especially diagnostic for myocardial infarction because this enzyme exists in 5 closely related, but slightly different forms (isozymes). The 5 types and their normal distribution and levels in non-disease/injury are listed below. [128]

LDH 1 - Found in heart and red-blood cells as the range 17% - 27% of the total normal serum.
LDH 2 - Found in heart and red-blood cells as the range 27% - 37% of the total normal serum.
LDH 3 - Found in a variety of organs and is 18% - 25% of the total

normal serum .
LDH 4 - Found in a variety of organs as the range 3% - 8% of the total normal serum .
LDH 5 - Found in liver and skeletal muscle as the range 0% - 5% of total the normal serum .

Especially diagnostic is a comparison of the LDH-1/LDH-2 ratio. Normally, this ration is less than 1, a reversal of this ratio is referred to as a "flipped LDH.". Following an acute myocardial infarction the flipped LDH ratio will appear in 12-24 hours and is definitely present by 48 hours in over 80% of patients. It is important to know the fact that persons suffering chest pain due to angina only will not likely to have altered LDH levels [128].

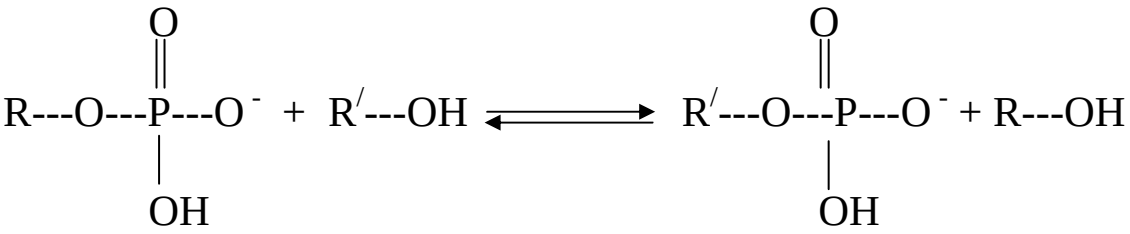
Creatine kinase, CK (also called creatine phosphokinase, CPK); is found primarily in heart and skeletal muscle as well as the brain. Therefore, measurement of serum CPK levels is a good diagnostic for injury to these tissues. [129]

1-4-1 : Alkaline Phosphatase (ALP) and acid phosphatase (ACP):

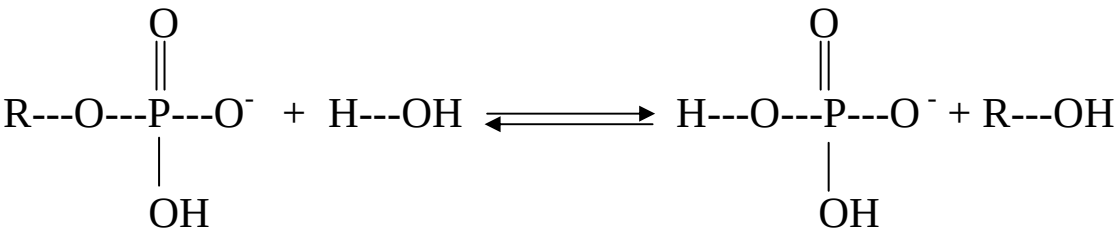
Great interest has been focused on ALP orthophosphoric monoester phosphohydrolase (Ec.3.1.3.1) and acid phosphatase (Ec.3.1.3.2) which is a group of enzymes that catalyzes the hydrolysis of some of organic ester at an alkaline and acid pH ,with the liberation of inorganic phosphate and an organic radical [130] .

The activity of ALP and ACP has been elevated in the bone and liver diseases such as paget's disease ,rickets , ostemalacia , carcinoma with osteoblastic metastases and malignant disease [131] .

Phosphatase enzymes transfer a phosphate moiety [132,133] from one group to another forming an alcohol and a second phosphate compound .



When water is the phosphate acceptor , inorganic phosphate is formed :



This may indicate that two hydroxyl groups on the phosphate radical are needed for activity of ALP.

It is found that the ions (Mg^{+2},Mn^{+2},Co^{+2}) work as activators of ALP and ACP, therefore any substance such as EDTA works to eliminate these ions from the reaction will inhibit the enzyme and block the reaction [134] .

There are many functions for these enzymes , bone Phosphatase participates in ossification process by precipitation calcium phosphate forming large quantities of inorganic phosphate (pi) ,or the enzyme helps in the process of the crystal growth by removing the diphosphate (ppi) which is considered crystal growth inhibitor [135] . Therefore any change of calcium precipitation velocity will cause a change in enzyme activity in the serum , this can be observe in the paget's disease in normal children [136,137].Also this enzyme plays an important role in the process of absorption,transfer of nutrients through epithelial membrane. [138,139]

1-4-2 :Acetyl Choline esterase (AChE) :

Cholinesterase is one of many important enzymes needed for the proper functioning of the nervous systems of humans, other vertebrates, and insects. Certain chemical classes of pesticides, such as organophosphates (OPs) and carbamates (CMs) work against undesirable bugs by interfering with, or 'inhibiting' cholinesterase. While the effects of cholinesterase inhibiting products are intended

for insect pests, these chemicals can also be poisonous, or toxic, to humans in some situations.Human exposure to cholinesterase inhibiting chemicals can result from inhalation, ingestion, or eye or skin contact during the manufacture, mixing, or applications of these pesticides [157].

Electrical switching centers, called 'synapses' are found throughout the nervous systems of humans, other vertebrates, and insects. Muscles, glands, and nerve fibers called 'neurons' are stimulated or inhibited by the constant firing of signals across these synapses. Stimulating signals are usually carried by a chemical called 'acetylcholine'. Stimulating signals are discontinued by a specific type of cholinesterase enzyme, acetylcholinesterase, which breaks down the acetylcholine. These important chemical reactions are usually going on all the time at a very fast rate, with acetylcholine causing stimulation and acetylcholinesterase ending the signal. If cholinesterase-affecting insecticides are presentin the synapses, however, this situation is thrown out of balance. The presence of cholinesterase inhibiting chemicals prevents the breakdown of acetylcholine. Acetylcholine can then build up, causing a "jam" in the nervous system. Thus, when a person receives to great an exposure to cholinesterase inhibiting compounds, the body is unable to break down the acetylcholine[158].

Let us look at a typical synapse in the body's nervous system, in which a muscle is being directed by a nerve to move. An electrical signal, or nerve impulse, is conducted by acetylcholine across the junction between the nerve and the muscle (the synapse) stimulating the muscle to move. Normally, after the appropriate response is accomplished, cholinesterase is released which breaks down the acetylcholine terminating the stimulation of the muscle. The enzyme acetylcholine esterase accomplishes this by chemically breaking the compound into other compounds and removing them from the nerve junction. If acetyl cholinesterase is unable to breakdown or remove acetylcholine, the muscle can continue to move uncontrollably.

Electrical impulses can fire away continuously unless the number of messages being sent through the synapse is limited by the action

of cholinesterase. Repeated and unchecked firing of electrical signals can cause uncontrolled, rapid twitching of some muscles, paralyzed breathing, convulsions, and in extreme cases, death. This is summarized below [159].

Exposure to:

- carbamates
- organophosphates
- chlorinated derivatives of nicotine

May result in:

- build-up of acetylcholine
- cholinesterase inhibition
- constant firing of electrical messages
- potential symptoms of: twitching, trembling, paralyzed breathing, convulsions, and in extreme cases, death.

1.4.3:Type of cholinesterase :

Humans have three types of cholinesterase: red blood cell (RBC) cholinesterase, called **"true cholinesterase;"** plasma cholinesterase, called **"pseudo cholinesterase;"** and **brain cholinesterase**. Red blood cell cholinesterase is the same enzyme that is found in the nervous system, while plasma cholinesterase is made in the liver.

When a cholinesterase blood test is taken, two types of cholinesterase can be detected. Physicians find plasma cholinesterase readings helpful for detecting the early, acute effects of organophosphate poisoning, while red blood cell readings are useful in evaluating long-term, or chronic, exposure.

The cholinesterase test is a blood test used to measure the effect of exposure to certain or cholinesterase-affected insecticides. Both plasma (or serum) and red blood cell (RBC) cholinesterase should be tested. These two tests have different meanings and the combined report is needed by the physician for a complete understanding of the individual's particular cholinesterase situation. Laboratory methods for cholinesterase testing differ greatly, and

results obtained by one method cannot be easily compared with results obtained by another. Sometimes there is also considerable variation in test results between laboratories using the same testing method. Whenever possible, cholinesterase monitoring for an individual should be performed in the same laboratory, using a consistent testing method [160].

The aim of the study :

1- Synthesis of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid : (19)
2- Synthesis of 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20)
3- Synthesis of 2,3,5,6-Tetra(acetyl salicyloyl)-*L*-ascorbic acid : (21)
4- Drug released study : Hydrolysis of the compounds (19, 20, 21) in different pH (2, 4, 6, 8, 10, 12).
5- Effect of compounds (19, 20, 21) on acetyl choline esterase (AChE) activity.
6- Effect of compounds (19, 20, 21) on Alkaline phosphatase (ALP) activity.
7- Effect of compounds (19, 20, 21) on Acid phosphatase (ACP) activity.
8- Effect of compounds (19, 20, 21) on different type of bacteria.
9- In vivo study (lab. Animal system).

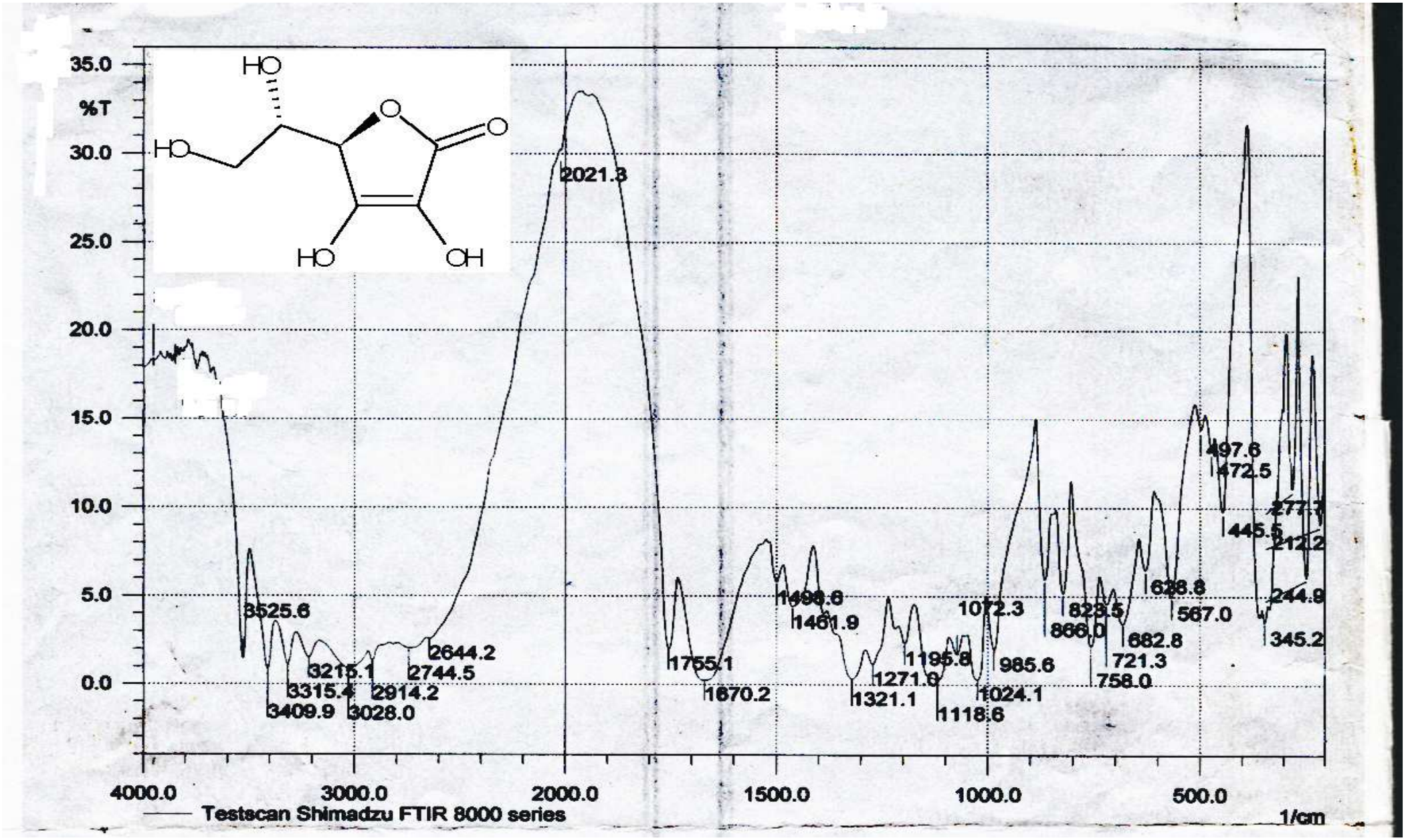


Figure (1) IR spectrum of Vitamin C (8)

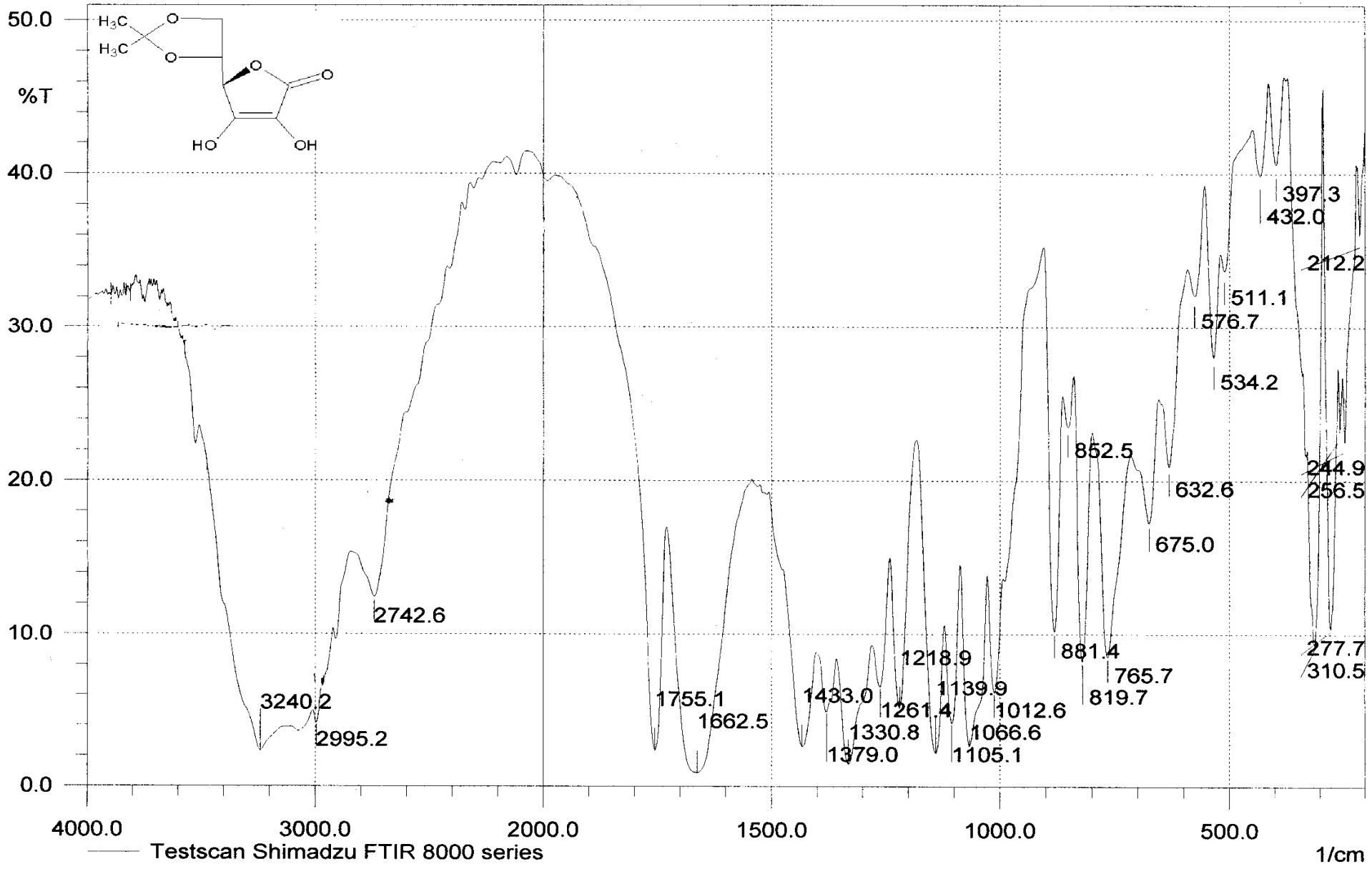


Figure (2) IR spectrum of 5,6-O-isopropylidene-L-ascorbic acid (10)

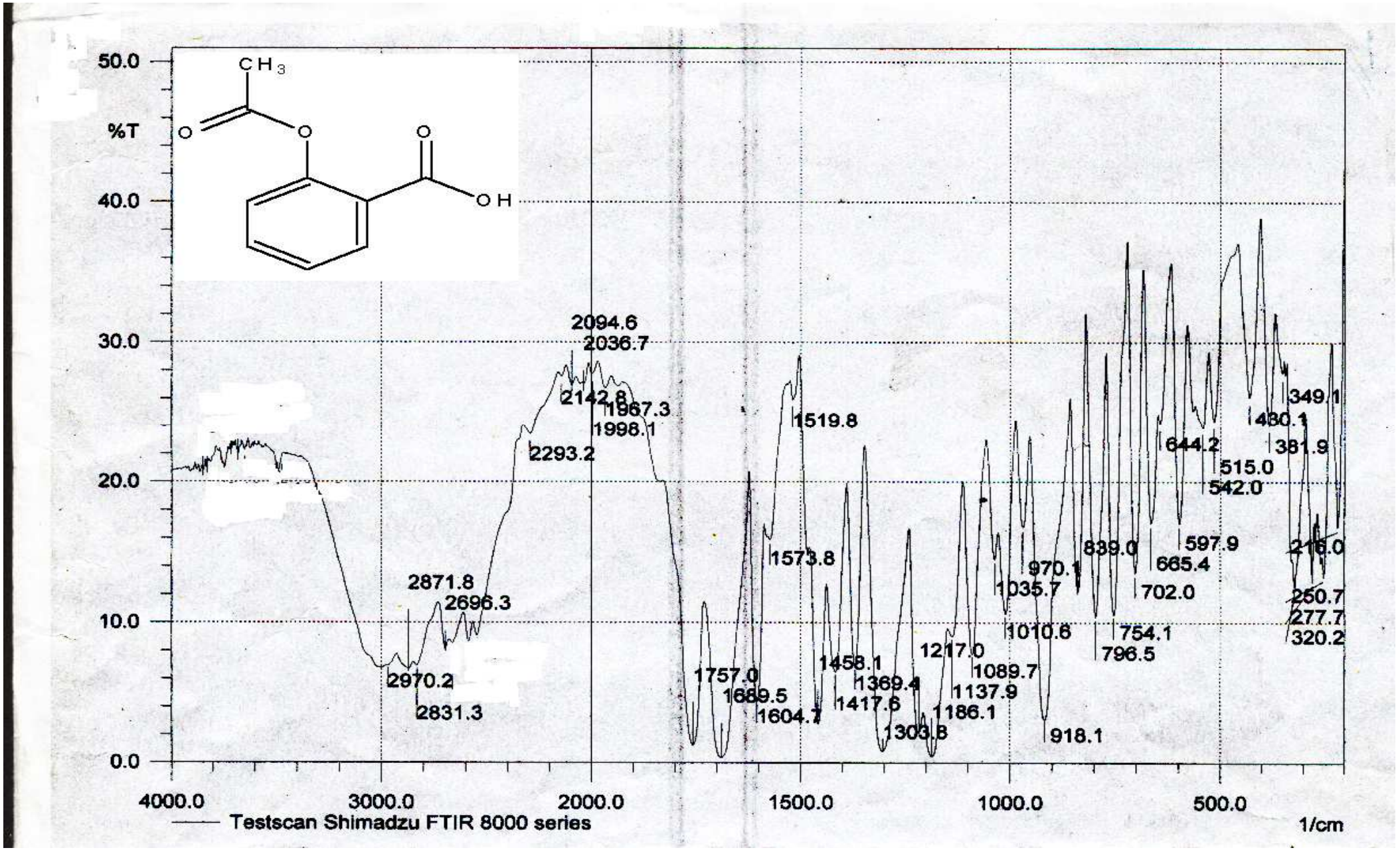


Figure (3) IR spectrum of Acetyl Salicylic acid (17)

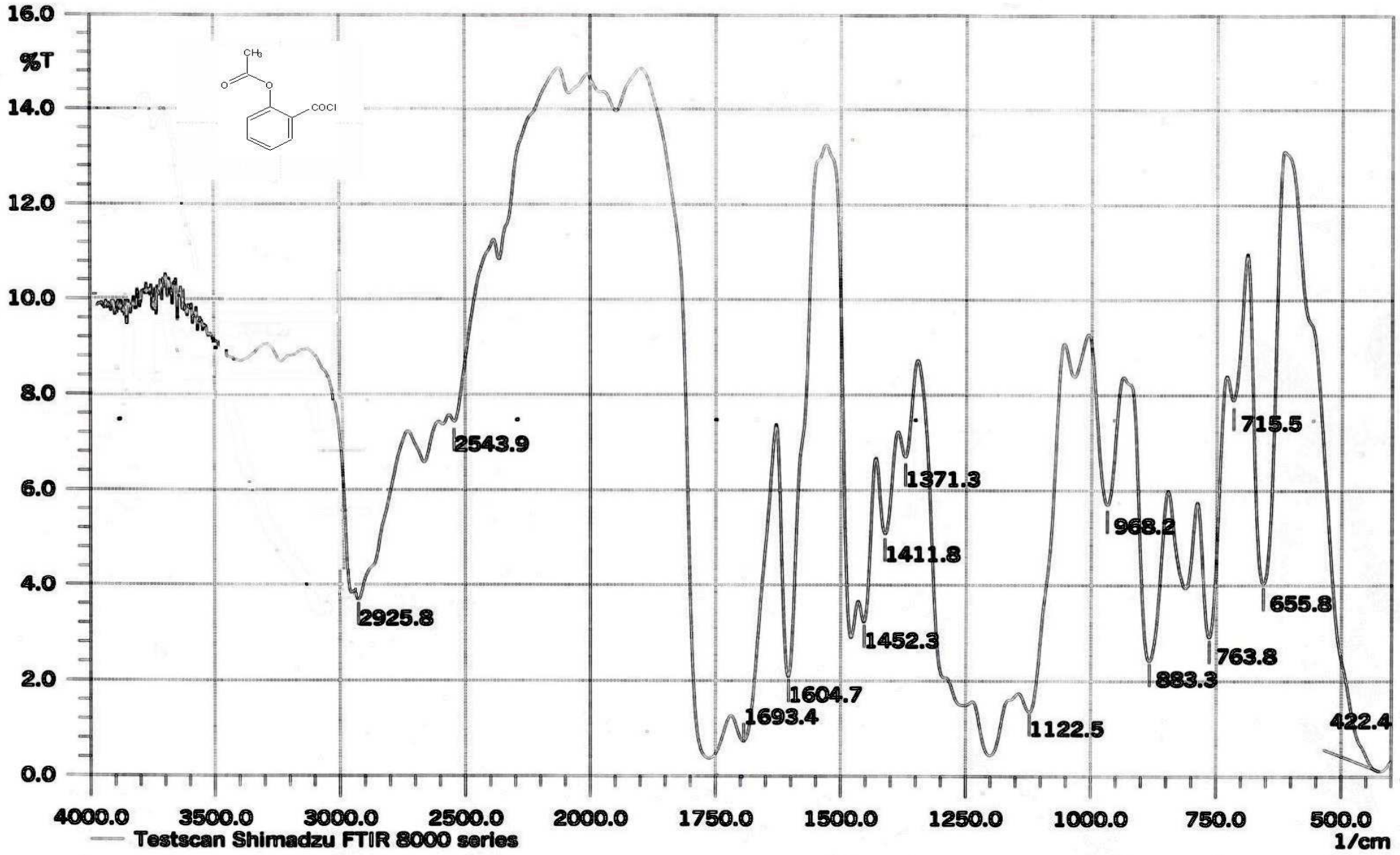


Figure (4) IR spectrum of Acetyl Salicyloyl Chloride (18)

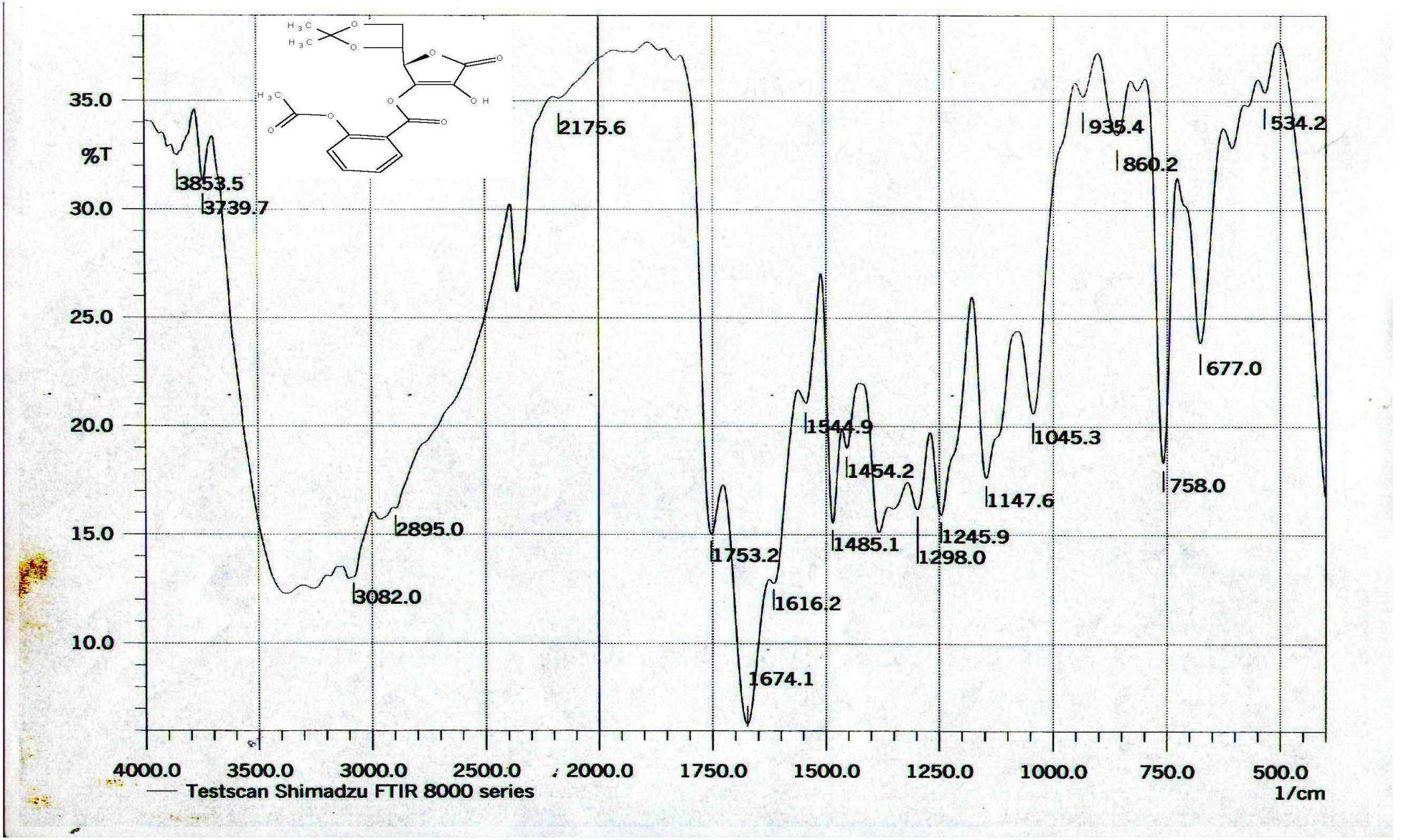


Figure (5) IR spectrum of 3- (acetyl salicyloyl)-5,6-O-isopropylidene- L-ascorbic acid (19)

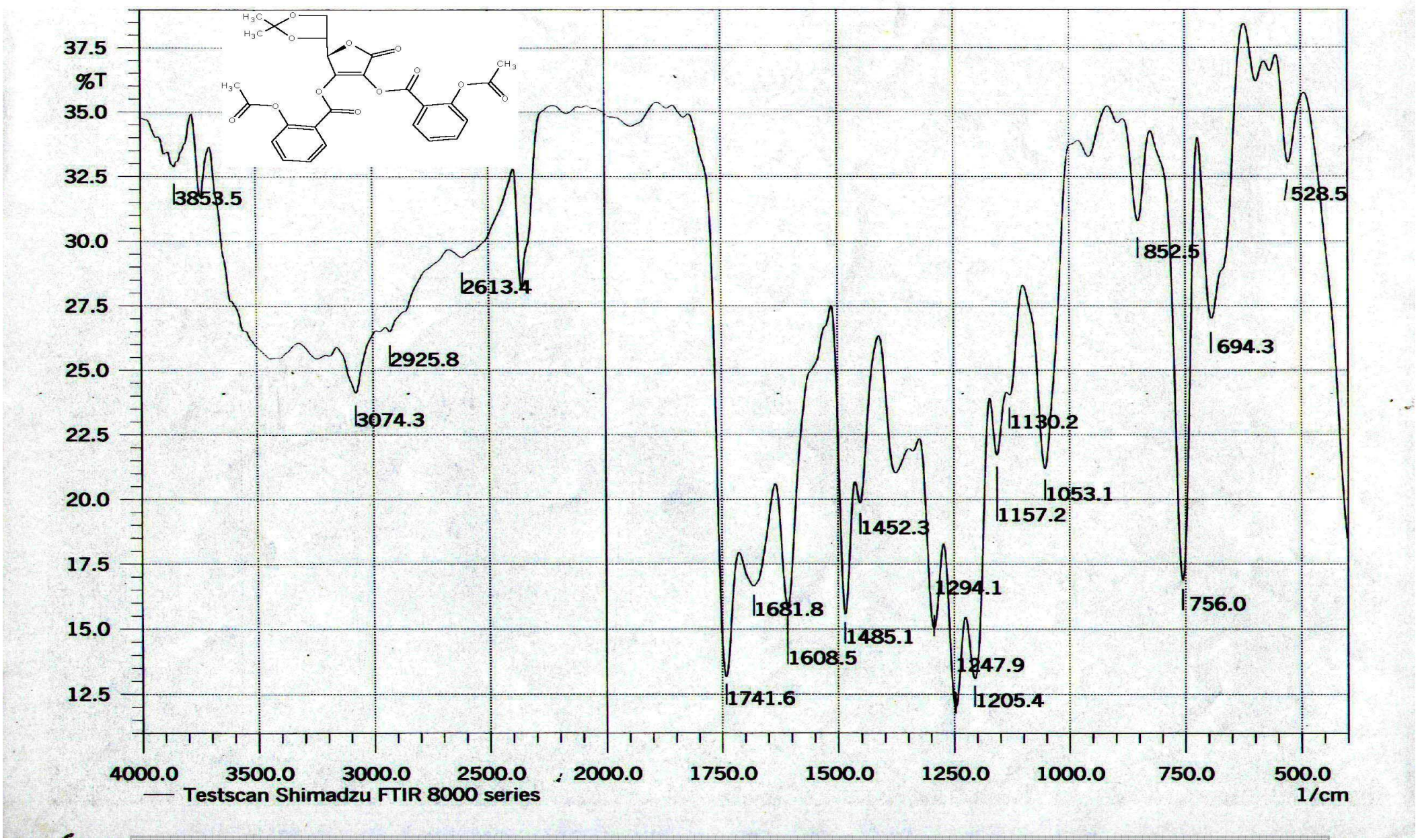


Figure (6) IR spectrum of 2,3-di (acetyl salicyloyl)-5,6-O-isopropylidene- L-ascorbic acid (20)

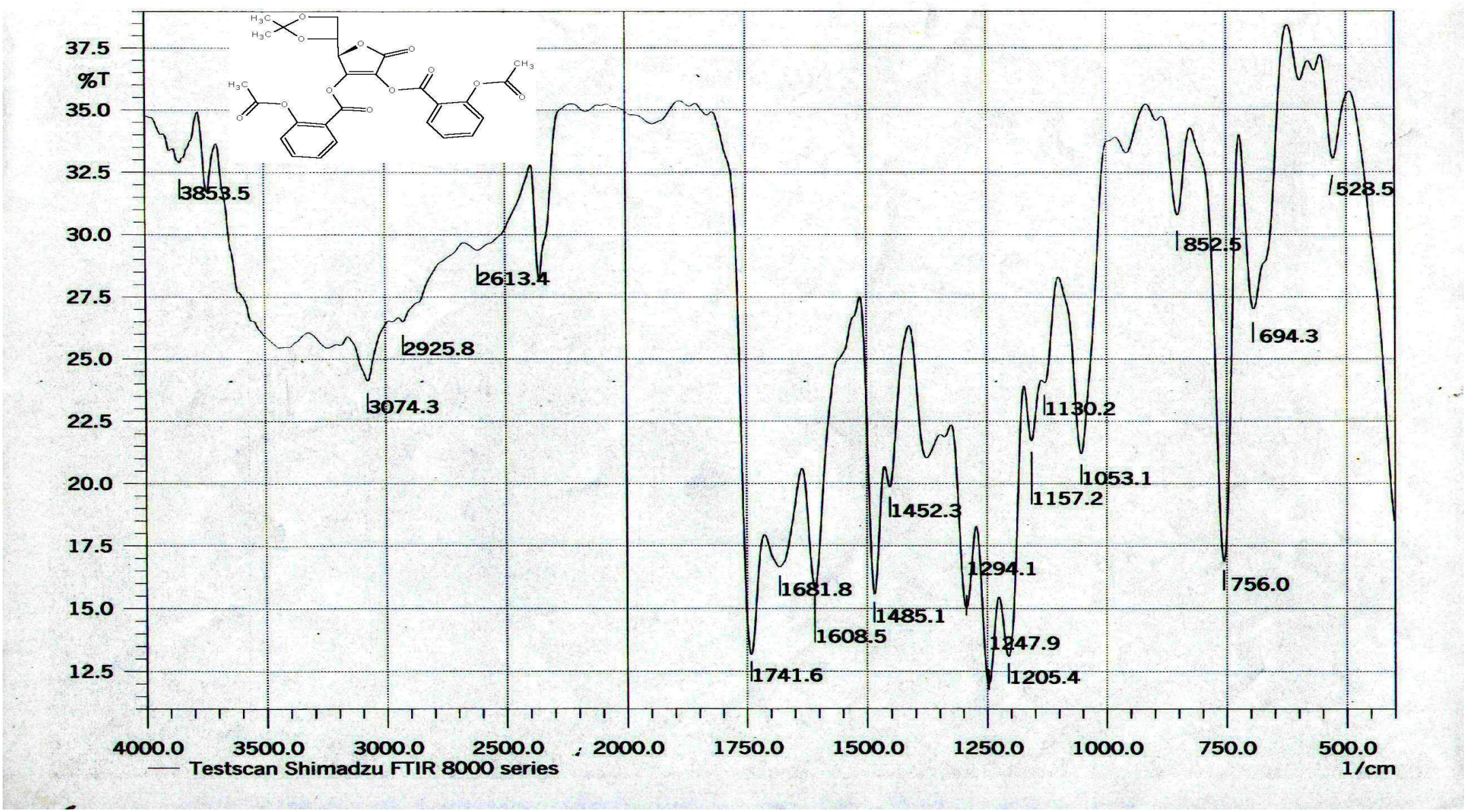


Figure (7) IR spectrum of 2,3,5,6-Tetra (acetyl salicyloyl)-L-ascorbic acid (21)

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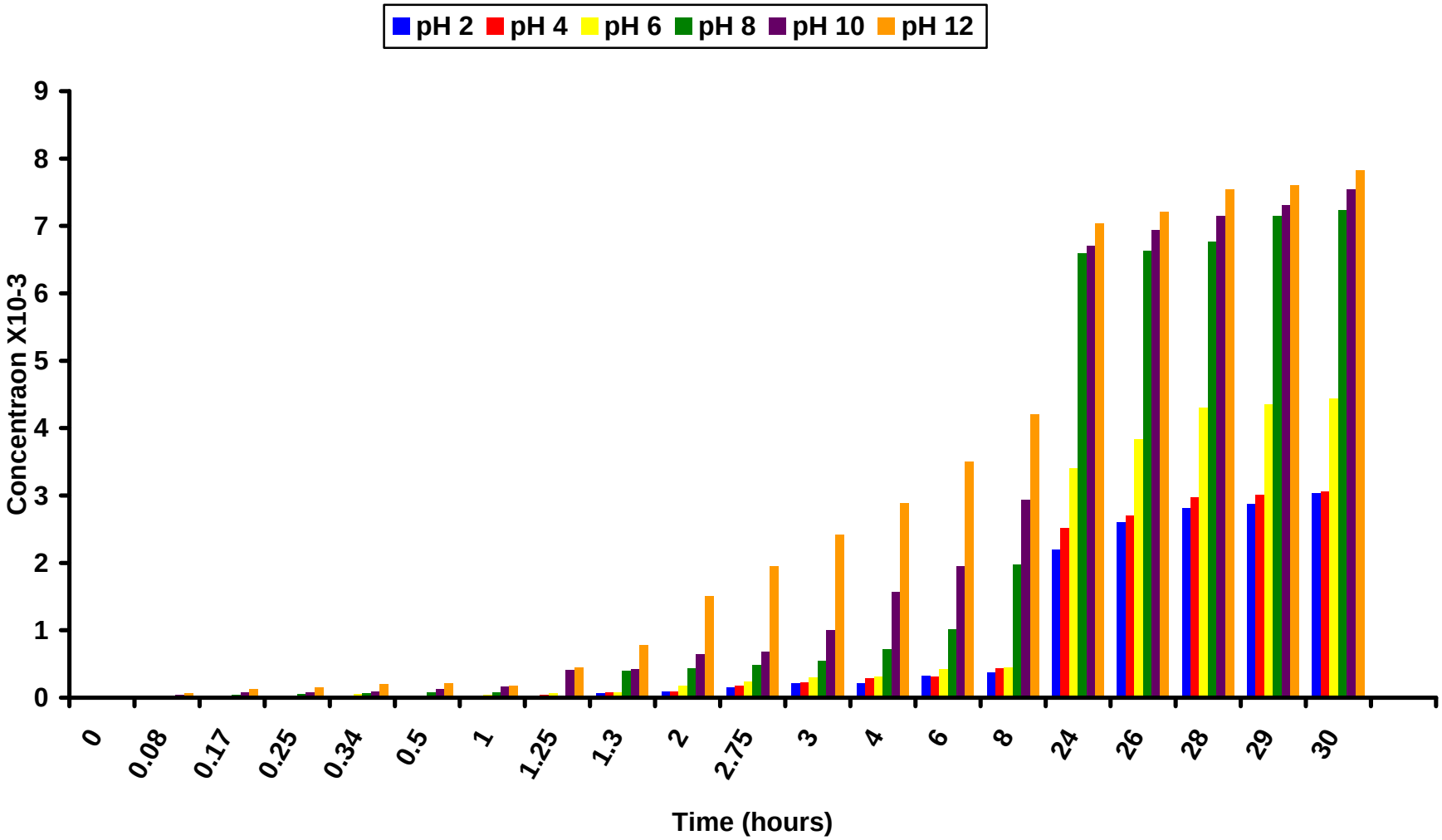

Figure (26) Concentration of aspirin released of compounds (19) in pH (2 , 4 ,6, 8 ,10 ,12)

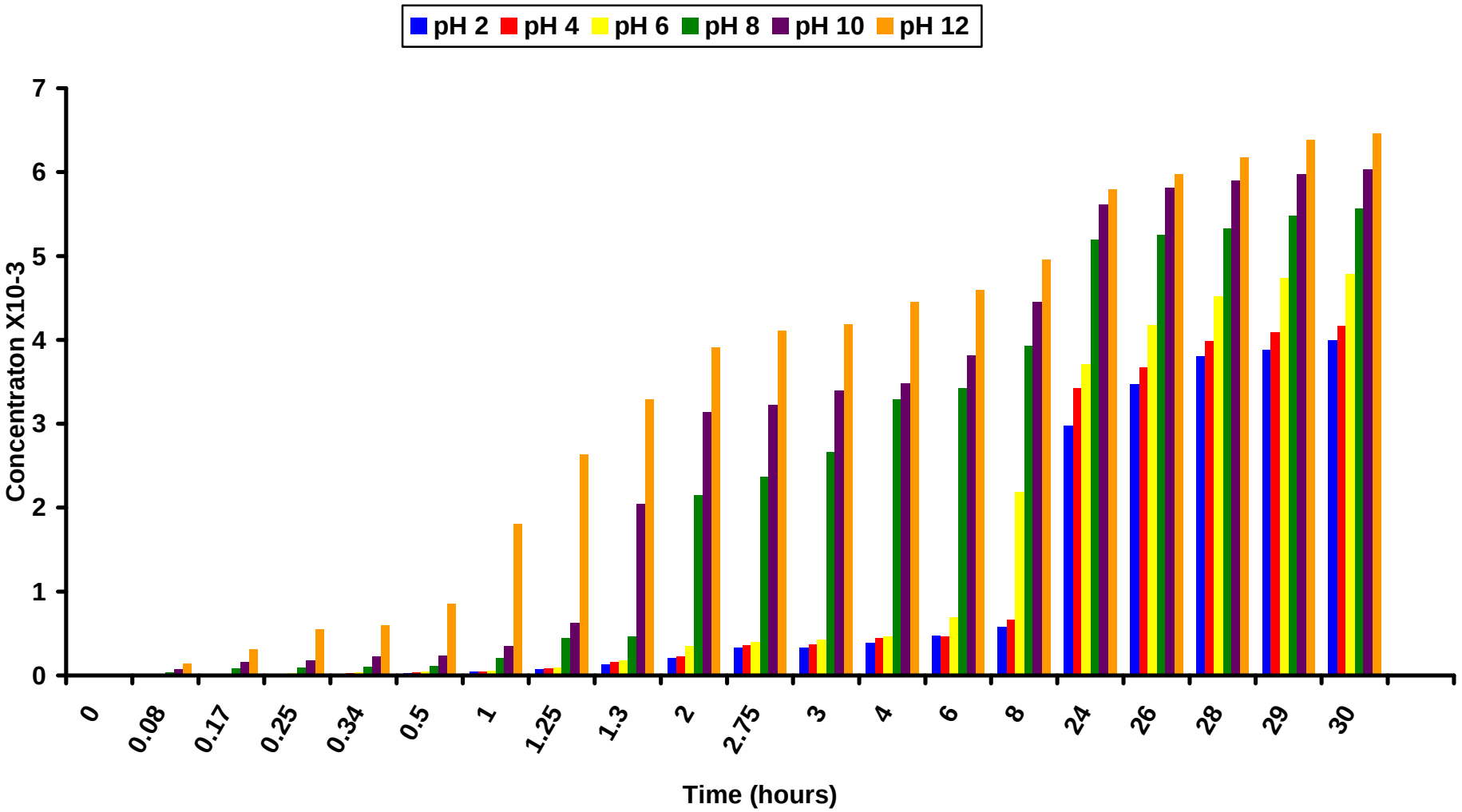

Figure (27) Concentration of aspirin released of compounds (20) in pH (2 , 4 ,6, 8 ,10 ,12)

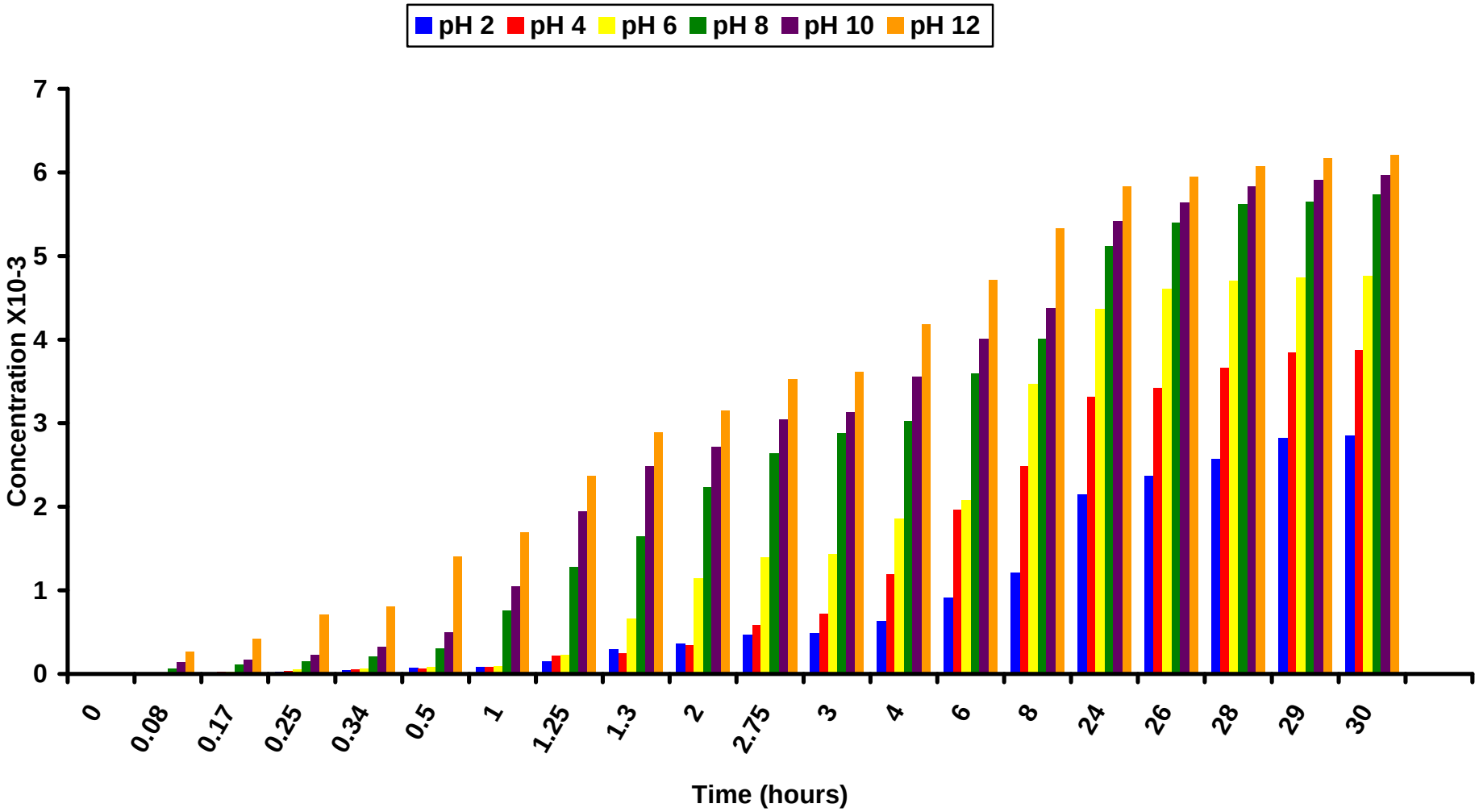

Figure (28) Concentration of aspirin released of compounds (21) in pH (2 , 4 ,6, 8 ,10 ,12)

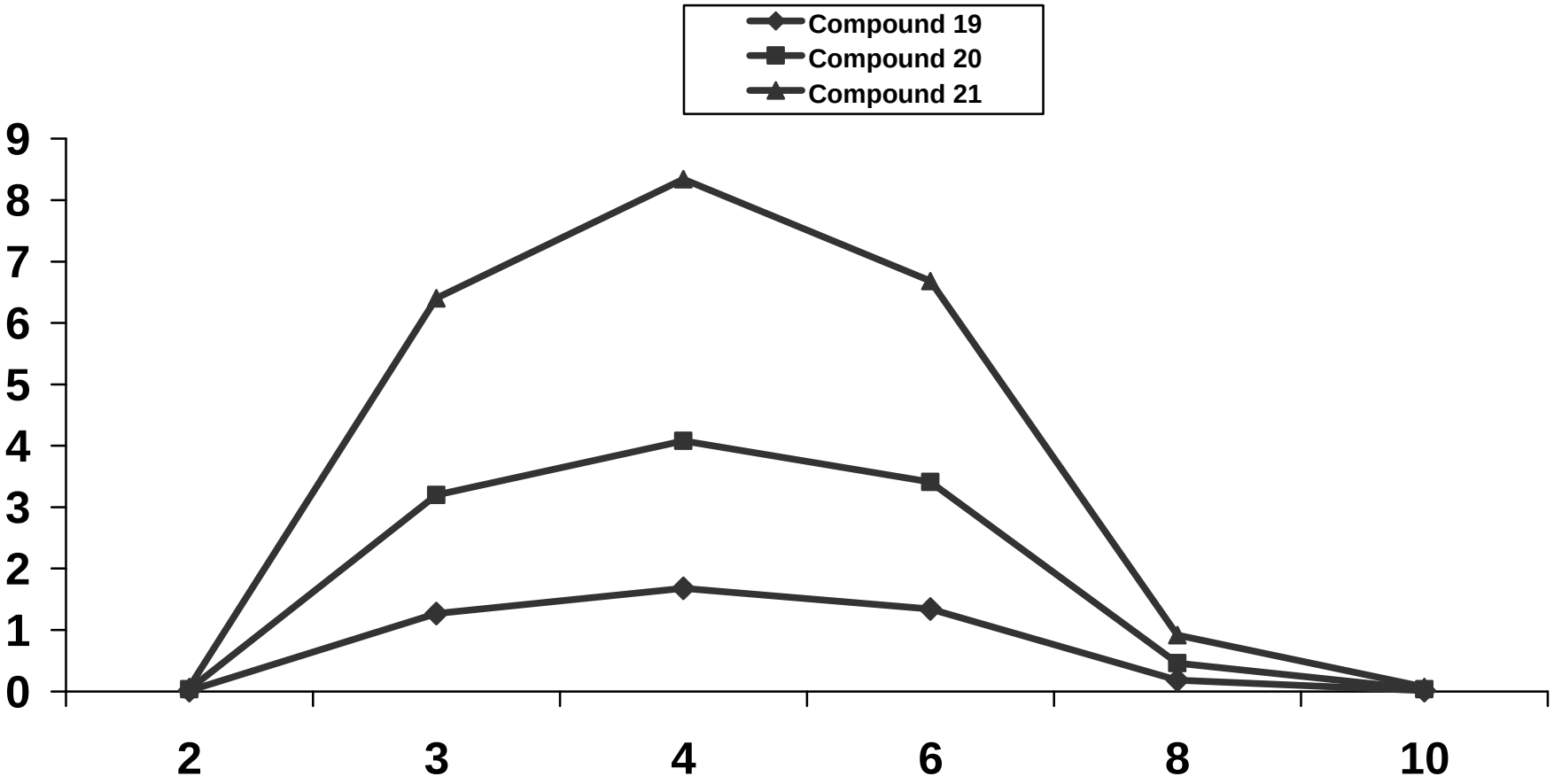

Figure (3٢) concentration of aspirin in rabbits blood serum [compounds (19),(20),(21)]

Recommended Future Studies :

1. More kinetic studies should be conducted in the relation to the rate of hydrolysis of the compounds in blood and with specific colon's enzyme .
2. Toxicity studies should carried out intensively , as acute and chronic exposure .
3. Testing the effect of these compounds on cancer cell.
4. Testing the effect of these compounds on cyclooxygenase activity.

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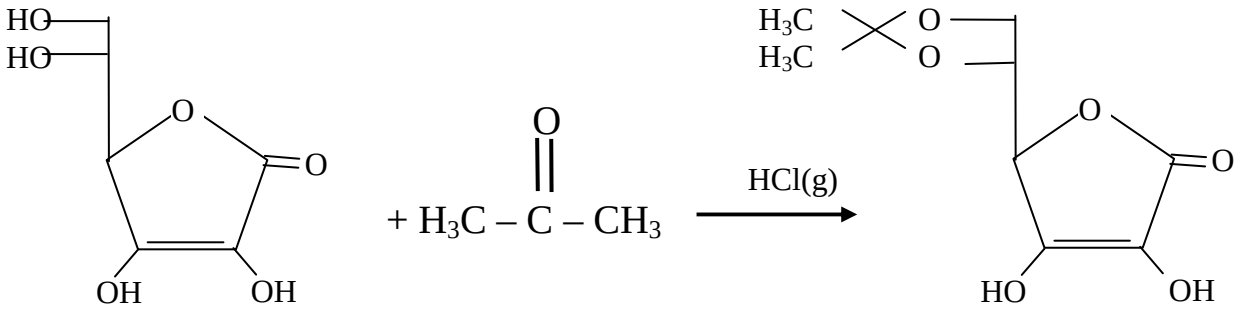
Results and Discussion

3.1: Synthesis of 5,6-O-isopropylidene-*L*-ascorbic acid (10)

L-ascorbic acid molecule has four hydroxyl groups, and all these groups are active for classical esterification and other reactions . Synthesis of L-ascorbic acid derivatives at 2 and 3-position need first conversion of L-ascorbic acid into its 5,6-isopropyliden derivative (10) because carbon - 6 hydroxyl group (a primary hydroxyl group) is the most reactive group [77,78] .

The acetal is stable toward alkaline conditions but it is readily hydrolyzed by dilute acid [80,149], hence it is very useful as blocking agent , and was used in this work to protect the hydroxyl group at **C- 5** and **C- 6** leaving the hydroxyl group at **C – 2** and **C-3** free for the required chemical modification . Accordingly , 5,6-O-isopropyldene-*L*-ascorbic acid (10) was prepared from the reaction of *L*-ascorbic acid (8)[IR Fig 1] with acetone in acidic media , according to the literature [61] .The IR spectrum of (10) show stretching band at : 3240 cm^{-1} for (O-H), 2995 cm^{-1} for (C-H aliphatic) (acetal linkage) , 1755 cm^{-1} for (C=O) lactone . [Fig. 2].



(8) (10)

3.2 : Synthesis of the acetyl salicyloyl chloride (18) :

The salicyloyl chloride (18) was prepared by reaction of thionyl chloride with o-acetyl salicylic acid (17) .

(17) (18)

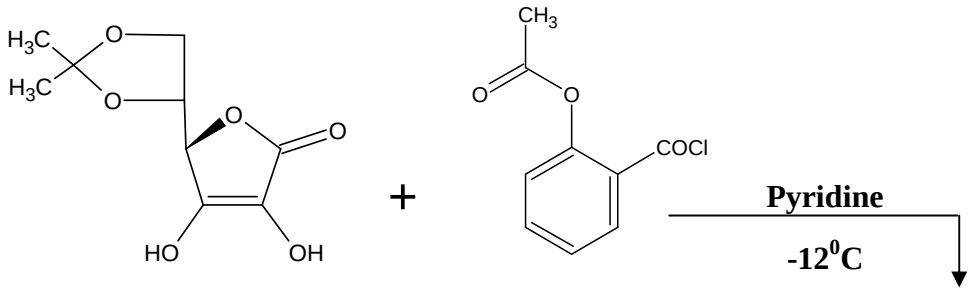
The IR spectrum of o-acetyl salicylic acid (17) showed stretching bands at 3150 cm^{-1} for (O-H) , and 1757 cm^{-1} for (C=O) of the carboxylic acid [Fig.3].

The IR spectrum for acetyl salicyloyl chloride (18) showed the stretching bands at 1755 cm^{-1} for (C=O) carboxylic acid chloride and 883 cm^{-1} for (C-Cl) , and disappearance of the stretching bands for (O-H) carboxylic acid .[Fig. 4].

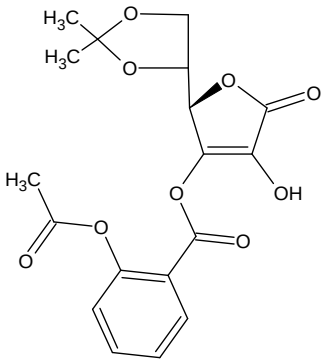
3.3:Synthesis of 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid : (19)

The acidic character of aspirin is the reason for stomach and small intestine irritation [1] . If the carboxyl hydrogen atom is replaced and converted to a pro-drug, this pro-drug may gave synergetic effect when it is hydrolysis in vivo . Vitamin C was chosen for this purpose because it is medically known to be "**virtually non toxic**"[17].

The reaction of (10) with acetyl salicyloyl chloride (18) in pyridine at (-12^0 C) for 24 hours gave 3-(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (19) as syrup 86.38 % yield, the IR spectrum of (19) showed stretching bands 3400-3082 (O-H), 2895 (C-H) aliphatic 1753 (C=O) ester , 1616,1485 (C=C) aromatic , 758 (C-H) out of plan . [Fig.5] .



10 18

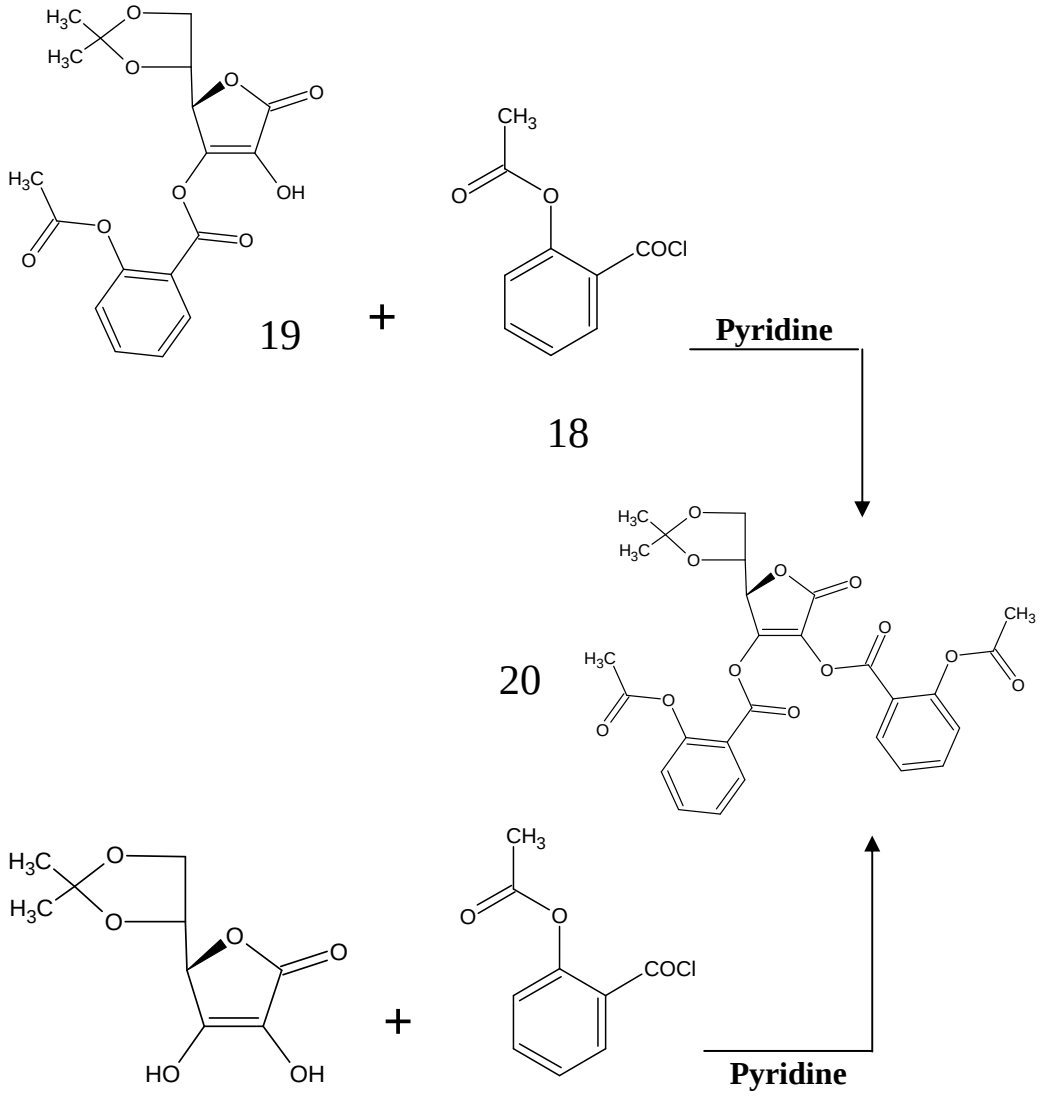


19

3.4:Synthesis of 2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid : (20)

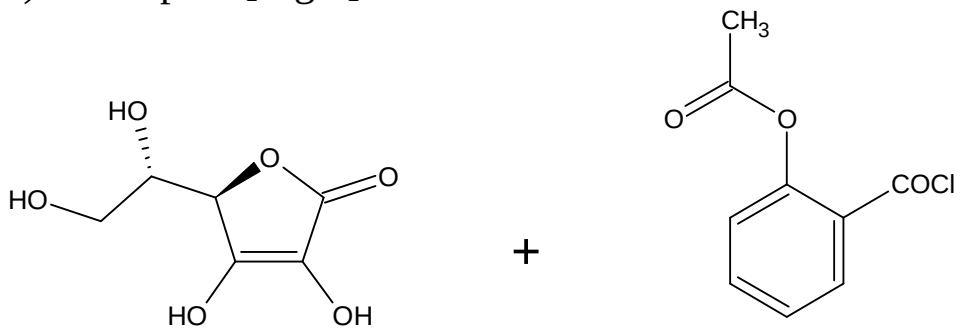
Treatment of (19) with acetyl salicyloyl chloride (18) in pyridine at room temperature for 24 hours gave 2,3-di (acetyl salicyloyl)-5,6 –O-isopropylidene-*L*-ascorbic acid (20) as semi solid in 81.63% yield (Reaction of 2 mole from (18) with 1 mole of 10 gave the same results) .

Hence the C-2 and C-3 hydroxyl groups of vitamin C have been substituted with acetyl salicyloyl group. The structure of (20) was characterized the IR spectrum which showed stretching bands at 2925 (C-H) aliphatic 1741 (C=O) ester , 1608,1485 (C=C) aromatic , 756 (C-H) out of plan [Fig. 6]



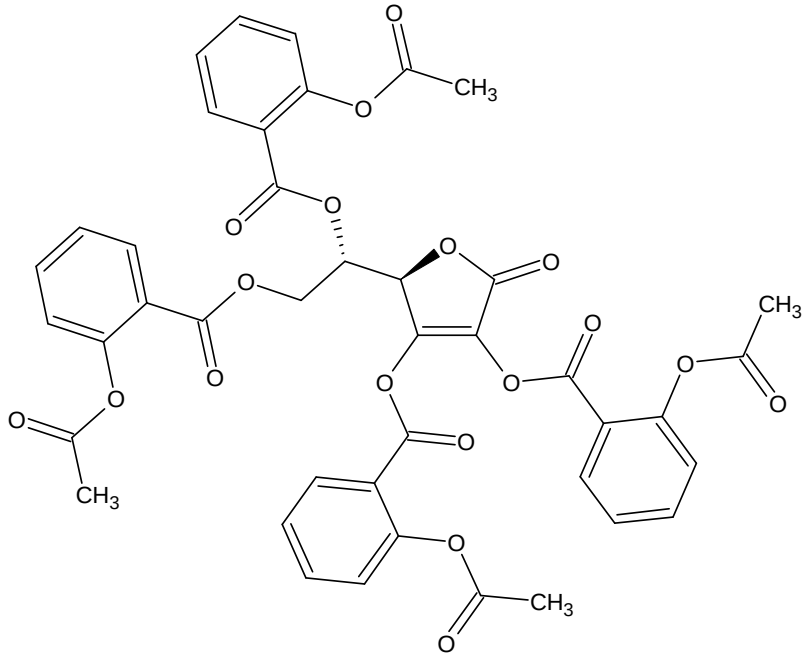
3.5 : Synthesis of 2,3,5,6 -tetra (acetyl salicyloyl)-*L*-ascorbic acid : (21)

The reaction of (8) with 4 mole acetyl salicyloyl chloride (18) in pyridine for 24 hours gave 2,3,5,6-tetra(acetyl salicyloyl)-*L*-ascorbic acid (21) as syrup 86.38 % yield the IR spectrum of (21) showed stretching bands at 2925 (C-H) aliphatic 1741 (C=O) ester , 1608,1485 (C=C) aromatic , 756 (C-H) out of plan [Fig.7] .



8 18

4 mole



21

3.6: Identification of compounds (19),(20),(21) by TLC:

The qualitative determination of compounds (19),(20),(21) was carried out using silica gel as stationary - phase for TLC while the mobile phase consist of a mixture of hydrocarbon carriers (toluene, chloroform dioxane or benzene) and polar organic modifiers (acetone, butanol, ethanol or acetic acid). The advantages in screening the sample by TLC prior to HPLC are the determination the contaminants that may absorb to the stationary phase in the HPLC column, and determination of solvent conditions necessary for a successful separation of compounds (19),(20),(21). The TLC plates were developed using UV lamp.

Table (2) physical properties for derivatives

Comp. No.	Formula	Physical state	R_f.	Yield%
8	$C_6H_8O_6$	White crystals	0	
10	$C_9H_8O_6$	White crystals	0.02	92%
17	$C_9H_8O_4$	White crystals	0.183	
19	$C_{18}H_{18}O_9$	Brown crystal	0.056	86.3%
20	$C_{27}H_{24}O_{12}$	Semi solid	0.718	81.6%
21	$C_{42}H_{32}O_{18}$	Brown crystal	0.934	84.38%

Mobile phase is (Benzen : ether) (8:2)

SUMMARY

In this work new derivatives of *L*-ascorbic acid (Vitamin C) have been stynthezized . These derivatives have been obtained by the esterfication .

Selective esterfication of *C*-3 and *C*-2 hydroxyl group required protecting the two hydroxyl group at 5,6-position by conversion of vitamin C to 5,6-*O*-isopropylidene derivative (10)

3- (acetyl salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid (19) was synthesized by treatment of (10) with acetyl salicyloyl Chloride (18) .

2,3-di(acetyl salicyloyl)-5,6 –O-isopropylidene-L-ascorbic acid (20) was synthesized by treatment of (10) with two moles of acetyl salicyloyl Chloride (18) or treatment of (19) with one mole of acetyl salicyloyl Chloride (18).

2,3,5,6 -Tetra (acetyl salicyloyl)-5,6-*L*-ascorbic acid (21) was synthesized by treatment of vitamin C with four moles of acetyl salicyloyl Chloride (18)

The purity of the compounds were characterized by thin later chromatography (TLC) and infrared spectroscopy (IR).

The drug released study for hydrolysis of compounds (19,20,21) was carried out using different buffer at pH (2,4,6,8,10,12) over 30 hours at different intervals using UV spectroscopy which showed that these compounds were hydrophobic , but a period of 8-13h post starting to be hydrophilic . The released aspirin was increased as the pH is increased and extended time

The biochemical tests revealed that the evaluated compounds (19,20,21) showed non competitive inhibition behavior to the activity of acetyl choline esterase (AChE)

enzyme while these compounds showed an activation on alkaline phosphatase and acid phosphatase enzymes activity .

Bacterial inhibition zone revealed a positive inhibitory impact .

The *In vivo* study was carried out by (1.5 g) oral administration of the three compounds (19,20,21) and measurement of aspirin concentration in rabbits blood after (2,3,4,6,8,10) hrs. showing that the highest aspirin concentration was found after 4 hrs. of administration .

الخلاصة

تم في هذه الدراسة تخليق عدد من المشتقات الجديدة لحامض الاسكوربيك (فيتامين C) عن طريق تفاعل الاسترة .
تطلب دخول الموقع (٣) تحوير الموقعين (٥،٦) الى مشتق الايزوبروبلدين (١٠) .

تم الحصول على حامض ٣-احادي اسيتايل ساليسلويليل -٦،٥ –O- ايزوبروبلدين –L-اسكوربيك (١٩) من تفاعل مشتق الايزوبروبلدين (١٠) مع كلوريد الاسيتايل سالسيلويل (١٨)

تم الحصول على حامض ٣،٢-ثنائي اسيتايل ساليسلويليل -٦،٥ –O- ايزوبروبلدين –L-اسكوربيك (٢٠) من تفاعل مشتق الايزوبروبلدين (١٠) مع مولين من كلوريد الاسيتايل سالسيلويل (١٨) . او عن طريق مفاعلة مول واحد من كلوريد الاسيتايل سالسيلويل (١٨) مع حامض ٣-احادي اسيتايل ساليسلويليل -٦،٥ –O-ايزوبروبلدين –L-اسكوربيك (١٩) .

تم الحصول على حامض ٦،٥،٣،٢-رباعي اسيتايل ساليسلويليل L-اسكوربيك (٢١) وذلك بمفاعلة حامض الاسكوربيك مع اربعة مولات من كلوريد الاسيتايل سالسيلويل (١٨)

تم تشخيص المركبات بكروموتكرافيا الطبقة الرقيقة (TLC) وطيف الاشعة تحت الحمراء (IR) .

تم دراسة تحرر الاسبرين من المركبات الثلاثة(١٩،٢٠،٢١) بأستخدام جهاز الاشعة فوق البنفسجية (UV) وبأستخدام محاليل منظمة مختلفة باس هيدروجيني مختلف (pH) (٢،٤،٦،٨،١٠،١٢) وخلال فترة ٣٠ ساعة وجد من خلال هذه الدراسة ان المركبات المحضرة تكون كاره للماء ولكن بعد مرور ٨-١٣ ساعة يتحول الى مركي محب للماء كما لوحظ ان تفكك المركب الى الاسبرين وفيتامين C يزداد بزيادة قاعدية المحلول المنظم وزيادة الوقت .
تم قياس فعالية المركبات الثلاثة (١٩،٢٠،٢١) على فعالية انزيم الكولين استريز والفوسفاتيز القاعدي والحامضي حيث اظهرت النتائج ان المركبات تظهر تأثيراً مثبطاً غير تنافسي على فعالية انزيم الكولين استريز بينما اظهرت تأثيره منشطا على فعالية انزيمات الفوسفتيز القاعدي والحامضي ويتناسب التثبيط والتنشيط مع التؤكيز المستخدم تناسبا طرديا .

اعتمدت بعض التجارب البابولوجية للمركبات (١٩،٢٠،٢١) على خمسة انواع من البكتريا حيث كانت مثبطة لاغلب انواع البكتريا ويتناسب التثبيط مع التركيز المستخدم تناسبا طرديا .

اجريت دراسة في داخل جسم الكائن الحي (*Invivo*) وقياس تركيز الاسبرين في مصل الدم بعد مرور (٢،٣،٤،٦،٨،١٠) ساعات من اعطاء الحيوان الجرعة حيث اظهرت النتائج ان اعلى تركيز للاسبرين كان بعد اربع من اعطاء الحيوان الجرعة وهذا يتطابق مع المراجع العلمية .